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RESEARCH ARTICLE

# Geospatial Green Cover Assessment for Chennai using Cartosat data in Changing Climate Scenario

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## ABSTRACT

Urbanization is an index of transformation from traditional rural economies to modern industrial area. It is a progressive congregation and dwelling of population in urban unit. In India, Tamil Nadu is one among the fast developing state that needs attention in the planning and development of urban areas such as smart cities in intention of (urban space index). Remote sensing and geospatial tools includes satellite based systems together with GIS enable a planner to spatially map the urban region inn near real time condition and provide management and decision support system for urban planning. Chennai Corporation the capital of Tamil Nadu and one of the major metropolitan cities, in India expanding horizontal and vertical in the recent years. An initiative was taken up to map thematic layers such as tree cover, crown area estimation, buildings, Temples, parks and water bodies of Chennai Corporation using high resolution Cartosat-1 satellite data and cross verify with google earth data. Visual interpretation techniques were employed to delineate spatial features in the form of Point, line and polygon from pan chromatic satellite data. Thematic maps on green cover, built up land, water bodies, temples, parks and other regions was derived. Ground truth Information of selected locations was taken up of field verification. Results of the analysis exhibited that Chennai has a built up cover of 52% and 16 % of green cover. An additional other area of 22 % can be taken for improving the green cover of Chennai city in

order to overcome changing climate scenario such as frequent floods and droughts. This study observed a close relationship of thematic boundaries acquired from satellite data /Google earth.

**Keywords:** Chennai Corporation, Green cover, Crown Area, Remote Sensing, GIS.

## INTRODUCTION

Urbanization is one of the most evident global land use land cover changes. During the last 200 years, the world population has increased six times, whereas urban populations have increased to over 100 times their original count (Leao et al., 2004). Nearly 50% of the world's population lives in urban areas especially all along the coastal regions. The urban population of India has rapidly increased in recent years due to industrialization. Frequent droughts in rural region forced peasants to migrate from rural to urban for new avenues on the account of socio economic development. Landuse /land cover changes frequently in urban region /semi urban region especially due development activities (Nethaji Mariappan, V.E. et al., 2010). Establishment of Special Economic Zones (SEZ) has lead to urban sprawl of Kanchipuram district in Tamil Nadu (Nethaji Mariappan, V.E. & Mohana P., 2011; Mohsen et al., 2104). Therefore unbalanced urban sprawl is a serious threat for sustainable urban development. Urban development lead to the encroachment of all water bodies as slums & for the development of urban infrastructure reduces the rain water carriage capacity of the few existing water ways (Lavanya, 2012).

Urban green spaces are defined as public and private open spaces in urban areas, primarily covered by vegetation which is directly or indirectly available for the user to combat pollution in urban region (Manlun, 2003). Green spaces are usually available at parks, gardens, institution, Industries, schools and open areas of public property. Green spaces play a major role in urban areas through their environmental, aesthetic, social and economic contributions to residents' health and well being. Such green spaces plays a vital role on the sustaining the urban temperature, pollution and ecological balance of urban region. In this context, assessing urban green space by field is time consuming and laborious.

Remote sensing is a new technology that can gather information on a target without coming in direct contact, and usually refers to the acquisition and processing of information on the earth's surface. Satellite data of the study site is utilized for deriving green space. Land use pattern changes using remotely sensed data in a time domain manner is for comparison of sequential changes in land use/land cover of urban environment Sanem et al., (2010); Deng et al. 2009; Jothimani, 1997. Visual interpretation is one of the successful methods of delineating spatial features of the earth by a geospatial expert. From this perspective, green space planning should be an integral part of any urban development or remodelling endeavour. GIS tools are necessitated for enumerating themes on built up land, tree cover, crown cover, water bodies, park and temples. Spatial integration of all the themes provides useful information on urban discomfort index of any region. Therefore purpose of this study is to ascertain the tree cover area of Chennai Corporation using high resolution satellite data.

### STUDY AREA

### Chennai Municipal Corporation

Chennai district is underlain by various geological formations from the ancient Achaeans to the Recent Alluvium. Most of the geological formations are concealed since overlain by the alluvial materials excepting for a few exposures of crystalline rocks like Charnockites. Chennai is the capital of Tamil Nadu, is one of the industrialized cities referred to as Detroit of India. The spatial extent of Chennai is of 176 Sq km in 2001 and 426 sq Km in 2011(CMDA). Chennai District is bounded by North Latitudes 12° 59' 10" and 13° 08' 50" and East Longitudes 80° 12° 10" and 80° 18' 20" and covered by Survey of India Topographical Maps Nos. 66 C / 4 & and 66 D 1. Sub tropical

climate prevails with a mean annual minimum temperature of 24.3°C to 32.9°C maximum temperature and occasionally rises to 41.2°C in the month of April / May. Humidity is usually higher during winter, monsoon season and lower in summer season that are in the range of 58 to 84%. Land breeze in the early morning and Sea-breeze in the late evening usually subside the surface temperature that is relatively higher during summer months. Chennai receives rainfall during both South West and North East monsoon, whereas North East monsoon coinciding in the months of October to December contributes maximum rainfall 1285.6 mm for this district. Chennai is expanding both vertically and horizontally due to rapid industrialization. Recently Avadi and Tambaram municipality has been included in under Chennai Metropolitan Development Authority (CMDA) in fig. 1.

## MATERIALS AND METHODS

Urban area map under the ambit of Chennai Corporation is procured from COC office. These maps are georeferenced under Universal Transverse Mercator (UTM) projection using ArcGIS Desktop Software using Ground Control Points collected using Global Positioning System (GPS) in the field. Geocorrected images are used as reference images for this study. Cartosat-1 data correspond to Chennai Corporation was covered by two scenes of path and row 0559-335, 0558-335, 0544-344, 0553-345, 0551-349 was procured from National Remote Sensing Centre (NRSC), Balanagar, Hyderabad. It is a Panchromatic image of 2.5m resolution available as two scenes were mosaicked using ERDAS IMAGINE software. Date of acquisition falls on 3rd August, 2009, Jun 6th 2011, February 8th 2011, February 8th 2011, August 21st 2012 as data was imported from native TIFF file format to ERDAS compatible (.img) format for visualization. Image is processed for image processing analysis for distinction in image visibility. The digital image processing analysis steps can be classified into i) Pre-processing ii) Rectification iii) Mosaic iv) Sub setting and v) Digitization of selected themes. Study area was subset from the whole scenes using corporation boundary in shape file format using ArcGIS software. Digitization of themes using Cartosat-1 satellite data include Built up area, Tree cover area, Water bodies, Parks and Temple area are derived from Cartosat-1 satellite data.

### Visual interpretation and Digitization of Cartosat 1 images

Visual Interpretation technique is one the familiar and most promising techniques usually used for land use/land cover analysis. Image interpretation keys such as such as tone, texture, shape, pattern, and relationship forms an idea for an expert in discriminating unfamiliar surface features. Cartosat 1 data black and white image is used here for discriminating land use /land cover features. Digitizing involves delineating surface features from Cartosat-1 image, features are identified based on tonal variation Road- linear feature, Buildings- rectangle or square feature, parks and schools - square /rectangular pattern, trees that are irregular size and shape.

### Tree Cover Mapping

Trees are referred as green belt for urban municipalities. These trees are naturally providing shade to residents of the Chennai region. They also act as a barrier in abatement of pollution from vehicles and industries and from other sources. Such trees are needed to be enumerated for Entire Corporation of Chennai to understand the green belt in proportion to the industries and building cover. Tree cover analysis was performed by two thronged approach. i) An expert was involved to digitize individual tree that are visible at the scale of 1:10,000. Identified trees are marked and plotted as point data in ArcGIS format within the framework of Chennai corporations. These usually exists as avenues trees or road side trees isolated trees in the parks, open land, industrial belts etc., Enumeration of trees were possible through visual interpretation. ii) Due to over growth of trees, there could be merging of tress spatially, where physical enumeration is not possible by the software. In such situations, individual tree enumeration seems to be practically not possible; relatively the trees were grouped together. In that scenario, boundary of the grouped trees was demarcated spatially and its spatial extent was calculated. Expertise available from the individual and the

ground truth information the tree diameter was identified. Aggregating the trees diameter to total demarcated area will provide us the number of trees within the spatial extent in fig 2.

## Crown Area Mapping

Crown area is the actual area of a tree (ie) spatial diameter of a tree. Crown area is also estimated in the field by using sky camera. The Cartosat 1 satellite data used to derive the crown diameter of individual tree for a single tree. They are usually in the range of 8m -28m. In spite of the fact that among the grouping of trees, a strategy was adopted of deriving the trees area of individual tree in shape file. Gaps between the trees are manipulated in accounting for the crown area. Enumerated each tree and the area calculated (circle area= $\prod r^2$). Approximate radius was taken as 3m and each tree area is calculated as (3.14*3*3). A total tree area was finally calculated by considering total number of trees from total crown area divided by each tree area. A separate analysis was taken up on how to deduce the gaps within and between trees to derive the actual crown area. From the analysis, we derived a strategy of deducing eight percent cover from the derived cover to get the actual crown cover from the total area.

## Mapping of Water bodies

Water bodies include tanks, ponds, lakes, rivers, streams and artificial storage structures that are spatially visible on the satellite images. These features are dynamic and temporal in nature. The size of the water bodies may increase in width and length depending on the seasonal inflow of water based on monsoon rains and may decrease in size during non monsoon seasons. Cartosat 1 data acquired during the non monsoon (with less cloud cover) was used in this study was adopted to extract the following information without any loss in deriving the spatial extent. Chennai usually contains Chembarampakkam lake, Puzhal lake, Redhills lake, Avadi, Ambattur lake as major zones for water storage. In addition to that there are large number of small lakes and tanks in and around Chennai. The bunds of these lakes are considered as boundary for digitization of water bodies.

### Mapping of Built up Lands

Chennai is one of the major cosmopolitan cities in India. Due to its nature aerial extent of the city spread across all direction except east. New thermal and auto industries are mushrooming in north Chennai and information technology and information technology enabled industries are emerging along south Chennai. In addition as the Capital of Tamil Nadu, Chennai houses all Government offices, residential complex and business centres, malls etc., Mapping such features by an expertise is so fascinating that concrete structure has higher reflectance behaviour that are usually delineated by light tonal variation. Such features are delineated based on reflectance properties of the satellite images that are represented by light tonal variation due to highest reflectance.

## Mapping of Parks and Temples

Features such as parks and temples usually have a pattern in the form of rectangle or square which will be a minimum requirement for delineation of these boundaries. All these boundaries are delineated in shape file format in temples. Parks are derived from satellite data considering more open spaces with major trees, shrubs, herbs and structures (fig.4). Temples possess a large area mostly of concrete structures with a minimum number of trees all along the boundaries of the temples (fig. 5).

### Mapping of open area

Areas other than aforementioned features are derived as open area in the form marshy land, swamp land, solid waste disposal site etc.,

## RESULTS

Urban Development Plans (UDP), City Development Plans (CDP) and master plan are developed by employing GIS software for proper management and action plans. Henceforth, in a view develop such strategy, themes on tree cover, crown area, water resources (Ponds, lakes, small reservoirs), City parks, Urban forest cover, (Commercial, residential and industrial area) as represented as built up lands and enumeration of total building within the city area are performed using GIS software and validated in the field by random surveys by staff of FC&RI. Map statistics were created from remote sensing images for Municipal Corporation of Chennai for identification and area calculation of building, trees, water bodies, temple and parks. The combined area of Chennai corporations occupied area 464 sq,km.

**Trees**

These trees are identified and marked in GPS point data format as shape file as individual trees as shown in the figure 6. Grouping of trees are made into polygon format by deriving the aerial boundary all the trees in GIS based on their shape and distribution density as either sparse or high dense. In dense region, a composite map is derived including dense grouping of trees. In case of sparse distribution, grouping takes place depends on the level of distribution as segments. Sample points were selected to identify centre point of each tree; radius and diameter of tree crown were also performed for most of the trees. An average tree diameter was arrived, that helped to us to derive circumference of individual tree. The average diameter value was used as an input to derive number of trees from each grouped shape file. Thus tree number was enumerated for Chennai Municipal Corporation (fig. 6) and area was of 72.82 sq.km.

**Crown Area**

Cartosat 1 data used to derive the crown diameter of individual tree. Each tree area was delineated in GIS format and the area was arrived as follows (circle area= $\prod r^2$). Based on the expertise approximate tree radius was taken as 3m for each tree area as (3.14*3*3=28.26 sq.m.). Considering the total number of trees and aggregating all the tree area. Finally, value is arrived as total number of trees from total crown area divided by each tree area. The total number of trees in Chennai corporation possess 23,70,504 trees and Crown area sq.km. was of 66.99 sq.km.

**Water bodies**

Delineation of tanks was taken up for Chennai Corporation by digitizing boundary of tanks, ponds, lakes, river from Cartosat 1 data within the study region. Bund of the tank , lakes are considered as boundary as the actual boundary for this study and not water status of the tank as tank boundary, as water level of the tank varies according to the monsoon rainfall. Generally tanks possess uniform shape either as rectangle or square or circle as viewed through satellite data. Chembrabakkam, Puzhal, Porur, Karanodai, are some of the larger lake that supplies drinking water to Chennai city. Some of these lakes are inter linked lakes /reservoir from other districts and states. Likewise a tank layer was generated for Chennai region that accounts for (35.73 sq.km). It is understood that most of the lakes are shrink due to new settlements that are emerging all along the boundaries of the lakes.

**Built up Lands**

Chennai Metropolitan Development Authority map has been used for deriving boundary considered as Greater Chennai (including Tambaram and Avadi municipality). The total area comprise of 464.65 Sq.km. Layers were derived from Cartosat 1 imagery based on themes. Buildings are much congregated at the central part of Chennai and therefore buildings were digitized from imagery. During the course of the mapping techniques, area of buildings

as view from space a minimum size of 2m*2m size can be mapped. Larger buildings could be spatially mapped in a convenient manner. At all point of time the map scale has been fixed to 1:10000 scales in order to derive maximum features with precise accuracy. Mapping of individual buildings were performed at most of the occasions as shown in the figure 7. In places where there a congregation of buildings, a combined unit of shape file was created in order to minimise the loss of area comprising all the buildings. All the individual shape file of buildings was later merged as a single polygon. Area calculation was carried out in ArcGIS software to derive total area of the Chennai buildings (241.57 Sq.km) (fig. 7).

**Parks**

These features have a similar pattern as seen through Cartosat 1 satellite imagery. Surface feature may be represented by the open spaces at the centre of parks and continuous plantation of trees at the boundaries. A vey minimum concrete structure is available in the parks for recreation purpose. These structures are very useful for the human well being for combating pollution level load in the corporations. Such structure are delineated as polygon feature from satellite imagery and aggregated to a single layer to derive the park area of Chennai Municipal Corporation that accounted for 9.29 Sq.km.

**Temples**

Area of feature that is greater than 3m*3m with square or rectangular pattern were delineated as temple boundary from Cartosat 1 satellite image. Temples are widely distributed within the Chennai municipal corporation. The older temples possess larger boundary with more open spaces and little number of trees. New temples are smaller in size having less open spaces and greenery. All the temples were derived from satellite imagery in shape file format merged to a single layer. Total area of temples was of 0.85 sq.km of the total area of 464.65 sq.km.

**Others**

The regions that a left other than the aforementioned themes were classified as other regions. Such regions were not derived from satellite imagery. However, the open spaces left out after delineating all the themes are referred as other regions. These regions include both government parcels and private parcels that are suited for development of greenery within the Chennai municipal corporation (104.38 sq.km).

## DISCUSSION

This study clearly depicts a picture on the status of green cover of Chennai for the 2011 derived from Cartosat 1 data. Tree cover area of Chennai was 72.82 sq.km. (15.67%). Built up area seems to contribute more on Chennai corporation 241.58 sq.km. (51.99%) of the total area. Parks and temples combined together occupy 10.15 sq.km. (2.18%) of the total area. Water bodies that supply drinking water to Chennai residents spatially spread across 35.73 sq.km. (7.69%) of the total area. Other area significantly occupies 104.39 sq.km. (22.53) becomes the only source for the policy planners to convert open land to greenery within Chennai city limits to combat pollution and to improve rainfall in order to overcome frequent drought in the changing climate scenario.

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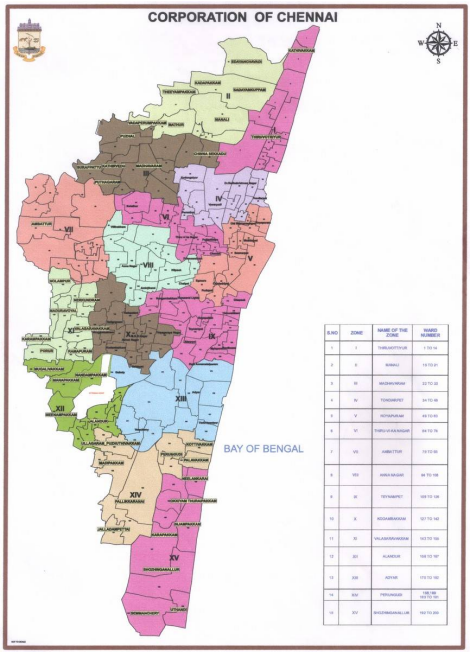


**Fig-1: Location map of the Chennai Corporation Study Area**

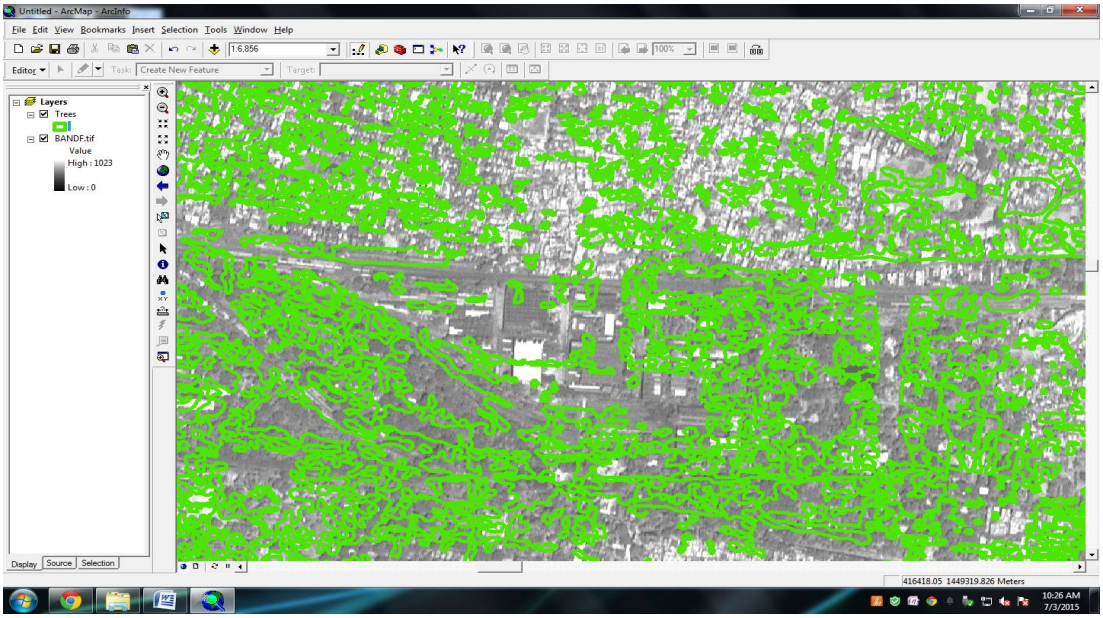


**Fig. 2 : Tree Cover mapping**

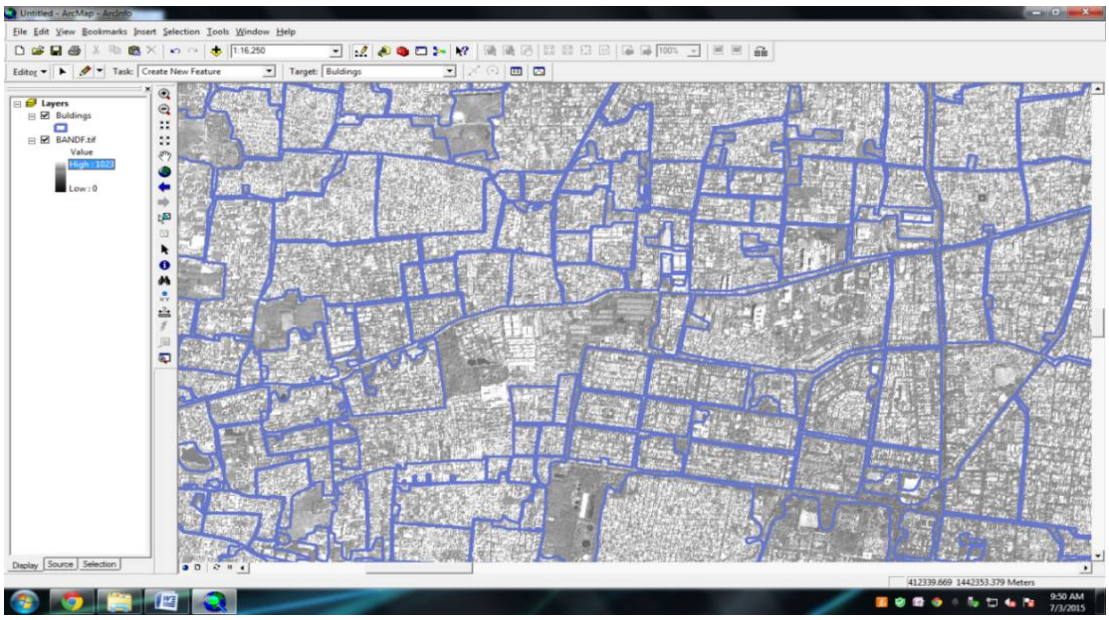


**Fig 3: Mapping of Built up Lands**

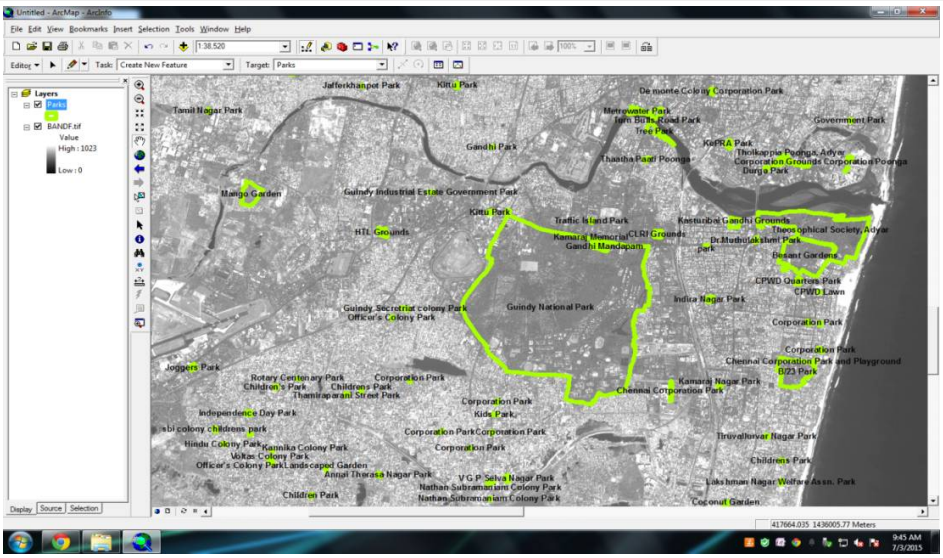


**Fig 4 : Mapping of Parks**

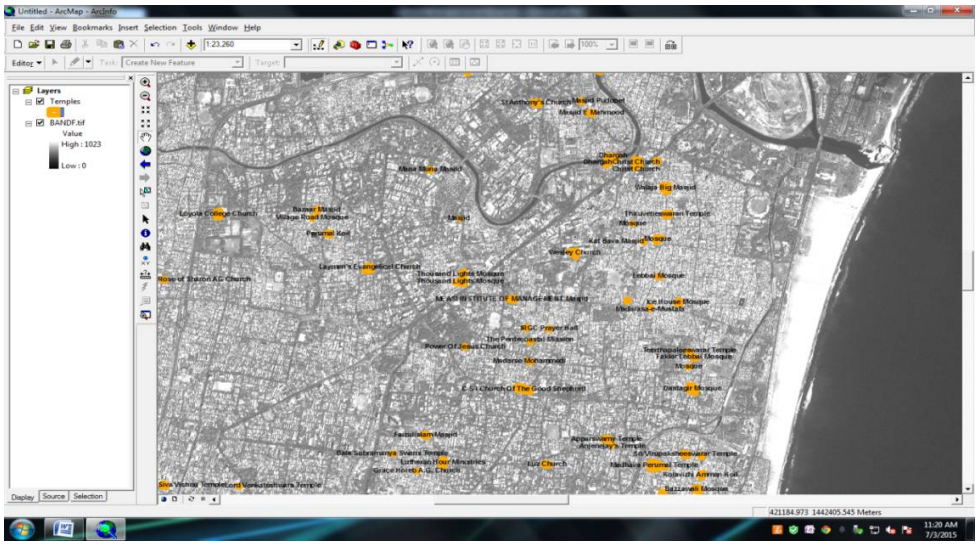

**Fig 5 : Mapping of Temples**

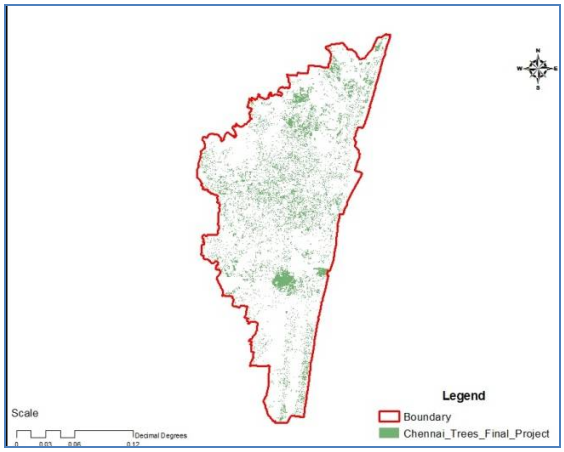


**Fig 6: Tree covers area derived from Cartosat 1 Satellite data**

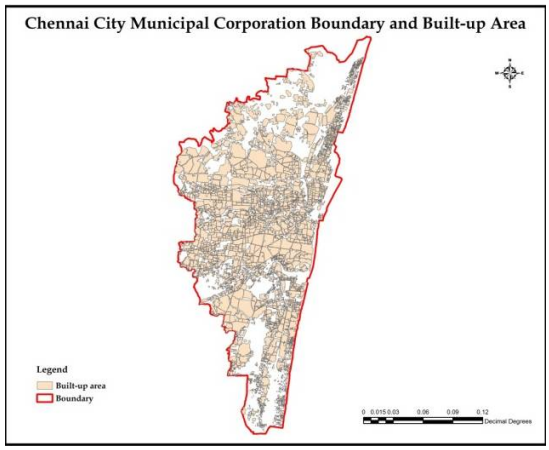


**Fig 7: Built up area derived Cartosat 1 Data**

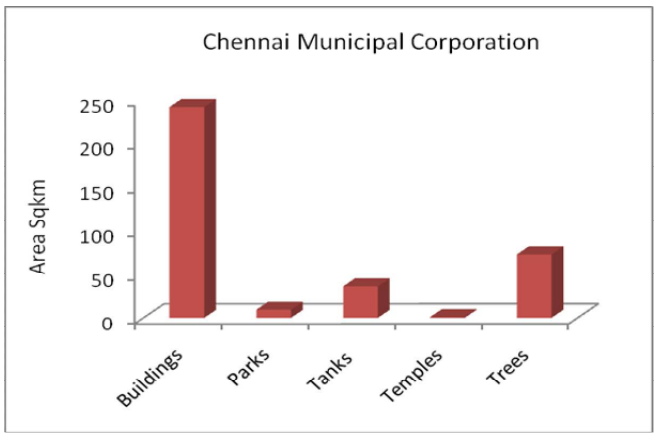


**Fig 8 : Chennai Municipal Corporation occupied urban area values**

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REVIEW ARTICLE

# Modelling Air Pollutant Emissions from Brick Kilns Industry using Geo-Spatial Techniques

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## ABSTRACT

Due to increasing urbanization and absence of proper air pollution monitoring mechanisms, the brick kiln industry has the adverse effects on the environment. This research study aimed to assess the impact of brick kilns emissions on air quality and environment of Islamabad and Rawalpindi using geospatial techniques. Air quality index (AQI) for 142 brick kilns were calculated based on the emissions of SO2, CO2 and PM10. The larger the value of AQI means more air pollution and vice versa. Total 19, 62, 44 and 17 brick kilns were categorized as moderate, poor, very poor and severe air quality respectively. The direction distribution of brick kilns having severe AQI was East-South to West-North. Further hotspot analysis showed that total 21 brick kilns are causing major air pollution in the area with 95 to 99% confidence level. This study can be served as base line to take measures like shutting or replacement illegitimate kilns.

**Keywords :** Brick kiln, air quality index, Indo-Pak, environmental pollution, hot spot analysis, geospatial techniques.

## INTRODUCTION

Brick production is an extensive and old-fashioned industry in many parts of world (Zhang et al., 2009). More than a trillion baked-clay bricks are formed every year, in 300,000 brick kilns around the world (Gidwani, 2006). The brick kiln industry is very popular in Asia, as bricks are mainly produced locally in small enterprises at the village and rural scale. India, Pakistan and Bangladesh are contributing for a quarter of the global production (Ahmad et al., 2012). The increasing demand of bricks in emerging and developing economies is due to rising populations, urbanization, increasing pace of development and modernization. Solid fired clay bricks are the most widely-used construction materials in Pakistan. The contribution of clay brick industry to the gross domestic product (GDP) of

Pakistan is about 1.5% (Batcheller, 2008). The industry is nursing in Pakistan on a widespread. The informal, small-scale business sector is often unlicensed, unregulated and commonly lack in the control of pollution that eventually lead to adverse environmental consequences (Co et al., 2009). Coal is the main energy source for brick kilns, in addition to coal, different biomass fuels, for example, wood, dry dung, rice husk bagasse and other agro-residues are used for firing bricks (Reddy and Venkataraman, 2002). Typical brick production procedure in Pakistan and other developing nations is of less energy efficient that leads to high levels of pollution. Besides these emissions from ignition, the life cycle of brick making comprises of important fugitive emissions (Rajarathnam et al., 2014). These brick kilns are deteriorating air quality and degrading people's health nearby the brick kilns. Several studies have found that the concentrations of particulate matter (PM) in air nearby the brick kilns areas are 3 times greater than the offseason of brick kilns (Raut, 2006). Particulate matter doesn't contain one compound or component but rather, it is a composite fusion of several organic and inorganic materials, a lot of those are dangerous to human health (Smith, 2008).

The World Health Organization (WHO) approximates that PM2.5 concentration adds to almost 800,000 early deaths per year, which causing it the 13th prominent cause of mortality level across global. Old people and new-born babies are mainly suspected to hazardous impacts of Sulphur dioxide ($SO_2$) generated from the brick kiln industry. All these pollutants are directly emitted from brick kilns during bricks production. Further environmental costs of the brick kilns are decline in soil productiveness, reduced visibility and drying the ground water sources. Incompliance with the urbanization, these brick kilns industries are speedily growing and migration of people into the metropolitan area causing to create more brick kilns. According to GEFONT (2008), 400,000 labours are working in the brickfields only in Nepal. Because of these and many other negative effects the air pollution has been widely studied all over the globe especially in Asia pacific with many techniques. In the past few decades new technologies and methods have been presented for analysing repeated varying levels of air pollution. These methods ranges from statistical analysis (regression) to using numerical models (REGINA (REGIonal high resolutioN Air pollution model), AERMOD (atmospheric dispersion modelling system) etc.). Geospatial techniques are the use of GIS, statistical analysis and other analytic techniques to data which has a geographical or spatial aspect. Spatial techniques such as buffering, directional distribution, hotspot analysis, kernel density, spatial interpolation etc. are very productive to map the not only the sources of pollution but also to assess its impact on nearby regions.

Geospatial technologies manages statistical and spatial data to provide a tool that shows the relationship between poor air quality and occurrences of deficient human and environmental health. In this way geospatial techniques aids in monitoring pollutants emissions. These techniques enhances the users' understandings of regional opportunities by allowing them to visualize the spatially distributed nature of the data. Several researchers used these techniques to map (Briggs et al., 1997; Craglia and Maheswaran, 2004 and Kumar et al., 2016), model (Gulliver and Briggs, 2005; Jerrett et al., 2004 and Proietti et al., 2016), analyse (Jerrett et al., 2001; Guttikunda and Calori, 2013), interpolate (Janssen et al., 2008) and decision making (Elbir, 2004; Zhang et al., 2015and Hacıoğlu et al., 2016). Air pollution in conjunction with the insufficient health care facilities and the absence of proper law to make sure workplace safety represent the main sources of high rate of causalities, diseases, and damages in brick kiln workers in Pakistan and other developing countries. Keeping in view the harmful effects of brick kilns on the people, atmosphere and environment a study was designed with the objectives to: 1) to develop a comprehensive inventory of brick kilns as geodatabase for Islamabad and Rawalpindi; 2) to assess the impact of brick kilns emissions on air quality and environment.

**Study Area**

Islamabad is the capital of Pakistan and Rawalpindi is its adjacent district (Figure 1). The region lies in a humid subtropical weather, hot summers followed by monsoon season and then by mild and wet winters. A survey conducted by The Express Tribune (Anwar, 2015) revealed more than 100 brick kilns located in twin cities and outskirts. Existence of these smoke-emitting brick kilns in the fringes of Rawalpindi and the federal capital is clearly deteriorating the air quality of the twin cities.

## MATERIALS AND METHODS

### Data Collection

Two types of data were used in this research study i.e. the satellite data to make landcover and digitize all the vector layers (roads, settlements, waterways etc.) in the study area and the field data to conduct survey and mark all the brick kilns in the area.

### Satellite Data

Satellite data is very important to conduct the GIS based studies as it provides a detail and up-to-date information of the study area (Blaschke, 2010). In this study Landsat satellite data was used as Landsat started in 1972 with its first satellite Landsat 1 and Landsat 8 is the latest. Because of its freely availability, medium resolution and multispectral bands Landsat is the most appropriate satellite for land cover classification (Halabisky et al., 2016). The satellite image of March 2016 was acquired and pre-processed in terms of atmospheric and radiometric corrections to remove any possible errors. The main focus was on the derivation of vegetation class present in the study area (Figure 2). Recent studies on brick kilns have shown some major disastrous impacts on the surrounding vegetation. Because many air pollutants especially sulfur dioxide shows harmful effects in terms of foliar injury, physical and biological alterations on vegetation (Dominick et al., 2012). Degradation in air quality causes in decrease in chlorophyll concentration in plants (Jahan et al., 2016).

### Field Data

An extensive field survey was conducted to collect the spatial and non-spatial data of the brick kilns in the twin cities. Garmin eTrex 10 handheld GPS was used to collect the data (Ali et al., 2012). The accuracy of GPS was ± 3 to 5 m in clear weather and open space which were later adjusted as data clearing process. All the brick kilns locations were noted and unique ID was assigned to each brick kilns. The number and exact location of brick kilns fluctuate constantly. Each kiln and the surrounding clay pit occupied about average 1 ha. In the metropolitan area, about 37.5 ha of land per year is consumed for the manufacture of bricks along with the location component, the other information including the daily bricks production, number of workers, their ages, gender, literacy level, available of facilities, kiln energy sources, alternate energy sources, environmental safety measures etc. were collected through a comprehensive field survey.

### Air Quality Index (AQI)

The most useful and up to date method for describing atmospheric pollution is the Pollution Standards Index (PSI) which was first referred by Ott and Hunt (1976). This initial index is based on five pollutants, namely O3, NO2, CO, SO2 and PM10. In June, 2000, Environmental Protection Agency (EPA) improved PSI and renamed it to Air Quality Index (AQI) (Plaia and Ruggieri, 2011). An air quality index (AQI) is a number used by organizations to communicate the people for how contaminated the air at present is or prediction of how polluted it can be. More the value of AQI, more will be the pollution in the atmosphere and vice versa (Büke and Köne, 2016). Several countries have their own air quality indices, corresponding to various national air quality standards. Few of them are the Air Quality Health Index (Canada) (Abelsohn and Stieb, 2011), the Air Pollution Index (Malaysia) (Dominici et al., 2006), and the Pollutant Standards Index (Singapore) (Kusumaningtyas and Aldrian, 2016). For this research we used the Air Quality Index developed by Central Pollution Control Board (CPCB) (Chelani et al., 2002 and Tanday, 2015) as India is the neighbouring country of Pakistan and both have the same geographical and atmospheric conditions. CPCB AQI is based on five pollutants i.e. PM10, PM2.5, SO2, NOx, CO (mg/m3), O3 and NH3. To calculate the value of AQI at any place concentrations of minimum three pollutants are required and one of them should be PM10 or

PM2.5. The final values of AQI is divided in six categories (Table 1) ranging from minimum 0-50 as good and maximum >401 as severe.

**Quantification of Air Pollutants**

To calculate the amount of air pollutants for each brick kiln site a methodology based on the observations of (Skinder et al., 2014) was used. The author observed that for an average emission factors per 1,000 bricks were; 6.35–12.3 kg of CO, 0.52–5.9 kg of SO2 and 0.64–1.4 kg of Particulate Matter (PM10). Owing to these results calculations has been carried on for the concentration of SO2, CO2 and PM10 using the bricks produced per day data. The value is further refined based on the fuel type used and the brick kilns size. For example the brick kiln with an average production of 10,000 bricks, coal as fuel and size of 15 ha produced SO2, CO2 and PM10 as 64.2 kg, 186 kg and 20.4 respectively.

**Geodatabase Development**

The satellite and field data was imported to ArcGIS to develop a geodatabase. A geodatabase is the native data structure for ArcGIS and is the primary data format used for editing and data management (5). A geodatabase stores GIS data in a central location for easy access and management. The brick kilns data were analyzed spatially using nearest neighbor analysis, spatial autocorrelation, and hotspot analysis. The inverse distance weighted interpolation technique was used to generate continues surfaces of all the pollutants. Further the kernel density was applied to assess the per square kilometers distributions of pollutants and AQI.

## RESULTS AND DISCUSSION

Total 142 brick kilns situated around the Islamabad and Rawalpindi were surveyed and marked on the map (Figure 3). The spatial distribution has been divided into two main clusters one is on the south-east and the other on north-west with 60 and 82 brick kilns respectively.The data shows that average production of bricks was 24,303 bricks in a week. The production distribution is highly variable in the study area (Figure 4). The main type of fuel used was coal used by 116 kilns. 14 kilns are using coal & rubber and 8 coal & wood. Only 4 kilns are using coal, wood and rubber together as their major fuel.PM10, SO2 and CO levels were found to be increased in the area and same trend was observed for air quality index (Figure 5). The same trend was also observed for Indian megacities like Delhi, Mumbai and Kolkata by Gurjar et al., (2016), In general the higher values of air quality index shows the more severe the air quality is.

Similarly the correlation analysis measures the strength of the relationship between selected independent and dependent variables. It is a simple measure to exhibit how well one variables predicts the other (Rahman and Zhang, 2016). The Pearson's correlation matrices between five air quality parameters were computed (Table 2) for 142 brick kilns.The results shows that the AQI has strong positive correlation with production capacity, fuel consumption, PM10,SO2 and CO. It means increase in these parameters will cause increase in AQI and vice versa. In this study, correlation coefficient ($r \geq 0.8$) are considered to be strongly correlated, where, r values $\geq 0.7$ and $< 0.8$ shows moderated correlation at a significant level P = 0.05.

**Air Quality Index Estimation**

The air quality index was calculated by using the air quality index calculator as mentioned in the methodology. The AQI was categorized as moderate, poor, very poor and severe air quality based on AQI values. The same type of categorization was also used by several researchers in their research (Nagendra et al., 2007; Sharma et al., 2003 and Qiao et al., 2015). The lower values of AQI indicates moderate air pollution and higher values represents the severe air quality which is injurious to health (Kyrkilis et al., 2007 and Sowlat et al., 2011). The spatial distribution of air

quality index shows that 19, 62, 44 and 17 brick kilns was categorized as moderate, poor, very poor and severe respectively.

**Directional Distribution of AQI**

Directional distribution is a common way of measuring the trend for a set of points or areas is to calculate the standard distance separately in the x and y directions. The directional map (Figure 6) with standard deviational ellipses obtained by executing the Directional Distribution tool offered by ArcGIS. It is recommended by the literature that tool should be run on first standard deviation ellipse (Wong et al., 2005; Boruff et al., 2012 and Wang et al., 2015) i.e. 68% data of air quality index.The direction of brick kilns causing moderate air quality index is East-South to West-North whereas the direction of brick kilns responsible for poor and very poor air quality are East to West. The direction of brick kilns causing severe air quality index is same as moderate i.e. East-South to West-North. Also the size of ellipse reveals that the brick kilns are not dispersed as much and clustered in groups.

**Hot Spot Analysis**

Hotspot analysis uses vectors to identify the locations of statistically significant hot spots and cold spots in data (Mahboubi et al., 2015). It produces Z scores and P values of the dataset. A high positive Z score and small P value and low negative Z score and small P for a feature indicates a significant hot and cold spot respectively (Geletic, 2013 and Mahmood and Gloaguen, 2011). The higher (or lower) the Z score, the more intense the clustering. The value of Z score closer to zero means no spatial clustering. In this research study the hot spot analysis was performed on the air quality index (AQI). The hotspots spatial estimated for the AQI (Figure 7) shows high variability in the hotspot and cold spot.

3 brick kilns were found as cold and 7 as hot spots of AQI with 95% confidence level. On the other hand 3 and 8 brick kilns were found as cold and hot spots with 90% confidence level. The brick kilns with 90% and 95% confidence level of cold and hot spot of AQI has both moderate and severe quality of air pollution respectively. Another important thing observed was that the major fuel type of those brick kilns were coal and there production capacity is large as compare to the others. They have double shift i.e. production day and night. With the hot spot analysis total 21 brick kilns were found statistically significant that cause major air pollution in the area. Whereas 121 brick kilns were found not significant statistically.

## CONCLUSION AND RECOMMENDATIONS

Several important quantities of particulates and gaseous pollutants emitted from the brick kilns causing the environmental degradation. The research study clearly shows that the brick kilns working in Islamabad and Rawalpindi are producing large amount of air pollutants. The field data was surveyed and collected from the study area. Advance geospatial techniques were applied to map the air pollution on the area and on the basis of data air quality index (AQI), was generated. Total 19, 62, 44 and 17 brick kilns was categorized as moderate, poor, very poor and severe air quality on the basis of AQI. With the hot spot analysis total 21 brick kilns were found statistically significant that cause major air pollution in the area. In general, the larger the AQI, the more is the pollution. A lower AQI therefore is beneficial for human health and the environment. Measures like shutting illegitimate kilns, introduction of cleaner machineries such as vertical shaft kilns and static funnel kilns, replacement of outdated kilns with newer technologies are decreasing air pollution. The other very important measure is that brick kilns should be built away from the residential areas.

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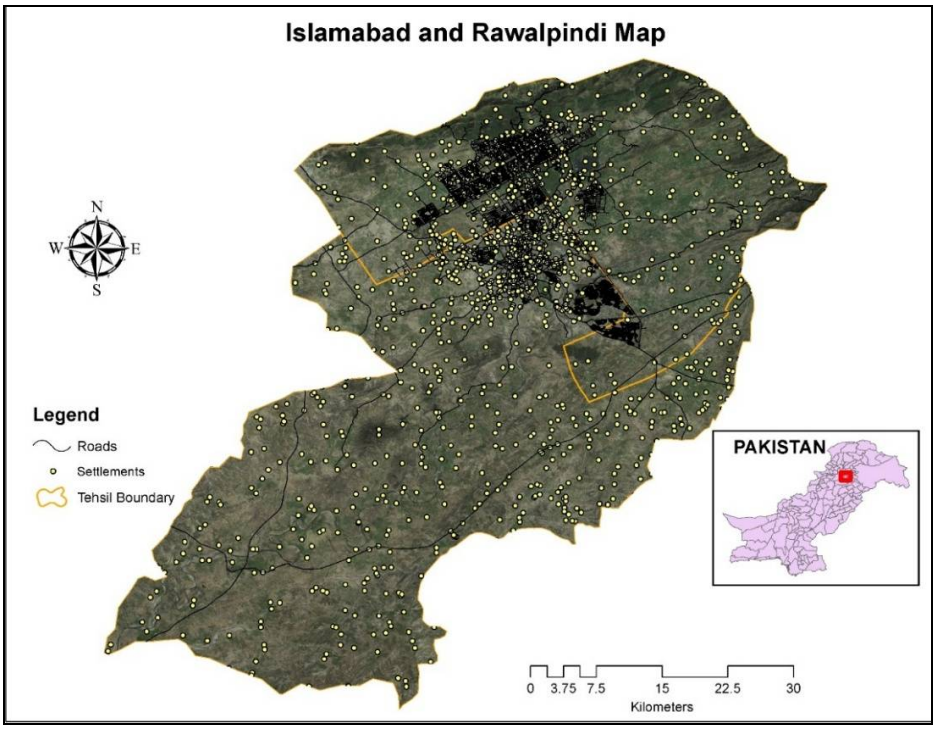


**Figure 1. The study area map of the research.**

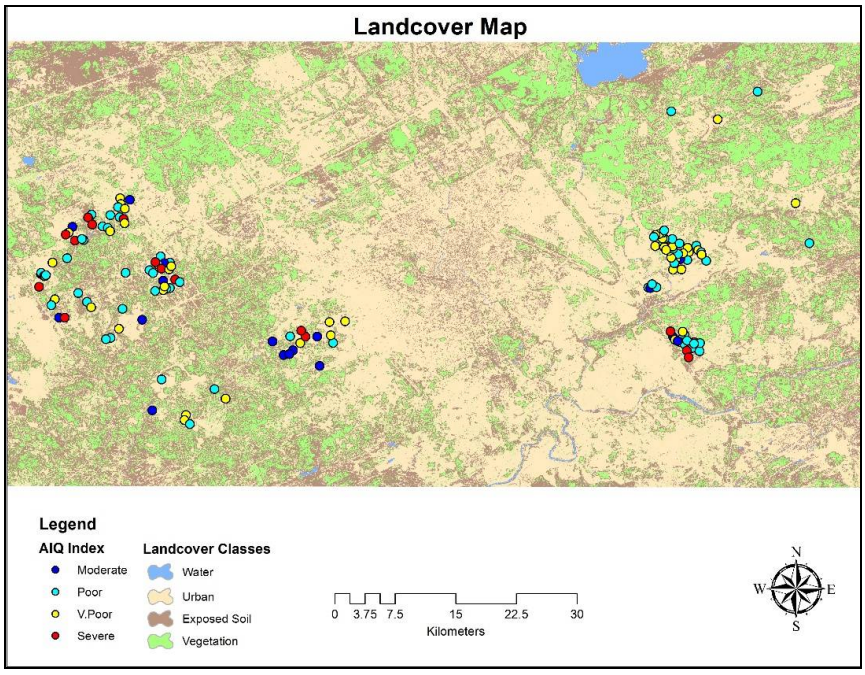


**Figure 2. Landcover classes derived from satellite imagery.**

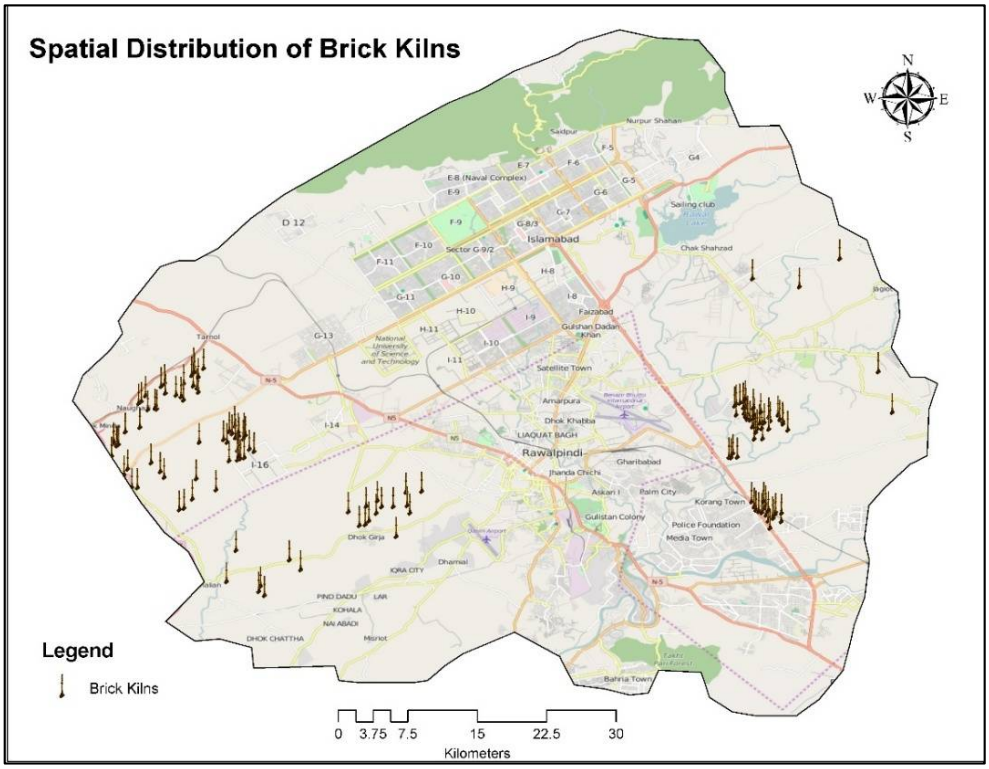


**Figure 3. Spatial distributing of brick kilns in the study area.**

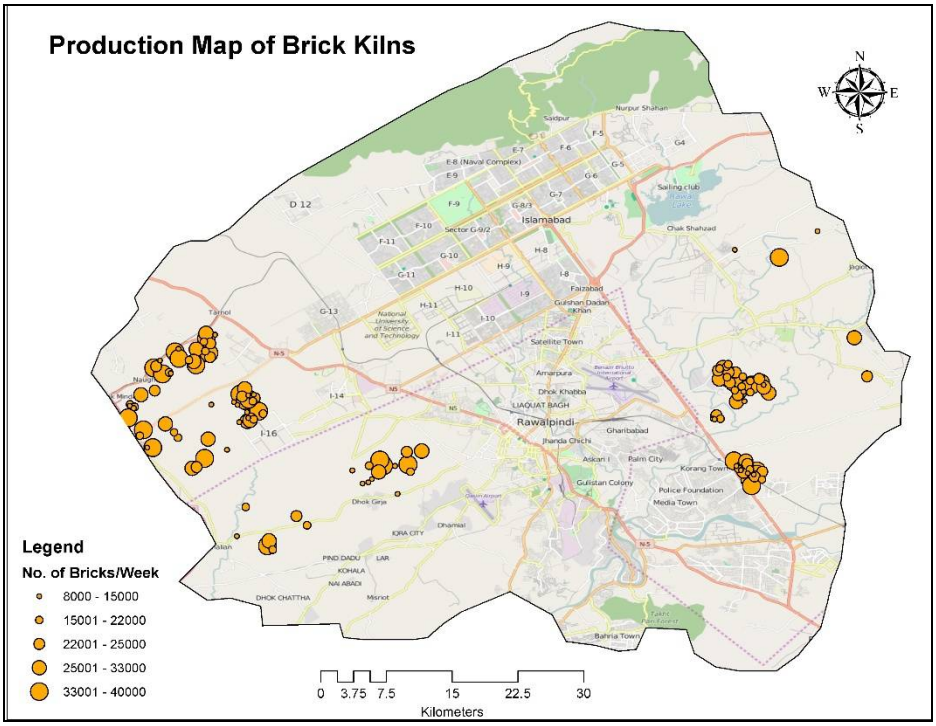


**Figure 4. Brick kilns production map.**

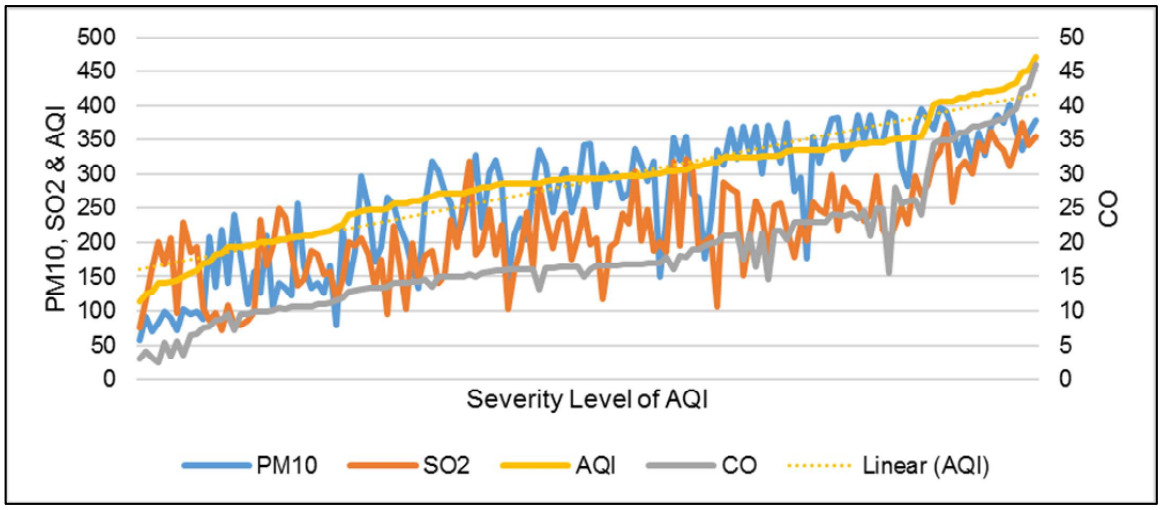


**Figure 5. General trend of air pollutants & air quality index.**

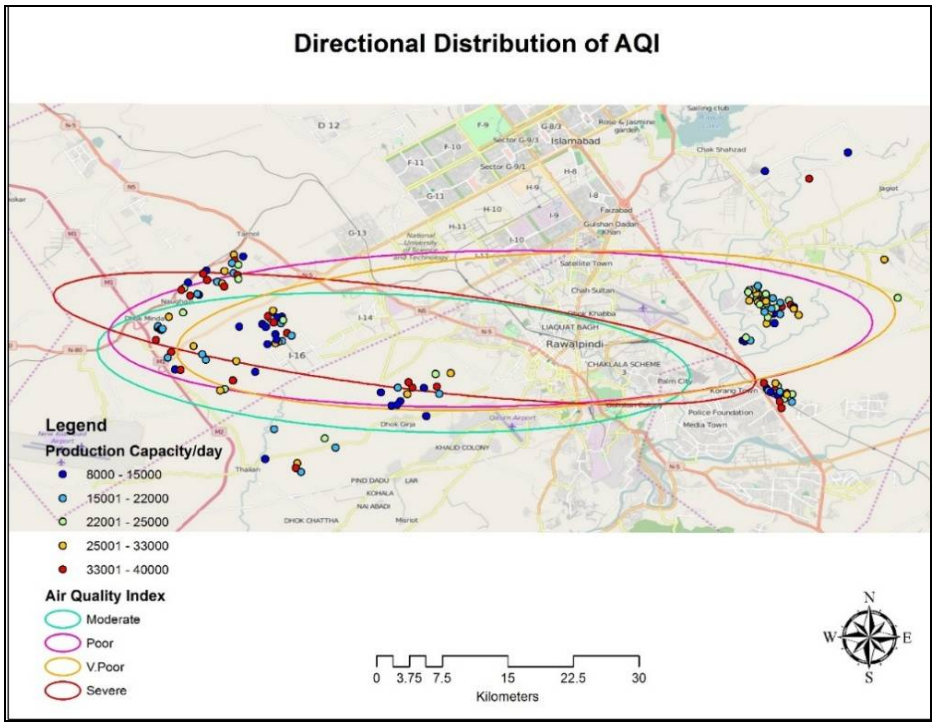


**Figure 6. Directional distribution of air quality index in the study area.**

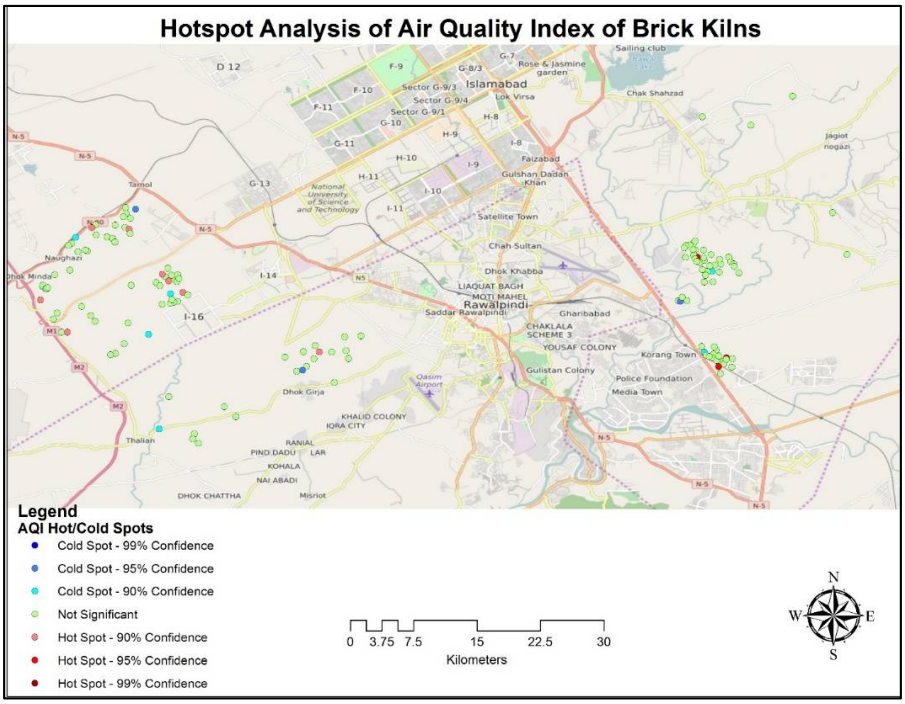


**Figure 7. Spatial distribution of hot and cold spots of air quality index.**

**Table 1. Categories of Air Quality Index (AQI) with respect to their impacts on health.**

| Category | Description | Category | Description |
|---|---|---|---|
| Good (0–25) | Minimal Impact | Poor(76–100) | Breathing discomfort to people on prolonged exposure |
| Satisfactory (26–50) | Minor breathing discomfort to sensitive people | Very Poor(101–125) | Respiratory illness to the people on prolonged exposure |
| Moderate (51–75) | Breathing discomfort to the people with lung, heart disease, children and older adults | Severe(>126) | Respiratory effects even on healthy people |

**Table 2. Pearson's correlation matrices between five air quality parameters.**

| | **Production Capacity** | **Fuel Consumption** | **PM10** | **SO2** | **CO** | **AQI** |
|---|---|---|---|---|---|---|
| Production Capacity | 1 | | | | | |
| Fuel Consumption | 0.98 | 1 | | | | |
| PM10 | 0.88 | 0.85 | 1 | | | |
| SO2 | 0.77 | 0.76 | 0.63 | 1 | | |
| CO | 0.87 | 0.87 | 0.74 | 0.76 | 1 | |
| AQI | 0.91 | 0.89 | 0.85 | 0.75 | 0.96 | 1 |

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RESEARCH ARTICLE

# Use of Botanical for the Management of Pulse Beetle, *Callosobruchus chinensis* (Linn.) on Cowpea under Storag Condition

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## ABSTRACT

The different plant products *viz*., neem oil, castor oil, mustard oil, groundnut oil, karanj oil (0.1 & 0.5 ml or g / 100 g of seeds) and neem leaf powder, karanj leaf powder, aak leaf powder and datura leaf powder (1.0 and 2.5 g/ 100 g seeds) when admixed with cowpea grains proved to be causing adverse effect on adult emergence of *C. chinensis* and reduces grain damage and weight loss by this pest. The neem oil was the best treatment to enhance the developmental period and reducing the adult emergence and ovipositional potential, while datura leaf powder was least effective treatment. The per cent grain damage and weight loss were minimum in neem oil and maximum in datura leaf powder. No adverse effect of tested plant products was observed on the germination of cowpea seeds up to 90 days of treatment.

**Keywords :** Pulse beetle, cowpea, *Callosobruchus chinensis,* management, plant product.

## INTRODUCTION

Pulses the "wonderful gift of nature" play an important role in Indian economy and are a rich source of supplementary protein to daily diets based on cereals and starchy food for a predominantly vegetarian population and for those who cannot afford expensive animal protein. Pulses are therefore often regarded as poor man's meat. In India 17 species of bruchids belonging to 11 genera have been recorded infesting different pulses (Arora, 1977). The members of genus *Callosobruchus* (Coleoptera, Bruchidae) is a cosmopolitan field-to-store pest ranked as the principal post harvest pest all over the world, but in India, *C. maculates* (Fab.), *C. analis* (Fab.) and *C. chinensis* (Linn.) are the predominate pest species of the genera (Dias, 1986). Bruchids causes substantial quantitative and qualitative losses

manifested by seed perforation, reduction in weight, market value and germ inability of seeds. The insects spend its entire immature stage in individual legume seeds.

## MATERIALS AND METHODS

The bio-efficacy of different plant products were evaluated against *C. chinensis* in complete randomized design. The treatments were replicated three times. The details of different plant products were use in the experiment are given in table 1. The fine powders of different plant products were prepared by drying them in shade and then grinded in electric grinder. The powders were sieved through 60 mesh sieve and mixed with seeds @ 1.0 and 2.5 g/100 g seeds. For mixing the powder with seeds, 100 g seeds were placed in glass vial and desired doses of powder were added to each vial. The powders were mixed thoroughly with seeds by shaking the vials. Samples of seed from each treatment were transferred to specimen tubes. A control (untreated) was also kept simultaneously. The observations (ovipositional potential, developmental period, adult emergence, grain damage, weight loss and germination test) were recorded.

## RESULTS AND DISCUSSION

**Effect on ovipositional potential (Table 1)**

The significant difference existed between the doses of plant product in reducing the ovipositional potential of test insect in comparison to control. The ovipositional potential of test insect got progressively decreased with the increase in dose level of each treatment. The mean number of eggs laid per female at different dose levels ranged from 27.89 to 29.52. Comparing the results obtained in different plant product, the neem oil was found to be most effective in which minimum mean number of eggs was laid (24.67 eggs) by the female and significantly superior to rest of the treatments. It was followed by castor oil, mustard oil, neem leaf powder, groundnut oil, karanj oil, karanj leaf powder, aak leaf powder and datura leaf powder with 27.50, 27.83, 28.17, 28.83, 29.17, 29.83, 30.67 and 31.67 eggs/ female, respectively, however, no significance difference among the treatments of castor oil, mustard oil, neem leaf powder, groundnut oil, karanj oil and karanj leaf powder; karanj oil, karanj leaf powder, aak leaf powder and datura leaf powder. The present results get fully support from the study of Bhargava (1997), Bhatnagar *et al.* (2001), Bhargava and Meena (2002) and Singh and Sharma (2002) who found that different plant product mixed with pulses was found effective for minimizing the ovipositional potential of *Callosobruchus* spp.

**Effect on developmental period (Table 2)**

When the newly hatched larvae fed with grains admixed with different plant products, all the doses were found to be significantly better in increasing the developmental period when compared with control. The mean duration of development ranged from 27.26 to 32.92 days at different dose levels of plant product, while it was 25.33 days in the control. Considering the results observed in different treatments, the mean duration of developmental period ranged from 28.67 to 32.33 days, being minimum in grains treated with datura leaf powder (28.67 days), at par with aak leaf powder (29.00 days), karanj oil (29.17 days) and karanj leaf powder (29.33 days). The maximum developmental period was recorded in neem oil (32.33 days), which was at par with castor oil (31.50 days). These findings are in accordance with the results obtained by Naik and Dumbre (1984), Singh *et al*. (1993), Bhargava and Meena (2002) and Bajiya (2010) who have also observed to prolonged the developmental period of *Callosobruchus* spp. by treatment with different plant products.

**Effect on adult emergence (Table 3)**

The adult emergence of C. chinensis from grain treated with different plant products showed significant difference as compared to control. The per cent adult emergence decreased with increase in dose levels of the test compounds. The adult emergence at different dose levels varied from 26.91 to 32.54 per cent as against 77.33 per cent in control. In

neem oil the adult emergence was 28.66 per cent at lowest dose level which decreased to 23.16 per cent at highest dose level, whereas, in control it was 77.33 per cent. Similar trend of adult emergence was recorded in other treatments.

Comparing the results obtained in various plant products, it was found that neem oil was most effective in which minimum percentage of adult emergence (25.91%) occurred and significantly superior to rest of the treatments. The next effective treatment was castor oil (26.92 days) followed by neem leaf powder (27.50 days), mustard oil (27.58 days) and groundnut oil (32.74 days), however no significant difference was observed among them. The maximum adult emergence was recorded in the treatment of dathura leaf powder (34.00%), which was statistically comparable with aak leaf powder (33.17%). The present findings are conformity with Singh and Sharma (2002), Haghtalab *et al*. (2009), Bajiya (2010) and Ratnasekera and Rajapakse (2012) who tested different plant products against *C. chinensis* and found that mixing of plant products *viz*., neem, castor, mustard with different pulses seed which causing inhibitory effect on adult emergence.

**Effect on grain damage (Table 4)**

The per cent of damaged grains in all the doses of different treatments were significantly less than the per cent of damaged grains in control. In neem oil, the grain damage was 30.80 per cent at the initial dose level, which reduced to 24.00 per cent at highest dose. Similar trends were recorded in other treatments. The mean grain damage at different dose levels ranged from 27.66 to 33.83 per cent. While, assessing the results obtained in different plant product, the average grain damage ranged from 27.40 to 33.90 per cent, being minimum in neem oil (27.40%) and maximum in datura leaf powder (33.90%). However, no significant difference was observed among the treatments of neem oil, castor oil and mustard oil; mustard oil, neem leaf powder, groundnut oil and karanj oil and karanj leaf powder, aak leaf powder and datura leaf powder.

**Effect on weight loss (Table 5)**

The relative efficacy of different treatments in per cent reduction in net weight of cowpea grains were found significant. The mean per cent weight loss at different dose levels ranged from 8.85 to 9.65 per cent, as against 13.10 per cent in control. A significant difference was observed between dose levels. Considering the results observed in different treatments, the minimum per cent weight loss (7.64%) was recorded in neem oil, at par with castor oil (8.37%) and mustard oil (8.89%). Maximum per cent weight loss (10.85%) was observed in datura leaf powder, followed by aak leaf powder (10.16%), karanj leaf powder (9.77%), karanj oil (9.55%) and these were at par with each other. However, no significant difference was also recorded between the treatments of mustard oil, neem leaf powder, groundnut oil, karanj oil, karanj leaf powder and aak leaf powder. The present findings are corroborate with the findings of Kumari et al. (1990), Choudhary (1990), Sundria et al. (2001), Raghavani and Kapadia (2003), Singh and Sharma (2002), Khalequzzaman et al. (2007) and Bajiya (2010) who observed that different oils tested against C. chinensis proved effective for the reduction in percentage damaged grains by number as well as by weight.

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**Table 1 : Effect of different plant products (oils/leaf powders) on ovipositional potential of *C. chinensis***

| Dose | Ovipositional potential (eggs/female)* | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant oils and leaf powders | | | | | | | | | |
| | Neem oil | Karanj oil | Castor oil | Mustard oil | Ground nut oil | Datura leaf powder | Neem leaf powder | Karanj leaf powder | Aak leaf powder | |
| $C_1$ | 25.33 | 30.00 | 28.66 | 29.00 | 29.66 | 32.00 | 29.00 | 30.66 | 31.33 | 29.52 |
| $C_2$ | 24.00 | 28.33 | 26.33 | 26.66 | 28.00 | 31.33 | 27.33 | 29.00 | 30.00 | 27.89 |
| Mean | 24.67 | 29.17 | 27.50 | 27.83 | 28.83 | 31.67 | 28.17 | 29.83 | 30.67 | - |
| Control | 51.33 | | | | | | | | | |
| | | S.Em± | C.D. at 5% | | | | | | | |
| Treatment | | 0.87 | 2.65 | | | | | | | |
| Dose | | 0.59 | 1.81 | | | | | | | |
| Treatment X Dose | | 1.99 | 6.02 | | | | | | | |

| Used treatment | Used dose | |
|---|---|---|
| | $C_1$ | $C_2$ |
| 1. Plant oils (ml / 100 g seeds) | 0.10 | 0.50 |
| 2. Plant leaf powders (g / 100 g seeds) | 1.00 | 2.50 |

* Data based on 120 individuals (three replications of 40 in each).

**Table 2 : Effect of different plant products (oils/leaf powders) on total developmental period of *C. chinensis***

| Dose | Total developmental period (days)* | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant oils and leaf powders | | | | | | | | | |
| | Neem oil | Karanj oil | Castor oil | Mustard oil | Ground nut oil | Datura leaf powder | Neem leaf powder | Karanj leaf powder | Aak leaf powder | |
| $C_1$ | 29.00 | 26.33 | 28.33 | 28.00 | 27.33 | 26.00 | 27.33 | 26.66 | 26.33 | 27.26 |
| $C_2$ | 35.66 | 32.00 | 34.66 | 33.33 | 32.66 | 31.33 | 33.00 | 32.00 | 31.66 | 32.92 |
| Mean | 32.33 | 29.17 | 31.50 | 30.67 | 30.00 | 28.67 | 30.17 | 29.33 | 29.00 | - |
| Control | 25.33 | | | | | | | | | |
| | | S.Em± | C.D. at 5% | | | | | | | |
| Treatment | | 0.37 | 1.15 | | | | | | | |
| Dose | | 0.34 | 1.07 | | | | | | | |
| Treatment X Dose | | 1.38 | 4.20 | | | | | | | |

| Used treatment | Used dose | |
|---|---|---|
| | $C_1$ | $C_2$ |
| 1. Plant oils (ml / 100 g seeds) | 0.10 | 0.50 |
| 2. Plant leaf powders (g / 100 g seeds) | 1.00 | 2.50 |

* Data based on 120 individuals (three replications of 40 in each).

**Table 3: Effect of different plant products (oils/leaf powders) on adult emergence of *C. chinensis***

| Dose | Adult emergence (%)* | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant oils and leaf powders | | | | | | | | | |
| | Neem oil | Karanj oil | Castor oil | Mustard oil | Ground nut oil | Datura leaf powder | Neem leaf powder | Karanj leaf powder | Aak leaf powder | |
| $C_1$ | 28.66 | 34.60 | 29.00 | 30.16 | 32.33 | 36.66 | 30.00 | 35.10 | 36.33 | 32.54 |
| | (32.37) | (36.03) | (32.58) | (33.31) | (34.65) | (37.26) | (33.21) | (36.33) | (37.07) | (34.78) |
| $C_2$ | 23.16 | 27.81 | 24.83 | 25.00 | 26.16 | 31.33 | 24.60 | 29.33 | 30.00 | 26.91 |
| | (28.77) | (31.83) | (29.89) | (30.00) | (30.76) | (34.04) | (29.73) | (32.79) | (33.21) | (31.25) |
| Mean | 25.91 | 31.21 | 26.92 | 27.58 | 29.25 | 34.00 | 27.30 | 32.22 | 33.17 | - |
| | (30.60) | (33.96) | (31.25) | (31.68) | (32.74) | (35.67) | (31.50) | (34.58) | (35.16) | |
| Control | 77.33 (61.57) | | | | | | | | | |
| | | S.Em± | C.D. at 5% | | | | | | | |
| Treatment | | 0.36 | 1.12 | | | | | | | |
| Dose | | 0.19 | 0.54 | | | | | | | |
| Treatment X Dose | | 1.30 | 3.92 | | | | | | | |

| Used treatment | Used dose | |
|---|---|---|
| | $C_1$ | $C_2$ |
| 1. Plant oils (ml / 100 g seeds) | 0.10 | 0.50 |
| 2. Plant leaf powders (g / 100 g seeds) | 1.00 | 2.50 |

* Data based on 120 individuals (three replications of 40 in each).

**Figures in parentheses are the angular transformation values

**Table 4: Effect of different plant products (oils/leaf powders) on grain damage of *C. chinensis***

| Dose | Grain damage (%)* | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant oils and leaf powders | | | | | | | | | |
| | Neem oil | Karanj oil | Castor oil | Mustard oil | Ground nut oil | Datura leaf powder | Neem leaf powder | Karanj leaf powder | Aak leaf powder | |
| $C_1$ | 30.80 | 34.10 | 31.53 | 32.33 | 33.70 | 36.70 | 33.80 | 35.00 | 36.50 | 33.83 |
| | (33.71) | (35.73) | (34.16) | (34.65) | (35.49) | (37.29) | (35.55) | (36.27) | (37.17) | (35.56) |
| $C_2$ | 24.00 | 28.30 | 25.10 | 26.66 | 27.20 | 31.10 | 27.50 | 29.10 | 30.00 | 27.66 |
| | (29.33) | (32.14) | (30.07) | (31.09) | (31.44) | (33.90) | (31.63) | (32.65) | (33.21) | (31.73) |
| Mean | 27.40 | 31.20 | 28.32 | 29.50 | 30.45 | 33.90 | 30.65 | 32.05 | 33.25 | - |
| | (31.56) | (33.96) | (32.15) | (32.89) | (33.49) | (35.61) | (33.62) | (34.48) | (35.21) | |
| Control | 41.20 (39.93) | | | | | | | | | |
| | | S.Em± | C.D. at 5% | | | | | | | |
| Treatment | | 0.44 | 1.35 | | | | | | | |
| Dose | | 0.21 | 0.61 | | | | | | | |
| Treatment X Dose | | 0.81 | 2.46 | | | | | | | |

| Used treatment | Used dose | |
|---|---|---|
| | $C_1$ | $C_2$ |
| 1. Plant oils (ml / 100 g seeds) | 0.10 | 0.50 |
| 2. Plant leaf powders (g / 100 g seeds) | 1.00 | 2.50 |

* Data based on 120 individuals (three replications of 40 in each).

**Figures in parentheses are the angular transformation values

**Table 5 : Effect of different plant products (oils/leaf powders) on weight loss of *C. chinensis***

| Dose | Weight loss (%)* | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| | Plant oils and leaf powders | | | | | | | | | |
| | Neem oil | Karanj oil | Castor oil | Mustard oil | Groundnut oil | Datura leaf powder | Neem leaf powder | Karanj leaf powder | Aak leaf powder | |
| $C_1$ | 8.06 | 10.00 | 8.90 | 9.30 | 9.50 | 11.18 | 9.31 | 10.20 | 10.44 | 9.65 |
| | (16.49) | (18.43) | (17.36) | (17.76) | (17.95) | (19.53) | (17.77) | (18.63) | (18.85) | (18.10) |
| $C_2$ | 7.21 | 9.10 | 7.84 | 8.47 | 8.80 | 10.51 | 8.50 | 9.33 | 9.87 | 8.85 |
| | (15.58) | (17.56) | (16.26) | (16.92) | (17.26) | (18.92) | (16.95) | (17.79) | (18.31) | (17.30) |
| Mean | 7.64 | 9.55 | 8.37 | 8.89 | 9.15 | 10.85 | 8.91 | 9.77 | 10.16 | - |
| | (16.04) | (18.00) | (16.82) | (17.34) | (17.61) | (19.23) | (17.36) | (18.21) | (18.58) | |
| Control | 13.10 (21.20) | | | | | | | | | |

| | | S.Em± | C.D. at 5% | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | | 0.44 | 1.34 | | | | | | | |
| Dose | | 0.20 | 0.59 | | | | | | | |
| Treatment X Dose | | 0.79 | 2.40 | | | | | | | |

| Used treatment | Used dose | |
|---|---|---|
| | $C_1$ | $C_2$ |
| 1. Plant oils (ml / 100 g seeds) | 0.10 | 0.50 |
| 2. Plant leaf powders (g / 100 g seeds) | 1.00 | 2.50 |

* Data based on 120 individuals (three replications of 40 in each).

**Figures in parentheses are the angular transformation values

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RESEARCH ARTICLE

# Evaluation of Phytochemical Analysis and Antithrombolytic Activities of "Dual Organisms Lichens" – *Parmotrema perlatum*

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## ABSTRACT

Lichens are known for their extraordinary secondary metabolites and thrombolytic activity. *Parmotrema perlatum* commonly called as Canary moss or Mangalore spices.This lichen is used as the spices in the food in the Indian tradition. The use of *P.perlatum* in medicine is based on the fact that they contain unique and varied biologically active substances, as natural Antioxidant, Antimicrobial and Anti-coagulant. Since they are natural antibiotics, their metabolites exert a wide variety of biological actions including anti-mycotic, antiviral, anti-inflammatory, analgesic, antipyretic, anti-proliferative, and cytotoxic effects, they are considered as potential drugs. They contain a variety of secondary metabolites flavonoids, triterpenoids, saponins and phytosterols. It also acts as an anti-thrombolytic for the cardiovascular disease by preventing the clotting of blood in the veins and arteries by the action of *P.perlatum.* The lichens can be used as active ingredient for the preparation of drugs for its broad range of activity. The need for the control of cardiovascular diseases is in high rate. The use of synthetic drugs results in the adverse effect along with the temporary relief. But the implementation of traditional herbs on the cardiovascular patient's results will be astonished. This paper is a tribute to the wealth of Indian traditional knowledge that exists about lichens.

**Keywords:** *Parmotrema perlatum*, Secondary metabolites, Anti-coagulant, Thrombolysis.

## INTRODUCTION

Lichens are symbiotic organisms which are composed of fungi and algae. Lichens have been used in various fields, especially as a source of natural drugs in pharmaceutical industry and food supplement. Lichens are effective in the treatment of bronchitis, tuberculosis, and haemorrhoids. Lichens are important traditional medicines in many different cultures. This information has been made available to us from the contributions of hundreds of traditional knowledge holders in communities across the world. It is our responsibility to respect and value the knowledge that has been given to us. Lichens produce a wide range of organic compounds that can be divided into two groups, called primary metabolites and secondary metabolites. Primary metabolites are proteins, lipids, carbohydrates, and other organic compounds that are essential to the lichen's metabolism and structure. Some of these metabolites are produced by the lichen's fungal partner and algal or cyano bacterial partners. Secondary metabolites are produced by the fungus alone and secreted onto the surface of hyphae either in amorphous forms or as crystals. If these substances are only found in lichens, then they are called lichen substances [1].

Once such a lichens is Kalpasi *(Parmotrema perlatum*) which belongs to the family of parmoleaceae is a foliose lichens used as traditional medicines. Extracts and metabolites from this lichens exhibit pharmacological properties such as anti-inflammatory, antiulcer, anthelmintic, antibacterial, and free radical scavenging activity. Beside medicinal uses, this lichens has high economic value due to its spice properties and provides substantial livelihood support to local inhabitants. A wide range of chemical compounds including atranorin, chloroatranarin, salazinic acid, lecanoric acid, imbricaric acid, lecanora. Two terpenes, parmelanostene and permelabdone and usnic acid have been isolated from this species. The present study summarizes the information concerning the traditional uses, phytochemistry and thrombolytic activity of hydroalcoholic extract of *Parmotrema perlatum* [2].*Parmotrema perlatum (*parmelacieae), commonly known as kalpasi has a long, rich history in herbal medicine with a lengthy recorded indigenous use. It had also been found to be a promising new anti-tumor agent in numerous *in vitro* studies.

*Parmotrema perlatum* is frequently utilized as a spice to improve the taste and flavour of the foodstuff and compound similar to usnic acid, 3-ketooleanane, tridecyl myristate, icosan-1-ol, azolitmin, erythrolein, orcin, spaniolitmint, atranorin and parmelanostene permelabdone were also present. Occurrences of phytochemical components in the traditional are responsible for healing of human syndrome and they are the primary and secondary metabolites [3]. An acidic phytochemical substance present in this lichen has also been exploited as an existing antibiotic for treating skin diseases in human beings [4]. The antibacterial properties of several lichens have been investigated in 1940s and 1950s targeting the invention of antibiotic penicillin by utilizing a fungus. Numerous secondary metabolites have been screened from lichens and usnic acid is an incredibly potent material employed in pharmaceutical treatment in relation to viruses along with analgesics and antipyretics [5].

In this present study preliminary phytochemical analysis and thrombolytic activity was carried out to find out the major chemical components available in this lichens and their potent thrombolytic activity. Lichens are utilized for detecting and examining of the phytochemical components that are beneficial for production of novel drugs and also significant in research organizations as well as pharmaceutical companies for the treatment of various diseases.The development of medicine from the traditional herbs and plants to prove the traditional spices has its efficiency in healing the disease without creating any side effects. There is no systematic work done it in this lichens and this is the first report of phytochemical and antithrombotic properties of hydroalcholic extract of *Parmotrema perlatum.*

## MATERIALS AND METHODS

### Collection and Identification

*Parmotrema perlatum* used in the study was identified and the reference material has been kept under reference GRD/SC/05/16-17. Lichens of *Parmotrema perlatum* was collected randomly from the region of in around Mangalore forest, Karnataka.

### Preparation of Extract

The shade dried coarsely powdered lichens of *Parmotrema perlatum* (25g) was extracted with 250 ml of 70% ethanol and 30% aqueous by soxhlet apparatus at room temperature for 72 hours. After extraction, the extract was filtered, concentrated to dryness in rota vapour under reduced pressure and controlled temperature. Dark yellowish brown colour residue was obtained and it was coded as *Parmotrema perlatum*. The residue was then stored in desiccators. The extractive value of hydro alcohol extract of *Parmotrema perlatum* was found to be 5g.

### Phytochemical Screening Analysis of *Parmotrema perlatum*

### Qualitative Phytochemical Analysis

Preliminary phytochemicals analysis was carried out for all the extracts as per standard methods described by Brain and Turner 1975 and Evans 1996.*Parmotrema perlatum* extracts obtained by the above method was subjected to qualitative analysis for the presence of Phenolic groups, Glycosides, Alkaloids, Flavonoids, Tannins, Terpenoids, Saponins, Oils and gums as described by the method of and also as specified in the book of Practical Pharmacognosy [6].

### Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

- **Mayer's test**: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.
- **Wagner's test**: Filtrates were treated with Wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

### Detection of Flavonoids

- **Lead acetate test**: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.
- **$H_2SO_4$ test**: Extracts were treated with few drops of $H_2SO_4$. Formation of orange colour indicates the presence of flavonoids.

### Detection of Steroids

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of $H_2SO_4$. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

### Detection of Terpenoids

- **Salkowski's test**

0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated $H_2SO_4$ (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

### Detection of Anthraquinones

- **Borntrager's test**

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% $NH_3$ were added to the mixture and heated. Formation of pink color indicates the presence anthraquinones.

### Detection of Phenols

- **Ferric chloride test**: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.
- **Lead acetate test**: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

### Detection of Saponins

About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

### Detection of Tannins

A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

### Detection of Carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

### Detection of Oils and Resins

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

### Thrombolytic Assay

*In vitro* clot lysis activity of test drug *was* carried out according to the method of Prasad *et al.,* 2006 with minor modifications. Briefly, venous blood drawn from the healthy volunteers was distributed in different pre weighed sterile micro centrifuge tube (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube –weight of tube alone). To each micro centrifuge tube containing

pre-weighed clot, 100 µl of test drug was added separately. As a standard, 100 µl of Streptokinase (SK) and as a non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered I and II. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated with the blood samples of the 5 volunteers.

## RESULTS AND DISCUSSION

As a result further fractionation and scrutiny of the effective portions may be initiated along with the usage of these solvents (aqueous, hydroalcolhol, ethanol, petroleum ether and chloroform). In the preliminary phytochemical analysis the occurrence of phytochemical was confirmed in the extracts that are displayed in the Table 1.

Phytochemical studies of *P.perlatum* have led to the isolation of various chemical constituents such as atranorin, chloroatranarin, salazinic acid [8] ,lecanoric acid, imbricaric acid [9], and lecanora.[10].Two terpenes, parmelanostene and permelabdone and usnic acid have also been isolated from this lichen.Results of the phytochemical screening of hydro alcoholic extract *Parmotrema peraltum* showed the flavonoids, saponins, triterpenoids, tannins, polyphenol and sterols were present and absence of and alkaloids, protein, carbohydrate and glycosides [11].The interaction between platelets and blood vessels is important in the development of thrombosis and cardiovascular diseases. The treatment and prevention of these cardiovascular diseases the inhibition of platelet aggregation is of fundamental importance [11]. The ability of *P.peraltum* to lysis of blood clot is recorded in this report.Medicinal plants contain different therapeutic agents which may have thrombolytic activity, cytotoxic effect etc. Working with different extract showed that they can lyses thrombus as streptokinase [12]

Traditional drugs have a long history of utilization for the prevention and treatment of human illnesses. Today, numerous pharmaceuticals at present sanction by the Food and Drug Administration (FDA) have inceptions to natural sources. A major role for natural-derived compounds based on the reported immunemodulatory effects has emerged in recent times and has prompted the thorough experimental examination to focus viability and wellbeing.Nowadays phyto pharmacological investigation has created a new field to discovery spices derivative drugs, which are effective in remedial of certain diseases, and renewed the attention in herbal medicines. It is estimated that about 30% of the pharmaceuticals are prepared from plants derivatives.

A failure of hemostasis and consequent formation of blood clots in the circulatory system can produce severe outcomes such as stroke and myocardial infraction. Pathological development of blood clots requires clinical intervention with fibrinolytic agents such as urokinase, tissue plasminogen activator and streptokinase. A number of research works have been conducted to discover the plants and natural food sources and their supplements having antithrombotic (anticoagulant and antiplatelet) effect and there is indication that consuming such food leads to prevention of coronary events and stroke. In the present study hydroalcholic extract of *parmotrema perlatum* showed significant thrombolytic activity this effect may be possibly due to phyto constituents present in the lichens extract affecting activation of plasminogen both by fibrin-dependent and fibrin-independent mechanisms similar to Streptokinase which causes extra production of plasmin which breaks down fibrin the major constituent of thrombi, to dissolve unwanted blood clots.

## CONCLUSION

The present study may be useful to supplement the information with regard to its standardization and identification and in carrying out further research as a significant new source for novel bioactive substances. It can be concluded that *Parmotrema perlatum* has got the potential as a candidate for future thrombolytic agent. It can also be investigated

as a possible source of antithrombotic drugs. This is only a preliminary study and investigated be phytochemically and to exploit their medicinal and pharmaceutical potentials. Moreover, these *Parmotrema perlatum* lichen is also used as fodder for animals and in spices for human consumption where it could play major role for possible formulation of new drug to fight against pathogens.

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**Fig 1. Fresh *Parmotrema perlatum***

**Fig 2. *Dried Parmotrema perlatum***

**Table 1:Phytochemical Analysis of Different Extract of *Parmotrema perlatum***

| S.No. | | Aqueous | Hydro alcohol | Ethanol | Petroleum ether | Chloroform |
|---|---|---|---|---|---|---|
| 1 | Alkaloid | | - | + | ND | ND |
| 2 | Flavonoid | | +++ | - | + | ND |
| 3 | Triterpenoid | + | ++ | - | ND | + |
| 4 | Phenolic compound | + | ++ | + | + | + |
| 5 | Protein | ND | - | - | ND | ND |
| 6 | Carbohydrates | ND | - | - | ND | ND |
| 7 | Saponin | ++ | +++ | - | ND | ++ |
| 8 | Steroids | ND | ++ | + | + | ND |
| 9 | Glycosides | + | - | - | ND | ND |
| 10 | Amino acid | ND | - | - | ND | ND |
| 11 | Tannin | + | - | ++ | + | + |
| 12 | Oil | ND | - | - | ND | ND |
| 13 | Gums & Musilage | + | - | - | ND | ND |
| 14 | Chlorogenic compound | ND | - | - | ND | ND |

+++ = high; ++ = moderate; + = low; ND = not detectable.

**Table2 : Behaviour of drug powder of hydroalcoholic extract *Parmotrema perlatum* with various chemical reagents**

| S.No | Test for | Reagents | Reaction | Observation |
|---|---|---|---|---|
| 1. | Flavonoid | Mg bits + HCl | Magenta colour | (+)ve |
| 2. | Alkaloids | Mayer's reagent | Cream precipitate | (-)ve |
| | | Dragendroff's reagent | Reddish brown precipitate | (-)ve |
| | | Hager's reagent | Yellow precipitate | (-)ve |
| | | Wagner's reagent | Reddish brown precipitate | (-)ve |
| 3. | Saponin | Water shake | Leather formation | (+)ve |

| | | | | |
|---|---|---|---|---|
| 4. | Tannins and Phenolic compound | Ferric chloride | Brownish green or blue-black | (+)ve |
| 5. | Sterol | Acetic anhydride + Sulphuric acid | Bluish green | (+)ve |
| 6. | Triterpenoids | Chloroform + $H_2SO_4$ | Reddish brown | (+)ve |
| 7. | Protein and Amino acids | Ninhydrin | No blue colour | (-)ve |
| 8. | Carbohydrates | Fehling's A and B solution | Brick red precipitate | (-)ve |
| 9. | Glycosides | Sodium nitroprusside solution | Pink to red colour | (-)ve |

**Table 3.Thrombolytic Activity of Hydroalcoholic Extract *Parmotrema perlatum***

| S.No. | Weight of empty tube A(g) | Weight of tube with clot B(g) | Weight of clot C (B-A) (g) | Weight of tube with clot after lysis D(g) | Weight of lysis E (B-D)(g) | % of clot lysis | Average % of clot lysis |
|---|---|---|---|---|---|---|---|
| 01 | 1.093 | 1.384 | 0.291 | 1.260 | 0.124 | 42.61 | |
| 02 | 1.017 | 1.571 | 0.554 | 1.307 | 0.264 | 47.65 | |
| 03 | 1.062 | 1.459 | 0.397 | 1.238 | 0.221 | 55.66 | |
| 04 | 1.015 | 1.587 | 0.572 | 1.342 | 0.245 | 42.83 | |
| 05 | 1.010 | 1.544 | 0.534 | 1.298 | 0.246 | 46.06 | **46.96** |

% of clot lysis = (wt of released clot /clot wt) × 100.**Negative control: 2.048, Positive control: 56.12,Test drug: 46.96**

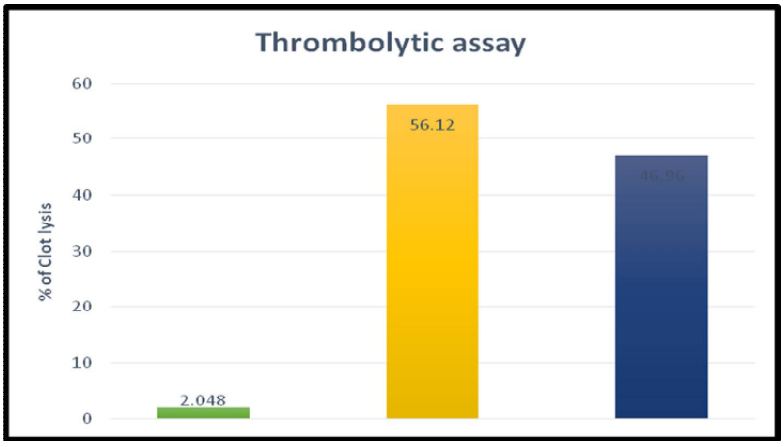


**Fig - 3: Percentage of Clot Lysis by Distilled Water, Streptokinase and Hydroalcoholi Extract *Parmotrema Perlatum.***

1. Control (sterile distilled water)
2. Standard drug-**Streptokinase**

**3 to 7-** Healthy volunteers treated with Hydroalcoholic extract *parmotrema peraltum*

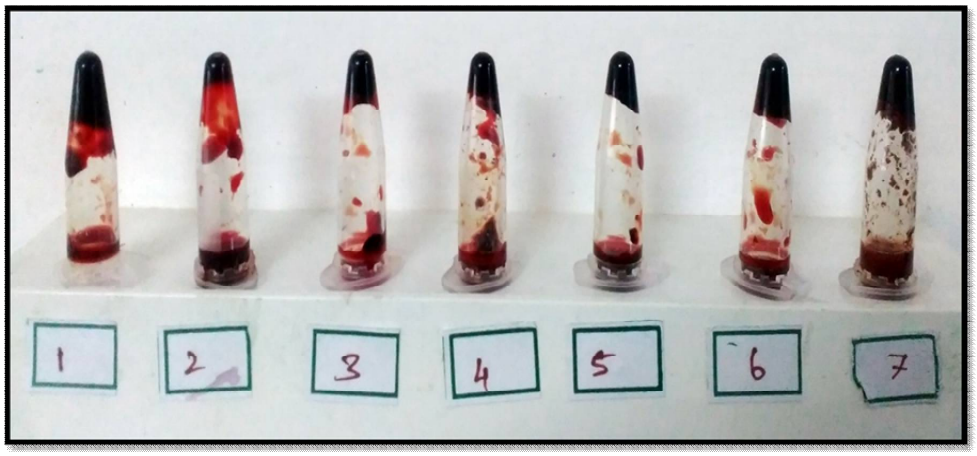


**Fig – 4: Dissolved Clots after Treating with Hydroalcoholic Extract *Parmotrema perlatum***

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RESEARCH ARTICLE

# Effect of Treated Distillery Effluent and Biocompost on Soil Urease and Dehydrogenase Activity in Paddy (*Oryza sativa*.L.,)

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## ABSTRACT

In India, sugar industry is the second largest agro-based industry producing enormous quantities of by-products like molasses, pressmud *etc*. The Treated Distillery Effluent (TDE) is waste water, which could be recycled in agriculture both as irrigation water and as a source of plant nutrients. The beneficial effect of organic matter for enhancing the soil fertility and thereby improving the crop productivity is well established. Thus, the soil application of TDE could offer the double benefit of safe disposal of wastes and its effective utilization for agricultural production. A field experiment was conducted to study the effect of Treated Distillery Effluent (TDE) and biocompost on soil urease and dehydrogenase activity using paddy as a test crop (ADT- 43). The application of TDE @ 1.0 lakh litres $ha^{-1}$(M3) or TDE @ 1.5 lakh litres $ha^{-1}$ (M4) along with 37.5% N as urea + 37.5 % N as biocompost (S7) increased the soil enzyme activity. Based on the increase in soil enzyme activity, it can be concluded that TDE @ 1.0 lakh litres $ha^{-1}$(M3) or TDE @ 1.5 lakh litres $ha^{-1}$ (M4) along with 37.5% N as urea + 37.5 % N as biocompost (S7) can be recommended as a nutrient source for paddy crop.

**Keywords :** Treated distillery effluent, Biocompost, Paddy, Enzyme activity, Nutrient source, Yield

## INTRODUCTION

Application of TDE on soils is one of the most economical resources for the soil fertility amelioration through improvement in soil water holding capacity, texture, structure and nutrients retention. Now days in our country due to the increasing number of sugar mills and distillery units, application of distillery effluent on soil nearly become

mandatory. These compounds may change soil physico- chemical properties and soil enzyme activities. Soil enzymes activities play an essential role in catalyzing reactions, which are necessary for the decomposition of organic matter and nutrient cycling in ecosystems, involving a range of plants, microorganisms, animals and their debris. Therefore, changes in enzymes activity could alter the availability of nutrients for plant uptake and these changes are potentially sensitive indicators of soil quality. Therefore, the main objectives of the present study were to evaluate the effect of different application rates of distillery effluent, on soil urease and dehydrogenase activities.

## MATERIALS AND METHODS

Field experiment was conducted using paddy as a test crop (ADT- 43). The experiment was conducted in a split plot design with four main plots *viz*., control; TDE @ 0.5 lakh litres $ha^{-1}$; TDE @ 1.0 lakh litres $ha^{-1}$; TDE @ 1.5 lakh litres $ha^{-1}$. Different levels of N fertilizers *viz.,* 100 per cent N as urea, 75 per cent N as urea, 100 per cent N as biocompost, 75 per cent N as biocompost, 75 per cent N as urea and 25 per cent N as biocompost, 37.5 per cent N as urea and 37.5 per cent N as biocompost and control were imposed as seven subplot treatments and the treatments were replicated twice. TDE was uniformly applied to each plot as per the treatment schedule at 45 days before planting.

The soil of the experimental field belong to Poovalur series (*Typic haplustert*)*,* neutral in pH (pH 7.58) and low in EC (0.30 $dSm^{-1}$). The organic carbon content (4.00 g $kg^{-1}$) and the alkaline $KMnO_4$-N (162 kg $ha^{-1}$) were found to be low. The Olsen-P level (16 kg $ha^{-1}$) and the $NH_4OAc$-K (205 kg $ha^{-1}$) were medium. The bacterial, fungal and actinomycetes population were found to be 10.2 x $10^6$ CFU $g^{-1}$ of soil, 14 x $10^4$ CFU $g^{-1}$ of soil, 5.1 x $10^3$ CFU $g^{-1}$ of soil respectively. The urease and dehydrogenase activity were found to be 4.5 µg $NH_4$-N $g^{-1}$ dry soil $hr^{-1}$ and 2.5 µg TPF $g^{-1}$ dry soil $hr^{-1}$ respectively.

The enzymes viz., Dehydrogenase and urease activities were estimated by Chendrayan *et al.,* (1980) [1] and Bremner and Mulvany (1978) [2] respectively. The data on various characters studied during the investigation were statistically analyzed by the method given by Gomez and Gomez (1984) [3]. The critical difference was worked out at 5 per cent (0.05) probability levels.

## RESULTS AND DISCUSSION

The impact of TDE and biocompost as well as the different levels of fertilizers on soil urease and dehydrogenase activity was very well pronounced and the results are as follows.

### Soil enzymes activity as influenced by different levels of TDE and biocompost

Enzyme activity in soil is an indirect indication of the microbial activity, which is directly correlated with soil microbial population. In the present investigation, greater activities of dehydrogenase and urease were associated with the TDE application.Application of TDE and biocompost significantly increased the soil enzyme activity. Among the main plot treatments, application of TDE @ 1.5 lakh litres ha-1 (M4) recorded highest urease and dehydrogenase activity of 7.12 µg NH4-N g-1 dry soil hr-1 and 24.05 µg TPF g-1 dry soil hr-1 respectively and this treatment was being on par with the application of TDE @ 1.0 lakh litres ha-1 (M3). The TDE being liquid organic manure increased the organic matter and nutrients content of the soil and subsequently enhanced the microbial biomass. The high dose of TDE along with the recommended dose of NPK recorded the highest value. It implies that organic and inorganic nutrient inputs provided a nutrient rich environment, which is essential for the development of microbes and synthesis of enzymes.Engracia Madejon *et al*. (2003) [4] found a positive correlation between the organic residues and dehydrogenase, β-glucosidase, urease and protease activities of the soil. Ramana et *al*. (2002) [5] also reported that the enzyme activities were increased due to the application of distillery effluent. Generally, organic manure addition was found to enhance the microbial activities which in turn favoured the synthesis of various enzymes in soil. These three enzymes play a significant role in the bio-transformation of nutrients in soil, and thus influence the nutrients availability in soil and uptake by crops. The mineralization rate of organic P is relevant to both

P nutrition of crops and phosphatase activity in soil. Therefore, higher enzyme activities in soil suggested that the mineralization of N and P was greater due to the application of spent wash. Similar results was reported by Dinesh (2011) [6] and Previna (2012) [7].

Among the N fertilizer levels, S6 (75% N as urea +25% N as biocompost) recorded higher urease and dehydrogenase activity of 6.32 µg NH4-N g-1 dry soil hr-1 , 21.30 µg TPF g-1dry soil hr-1 respectively which was being comparable with S7 (37.5% N as urea + 37.5% N as biocompost).The interaction effect of M × S treatment was found to be significant. Application of TDE @ 1.0 lakh litres ha-1 along with 37.5% N as urea + 37.5% N as biocompost (M3S7) recorded higher urease and dehydrogenase activity of 7.50 µg NH4-N g-1 dry soil hr-1 and 25.30 µg TPF g-1dry soil hr-1 respectively. This was followed by the application of TDE @ 1.5 lakh litres ha-1 along with 37.5% N as urea + 37.5% N as biocompost (M4S7) which recorded higher enzyme activity. Therefore, higher enzyme activities in soil suggested that the mineralization of N was greater due to the application of distillery effluent (Rajannan, 1998) [8].

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**Table 1. Characteristics of Initial Soil**

| Parameters | Values |
|---|---|
| 1. **Mechanical composition** | |
| Textural class | Sandy Clay |
| Soil series | Poovalur Series |
| Soil taxonomy | *Typic haplustert* |
| **2.Chemical composition** | |
| pH (1: 2.5 soil water suspension) | 7.58 |
| EC ($dSm^{-1}$) (1: 2.5 soil water extract) | 0.30 |
| Organic carbon ($g\ kg^{-1}$) | 4.00 |
| $KMnO_4$ - N ($kg\ ha^{-1}$) | 162 |
| Olsen - P ($kg\ ha^{-1}$) | 16 |
| $NH_4OAc$ - K ($kg\ ha^{-1}$) | 205 |

| **3.Biological properties** | |
|---|---|
| Bacteria (x $10^6$ CFU $g^{-1}$ of soil) | 10.2 |
| Fungi (x $10^4$ CFU $g^{-1}$ of soil) | 14.0 |
| Actinomycetes (x $10^3$ CFU $g^{-1}$ of soil) | 5.1 |
| Dehydrogenase activity (µg TPF $g^{-1}$ of soil $hr^{-1}$) | 2.5 |
| Urease activity (µg $NH_4$-N $g^{-1}$ of soil $hr^{-1}$) | 4.5 |

$dSm^{-1}$– deci Siemen per metre ,CFU- Colony forming units, TPF- Triphenylformazan

**Table 2. Soil Dehydrogenase Activity as Influenced by Different Levels of Tde and Biocompost**

| **Treatments** | **Dehydrogenase activity (µg TPF $g^{-1}$ soil $hr^{-1}$)** | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** | **S7** | **Mean** |
| **M1** | 1.27 | 1.45 | 1.43 | 1.40 | 1.38 | 1.49 | 1.41 | **1.40** |
| **M2** | 1.81 | 1.95 | 1.95 | 1.90 | 1.88 | 1.99 | 1.93 | **1.92** |
| **M3** | 2.10 | 2.31 | 2.28 | 2.21 | 2.17 | 2.36 | 2.44 | **2.27** |
| **M4** | 2.21 | 2.32 | 2.34 | 2.28 | 2.25 | 2.38 | 2.40 | **2.31** |
| **Mean** | **1.85** | **2.01** | **2.00** | **1.95** | **1.92** | **2.06** | **2.04** | **1.97** |

| | **M** | **S** | **M at S** | **S at M** |
|---|---|---|---|---|
| SEd | 0.03 | 0.01 | 0.03 | 0.01 |
| CD(5%) | 0.10 | 0.02 | 0.10 | 0.02 |

**M1** - Control; **M2** - application of TDE @ 0.5 lakh litres $ha^{-1}$; **M3** - application of TDE @ 1.0 lakh litres $ha^{-1}$;

**M4** - application of TDE @ 1.5 lakh litres $ha^{-1}$

**S1** - Control; **S2**- 100 % N as urea; **S3** - 75 % N as urea; **S4**- 100 % N as biocompost ; **S5**- 75 % N as biocompost ;

**S6** - 75 % N as urea and 25 % N as biocompost ;**S7** - 37.5 % N as urea and 37.5 % N as biocompost

**Table 3. Soil Urease Activity as Influenced by Different Levels of Tde and Biocompost**

| Treatments | Urease activity (µg NH4-N $g^{-1}$ soil $hr^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** | **S7** | **Mean** |
| **M1** | 3.91 | 4.47 | 4.42 | 4.31 | 4.25 | 4.60 | 4.36 | **4.33** |
| **M2** | 5.56 | 6.02 | 6.02 | 5.86 | 5.80 | 6.13 | 5.94 | **5.90** |
| **M3** | 6.46 | 7.12 | 7.03 | 6.79 | 6.69 | 7.25 | 7.50 | **6.98** |
| **M4** | 6.81 | 7.15 | 7.22 | 7.03 | 6.94 | 7.32 | 7.40 | **7.12** |
| **Mean** | **5.68** | **6.19** | **6.17** | **6.00** | **5.92** | **6.32** | **6.30** | **6.08** |

| | **M** | **S** | **M at S** | **S at M** |
|---|---|---|---|---|
| SEd | 0.10 | 0.01 | 0.10 | 0.01 |
| CD(5%) | 0.30 | 0.02 | 0.30 | 0.02 |

**M1** - Control; **M2** - application of TDE @ 0.5 lakh litres $ha^{-1}$; **M3** - application of TDE @ 1.0 lakh litres $ha^{-1}$;

**M4** - application of TDE @ 1.5 lakh litres $ha^{-1}$

**S1** - Control; **S2**- 100 % N as urea; **S3** - 75 % N as urea; **S4**- 100 % N as biocompost ; **S5**- 75 % N as biocompost ;

**S6** - 75 % N as urea and 25 % N as biocompost ;**S7** - 37.5 % N as urea and 37.5 % N as biocompost

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RESEARCH ARTICLE

# Comparitive Morphometry and Histomorphological Study on Superficial Lymph Nodes of Head Region in Deccani Sheep (*Ovies aries*) and Bidri Goat(*Capra hircus*)

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## ABSTRACT

The present study provides a baseline data on morphometry and histomorphology of superficial lymph nodes of head region namely Parotid and mandibular in Deccani sheep and Bidri goat. Parotid and Mandibular lymph nodes of six Adult Deccani Sheep and Bidri goat collected were collected and linear parameters like length, width, thickness and weight were recorded and studied using light microscopy. The nodes were surrounded with a capsule of dense connective tissue and smooth muscles. Subcapsular and trabecular lymphatic sinuses were present to convey lymph through them. The cortex formed lymphoid follicles were primary and secondary lymphoid follicles are present. Medulla consists of medullary trabeculae, sinuses, blood vessels and cosisits of diversified cell popullation like plasma cells, lymphoblast and macrophages.

**Keywords:** morphometry, histomorphology, Deccani sheep, Deccani sheep, lymphoblast.

## INTRODUCTION

Small ruminant like sheep and goat form an important economical and ecological niche of agricultural system in India. Goat and sheep seem to be best choice of livestock component to provide security to the growing population and wellbeing of society. There has been considerable progress in science and technology to understand small ruminant biology and knowledge in this field need to be transformed into practice for sustainable production. Bidri goat and Deccani sheep are the most versatile breeds of Bidar district of Karnataka state. These animals are mostly kept for meat and it is strongly felt that detailed anatomical data should be established pertaining to its superficial lymph nodes as these play an important and indispensable role in the process of meat inspection and diagnosis of different disease processes. Therefore the aim of this investigation, the first one of this kind on Bidri goat and Deccani sheep was to elucidate the histomorphological aspects of certain superficial plalpable lymph nodes of head region namely Parotid and Mandibular in these animals.

## MATERIALS AND METHODS

The tissue samples required for the work were collected from different local slaughter houses from six adult Bidri goats and six adult Deccani sheep. The collected lymph nodes namely Parotid and Mandibular lymph nodes were cleaned by removing extra fascia and linear parameters like length, width, thickness and weight of each fresh lymph node of sheep and goat were measured (cm) by vernier caliper and digital weight balance and readings were recorded and a small sample of 1cm was cut from each lymph node of sheep and goat and washed in normal saline and later fixed in fixative 10% Neutral buffered formalin. The samples were then processed in isopropyl alcohol-xylene sequence and embedded in paraffin by routine method (Luna, 1968). Sections were cut at 5-6µm thickness by using microtome and were utilized for histological studies.

### Methods

The following staining techniques were carried out to study histological features of Parotid and Mandibular lymph nodes in Deccani sheep and Bidri goat.

### Histological staining techniques

1)Ehrlich's Haematoxylin and Eosin stain for general histological observations (Luna, 1968), 2)Van Geison's stain for collagen fibres (Bancroft et al., 2008), 3)Gomori's method for reticular fibres (Luna, 1968), 4)Verhoeff's stain for Elastic fibres (Bancroft et al., 2008), 5)Toludine blue method for mast cells (Singh and Sulochana, 1996).

### Statistical analysis

The thickness of capsule, diameter of subcapsular sinus, primary lymphatic nodules on its long axis and secondary lymphatic nodule on its long axis were measured by using image analyzer software carton images. Data collected regarding various parameters of Parotid and Mandibular lymph nodes in Bidri goat and Deccani sheep were tabulated and the available data were tested for statistical significance by simple t-Test using software SAS. SAS/START (2012).

# RESULTS

### Gross Morphometry

Different gross values like length(cm), width(cm), thickness(cm) and weight(gm) of Parotid and Mandibular lymph nodes of six Bidri goats and Deccani sheep were recorded in Table 1.

### Histomorphology

### Capsule and trabeculae

In the present study Parotid and Mandibular lymph nodes in both Bidri goat and Deccani sheep were surrounded by connective tissue capsule measuring 37.72±3.649μm and 51.25±3.911 μm in diameter respectively in Bidri goat and 40.26±2.921μm and 50.179±4.117 μm in diameter respectively in Deccani sheep (Table 2) which surrounded the whole parenchyma of node except at hilus. The trabeculae originated from capsule and entered the parenchyma of node(Figure 1). In both Bidri goat and Deccani sheep subcapsular sinus was present, which separates the capsule and parenchyma of node and further continued as trabecular sinus and medullary sinus and subcapsular sinus was traversed by reticular fibres(Figure 1). The capsule and trabeculae consisted of dense connective tissue namely collagen fibres, elastic fibres and reticular fibres and Reticular fibres formed an inner lamina in capsule and borders of trabeculae. Smooth muscle fibres were also observed in capsule and trabeculae in both species(Figure 1,2&3).

### Cortex

Cortex of both Bidri goat and Deccani sheep was characterized by dense population of lymphocytes which was organized in to primary and secondary lymphatic nodules. Primary lymphatic nodules showed uniform mass of small matured lymphocytes whereas secondary lymphatic nodules had central pale area called germinal center which was surrounded by small lymphocytic area called corona(Figure 4). The germinal centre of the secondary lymphatic nodules and observed a different cell population like few lymphoblasts, lymphocytes, plasma cells while reticular fibres were distributed to the periphery of secondary lymphatic nodules(Figure 1).

### Medulla

In the present study medulla of Bidri goat and Deccani sheep lymph nodes were diffused areas and had network like cords of the dense lymphatic tissue medullary cords, medullary trabeculae in branching pattern and medullary sinuses consisted of empty spaces which were traversed by reticular fibres. All these aspects formed inner part of the lymph node after the cortex towards the hilus region were blood vessels, nerve fibres and lymphatic vessels were present(Figure 4). Medulla of both species contained population of mast cells which are present in conjunction with plasma cells, reticular cells, lymphocytes and macrophages(Figure 5).

# DISCUSSION

### Gross Morphology

In the present study on the basis of linear measurements Parotid lymph node in adult Deccani sheep and Mandibular lymph node in Bidri goat was largest. However, in Deccani sheep weight of Parotid was slightly more than the Mandibular lymph node, studied in the present research. There was significant difference ($P< 0.05$) in the length of Mandibular lymph node, between Deccani sheep and Bidri goat.

### Histomorphology

### Capsule and trabeculae

In the present study Parotid and Mandibular lymph nodes in both Bidri goat and Deccani sheep were surrounded by connective tissue capsule which surrounded the whole parenchyma of node except at hilus. The trabeculae originated from capsule and entered the parenchyma of node. These findings were similar to earlier statements Faroon et al. (1989) in caprines, Sarma & Sarma (2001) in Assam goat. Comparatively Parotid lymph node in Deccani sheep and Mandibular lymph node in Bidri goat were having thicker capsule. Gadre (1982) in cow calf and Sarma *et al*.(2008) in kagani goat noticed less elastic fibres in capsule than trabeclae. Similar observations were also recorded in the present study in both species.

### Cortex

Cortex of both Bidri goat and Deccani sheep was characterized by dense population of lymphocytes which was organized in to primary and secondary lymphatic nodules. Primary lymphatic nodules showed uniform mass of small matured lymphocytes whereas secondary lymphatic nodules had central pale area called germinal center which was surrounded by small lymphocytic area called corona(Figure 4). This was in accordance with the findings of Makoto Sugimura (1962) in cat and Sarma & Sarma (2001) in assam goat. Kalita *et al*. (2014) Observed reverse pattern cortex and medulla of lymph nodes of Mizo Local Pig where cortex was diffusely distributed in central area of lymph nodes. These variations between Mizo Local Pig and present study might be due to the species variations. Sarma *et al*. (2008) in kagani goat, Gadhave *et al.* (2011) in murrah buffalo observed the germinal centre of the secondary lymphatic nodules and observed a different cell population like few lymphoblasts, lymphocytes, plasma cells while reticular fibres were distributed to the periphery of secondary lymphatic nodules. Similar observations were also recorded in the present study in both species in both lymph nodes.

### Medulla

In the present study medulla of Bidri goat and Deccani sheep lymph nodes were diffused areas and had network like cords of the dense lymphatic tissue medullary cords, medullary trabeculae in branching pattern and medullary sinuses consisted of empty spaces which were traversed by reticular fibres. All these aspects formed inner part of the lymph node after the cortex towards the hilus region were blood vessels, nerve fibres and lymphatic vessels were present. These findings were accordance with the findings of Faroon *et al.(*1989) in caprines, Sarma & Sarma (2001) in assam goa, Gadhave et al. (2011) in murrah buffalo. Gadre (1982) in cow calf, Sarma *et al.* (2008) in kagani goat observed that medullary trabeculae were richly supplied by blood vessels than cortical trabeculae. Similar observations were also recorded in the present study in both lymph nodes of both species. Medulla of both species contained population of mast cells which are present in conjunction with plasma cells, reticular cells, lymphocytes and macrophages. These findings were in accordance with observations made by Makoto Sugimura (1962) in cat, Gadre (1982) in cow calf, Faroon *et al*. (1989) in caprines.

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**Table 1: Mean±SE of linear parameters of Parotid and Mandibular lymph nodes of adult Deccani sheep and adult Bidri goat**

| LYMPH NODE | DECCANI SHEEP | | | | BIDRI GOAT | | | |
|---|---|---|---|---|---|---|---|---|
| | L(cm) | W(cm) | T(cm) | Wt(gm) | L(cm) | W(cm) | T(cm) | Wt(gm) |
| PAROTID | 1.98±0.09$^{a}$ | 1.31±0.14$^{a}$ | 0.78±0.10$^{a}$ | 1.66±0.09$^{a}$ | 1.76±0.04$^{a}$ | 1.13±0.04$^{a}$ | 0.88±0.13$^{a}$ | 1.43±0.11$^{a}$ |
| MANDIBULAR | 1.56±0.14 $^{a}$ | 0.96±0.06$^{a}$ | 0.53±0.03$^{a}$ | 1.23±0.11$^{a}$ | 1.66±0.04$^{b}$ | 1.1±0.06$^{a}$ | 0.66±0.07$^{a}$ | 1.23±0.12$^{a}$ |

Dissimilar superscripts differ significantly p<0.05

**Table 2: Mean ± SE of histometric parameters (µm) of Parotid and Mandibular lymph nodes in Deccani sheep and Bidri goat**

| LYMPH NODE | Maximum thickness and diameter | DECCANI SHEEP | BIDRI GOAT |
|---|---|---|---|
| | | Mean±SE | Mean±SE |
| PAROTID | Capsule | 40.26±2.921$^{a}$ | 37.72±3.649$^{b}$ |
| | Subcapsular sinus | 57.53±6.115$^{b}$ | 60.25±5.972$^{a}$ |
| | Primary lymphatic nodule | 273.59±12.005$^{a}$ | 275.52±12.159$^{a}$ |
| | Secondary lymphatic nodule | 325.01±14.539$^{a}$ | 325.11±14.057$^{a}$ |
| MANDIBULAR | Capsule | 50.179±4.117$^{a}$ | 51.25±3.911$^{a}$ |
| | Subcapsular sinus | 50.61±5.977$^{a}$ | 52.52±6.259$^{a}$ |
| | Primary lymphatic nodule | 280.21±12.992$^{a}$ | 279.17±13.592$^{a}$ |
| | Secondary lymphatic nnodule | 400.97±15.237$^{b}$ | 405.27±15.672$^{a}$ |

**Dissimilar superscripts differ significantly p<0.05**

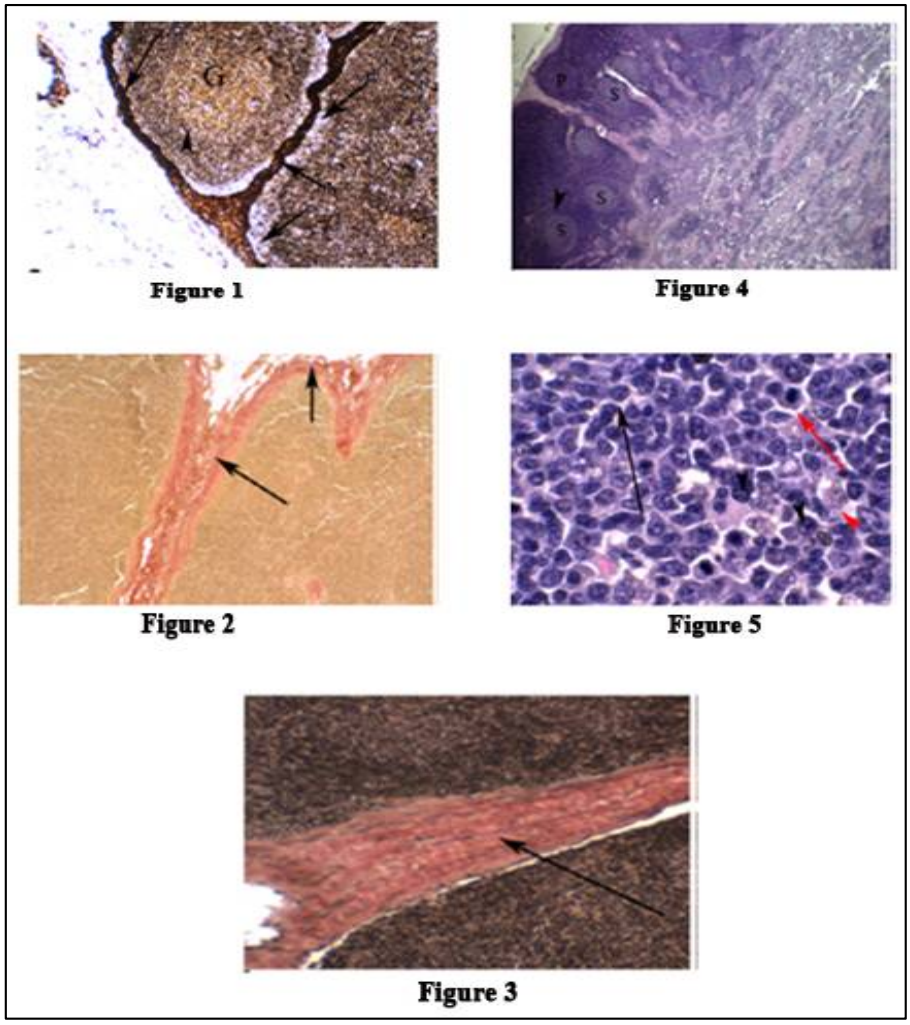


**Figure 1: Photomicrograph of Parotid lymph node of Bidri goat showingreticular fibres in the a) Capsule b) Trabeculae c) Subcapsular sinus d) Trabecular sinus and around germinal center (G)**
**Gomori's stain X 40**

**Figure 2: Photomicrograph of Mandibular lymph node of Deccani sheep showing collagen fibres (arrow) in the capsule and trabeculae**
**Vangieson's stain X 10**

**Figure 3: Photomicrograph of Parotid lymph node of Bidri goat showing elastic fibres (arrow) in the capsule and trabeculae**
**Verhoeff's stain X 10**

**Figure 4: Photomicrograph of Mandibular lymph node of Deccani sheep showing primary lymphatic nodule(P) and secondary lymphatic nodule (S), corona region (arrowhead), Medullary trabeculae, Medullary sinuses and Medullary cords.**
**H&E X 10**

**Figure 5: Photomicrograph of Parotid lymph node of Bidri goat showing lymphocytes (arrow), plasma cells (arrow head), macrophages (red arrow) and reticular cells (red arrow head) in the medulla. H&E X 100**

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RESEARCH ARTICLE

# Delineation and Assessment of Available Zinc Status in Tirunelveli District of Tamil Nadu using GIS and GPS Techniques

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## ABSTRACT

A study was undertaken in Tirunelveli district of Tamil Nadu with a view to assess the DTPA-Zn status of soils at block level. A sum of 1,798 geo-referenced surface soil samples from nineteen blocks of Tirunelveli district representing different soil units as per the soil map prepared on 1:50,000 scales was collected randomly at 0-15 cm depth using Global Positioning System. The soil samples were analysed for available DTPA- Zn and the DTPA-Zn content varied from 0.01 to 8.04 mg $kg^{-1}$ soil. Analytical results and the GPS data was used for the preparation of thematic maps showing spatial distribution of micronutrients status block wise in the district. Locations of soil sampling sites of Tirunelveli district were marked on base map on 1: 50,000 scales prepared from State Revenue Maps and digitized using Arc-info GIS. The delineation study thus clearly indicates that, DTPA – Zn was found to be deficient in 90 per cent of soils of Tirunelveli district.

**Keywords:** DTPA-Zn, GPS, GIS, Thematic maps.

## INTRODUCTION

Soil micronutrients play a vital role in the growth, development, yield of plant besides the information on the nutritional status of an area, and thus go a long way in planning judicious fertilizers and soil management practices to develop economically viable alternatives for farming community. The estimation, characterization and comparison

of micronutrients of soil are important issues in site-specific crop management, precision farming and sustainable agriculture (Deb, 1997)[1].

In the context of today changing scenario, there is a need to generate the spatial data of micronutrients using frontier technologies like Global Positioning System (GPS) and Geographical Information System (GIS). The GPS has revolutionized positioning concept though it started primarily as a satellite based radio navigation system providing precise, three dimensional position navigation and time information. The GIS provides scientists, planners, managers and decision makers an efficient way of combining and analyzing geo-referenced and descriptive data from different sources (soils, vegetation, geology, land covers and others) for better understanding and management of natural resources (Fernandez et al.1993)[2]. The thematic maps for individual nutrient (Zn, Fe, Cu and Mn) is prepared by using GIS software (Nayak et al, 2006)[3] and multi micronutrient maps are generated by integrating individual maps of Fe, Mn, Zn and Cu in the GIS (Sood et al. 2004)[4]. This will also help in monitoring changes in micronutrient status over a period of time. It can be revisited with help of GPS, which is otherwise not possible in the random sampling.
With this background, a study was conducted with revolutionary effort to examine soil available zinc status and delineate the DTPA- Zn status scrupulously at block level in the Tirunelveli district of Tamil Nadu.

### Study Area

Tirunelveli district in Tamil Nadu is bounded by Virudhunagar district in the North, Western Ghats in the West, Kanniyakumari district in the South and Thoothukudi district in the East. Tirunelveli district is comprised of 11 taluks, 19 blocks and 628 Revenue villages covering an area of 6, 81,065 ha of land. The Tirunelveli district lies between 8°.08' and 9°.25' of the Northern latitude and 77°.09' and 77°.59' of Eastern longitude. Major portion of the district is covered by plain topography. Red loam is the predominant soil type in the district accounting for 48.21 per cent followed by the black soil of 30.09 per cent.

## MATERIALS AND METHODS

### Collection of Soil Samples

Totally 1798 geo-referenced surface soil samples covering the entire village in nineteen blocks of Tirunelveli district were collected randomly at 0-15 cm depth by adopting the standard procedures of soil sample collection. The GPS data (Latitude ºN and Longitude ºE) were collected from each sampling sites distributed over the entire Tirunelveli district by using Garmin GPS 76CS model (Fig. 1). The collected soil samples were dried, gently bound, sieved (2 mm sieve) and preserved in polythene bags for DTPA extractable micronutrients (Lindsay and Norvell, 1978)[5]. Locations of soil sampling sites of Tirunelveli district were marked on base map on 1: 50,000 scales prepared from State Revenue Maps and digitized using Arc-info GIS (9.2).

### Generation of Map

The Tirunelveli district map (1:50,000) was vectorised by using Raster to Vector software (R2V), and then exported into Arc-GIS software. Database on DTPA- Zn status of the study area was developed using Microsoft Excel package. The database was exported to Arc GIS software and the thematic map on DTPA- Zn status was generated.

## RESULTS AND DISCUSSION

In Tirunelveli district, the two major soil groups exists are the red and black soils. Red loam is the predominant soil type accounting for 48.21 per cent followed by the black soil of 30.09 per cent. The other types of soils are lateritic soil, sandy coastal alluvium, red sandy soil and others.

### DTPA- Zinc

There were wide variations in DTPA-Zn content of different blocks of Tirunelveli district surveyed, which varied from 0.01 to 8.04 mg $kg^{-1}$ with a mean value of 0.59 mg $kg^{-1}$ soil. In Tirunelveli district, Malayankulam village of Cheranmahadevi block showed the highest mean value of 8.04 mg $kg^{-1}$ with respect to available Zn followed by Mannarkovil village of Ambasamudram block as 5.71 mg $kg^{-1}$ (Table 1).In the soils of Kalakadu, Manur and Tenkasi blocks, showed the lowest mean value (0.01 mg $kg^{-1}$) of available Zn content. This was due to low organic matter content in the soils of those blocks. The present results corroborate with the findings of Takkar *et al.* (1997)[6] in which they reported that when the soils are low in organic matter and not supplemented by mineral fertilization, there are prone to zinc deficiency.

Considering the critical limit of DTPA-Zn has 1.2 mg $kg^{-1}$, Krishnaswamy *et al.* (1994)[7] observed that about 90.4 per cent of samples in this district were deficient in zinc. This clearly indicates the need for regular supply of this nutrient to the crops for realizing the optimum yields.

### Thematic map

The DTPA extractable zinc of different blocks of Tirunelveli district was grouped into three categories based on the critical limits followed for availability of micronutrient in India and Tamil Nadu. The thematic map clearly identifies the blocks that are extremely deficient in micronutrient status which require utmost attention to sustain the soil productivity. The available Zn status suggests that out of nineteen blocks, eighteen blocks were found to be low (Fig.2) and the Ambasamudram block exhibits medium Zn status (1.76 mg $kg^{-1}$).

Thus, from the above investigation it is very clear that the soils of Tirunelveli district are severely deficient in available zinc. Therefore, to overcome the deficiency of zinc, the need for sustained application of organic manures or through supplementation with inorganic fertilizers is essential. Besides, the edaphic factors such as organic carbon content and free lime status are widely believed to be circumventing the availability of micronutrients, which also needs to be addressed to develop strategies for alleviation of micronutrient deficiencies in Tirunelveli district of Tamil Nadu. Micronutrient status plays a major role in increasing crop yields and soil productivity in general and hence, it is essential to adopt an integrated way of adding organic amendments with micronutrients, which will sustain the soil fertility and crop productivity.

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**Table 1.Range, mean values and percent sample category of dtpa-zn statusfor different blocks of tirunelveli district**

| S.No | Block name | Range and Mean Values | Percentage sample category | | |
|---|---|---|---|---|---|
| | | Zn (mg $kg^{-1}$) | DTPA- Zn | | |
| | | | L | M | H |
| 1. | Alangulam | 0.06 - 1.24(0.29) | 99.1 | 0.9 | 0.0 |
| 2. | Ambasamudram | 0.03 - 5.71 (1.32) | 56.9 | 10.3 | 32.8 |
| 3. | Cheranmahadevi | 0.26 -8.04(1.05) | 84.3 | 8.6 | 7.1 |
| 4. | Kadayanallur | 0.22 - 2.44(0.79) | 50.7 | 29.9 | 19.4 |
| 5. | Kadayam | 0.08 - 2.22(0.51) | 97.7 | 1.1 | 1.1 |
| 6. | Kalakadu | 0.01 - 1.78(0.60) | 96.2 | 3.8 | 0.0 |
| 7. | Keelapavoor | 0.13 - 1.41(0.47) | 97.6 | 2.4 | 0.0 |
| 8. | Kuruvikulam | 0.18 - 4.09(0.87) | 86.2 | 8.6 | 5.2 |
| 9. | Manur | 0.01 - 4.07(0.59) | 94.7 | 2.0 | 3.3 |
| 10. | Melaneelithanallur | 0.10 - 3.08(0.37) | 96.0 | 2.0 | 2.0 |
| 11. | Nanguneri | 0.08 - 0.57(0.21) | 100.0 | 0.0 | 0.0 |
| 12. | Pappakudi | 0.14 - 3.23(0.63) | 91.9 | 3.2 | 4.8 |
| 13. | Palayamkottai | 0.08 - 2.20(0.41) | 96.1 | 3.1 | 0.8 |
| 14. | Radhapuram | 0.11 - 1.25(0.41) | 99.0 | 1.0 | 0.0 |
| 15. | Sankarankovil | 0.03 - 4.05(0.72) | 86.2 | 8.6 | 5.2 |
| 16. | Senkottai | 0.08 - 2.04(0.64) | 83.0 | 10.6 | 6.4 |
| 17. | Tenkasi | 0.01 - 3.7(0.85) | 76.6 | 13.0 | 10.4 |
| 18. | Valliyoor | 0.16 - 0.96(0.38) | 100.0 | 0.0 | 0.0 |
| 19. | Vasudevanallur | 0.14 - 4.9(0.61) | 93.5 | 1.1 | 5.4 |
| | **Overall for District** | | **90.4** | **5.1** | **4.4** |

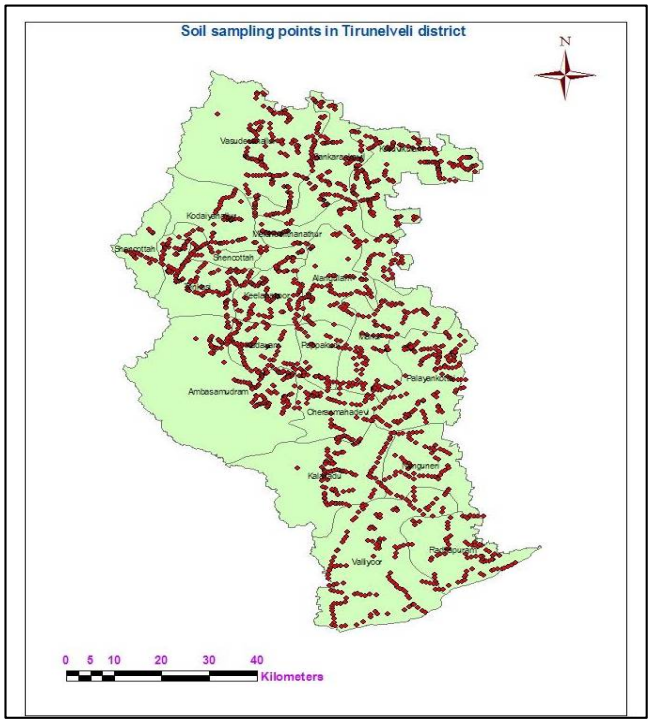


**Fig. 1. Soil Sampling Points of Tirunelveli District**

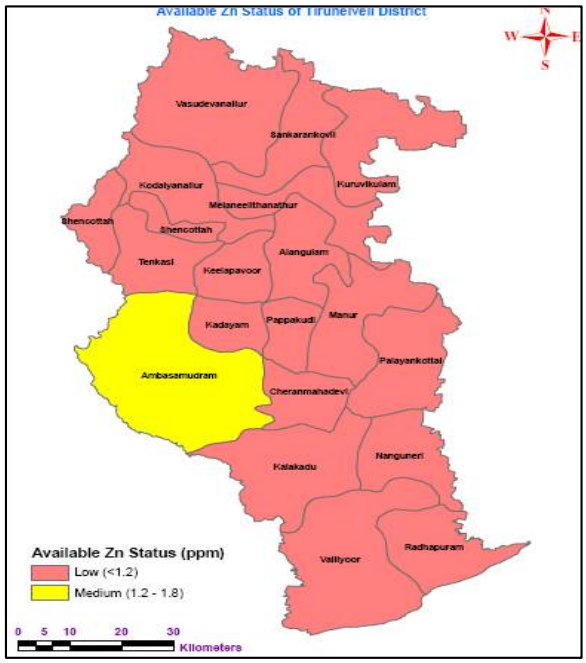


**Fig. 2. DTPA- ZN Status of Tirunelveli District**

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**RESEARCH ARTICLE**

# A Negotiation Model Based on Perspectives

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**ABSTRACT**

This article deals with negotiation preparation among buyers and different supplier types as well as examines factors that influence negotiations. Based on previous research, buyers classify suppliers into two types: strategic supplier or temporal supplier. The aim of the article is to examine how to manage negotiations to reach optimal results according to supplier type. Based on previous research, interview prompts for a focus group were prepare, which was then used to create a questionnaire for the quantitative analysis with 92 participants.The main contribution of the research study could assist negotiators in the negotiation process between a buyer and a supplier to reach optimal negotiation objectives, to better understand the supplier's perspective, and to guide negotiation based on whether a supplier is strategic or temporal.

**Keywords :** negotiation preparation, temporal supplier, questionnaire, quantitative analysis.

## INTRODUCTION

The effectiveness and efficiency of an organization depends onmany factors, which influence whether an objective is reached or not. One of them is negotiation betweenbuyers and suppliers. Conflicts can arise during the negotiating process because all the actors have different interests and objectives. Negotiation is mandatory for a buyer – supplier relationship. Thebasis for a successful collaboration is a compromise thatcan be gained through a great deal ofnegotiating based onknowledgeand experience. Malbašić and Brčić (2012, p. 104) argue that organizational values determine how to behave with partners and suppliers.Negotiation is a coordination of interests. All interests have to be converged and all interests have to be acceptable for the opposing side, both of which are necessary for a final agreement. Herbst, Voeth and Meister (2011, p. 976) found in their review of 78 studieson the negotiation processes that it is necessary to provide more attention to negotiation compared toother dailypractices. All negotiating partners have to be aware of the fact that a fair agreement is the best optionfor all business partners. The main objective of

thisresearch study isto determine how a supplier classifies the supplier during the negotiation process. We assumedthat a buyer has different negotiation objectivesbased on the type of supplier(i.e., strategic supplier or temporal supplier). We also expected different behaviour of suppliers depending ontheir position onbeing either a strategic or a temporal business partner. Interestingly, usually suppliers do not know the buyers' perspective of their role.

The buyer plans a negotiation tactic depending on theindividual supplier orthe type ofsupplier. The buyeralso defines negotiating objectives which are more or less in accordance with the suppliers' expectations. Normally, a buyer manages a negotiating process irrespective of the supplier's interest. Therefore, buyersdirectnegotiation towards their own goal.As such, our aim was to explorethe buyers' main objectives and their influential factors. We may expect from the supplier's perspective that the buyer's objective in the negotiation process is minimal price. We may also expect the same from the supplier – focus on price. As such, we would like to determineif buyers' objectivesdiffer from suppliers' objectives as well as which factors influence them.The goal was to determine if such factors are equally important for both sides and how they differ. Considering the afore-mentioned, the overarching research question is: "*How does one prepare for the negotiating process with suppliers and howdoes one reach optimal results depending on supplier type?*"

As such, we examined different negotiation approaches, whether these approaches refer to permanent or temporal suppliers, and factors influencing negotiation results. We wanted to discern influential negotiation factors, the main objectives of negotiation, and different negotiationperspective of buyers and suppliers. Based on these objectives, a mixed-methods approach was used that utilized triangulation of data. The data collected from focus groupswereanalysed qualitatively, i.e. coding and discovering relations between categories. Using Mesec's (1998, p. 109) coding method, the data from the focus groups were transformed from concepts to categories which were then transformed into the measurement tool, i.e. a questionnaire for part two of the research study.For the quantitative analysis, the questionnaire from part one of the research study was distributed to buyers and suppliers from different profitable organizations and analysed using different statistical methods. The results of the research are presentedbelow, where the differences in the average score of individual factors were assessed using Wilcox signed-rank test and Kruskal-Wallis test.

In these demanding economic conditions thistype of research is essential for management. The findings are useful for managers' decision-making when wanting to reach an optimal negotiation goal based on the type ofsupplier or when deciding whether to abandon or strengthen a negotiation based on influential factors.

## THEORETICAL FRAMEWORK

Fells (1996, p. 58) states that even when negotiators know the negotiating objectives quite well, they usually do not consider how they can reach these objectives. Liu, Huang, Luo and Zhao (212, p. 364) argue that the concept of justice between business partners influence the relationship between them. Mutual understanding in negotiating factors is crucial for the relationship and healthy business results. Lin and Chang (2012, p. 90) stress that trust is an essential factor for the relation between buyers and suppliers which consequently influences business risk that consequently leads to cost reductions. Face-to-face (F2F) communication increases the level of trust and that the buyer-seller relationship has a direct impact on business competitiveness (Ketkar, Parente and Verville 2012, p. 790; Verle, Markič, Kodrič and Gorenc Zoran, 2014, p. 932). We can increase negotiation efficiency and orientation towards objectives by understanding negotiating factors and knowing the negotiating perspective of buyers and suppliers.

Preparing for negotiationsis essential because it entails preparing theexpected results of the negotiation. Lumineau and Henderson (2012, p. 391) wrote that preparation fornegotiationsdepend on the type of suppliers and onthe expected competitive advantages. The buyers and suppliers usually prepare a plan for negotiation with check points

and as such direct the negotiation towards the expected results (Fells, 1996, pp. 50–51).Fells also stresses that planning is very important; if planning is not appropriate we cannot reach the expected results and we cannot reach a minimal point of agreement; on the other side, too ambitious goals are often a reason for the interruption or cancelation of negotiation. However, Gettinger, Koeszegi and Schoop (2012, p. 170) claim that it is possible to define or redefine the objectives duringnegotiation when the results of the opposite parties can be aligned, where small differences can evoke a feeling of a loser instead of a winner and vice versa. Ribbink and Grimm (2014, p. 115) argue that each research studytreats the behaviour of buyers and suppliers between negotiationdifferently. The viewsof buyers with regard to suppliers were examinedby many researchers. Ambrose, Marshall and Lynch (2010) state that buyers and suppliers in the supply chain have different perspectives concerning responsibility, mutual adaptation, communications, trust, uncertainty, power, and relation to success. Decreasing uncertainty is the foundation of a successful relationship for both sides. All business partners wantcollaboration with decreased uncertainty. In the afore-mentioned research study, senior managerial staff with more than ten years of experiences with suppliers participated in theresearch study, where their buyers stressed that they expect open interpersonal business activities ofgenuine relationships with anunderstanding of the risks. (pp. 1237−1283)

Most research studies that examine the relation between buyers and suppliers, such as Liu, Huang, Luo and Zhao (2012, p. 581) or De Sousa and Fairise (2014, p. 154), do not distinguish suppliers bydifferent categories.In the review of literature, we found suppliers' classification from a buyers' negotiating perspective. Byrne, Heavy, Blake, and Liston (2013, p. 1036) state that the classification is useful for negotiation due to greater efficiency and other benefits and the authorsalso suggest that it is a constructive and effective method for theselection of business partners and building partnerships.The final results of the study showed that the classification is positive for all negotiators. Many authors (e.g., Aissaouia, Haouaria, and Hassini, 2007, p. 3534) propose the model of elimination based on classification, where in the final phase a supplier is selected based on supplied raw material.Rezaei and Ortt (2013, p. 510)classified suppliers based on the material being supplied using the fuzzy logic approach. They measured capacity and willingness of suppliers, which could help managers to formulate strategic guidelines with suppliers. Deshamais (2000, p. 12) differentiates the following categories of negotiation:

- Negotiation on prices only. Theonly discussion is about prices and the result of negotiation is clearly defined. Both parties can win (a win-win result), one side can obtain more than the other, but the objective can be easily measured.
- Multi-negotiations with more open questions. The prioritized question is theprice;including other questions (delivery, warranty, additional functionality, and so forth) are also factors of negotiation. Such type of negotiations may have different outcomes and are focused on compromises where the goal is to come toan agreement. Different outcomes in negotiations are very challenging for negotiators, especially during these demanding economic conditions.

Based on the review of literature, the authors of this research study did not find any studies regarding the classification of suppliers based on the topic of negotiation or based on the suppliers categorization of a buyers' negotiation perspective. As such, this was determined as a relevant research gap and the working thesis defined is: "The buyers' negotiation approach depends on the type ofsupplier."

We are assuming that the objectives ofabuyer and supplier arein most cases different, very often even opposite. The objective depends on many factors and circumstances. Dobrijević, Stanisić and Masič (2011, p. 37) distinguish sixteen fundamental sources for negotiating power and its factors. The most important factor for an agreement is the Best Alternative to Negotiation Agreement (BATNA) by Fisher, Ury and Patton (1998, p. 120)that differs between buyers and suppliers. If the best buyer's alternative is better than the supplier's proposition, the negotiating agreement is not possible, and vice versa. In this situationone negotiating party will interrupt the negotiation process. Bogataj and Bogataj (2007, p. 11) stressed that a buyer has to have a supply chain secured by alternative suppliers or has to be secured by another manner. For this reason,the BATNA has to be exactly defined before the beginning of the negotiating process. Fells (1996, p. 58) states that 25% of negotiators are not able to continue with negotiation after a

deadlock or an interruption. The afore-mentioned studieshave stated that there are numerous factors that influence the negotiation process.

Whenever there is a high level of dependence between the buyer and supplier, the negotiating power is at equilibrium. Becauseother alternative solutions (e.g. BATNA) to reach the objectives do not exist, the only possible solution for both sides is a common agreement for both parties. The supplier becomes a partner, i.e. a strategic partner. The key for an agreement and building relationships is notnegotiating power but a common understanding and trust. Opentransparent communication and exchange of informationare essential for interdependence. Fells (1996, p. 58) further states that negotiators have to be cognizant on the importance of negotiation as well as be able to direct negotiations towardsfavourable negotiating results.A competent negotiating group or individuals are able to maintain anintegrative orientation towardsthe common goal. Such group or individuals are able to understand reactions of the opposite side and at the same time maintain mutual understanding to create a trustworthy relationship. Saorin-Iborra, Redondo-Cano and Revuelto-Taboada (2013) state that for a successful agreement both sides have to be interested, however, usually one side expressesa greater interest than the other. Normally, the initiator expressesa greater interest, especially in the initial phase of negotiation. This interestcould be changed during the negotiating process. Interest isan important influential factor for negotiating results. The understanding of interests could be an opportunity for negotiators to managenegotiation process to the"win-win" principle i.e. toward the most favourable negotiating outcome for both sides. Therefore, the selection ofcompetent negotiating individuals within a group is essential for maintaining and directing negotiating interests towards favourable results. (pp. 428–429)

Mohebbi and Shafaei (2012, p. 378) added the category of quality as an important negotiating factor. This factor is not included in this research study, namely because quality is a given factor and normal in business excellence and therefore, it is not theobject of negotiation. Lenarčič and Brcar (2014) wrote that negotiating factors such as preparation, the object of negotiation, negotiating power of position, relationships, and so forth, have a different impact on the negotiating process between buyers and suppliers. Therefore, negotiation could cause a positive or negative effect on one or more negotiating partiesbased on the intended objectives. The mood and confidence can influence negotiators. The mutual interactions are more positive in a friendly environment, behaviour is less controversial and collaboration is more intensive. The achieved objective increases satisfaction and desire for further cooperation. Anger has the opposite effect.Angry negotiators have a decreased desireto cooperate before or during the negotiating process.Anger among other factorsreduces confidence between business partners and disturbs the negotiating process or even leads to revenge. Angry negotiators are not able to take into account interests of the opposite negotiating parties. They also arenot able to assesstheir own interests, which consequentlythe opponents couldtake advantage of such a situation. Finally, common results are less favourable. (pp. 96–97)

Spaho (2013, p. 113) states that if a strategy cannot be reached or when the strategy and results are not aligned thana third partner is necessary or very often obligatory to interfere. The third partner is a mediator who offers a solution in a deadlock situation. Sometimes, objectives have to be redefined or a potential supplier might need to be replaced. A buyer and a supplier observe each another during a negotiating process and want to determine where the threshold of an agreement is. Van Poucke and Buelens (2002) studied the influence of prices on negotiating results. They concluded that the threshold of an agreement is defined by theBATNA. Suppliers with a clearly defined BATNA start with a25% higher price. They also state that the final price is 3.5% higher than the average of all the prices proposed by buyers and suppliers. However, buyers with a clearly defined BATNA start negotiation with a 20% lower price and the final result is 1% lower than the average of all negotiators. Their research confirmed that the final negotiation result is determined by who defines the initial price. The BATNA and acarefully explained negotiating area are crucial forsuccessful negotiations: therefore, this type of negotiation bringsmore favourable results. (pp. 69–72)

Based on the review of literature we can conclude that different authors take into account different factors which influence negotiation results. Aleksandrova (2012 p. 47) proposes to study allfactors which influencethe success of

relationships between business partners. However, most factors depend onthe BATNA and consequently on a power of negotiating position. In the article we wanted to determine other factors and their influence attributed by suppliers and buyers.To obtain an answer to the fundamental research question, we developed the following hypotheses:

- Hypothesis 1: The perspective of theinfluential power of individual factors on negotiation outcomes considering price and relationship differs between temporal suppliers and strategic suppliers.
- Hypothesis 2: The buyer's perspective of the influential power of individual factors on negotiation outcomesconsideringprice and relationshipdiffers depending on negotiation with temporal suppliers or strategic suppliers.
- Hypothesis 3: The perspective of theinfluential power of individual factors on negotiation out comes considering price and relationshipdiffers between buyers and suppliers.

## QUALITATIVE ANALYSIS

### Data Analysis

We carried out a semi-structured interview for data collection with focus group oftwo suppliers (S) and two buyers (B). The participants were chosen from profit organizations. The main goal of the focus group was to obtain information whether a buyer's approach to negotiation depends on the type ofsupplier, i.e. supplier's characteristics. We also were interested in which factors influence negotiation results depending on thetype ofsupplier chosen in advance.The data wereanalysed in seven steps. The duration of the focus group was 80 minutes. It was conducted with pre-prepared questions in a closed office, a quiet environment, and a relaxed atmosphere. The interview was not recorded as requestedby the participants. At the beginning, the purpose and the objective of the focus group wereexplained and the participants were introduced to one another. The guiding questions of the semi-structured interview were also introduced. A draft transcript was written during the session, which was further used for the summary of answers in a common transcript shown in Table 1. While defining open coding units certain parts of the transcript during the qualitative coding approach were split, merged, purified, synonyms were chosen, and so on. The non-relevant parts were not included. After we had determined relevant terms, we merged related terms into categories. The nextimportant step was to determine the relationship between categories. The result of the qualitative analysis was the foundation for the quantitative methodology, which was the questionnaire. The questions of the semi-structured interviewwithinthe focus group were as follows:

- Do you distinguish suppliers depending on duration of partnerships?
- Depending on suppliers' classification in which direction are negotiations directed?
- Do you know suppliers' classification depending on negotiations?
- Which factors influence negotiationoutcomes?

## RESEARCH MODEL

In the first phase, we were able to determine the relationship between levels. The firstlevel, the basic and the most influentialcategories are a) price and b) relationship. The second level category represents negotiation factors: preparation, BATNA, interest, experiences, and duration. The relationship between categories is shown in the research model (see Figure 1).

## QUALITATIVE RESULTS

During the interview we could feel contradictory interestsbetween buyers and suppliers who asked each other questions during certain moments. Based on their conversation and data analysis, we concluded that buyers have different negotiationapproaches depending on supplier type and that a buyer classifies suppliers into two groups:

- Supplier – partner: is a supplier who is well known to the buyer. He/shesupplies a buyer with products, material, technology and knowledge, and is involved inthe buyer's strategy. In a way, a buyer depends on the supplier and replacing the supplier with another is not straightforward.
- Supplier: is a temporal supplier who carries out supplies once or multiple times. In this research study,this type of supplier is coined as "supplier".A buyer does not depend onthesupplier and the replacement of a supplier is more straightforward.

The thesis that "a buyer has different negotiating approaches depending on supplier type, i.e. characteristics", is somewhat confirmed in the qualitative study. However, this thesis cannot be generalized due to the limited number of participants. Twointerviewed buyers (100% of all the interviewed buyers) and one supplier (50% of all the interviewed suppliers) from the focus group share the opinion that buyers approach negotiations based on supplier type. Other results of the qualitative data analysis are also:

- The buyer's classification of suppliers directs the results of negotiation: price and/or relationship.
- The supplier does not know the buyer's classification and does not distinguish the buyer's different negotiation approaches.
- The important influential factors on negotiation approaches are price, relationships, preparation, BATNA, interest, experiences and duration of negotiation. However, from the qualitative analysis, we cannot concludewhich factors are the most significant fromeither buyers' or suppliers' perspective.

### Questionnaire

In part two of the research study, a questionnaire was created from the qualitative analysis, which was based on Mesec's (1998, p. 109) rules of relations between categories. The results of the qualitative analysiswere converted into 11 questions used in the questionnaire (see Table 2). All of the variables are ordinal. The questionnaire participantswere requested to respond to a 5-point Likert-type scale ranging from 1 (no influence) to 5 (strong influence). We wanted to determine themulti-faceted opinion of buyers and suppliers, where negotiations occur with suppliers and with supplier-partner. Both groups, buyers and suppliers completed the questionnaire twice, from two perspectives. First, the buyer completed the questionnaireas ifnegotiationswere occurring with a "supplier" (BS)and then, as if the negotiations were occurring with a "supplier-partner" (BSP).Similarly, the suppliercompleted the questionnaire twice, first as the role of a "supplier" (SS) and second as the role of a "supplier-partner" (SSP). As such, we determined four types of perspectives:buyer and supplier (BS); buyer and supplier-partner (BSP); supplier and buyer (SB); and supplier-partner and buyer (SPB).

## QUANTITATIVE ANALYSIS AND DISCUSSION

### Data Collection

The questionnaire was piloted with ten randomly chosen commercial agents. Based on the feedback received, the questionnaire was edited and upon final testing was distributed to potential participants.The questionnaire was distributed to buyers and suppliers from medium and large for-profit organizations in Slovenia, Italy, and Germany. In September 2014, participants received a questionnaire together with a cover letter which described the purpose and the framework of the research study. Data collection occurred between September 2014 and October 2014. In total, 25 buyer agents and 25 supplier agents participated in the study. After carefully analysing the collected questionnaires, two buyer and two supplier questionnaires were eliminated because of non-completion of the questionnaire. The data were then transferred into table form and analyses were conducted in *R* (R Core Team, 2012). The data were separated into three distinguished groups: buyers, suppliers and combined data.Hereinafter, we present the results together with the discussion of the research study.

## Data Analysis

The difference of the average between groups is thecriterion for analysis. The level of statistical significance is 5% (*p*). Value *p*showsstatistical probability that thedifference between groups exists. If the value is:

- *p*> 0.05: alternative hypothesis is refused, null hypothesis is accepted.
- $p \leq 0.05$:alternative hypothesis is accepted, null hypothesis is refused.

Furthermore, questionnaire results are presentedin three parts, the results of buyers', suppliers', and all responses combined. The results are shown depending on the negotiationrole of business partners; whether a supplier acts as a strategic supplier (SPB) or a temporalsupplier (SB), and whether buyers negotiate with a strategic supplier (BSP) or a temporal supplier (BS). We examined the influence of individual factors on price and relationship depending onparticipants' perspective.Different perspectives and differences of the average values are significant as well. The Wilcoxon signed-ranks test and Kruskal-Wallis test were used for statistical analysis.

## Questionnaire Results

The results are combined with relevant hypotheses.Suppliers and suppliers-partners have different opinions on what is the influential power of individual factors on negotiation prices. The different perspectives on price areshown in Table 3. Preparation, BATNA and interest are emphasized by suppliers, i.e. temporal suppliers, when they refer to their influence on prices. However, the influence of experience and duration are less important. The perspectiveof suppliers-partners, i.e. strategic suppliers, is less. BATNA has the highest frequency. Influences of other four factors on pricesareless frequent.

Frequency on the influences of relationships is summarized in Table 4. Both temporal suppliers and suppliers-partners stress that during negotiation the most important influential factor on relationships are interest and experience. Other factors are less important whereas suppliers-partners emphasize the duration of negotiation as less important. Based on these finding, we emphasize the hypothesis, which is:

> H1: *The perspective of the influential power of individual factors on negotiation outcomes considering price and relationship differs between temporal suppliers and strategic suppliers.*

The frequency of buyers' perspective on negotiation price with temporal suppliers is shown in Table 5. Participants stressedthat BATNA and experiences are the most important factors influencing negotiations, less important are preparation and interest, whereas duration of negotiationis the least important factor. When negotiating with a supplier-partner, buyers emphasize factors of interest and experiences.

When the buyers negotiate with a temporal supplier and the relationship during the process is analysed the results differ. During negotiation with a temporal supplier, preparation, BATNA, and interest are important for buyers (see Table 6). Experience and duration are less important. When analysing buyers' perspective with suppliers-partners, none of the factors are quite important. According to the results, the following hypothesis is highlighted:

> H2: *The buyer's perspective of the influential power of individual factors on negotiation outcomes considering price and relationship differ depending on negotiation with temporal suppliers or strategic suppliers.*

We can conclude that the influences of factors differ, which depend on negotiationobjectives.Price and relationship are defined as negotiationobjectives. The mean influence of individual factors depending on the type of suppliers and negotiation results, price and relationship, are shown in Table 7, which relates to hypothesis 3.

H3: *The perspective of the influential power of individual factors on negotiation outcomes considering price and relationship differ between buyers and suppliers.*

## Hypotheses Verification

All the variables in the questionnaire were measured using a Likert-type scalefrom 1 (no influence) to 5 (stronginfluence). As such, these variablesare ordinal. The Wilcoxon signed-ranks test was used to verifytwo related samplesof the suppliers' questionnaire results. Kruskal-Wallis test was usedto verify statistically characteristic differencesamongseveral independent samples. The hypotheses are verified at a 5% significance level. The alternative hypothesis is accepted if the null hypothesis is rejected at this level of risk.

Accepting the first hypothesis (H1) is shown in Table 8. We analysed 46 questionnaires from suppliers. We have two testing aspects, i.e. two negotiating directions or objectives: price and relationship and two related groups: SB – the perspective of supplier and SPB – the perspective of a supplier-partner. The inferential non-parametric Wilcoxon signed-ranks test was used. The criterion of significance of $Z$-statistic is 5% ($p = 0.05$). The difference is not significant at factors Q2 and Q10. The perspective of suppliers and suppliers-partners is similar. Hypothesis 1 cannot be accepted in these two cases, for other factors, we can accept hypothesis 1. As such, there isa significant difference between theperspective of suppliers and suppliers-partners at individual influential negotiating factors.

The verification of the hypothesis ($Z$-statistics) is shown in Table 8. The factor Q5 (price) has the greatest difference; the mean of the sample SB is $M = 4.74$ and the mean of the sample SPB is $M = 2.74$. $Z$-statistics is –4.14, $p< 0.001$. On the contrary, the factor Q2 shows the smallest difference (the aspect of negotiation relationship) withthe mean of the sample SB ($M = 2.61$) and SPB ($M = 2.91$). Q8 (relationship) has the highest mean on the sample SPB ($M = 4.78$) and Q10 (relationship) shows the smallest meanon the sample SPB ($M = 1.52$). The smallest standard deviation is $SD = 0.42$ and the highest is $SD = 1.11$.

As such, we can state that with a 95% confidence level that the difference of perspectivesbetween temporal suppliers and strategic suppliers exists. The perspectives'includes negotiating results, negotiating direction or aspect: price and relationship. The perspectives are significant with factors Q1, Q3, Q4, Q5, Q6, Q7, Q8 and Q9. The influences of factors Q2 and Q10 are not significantly different between these two samples.

The second hypothesis (H2) is also verified with Wilcoxon signed-ranks test (see Table 9). In this case, the buyers' perspective is the object of analysis. The buyers can play two roles depending onthe negotiation, i.e. negotiation with a temporal supplier (BS) or with a strategic supplier (BSP). We are interested in differences between the two samples: BS and BSP. The hypothesis is verified at a 5% risk level. If the null hypothesis on population equality is rejected at this level, the alternative hypothesis H2 is accepted.

The differencesare significant for factors Q1, Q3, Q4, Q5 and Q6, therefore the alternative hypothesis is accepted with these factors. The perspectives of buyers negotiating with a temporal supplier and the perspectives of buyers negotiating with a strategic supplier are significantly different. The alternative hypothesis is rejected in other cases.

Table 9 depicts descriptive statistics and hypothesis testing ($Z$-statistics). Factor Q1, aspect price, has the greatest difference of mean among the samples BS ($M = 2.83$) and BSP ($M = 4.65$). $Z$-statistics is –4.20, $p< 0.001$. Factor Q10 (relationship) hasthe smallest difference among the samples BS ($M = 2.13$) and BSP ($M = 2.00$). The standard deviation is between 0.42 and 0.96. The most influential factor is Q3 (price) on the sample BSP ($M = 4.78$) and least influential factor is Q4 (relationship) on the sample BSP (a buyer negotiating with a supplier-partner).

We can say with a95% confidence level that the difference of perspectivesamong buyers negotiating with a temporal supplier and buyers negotiating with a strategic supplier exists. The perspectives are significant in factors Q1, Q3, Q4,

Q5 and Q6. The influences of other factors (Q2, Q7, Q8, Q9 and Q10) are not significantly different between these two samples.

Kruskal-Wallis Test is used to verify the third hypothesis (H3) to determine a statistically significant difference among four independent groups- samples: two suppliers (as a temporal supplier – SB and a supplier-partner – SPB) and two buyers (negotiating with a temporal supplier – BS and with a supplier-partner– BSP). The data are summarized in Table 10.The hypothesis is verified at a 5% risk level. If the null hypothesis on the population equality is rejected at this level, the alternative hypothesis H3 is accepted.

We can say that for all the factors different opinionsexistamong the four groups, except for Q2. Preparation of negotiation is not an influential factor of a relationship, $K\text{-}W(3) = 4.56$, $p > 0.05$. The mean of individual factors are: BATNA – relationship (Q4) $M = 2.14$; duration of negotiation – relationship (Q10) $M = 1.87$, etc. The other values are above average, the most important influential factor is BATNA – price (Q3) with amean of $M = 4.15$. The least volatile opinion is regarding importance onpreparing for negotiations – relationship (Q2) with a standard deviation $SD = 0.75$ and the most volatile factor is interest – price ($SD = 1.21$).Therefore, we can state with a 95% level of confidence that the difference of perspective amongthe four groups exists when the participants are: a buyer negotiating with a temporal supplier, a buyer negotiating with a strategic supplier, a temporal supplier or a strategic supplier. Thehypothesis is accepted for all the factors, except for Q2.

## DISCUSSION AND CONCLUSION

This article summarizes the analysis of influential factors of negotiation between buyers and suppliers. It combines two research methods: (1) qualitative: to determine influential factors, and (2) quantitative: toevaluate the power of factorsthat are dependent on negotiating objectives and the perspective of both parties. In addition to the list of influential factors, the result of the qualitative analysis is also that suppliers are classified by buyers into two groups, as temporal suppliers (suppliers) and strategic suppliers (suppliers-partners). Also, a very important findingis that negotiations by buyers are oriented into two directions, towards price (the most optimal financial benefits) and towardsrelationship (the objective is to maintain and improve the partnership).In this research study, we determined that the classification of two groups and two negotiating directions are unknown to the suppliers. The result of negotiation depends on: preparations, BATNA, interest, experiences, and duration. Withthequantitative analysis (n = 92)we confirmed that the perspectives of buyers and supplier on negotiation results differ. We also concluded that we have five influential factors on negotiation results, and that the power of influence is different among the factors. The Wilcoxon signed-rank test and Kruskal-Wallis test were implemented for hypotheses verification. The results of the test confirmed all three hypotheses which are then confirmed by the following:

- Factor Q1 (Do preparations for negotiations influence the price?): When testing the hypotheses (H1, H2 and H3) we accepted the alternative hypothesis. With this we can claim that preparations for negotiations influence the results of negotiations when negotiations are directed towards lowering the price.
- Factor Q2 (Do preparations for negotiations influence the relationship?): We rejected the alternative hypothesis (H1, H2 and H3). According to these findings we can claim that preparation for negotiations do not statistically influence the results of negotiations when a buyer directs negotiations towards maintaining or improving the relationship/partnership.
- Factors Q3 and Q4 (Does BATNA influence the relationship?): In all the cases of statistical testing we accepted the alternative hypothesis and we can confirm that BATNA has a very powerful influence on negotiations oriented towards lowering the price and maintaining the relationship between a buyer and a supplier.
- Factors Q5 and Q6 (Does interest influence the price and the relationship?): In all cases of statistical

testing we accepted the alternative hypothesis and we can confirm that interest has a very powerful influence on negotiations oriented towards lowering the price and maintaining the relationshipbetween a buyer and a supplier.

We would like to emphasizethe first finding thatsuppliers do not knowabuyers perspectiveregardingthe main objective of negotiations, i.e. prices or relationship, as well as not knowing that they are divided into two groups, i.e. temporal suppliers and strategic suppliers. These factors have an impact on both negotiating sides. The total mean of all the factors also differs between buyers and suppliers. The mean of buyers is *M* = 2.9 and the mean of suppliers is *M* = 3.2. All factors in general are more important for suppliers compared to buyers. This result is an additional confirmation of the thesis that buyers and suppliers have a different negotiating approach. Carter, Carter, Monczka and Scannell (2011, p. 23) also agree that a buyer and a supplier havedifferent opinions about common activities.

The research results could support buyers during negotiating processes. Similarly as Fells (1996, p. 51) that asked what kind of negotiating instructions have to be given to negotiators who plan the negotiating processes. The research results are presented by a list of factors which are important for success innegotiations. The power of individual factors on negotiating results also is important.Acceptable price isa very important negotiating result but we have to take into account other factors as well. Ambrose, Marshall and Lynch (2010, p. 1269) determined thatfor the success of a supply chain and the success of an organizationthe most important factor is relationship between a buyer and a supplier. Pache (2013, p. 127) wrote that all parties in the procurement chain are interconnected and all of them have a common purpose – the satisfaction of the end user / thefinal customer. Negotiation is an interaction between buyers and suppliers. Understanding both perspectives is vital in this demanding economic condition.It is also essential for a successful agreement and collaboration. Onwuegbuzie's (2003, pp.72-89) framework for possible external and internal validity threats to a study were used as a guide in this study. Possible threats to external validity were (a) ecological validity, which might have had a possible threat because the participants were limited to participants from specific geographic areas only in Europe; and (b) population validity, because the sample size may not be large enough to justify generalizations beyond the sample. Further, there were several threats to internal validity of the findings, including (a) data saturation point: data from a limited number of focus group participants may have yielded data that did not reach data saturation point; (b) researcher bias also was a threat that limited the results, in which certain categories might have been constructed or collapsed based on personal beliefs of the researchers (i.e., illusory correlation); and (c) finally, instrumentation threat was a threat pertaining to the reliability and validity of the coded data, although high inter-rater reliability obtained suggested that this threat was minimal.For further research, we propose a broader sample, a research of other influential factors, a comparison among different fields of industry, and a comparison among different economies and countries.

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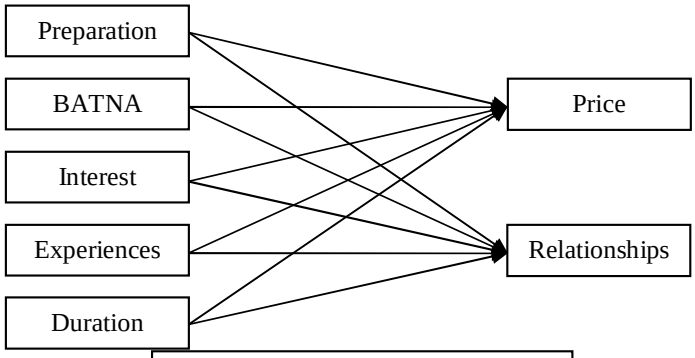


**Figure 1. Research model**

**Table 1. Coding units, terms and categories**

| Coding units | Person | Common purified transcript | Term | Category |
|---|---|---|---|---|
| Knowledge about suppliers' classification | B1 | Yes, the way of supplying, partner or supplier. | Supplier Type | Classification |
| | B2 | Yes, on partnership (outsourcing), No. of supply … | | |
| | S1 | I do not know. | | |
| | S2 | I know a classification depending on supplier's assessment. | | |
| Negotiation directions | B1 | Supplier – partner, new supplier, supplier of machines. | Supplier Price Relationship | Supplier-partner Supplier |
| | B2 | Supplier, supplier of technology, supplier expert. | | |
| | S1 | Supplier for production, relationship and price. | | |
| | S2 | Supplier of raw materials, price, temporal supplier. | | |
| Negotiation factors | B1 | Depending on supply: price, expectation orinterest, preparation. | Preparation Price Collaboration Power BATNA Quality Interest Relationship Influence Experiences Duration | Preparation Price BATNA Interest Relationship Experiences Duration |
| | | Negotiation, experiences,and duration of negotiation. | | |
| | | Collaboration, alternative of collaboration, investment. | | |
| | B2 | Result: price or relationship, negotiation deadline, duration of negotiation | | |
| | | Experience, origin of material, BATNA, reputation, power. | | |
| | | Knowledge, product warranty, interest. | | |
| | S1 | Price, preparation, interest, alternative solution, relationship | | |
| | | Perspective power, quality system, service. | | |
| | | Wishing for agreement, tactics, strategy, power. | | |
| | S2 | Price, quality, preparation, objective, BATNA, | | |
| | | Relationship, competitive advantage, benchmarking. | | |
| | | Experience, negotiation approach, duration … | | |

**Table 2. Questionnaire**

| Symbol | Question |
|---|---|
| Q0 | What is your role in negotiations (buyer or supplier)? |
| Q1 | Do preparations for negotiation influencethe price? |
| Q2 | Do preparations for negotiation influencethe relationship? |
| Q3 | Does BATNA influence the price? |
| Q4 | Does BATNA influence the relationship? |
| Q5 | Does interest influence the price? |
| Q6 | Does interest influence therelationship? |
| Q7 | Does experience influence the price? |
| Q8 | Does experience influence the relationship? |
| Q9 | Does duration of negotiation influence the price? |
| Q10 | Does duration of negotiation influence the relationship? |

**Table 3. Frequency on influence of prices – suppliers' perspectives**

| Type of a supplier | Response | Preparation | BATNA | Interest | Experience | Duration |
|---|---|---|---|---|---|---|
| **Supplier (SB)** | 5 | 12 | 12 | 17 | 0 | 2 |
| | 4 | 8 | 9 | 6 | 8 | 16 |
| | 3 | 3 | 2 | 0 | 15 | 5 |
| | 2 | 0 | 0 | 0 | 0 | 0 |
| | 1 | 0 | 0 | 0 | 0 | 0 |
| **Supplier-partner (SPB)** | 5 | 0 | 7 | 0 | 0 | 0 |
| | 4 | 1 | 5 | 2 | 3 | 7 |
| | 3 | 12 | 7 | 13 | 9 | 14 |
| | 2 | 10 | 4 | 8 | 7 | 2 |
| | 1 | 0 | 0 | 0 | 4 | 0 |

**Table 4. Frequency on the influence on relationships – suppliers' perspectives**

| Type of a supplier | Response | Preparation | BATNA | Interest | Experience | Duration |
|---|---|---|---|---|---|---|
| **Supplier (SB)** | 5 | 0 | 0 | 0 | 8 | 0 |
| | 4 | 1 | 0 | 8 | 8 | 0 |
| | 3 | 12 | 6 | 15 | 7 | 2 |
| | 2 | 10 | 14 | 0 | 0 | 15 |
| | 1 | 0 | 3 | 0 | 0 | 6 |
| **Supplier-partner (SPB)** | 5 | 1 | 0 | 14 | 18 | 0 |
| | 4 | 3 | 3 | 9 | 5 | 0 |
| | 3 | 12 | 10 | 0 | 0 | 2 |
| | 2 | 7 | 10 | 0 | 0 | 8 |
| | 1 | 0 | 0 | 0 | 0 | 13 |

**Table 5. Frequency on the influence on prices – buyers' perspectives**

| Type of supplier | Response | Preparation | BATNA | Interest | Experience | Duration |
|---|---|---|---|---|---|---|
| Supplier (BS) | 5 | 0 | 7 | 0 | 1 | 0 |
| | 4 | 3 | 4 | 2 | 9 | 0 |
| | 3 | 13 | 11 | 13 | 13 | 6 |
| | 2 | 7 | 1 | 8 | 0 | 13 |
| | 1 | 0 | 0 | 0 | 0 | 4 |
| Supplier-partner (BSP) | 5 | 0 | 0 | 1 | 3 | 0 |
| | 4 | 3 | 0 | 9 | 10 | 0 |
| | 3 | 9 | 10 | 12 | 10 | 7 |
| | 2 | 11 | 11 | 1 | 0 | 12 |
| | 1 | 0 | 2 | 0 | 0 | 4 |

**Table 6. The frequency of the influence on relationship – buyers' perspectives**

| Type of a supplier | Response | Preparation | BATNA | Interest | Experience | Duration |
|---|---|---|---|---|---|---|
| Supplier (BS) | 5 | 15 | 18 | 12 | 0 | 0 |
| | 4 | 8 | 5 | 8 | 7 | 1 |
| | 3 | 0 | 0 | 3 | 14 | 6 |
| | 2 | 0 | 0 | 0 | 2 | 14 |
| | 1 | 0 | 0 | 0 | 0 | 2 |
| Supplier-partner (BSP) | 5 | 0 | 0 | 0 | 3 | 0 |
| | 4 | 2 | 0 | 2 | 5 | 0 |
| | 3 | 8 | 1 | 9 | 11 | 4 |
| | 2 | 10 | 7 | 10 | 4 | 15 |
| | 1 | 3 | 15 | 2 | 0 | 4 |

**Table 7. Mean of influential factors**

| Negotiation objective | Type of supplier | Preparation | BATNA | Interest | Experience | Duration |
|---|---|---|---|---|---|---|
| Price | SB | 4.4 | 4.4 | 4.7 | 3.4 | 3.9 |
| | SPB | 2.6 | 3.7 | 2.7 | 2.5 | 3.2 |
| | BS | 4.7 | 4.8 | 4.4 | 3.2 | 2.3 |
| | BSP | 2.8 | 3.7 | 2.7 | 3.5 | 2.1 |
| Relationship | SB | 2.6 | 2.1 | 3.4 | 4.0 | 1.8 |
| | SPB | 2.9 | 2.7 | 4.6 | 4.8 | 1.5 |
| | BS | 2.4 | 1.4 | 2.5 | 3.3 | 2.0 |
| | BSP | 2.7 | 2.4 | 3.5 | 3.7 | 2.1 |

**Table 8. Test of the first hypothesis (H1)**

| Aspect | Factor | SB/SPB | Descriptive statistics | | | | | Wilcoxon signed-ranks test | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *N* | *M* | *SD* | *Min* | *Max* | *Z* | *p* | Hypothesis 1 |
| Price | Q1 | SB | 23 | 4.39 | 0.72 | 3 | 5 | -4.11 | <0.001 | Accepted |
| | | SPB | 23 | 2.6 | 0.58 | 2 | 4 | | | |
| Relationship | Q2 | SB | 23 | 2.61 | 0.58 | 2 | 4 | -1.63 | 0.102 | Rejected |
| | | SPB | 23 | 2.91 | 0.79 | 2 | 4 | | | |
| Price | Q3 | SB | 23 | 4.44 | 0.66 | 3 | 5 | -2.69 | <0.007 | Accepted |
| | | SPB | 23 | 3.65 | 1.11 | 2 | 5 | | | |
| Relationship | Q4 | SB | 23 | 2.13 | 0.63 | 1 | 3 | -2.59 | 0.009 | Accepted |
| | | SPB | 23 | 2.69 | 0.70 | 2 | 4 | | | |
| Price | Q5 | SB | 23 | 4.74 | 0.45 | 4 | 5 | -4.14 | <0.001 | Accepted |
| | | SPB | 23 | 2.74 | 0.62 | 2 | 4 | | | |
| Relationship | Q6 | SB | 23 | 3.34 | 0.49 | 3 | 4 | -4.04 | <0.001 | Accepted |
| | | SPB | 23 | 4.61 | 0.45 | 4 | 5 | | | |
| Price | Q7 | SB | 23 | 3.35 | 0.49 | 3 | 4 | -3.17 | 0.002 | Accepted |
| | | SPB | 23 | 2.48 | 0.95 | 1 | 4 | | | |
| Relationship | Q8 | SB | 23 | 4.04 | 0.82 | 3 | 5 | -2.93 | 0.003 | Accepted |
| | | SPB | 23 | 4.78 | 0.42 | 4 | 5 | | | |
| Price | Q9 | SB | 23 | 3.87 | 0.55 | 3 | 5 | -3.26 | <0.001 | Accepted |
| | | SPB | 23 | 3.22 | 0.60 | 2 | 4 | | | |
| Relationship | Q10 | SB | 23 | 1.83 | 0.58 | 1 | 3 | -1.70 | 0.088 | Rejected |
| | | SPB | 23 | 1.52 | 0.67 | 1 | 3 | | | |

**Table 9. Test of second hypothesis (H2)**

| Aspect | Factor | BS/BSP | Descriptive statistics | | | | | Wilcoxon signed-ranks test | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *N* | *M* | *SD* | *Min* | *Max* | *Z* | *p* | Hypothesis 2 |
| Price | Q1 | BS | 23 | 2.83 | 0.65 | 2 | 4 | -4.20 | <0.001 | Accepted |
| | | BSP | 23 | 4.65 | 0.49 | 4 | 5 | | | |
| Relationship | Q2 | BS | 23 | 2.65 | 0.71 | 2 | 4 | -1.19 | 0.233 | Rejected |
| | | BSP | 23 | 2.39 | 0.84 | 1 | 4 | | | |
| Price | Q3 | BS | 23 | 3.74 | 0.96 | 2 | 5 | -3.29 | <0.001 | Accepted |
| | | BSP | 23 | 4.78 | 0.42 | 4 | 5 | | | |
| Relationship | Q4 | BS | 23 | 2.35 | 0.65 | 1 | 3 | -3.64 | <0.001 | Accepted |
| | | BSP | 23 | 1.39 | 0.58 | 1 | 3 | | | |
| Price | Q5 | BS | 23 | 2.74 | 0.62 | 2 | 4 | -3.92 | <0.001 | Accepted |
| | | BSP | 23 | 4.39 | 0.72 | 3 | 5 | | | |
| Relationship | Q6 | BS | 23 | 3.43 | 0.66 | 2 | 5 | -3.31 | <0.001 | Accepted |
| | | BSP | 23 | 2.48 | 0.79 | 1 | 4 | | | |
| Price | Q7 | BS | 23 | 3.48 | 0.59 | 3 | 5 | -1.61 | 0.107 | Rejected |
| | | BSP | 23 | 3.22 | 0.60 | 2 | 4 | | | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Relationship | Q8 | BS | 23 | 3.70 | 0.70 | 3 | 5 | -1.66 | 0.097 | Rejected |
| | | BSP | 23 | 3.30 | 0.93 | 2 | 5 | | | |
| Price | Q9 | BS | 23 | 2.09 | 0.67 | 1 | 3 | -1.43 | 0.346 | Rejected |
| | | BSP | 23 | 2.26 | 0.69 | 1 | 4 | | | |
| Relationship | Q10 | BS | 23 | 2.13 | 0.69 | 1 | 3 | -1.73 | 0.083 | Rejected |
| | | BSP | 23 | 2.00 | 0.60 | 1 | 3 | | | |

**Table 10. Test of third hypothesis (H3)**

| Factor | *N* | *M* | *Min* | *Max* | *SD* | Kruskal-Wallis test | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *H*-test | *df* | *P* | Hypothesis 3 |
| Q1 | 92 | 3.62 | 2 | 5 | 1.10 | 63.62 | 3 | < 0.001 | Accepted |
| Q2 | 92 | 2.64 | 1 | 5 | 0.75 | 4.56 | 3 | 0.2 | Rejected |
| Q3 | 92 | 4.15 | 2 | 5 | 1.14 | 21.55 | 3 | < 0.001 | Accepted |
| Q4 | 92 | 2.14 | 1 | 4 | 1.19 | 30.96 | 3 | < 0.001 | Accepted |
| Q5 | 92 | 3.65 | 2 | 5 | 1.21 | 64.37 | 3 | < 0.001 | Accepted |
| Q6 | 92 | 3.47 | 1 | 5 | 1.17 | 55.15 | 3 | < 0.001 | Accepted |
| Q7 | 92 | 3.13 | 1 | 5 | 1.11 | 18.50 | 3 | < 0.001 | Accepted |
| Q8 | 92 | 3.96 | 2 | 5 | 1.12 | 32.80 | 3 | < 0.001 | Accepted |
| Q9 | 92 | 2.86 | 1 | 5 | 1.11 | 54.80 | 3 | < 0.001 | Accepted |
| Q10 | 92 | 1.87 | 1 | 3 | 1.15 | 9.70 | 3 | 0.02 | Accepted |

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RESEARCH ARTICLE

# Perception about Climate Change and Adaptation Pattern among Dairy Farmers' in Bengaluru Rural District

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## ABSTRACT

The study was conducted in purposively selected 30 villages in four taluks of Bengaluru Rural district considering the implementation RKVY project "Effective and Sustainable Information Reach of Farmers' Through Milk Producer Co-Operative Societies (MPCSs)". In each village, list of milk supplying farmers' to the MPCSs was prepared and from each MPCS four farmers' were randomly selected thus making total sample of 120. The data were collected by structured interview schedule. The findings reveals that majority (93.33 %) of farmers' perceived that there were less number of long dry spells and less number of rainy days (90.83 % ) before 2005. But, cent per cent of farmers' perceived that there was change in the rainfall during crop growth period, rainfall pattern and occurrence of more droughts after 2005. With regard to perception about the change in temperature, equal per cent of farmers' not experienced high temperature (95.00%) and winter getting more warmer (95.00%) before 2005. But, after 2005, cent per cent of farmers' perceived that there was increase in the temperature, experienced high temperature, scorching sunshine and summer getting more warmer. With respect to the negative effects of climate change on dairy farming, cent per cent farmers' perceived acute shortage of fodder, decrease in milk production and quality

With respect to the adaptation measures, cent per cent of dairy farmers' provided the shade, practiced stall feeding & maintained hygiene conditions, self prepared mineral mixture and readymade mineral mixture & concentrates. Majority (82.50%) of farmers' grown green fodder (NB21 & Co-3) and practiced silage (63.69%). Hence, there is a need to intensify extension educational activities to educate the farmers' about climate change and appropriate adaptation measures by the Milk Unions and Veterinary and Animal Husbandry Department. Further, they should take up measures to supply the required inputs and incentives at appropriate time in order to sustain the dairy enterprise under adverse climatic conditions.

**Keywords :** Perception,Climate Change and Adaptation Pattern.

## INTRODUCTION

Increasing population, rapid urbanization and the over increasing desire of human beings to raise their standard of living has led to technological innovations of all kinds. These innovations have made life more comfortable but at the cost of increased demand for food, air, water, minerals and energy. However, these resources are limited by to earth's capability to renew them. Rapid depletion of natural resources has caused unprecedented changes in the global climate resulting in serious implications on the survival of both human and animal species on earth (Deepika Kachhal, 2015).Climate change is the long term change in earth's climate due to natural mechanical and anthropological processes which result in emission of green house gases like $CO_2$, methane etc. These gases settle in the stratosphere and trap the heat within the atmosphere leading to global warming and changing climate patterns. Shifting of seasons, increasing global temperatures, rising sea levels, changing agricultural patterns have resulted in frequent disasters like landslides, tsunami, drought, famine, population migration and major health hazards, not only to present generation but also for future generations (Deepika Kachhal, 2015).

Climate change affects agriculture and agriculture also affects climate change. Higher temperature, reduced rainfall and increased rainfall variability reduce crop yield and threaten food security in low income and agriculture-based economics.Climate change poses a formidable challenge to the development of livestock sector in India. The anticipated rise in temperature between 2.3 and 4.8 $^0$C over the entire country together with increased precipitation resulting from climate change is likely to aggravate the heat stress in dairy animals, particularly hybrid animals, adversely affecting their productive and reproductive performance, and hence reducing the total area where high yielding dairy cattle can be economically reared. Milk is an important component of food that is significantly increasing in demand. Increased heat stress associated with climate change may, however, cause distress to dairy animals and possibly impact milk production (https://www.researchgate.net/pub.)

With unpredictable weather, dairy farmers' keep changing management practices and be prepared for constant change in the dairy farming practices. Impact of climate change is diversified and need to be understood, so as to workout strategies to mitigate ill-effects of climate change. With this back ground, the present study was initiated with the following objectives.

- To asses dairy farmers' perception about climate change
- To study the adaptation measures initiated by dairy farmers' due to climate change

## METHOLODOGY

The study was conducted in purposively selected Bengaluru Rural district. All the four taluks namely Hoskote, Devanahalli, Doddaballapura and Nelamangala were purposively considered based on the implementation RKVY project "Effective and Sustainable Information Reach of Farmers' Through Milk Producer Co-Operative Societies (MPCSs)". Thirty MPCS villages comprising of eight from Hoskote, nine from Devanahalli, eight from Doddaballapura and five from Nelamangala taluks were purposively considered for the study. In each village, list of milk supplying farmers' to the MPCSs was prepared in consultation with Chief Executive Officers (Secretaries) of MPCSs. From the list in each MPCS, four farmers' were randomly selected thus making total sample of 120. The data were collected by using structured interview schedule and analysed the data by using appropriate statistical tools.The mean annual rainfall (mm) and temperature ($^0$c) of Bengaluru Rural District 10 years before and after 2005 was collected from the All India Co-ordinated Research Project (AICRP) on Agro Meteorology, GKVK, UAS, Bengaluru.The details are mentioned in the Table1.The dairy farmers' perception about rainfall pattern and temperature change before 2005 were compared with the above data of AICRP on Agro-meteorology and interpreted the results.

## RESULTS AND DISCUSSION

### Dairy farmers' perception about climate change

Data with respect to dairy farmers' perception about changes in rainfall pattern was recorded twice and presented in two time intervals viz., before and after 2005.A cursory look at the Table 2 that, majority of farmers' (93.33 %) perceived short dry spell followed by equal per cent of them perceived more number of rainy days (90.83 %) and even distribution of rainfall (90.83 %). About 90.00 per cent of farmers' perceived there was no change in the rainfall during crop period while 89.17 per cent of them perceived there was no changes in the onset timing of rainfall, equal per cent of farmers' were perceived amount of rainfall was more and predictable rainfall (88.13%) . About 87.50 per cent perceived there were no changes in the rainfall pattern before 2005. The dairy farmers' perception about rainfall pattern change before 2005 was on for with data of AICRP on Agro-meteorology, University of Agriculture Sciences, Bengaluru. The probable reasons for this might be that , in earlier days farmers' were rearing local buffaloes/cows for dairy and growing more food grains which requires less water. Hence, they might have not faced water shortage. These findings are more or less in conformity with findings of Shankara (2010) and Vinaya Kumar (2015)

Contrary to the perception of rainfall pattern cent per cent of farmers' perceived that there was changes in the rainfall during crop growth period, changes in the rainfall pattern, occurrence of more droughts, drying more number of tube wells, digging more number of tube wells and decreased water yield in tube wells. Equal per cent of them perceived that dry spells were more (98.33%) and amount of rainfall was less (98.33%) after 2005. The dairy farmers' perception of about rainfall pattern change after 2005 was opposite to the meteorological data. The probable reasons for this might be, in recent years farmers' were more interested in cross breed cows for dairy and commercial crops which require more water. Even though increased in quantity of rainfall after 2005, farmers' were perceived as shortage. These findings are more or less in conformity with findings of Shankara (2010) and Vinaya Kumar (2015)

Data with respect to dairy farmers' perception about changes in temperature was recorded twice is presented in two time interval viz., before and after 2005. The data presented in Table-3 reveals that equal per cent farmers' not experienced high temperature (95.00) and winter was getting more warmer (95.00 %). Equal per cent of farmers' agreed that temperature not increased (94.17 %) and there were no changes in the temperature (94.17%). About 93.33 per cent of farmers' perceived summer was not getting more warm and 92.50 per cent of them were not experienced scorching sunshine before 2005. The dairy farmers' perception about temperature change before 2005 was contrary with meteorological data. The probable reasons for this might be, farmers' were more keen on changes in the rainfall pattern before 2005 than temperature. According to them food and milk production is in large extent depend on rainfall when compare to contribution of temperature. These findings are more or less in conformity with findings of Shankara (2010) and Vinaya Kumar (2015)

After 2005, cent per cent of the farmers' perceived that there was increase in the temperature, experienced high temperature, scorching sunshine, summer getting more warmer and there was no change in the temperature. Majority of the farmers' (96.67 %) perceived winter was getting more colder. The dairy farmers' perception about temperature change after 2005 was on for with meteorological data. The probable reasons for this might be, they were experiencing extremely higher temperature and came to know that dairy farming and crop growth were affected by temperature along with rainfall in an same extent based on their experience. These findings are more or less in conformity with findings of Shankara (2010) and Vinaya Kumar (2015).Negative effects of climate change as perceived by dairy farmers' presented in Table 4 reveals that cent per cent of the farmers' perceived acute shortage of fodder (green fodder), decrease in milk production and decrease in milk quality. About 86.67 per cent of farmers' perceived water security due to climate change on dairy farming. Other negative effects included were distress sale of cows and shifting of cows to the relatives houses. Some of these effects are not entirely due to climate changes but

a result of multiple factors such as more demand for water for agriculture, management of dairy animals and domestic purpose. These findings are in conformity with findings of Vinaya Kumar (2015)

### Adaptation measures initiated by dairy farmers' due to climate change

A variety of adaptation measures initiated by dairy farmers' in response to climate change are summarized in Table 5. It could be observed that cent per cent of farmers' were used self prepared mineral mixture, readymade mineral mixture & concentrates and provided shade, stall feeding & hygiene. About 82.50 per cent of farmers' were grown green fodder (NB-21, Co-3) in limited area (1-2 guntas) through drip irrigation followed by practiced silage in syntax/cement tanks (63.69 %), grown azolla (52.50), purchased silage fodder (44.57 %), grown linseed (Agase) fodder (42.50%) and stored dry fodder after harvesting the maize for seed production purpose (39.17%). The probable reason might be, dairy farmers' interested in more yield and quality which fetches more price and gained social status and also more demand for the milk and it's products in urban areas. These findings are more or less in conformity with findings of Shankara (2010) and Vinaya Kumar (2015)

## CONCLUSION

The study concluded that majority (93.33 %) of dairy farmers' perceived that there were short spells and more rainy days (90.83 % ) before 2005. But, cent per cent of farmers' perceived that there was change in the rainfall during crop growth period, rainfall pattern and occurrence of more droughts after 2005. With regard to perception about the change in temperature, equal per cent of farmers' not experienced high temperature (95.00%) and winter getting more warmer (95.00%) before 2005. But, after 2005, cent per cent of farmers' perceived that there was increase in the temperature and experienced high temperature. With respect to the negative effects of climate change on dairy farming, cent per cent farmers' perceived acute shortage of fodder, decrease in milk production and quality. With regard to the adaptation measures, cent per cent of dairy farmers' provided the shade, practiced stall feeding & maintained hygiene conditions. Hence, there is a need to intensify extension educational activities to educate the farmers' about climate change and appropriate adaptation measures by the Milk Unions and Veterinary and Animal Husbandry Department. Further, they should take up measures to supply the required inputs and incentives at appropriate time to the dairy farmers' at their door steps in order to sustain the dairy enterprise under adverse climatic conditions.

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**Table1.The mean annual rainfall (mm) and temperature (⁰c) of Bengaluru Rural District 10 years before and after 2005**

| Sl. No. | Parameters | 1994-2004 | 2005-2015 | 't' Value | Significant/Non Significant |
|---|---|---|---|---|---|
| 1 | Rainfall(mm) | 772 | 845 | 0.92 | Non Significant |
| 2 | Temperature (⁰c) | 24.6 | 25.6 | 0.70 | Significant |

**Table 2. Dairy farmers' perception about changes in the rainfall pattern**

n=120

| Sl. No. | Statements | Responses | | | | | |
|---|---|---|---|---|---|---|---|
| | | Before 2005 | | | After 2005 | | |
| | | A | SA | DA | A | SA | DA |
| 1 | Number of rainy days (> 2.5mm) were more | 109 (90.83) | 11 (17.30) | - | 1 (0.83) | 4 (3.33) | 115 (95.83) |
| 2 | Amount of rainfall was more | 106 (88.33) | 13 (10.83) | - | - | 2 (1.67) | 118 (98.33) |
| 3 | There was changes in the onset timing of rainfall | 4 (3.33) | 9 (7.50) | 107 (89.17) | - | 2 (1.67) | 118 (98.33) |
| 4 | Long dry spells | - | 7 (5.83) | 113 (93.33) | 118 (98.33) | 2 (1.67) | - |
| 5 | There was change in the rainfall during crop growth period (July –October) | 4 (3.33) | 4 (3.33) | 108 (90.00) | 120 (100.00) | - | - |
| 6 | There was changes in the rainfall pattern | 5 (4.17) | 10 (8.33) | 105 (87.50) | 120 (100.00) | - | - |
| 7 | Uneven distribution of rain fall | 6 (5.00) | 05 (4.17) | 109 (90.83) | 112 (93.33) | 08 (6.67) | - |
| 8 | Unpredictable rain fall | 4 (3.33) | 10 (8.33) | 106 (88.33) | 110 (91.67) | 6 (5.00) | 4 (3.33) |
| 9 | Occurrence of drought was more | 10 (8.33) | 6 (5.00) | 104 (86.67) | 120 (100.00) | - | - |
| 10 | Drying more No. of tube wells | 3 (2.50) | 16 (13.33) | 101 (84.17) | 120 (100.00) | - | - |
| 11 | Digging more No. of tube wells | 2 (1.67) | 16 (13.33) | 101 (84.17) | 120 (100.00) | - | - |
| 12 | Decreased water yield in the tube wells | 7 (5.83) | 11 (17.3) | 102 (85.00) | 120 (100.00) | - | - |

(Figures in parentheses depicts percentage) A=Agree, SA = Somewhat Agree, DA= Disagree

**Table 3. Dairy farmers' perception about changes in temperature**

n=120

| Sl. No. | | Statements | Responses | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Before 2005 | | | After 2005 | | |
| | | | A | SA | DA | A | SA | DA |
| 1 | | There was increase in the temperature | - | 7 (5.83) | 113 (94.17) | 120 (100.00) | - | - |
| 2 | | Experienced high temperature | - | 6 (5.00) | 114 (95.00) | 120 (100.00) | - | - |
| 3 | a | Experienced scorching sun shine | - | 9 (7.50) | 111 (92.50) | 120 (100.00) | - | - |
| | b | Summer was getting more warmer | - | 8 (6.67) | 112 (93.33) | 120 (100.00) | - | - |
| 4 | a | Winter was getting more colder | - | 6 (5.00) | 114 (95.00) | 116 (96.67) | 4 (3.33) | - |
| | b | There was no changes in the temperature | 113 (94.17) | 7 (5.83) | - | - | - | 120 (100.00) |

(Figures in parentheses depicts perecentage) **A=Agree, SA=Somewhat Agree, DA=Disagree**

**Table 4. Dairy farmers' perception about negative effects of climate change on dairy farming**

n=120

| Sl. No. | Particulars | No. | % |
|---|---|---|---|
| 1 | Acute shortage of fodder (green fodder) | 120 | 100.00 |
| 2 | Decrease in milk production (due to scarcity of fodder) | 120 | 100.00 |
| 3 | Decrease in milk quality (due to less nutritional fodder) | 120 | 100.00 |
| 4 | Distress sale of cows | 14 | 11. 67 |
| 5 | Shifting of cows to the relatives houses | 10 | 08.33 |
| 6 | Water scarcity due to droughts | 104 | 86.67 |

**Table 5. Adaptation measures initiated by dairy farmers' in response to climate change**

n=120

| Sl. No. | Adaptation measures | No. | % |
|---|---|---|---|
| 1 | Growing green fodder (NB-21, Co-3) under in limited area (1-2 guntas) through drip irrigation | 99 | 82.50 |
| 2 | Growing linseed (Agase) fodder | 51 | 42.50 |
| 3 | Growing azolla | 63 | 52.50 |
| 4 | Storing dry fodder after harvesting the maize for seed production purpose | 47 | 39.17 |
| 5 | Construction of farm ponds/ krishi honda to store rain water for fodder production | 29 | 24.17 |
| 6 | Adoption of agro forestry (Subabul/ glyricidea ) | 13 | 10.83 |
| 7 | Using self prepared mineral mixture (Broken maize/ragi, Bengal gram | 120 | 100.00 |

| | | | |
|---|---|---|---|
| | hask and wheat bran | | |
| 8 | Using readymade mineral mixture and concentrates | 120 | 100.00 |
| 9 | Using dry paddy straw as fodder | 26 | 21.67 |
| 10 | Providing shade, stall feeding and hygiene maintenance | 120 | 100.00 |
| 11 | Practicing silage in syntax /cement tanks | 76 | 63.69 |
| 12 | Cow grazing in the field | 13 | 10.83 |
| 13 | Purchasing of silage fodder | 53 | 44.57 |

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RESEARCH ARTICLE

# Fertilizer Prescription Equations for Desired Yield Targets of Wheat (*Triticum aestivum* L.) under Integrated Plant Nutrient System Based on Targeted Yield Model

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## ABSTRACT

Soil test crop response correlation involving integrated plant nutrition system (STCR-IPNS) studies on wheat (*Triticum aestivum* L) on an Inceptisol, (Vertic Ustropept) in Coimbatore district of Tamil Nadu were carried out following Ramamoorthy's 'inductive cum targeted yield model'. In order to develop a scientific base for prescribing fertilizer recommendations for wheat, two field experiments were carried out with maize as gradient crop and wheat [var Co W (w) -1] as test crop. From the soil test and crop response data, the basic parameters were computed and the fertilizer prescription equations for recommending fertilizer doses were developed for wheat. The fertilizer estimates under IPNS clearly indicated better utilization of added fertilizer nutrients by recording of high response ratio.Since the conjoint application of organics with fertilizers resulted in saving of 25, 16 and 18 kg $ha^{-1}$ of N, $P_2O_5$ and $K_2O$ respectively.

**Keywords :** Basic parameters, Fertilizer prescription equation, IPNS, STCR

## INTRODUCTION

Wheat is one of the most important cereal crops grown in India. India ranks second in global acreage (28.52 million hectares) and grain production (80.71 million tonnes) (FAOSTAT, 2012)[1]. At present Uttar Pradesh, Punjab and

Haryana are the three major wheat producing states. They account for nearly 70 per cent of the total wheat produced in the country. The soil test based fertilizer prescription equations for wheat with conjoint use of FYM with N, P, and K fertilizer result implies that fertilizer requirement increased with increasing yield target and decreased with increasing soil test values (Konde *et al*. 2008)[2]. In Soil Test Crop Response (STCR) Correlation method, the fertilizer doses are recommended based on fertilizer adjustment equations. These equations are developed after establishing significant relationship between soil test values and the added fertilizer levels and crop response for a particular soil type. The fertilizer recommendations based on STCR concept are quantitative, precise and meaningful because combined use of soil and plant analysis is involved in it. It gives a real balance between applied nutrients and the available nutrients already present in the soil.

So far STCR – IPNS studies have not been conducted for plain Wheat in Tamil Nadu. Hence, the present study was under taken with the objective to develop fertilizer prescription equations for desired yield targets of wheat with conjoint use of organic manure and chemical fertilizers.

## MATERIALS AND METHODS

The STCR field experiment was conducted at field No 36 C in the eastern block of TNAU farm on Inceptisols (Fig. 1). The farm is located in the Western agro climatic zone of Tamil Nadu at 11°12' North latitude and 77° 03' East longitude at an altitude of 426.74 m above MSL. The soil of experimental site belongs to Periyanaicken palayam series (Vertic Ustropept).The soil was clay in texture with pH of 8.51 and EC of 0.44 $dSm^{-1}$.The initial soil sample was low in available N, medium in available P and high in available K. The fertility gradient experiment was conducted adopting the Inductive methodology proposed by Ramamoorthy *et al*., (1967)[3] and the fertility gradients were created with respect to major nutrients by applying graded dose of fertilizer N, $P_2O_5$ and $K_2O$ (Table 1). An exhaust crop of fodder maize var (Co 1) was grown so that the fertilizers undergo transformation in the soil with plant.

After harvest of the exhaustive crop, each strip was divided in to 24 sub-plots without disturbing the strips. In this experiment, there were four levels of N, four levels of $P_2O_5$ and four levels of $K_2O$. For N, there were four treatment points at zero level, four at first level (50 kg N $ha^{-1}$), nine at second level (100 kg $ha^{-1}$), seven at third level (150 kg N $ha^{-1}$). For $P_2O_5$, three (60 kg $P_2O_5$ kg $ha^{-1}$) and five at third level (90 kg $P_2O_5$ $ha^{-1}$). For $K_2O$, here were four points at zero level, seven at first level (30 kg $K_2O$ $ha^{-1}$), Nine points at second level (60 kg $K_2O$ $ha^{-1}$) and four points at third level (90 kg $K_2O$ $ha^{-1}$). The treatment points were chosen so as to compute the response of each total effect of NPK treatments. The treatments also included two levels (5, 10 t $ha^{-1}$) of Farmyard manure (with a moisture content of 28 per cent, N-0.61 per cent, $P_2O_5$ - 0.37 per cent, $K_2O$ - 0.69 per cent along with fertilizers). In the 24 plots, all the 20 selected treatment combinations along with four controls were superimposed in each gradient strips adopting fractional factorial randomized block design as per the technical programme adopted by the All India Coordinated Research Project on Soil Test Crop Response Correlation studies. The IPNS component viz., NPK alone and NPK+FYM were applied across each strip. Grain yield of wheat was recorded plot wise.

Soil samples were collected from a depth of 0-15 cm before the application of fertilizers and after the harvest of both gradient and test crops. The soil samples were analyzed for available N (Subbiah and Asija, 1956)[4], P (Olsen et al., 1954)[5] and K (Stanford and English, 1949)[6]. Plant samples were also collected from both gradient and test crop and analyzed for N (Humphries, 1956)[7], P and K (Jackson, 1973)[8] contents and their uptake values were computed. From the soil test values, crop yield and uptake data, the basic parameters viz., nutrient requirement (NR), soil efficiency (Cs) fertilizer efficiency (Cf) and organic efficiency ($C_{FYM}$). From the basic parameters fertilizer prescription equation were developed for wheat. Based on the equation fertilizer recommendations were prescribed in the form of ready reckoner for desired yield target of 35 $qha^{-1}$ of wheat under NPK alone as well as for IPNS.

## RESULTS AND DISCUSSION

### Gradient experiment

For the present investigation all the needed variations in soil fertility level was obtained by deliberately creating it in one and the same field .The results showed that there was progressive increase in $KMnO_4$-N, Olsen-P and $K_2O$ with the increased doses of added NPK and this increase was found to be statistically significant (Table 2). The uptake of N, P and K also increased significantly from strip I to strip III. When all other production factors are kept at optimal levels, crop yield becomes a function of soil fertility. The statistical analysis showed the creation of fertility gradient in the experimental site due to graded dose of fertilizer application.

### Test crop experiment

### Basic parameters

The presowing soil test values, crop yield and nutrient uptake of wheat were used to calculate the basic parameters viz., Nutrient Requirement (NR) for producing one quintal of wheat, percent contribution from soil (Cs), fertilizer (Cf) and farm yard manure ($C_{FYM}$) (Table 3).

The average nutrient requirement to produce one quintal of wheat grain was found to be 2.86, 1.84 and 1.93 kg of N, $P_2O_5$ and $K_2O$ respectively. For wheat, the N requirement was high followed by K and P. Wheat var HD-2189 needs 2.51, 1.57 and 2.25 kg of N, $P_2O_5$ and $K_2O$ respectively to produce one quintal of produce (Tamboli and Sonar, 1998)[9]. Wheat removes 2.56, 0.64, 1.34 kg of N, $P_2O_5$ and $K_2O$ to produce one quintal of wheat. The above reports lent support for the results obtained in the present study.

The parameters *viz*., per cent contribution from soil and that from applied fertilizers were calculated respectively from the data of control plots and fertilizer treated plots. The per cent contribution from soil was 23.18, 31.24 and 5.37 respectively, for N, P and K. The efficiency of fertilizers to supply P was high followed by N and K. The per cent contributions from fertilizers were 32.45, 40.77 and 31.85 for N, $P_2O_5$ and $K_2O$, respectively. The per cent contribution from fertilizers in respect of $P_2O_5$ was more as compared to N and $K_2O$ and this might be due to application of FYM @ 5 and 10 t $ha^{-1}$ which might have helped in increasing the P availability. The results indicated that the nutrient efficiency was high for fertilizers than the soil. This may be due to the fixation of nutrients in soil and it's availability to plant is reduced, which is in close conformity with the results reported by Srinivas *et al.* (2001)[10].

Increase in available P may be due to the release of organic acid, which would have facilitated the solubility of P besides replacing a part of absorbed P. Further, the organic anions compete with phosphate ions and chelate or complex, the $Al^{3+}$, $Fe^{3+}$ and $Ca^{2+}$. This decreased the phosphate precipitation and increased P availability (Duraisami *et al.* (2000)[11].

### Optimisation of fertilizer doses through targeted yield model

The fertilizer prescription equations developed using the basic parameters as estimated by whole field method are presented below.

**NPK alone**

FN = 8.83 T – 0.71 SN

$FP_2O_5$ = 4.52 T – 1.75 SP

$FK_2O$ = 6.05 T – 0.20 SK

**NPK with farmyard manure**

FN = 8.83 T - 0.71 SN - 0.88 ON

$FP_2O_5$ = 4.52 T - 1.75 SP - 0.95 OP

$FK_2O$ = 6.05 T - 0.20 SK - 0.83 OK

Based on these above equations fertilizer doses were calculated for a yield target of 35 q $ha^{-1}$ at soil test value of 195, 24.0 and 440 kg $ha^{-1}$ of $KMnO_4$-N, Olsen P and $NH_4OAc$ K respectively. The fertilizer N, $P_2O_5$ and $K_2O$ requirements were 146, 100 and 106 kg $ha^{-1}$ without FYM. The integrated use of inorganics and organics considerably reduced the quantities of fertilizers applied. The Soil Test Crop Response studies for wheat under IPNS indicated the extent of saving of fertilizers. Santhi et al (2002a)[12] reported the same kind of reduction in chemical fertilizers under IPNS in onion.

### Ready reckoner for fertilizer recommendation for wheat under IPNS

Based on these equation ready reckoner was prepared for different soil test values for yield target of 35 q $ha^{-1}$ under NPK alone and IPNS (Table 4).The ready reckoners constructed from the fertilizer adjustment equations to obtain fertilizer doses for varying yield target and IPNS for wheat would be used to recommend the optimal quantity of fertilizer and organics applied for getting maximum yield of wheat. The yield was increased due to application of FYM @ 5 and 10 t $ha^{-1}$.

The increase in wheat yield with increase in FYM @ 15 t $ha^{-1}$. It may be ascribed to the addition of FYM, which improved the microbial activity and enhanced the availability of native and applied nutrients, which in turn increased the yield of crop.The results clearly revealed that, irrespective of yield targets, the response ratio increased under IPNS over NPK alone, exhibiting the efficient use of applied nutrients. However, the response ratio decreased with increase in yield targets. The fertilizer quantity required for a yield target of 35 q $ha^{-1}$ at soil fertility level of 195, 24 and 420 kg $ha^{-1}$ were reduced considerably with the integrated use of fertilizers, FYM @ 5 and 10 tones. Though the saving or the monetary returns were small with the integration of organics and chemical fertilizers than fertilizers alone it might be beneficial on long run basis (Sharma *et al*., 1999)[13].

The fertilizer estimates under IPNS clearly indicated better utilization of added fertilizer nutrients by recording of high response ratio. The increased availability of nutrient status observed in the post harvest soil showed the possibility of sustenance of soil fertility under IPNS practice. Thus the increased availability of plant nutrients and built up of soil fertility under IPNS prevented the mining of native soil nutrients. The conjoint application of organics with fertilizers resulted in saving of 25, 16 and 18 kg $ha^{-1}$ of N, $P_2O_5$ and $K_2O$ respectively.Soil test based balanced fertilizer recommendations under STCR - IPNS for wheat not only helped in achieving higher yield targets but also in the maintenance and built up of soil fertility.

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**Gradient crop: Fodder maize- *var* (Co 1)**

**Test crop : Wheat- var CoW(w)-1**


**Fig. 1. General Field Layout**

**Table 1.Graded doses of fertilizers applied to the gradient crop of fodder maize**

| Strip | Levels of Nutrients | | | Fertilizer doses (kg ha$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | N | $P_2O_5$ | $K_2O$ | N | $P_2O_5$ | $K_2O$ |
| I | $N_0$ | $P_0$ | $K_0$ | 0 | 0 | 0 |
| II | $N_1$* | $P_1$** | $K_1$** | 80 | 206 | 121 |
| III | $N_2$ | $P_2$ | $K_2$ | 160 | 412 | 242 |

* $N_1$ : As per blanket recommendation
**$P_1$ and $K_1$ : As per P and K fixing capacities of the experimental soil

**Table 2. Effect of Application of Graded Levels of N, $P_2O_5$ and $K_2O$ on Soil Fertility, Yield and Nutrient uptake of fodder maize VAR CO 1.**

| Strip | Nutrient uptake (kg ha$^{-1}$) | | | Yield (t ha$^{-1}$) | Soil available nutrients | | |
|---|---|---|---|---|---|---|---|
| | N | P | K | | N | P | K |
| I | 137.4 | 56.6 | 72.4 | 32.6 | 164 | 20 | 545 |
| II | 163.4 | 72.9 | 94.9 | 63.9 | 206 | 42 | 609 |
| III | 191.4 | 81.9 | 112.1 | 77.4 | 244 | 78 | 655 |
| SEd | 12.15 | 3.26 | 7.04 | 1.59 | 1.22 | 0.79 | 2.01 |
| CD(5%) | 26.07 | 7.00 | 15.11 | 3.41 | 3.41 | 1.70 | 4.33 |

**Table 3. Basic Parameter for Wheat**

| Parameters | Basic data | | |
|---|---|---|---|
| | N | $P_2O_5$ | $K_2O$ |
| Nutrient requirement (kg q$^{-1}$) | 2.86 | 1.84 | 1.93 |
| Per cent contribution from soil (Cs) | 23.18 | 31.24 | 5.37 |
| Per cent contribution from fertilizers Cf) | 32.45 | 40.77 | 31.85 |
| Per cent contribution from Farmyard manure ($C_{FYM}$) | 43.83 | 24.84 | 8.36 |

**Table 4. Ready reckoner for fertilizer recommendation of wheat based on targeted yield equation by IPNS approach**.

| Soil test values (kg ha$^{-1}$) | | | Fertilizer required for yield target of 35 q ha$^{-1}$ | | | | | |
|---|---|---|---|---|---|---|---|---|
| $KMnO_4$-N | Olsen P | $NH_4OAc$ K | Inorganics (kg ha$^{-1}$) | | | FYM (kg ha$^{-1}$) | | |
| | | | FN | $FP_2O_5$ | $FK_2O$ | FN | $FP_2O_5$ | $FK_2O$ |
| 185 | 20 | 400 | 178 | 123 | 132 | 153 | 107 | 114 |
| 190 | 22 | 410 | 171 | 119 | 130 | 146 | 103 | 112 |
| 195 | 24 | 420 | 164 | 116 | 128 | 139 | 100 | 110 |
| 200 | 26 | 430 | 156 | 112 | 126 | 131 | 96 | 108 |
| 205 | 28 | 440 | 149 | 109 | 124 | 124 | 93 | 106 |
| 210 | 30 | 450 | 142 | 105 | 122 | 117 | 89 | 104 |
| 215 | 32 | 460 | 135 | 102 | 120 | 110 | 86 | 102 |

FN- Fertilizer Nitrogen

$FP_2O_5$ -Fertilizer Phosphorus

$FK_2O$ – Fertilizer Potassium

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RESEARCH ARTICLE

# Appraisal of Critical Limits of Soil Available Sulphur for Sunflower

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## ABSTRACT

Delineation of sulphur status was carried out by collecting representative soil samples at two depths viz., surface and subsurface soil samples of 4 taluks in Coimbatore district. The study indicated 32 percent deficiency in the surface soils. Based on the analytical data, bulk soils were collected and pot culture experiment was conducted to determine the critical level of available sulphur.The result obtained indicated that drymatter yield .ranged from 11.2 to 23.0 g $pot^{-1}$ and it was significantly increased by sulphur application upto 60 $kg^{-1}$ sulphur over control. From the experiment, critical sulphur level for sunflower was fixed as 6.5 ppm by plotting the available soil sulphur value against Bray's percent yield.

**Keywords :** Sulphur, critical level, sunflower, drymatter yield.

## INTRODUCTION

Soil fertility evaluation of an area or region is an important aspect in context of sustainable agricultural production. In Indian Agriculture, nutrient mining is a great threat, as wide gap exists between the annual plant nutrient removal from the soil and addition of nutrients through external sources (Singh, 2008)[1]. Among the plant nutrients, sulphur is now called as the fourth major plant nutrient as it is required for the synthesis of oil, S containing amino acids (cystine, cysteine and methionine) and protein. Sulphur deficiency in soils of various Indian states varies from 5 to 83% with an overall mean of 41% (Singh 2001)[2] which may likely to increase in the years to come. A timely and

precise appraisal of sulphur deficiency is necessary for monitoring and identifying deficient areas for taking prompt and appropriate corrective measures to obtain the best crop yields as well as to increase the fertilizer use efficiency and better return from other costly inputs.

It has been estimated that oilseeds remove around 12 kg of S per ton of seed production Substantial scope for harnessing the potential of oilseeds exists only in terms of increasing the level of productivity. Sulphur deficiency can be corrected through application of proper amount of S based on various approaches including soil and plant test, proper source of S, right time of application, balanced fertilization and use of S efficient genotypes. Establishment of critical limit for soil and plant is appropriate step to monitor S deficiency. The level of response under different categories of availability including low, medium and high status of any nutrient is to be assessed based on the critical level concept. Hence, the pot culture experiment was conducted to fix the critical level of S for sunflower crop.

## MATERIALS AND METHODS

Soil samples were collected from different areas of Coimbatore district to delineate the soil sulphur status. Representative soil samples were collected as per the procedure at two depths viz., 0-15 cm and 15-30 cm and analyzed for available soil sulphur using the method given by Chesnin and Yien (1950)[3]. Based on the available soil sulphur status, bulk soils were collected from twenty locations covering low, medium and high soil available sulphur status (14 low: 3 medium: 3 high soil sulphur soils). The soils were processed, sieved through 2 mm sieve and filled in pots of 25 cm diameter and 21 cm height uniformly (7 kg each).The potculture experiment was conducted with sunflower variety CO4 in Completely randomized design with two replications. Sulphur was applied as basal @ 0,20,40,60 and 80 kg $ha^{-1}$ along with the recommended dose of N, $P_2O_5$ and $K_2O$ (40:20:20 kg $ha^{-1}$ as Urea, DAP and Muriate of Potash) for sunflower. Three seeds were sown in each pot and after germination two seedlings were maintained in each pot. Plant samples were collected at flowering and harvesting stages, while soil samples were collected at harvest stage. Based on the dry matter production, the critical soil sulphur content was fixed for sunflower as per the procedure given by Cate and Nelson (1965)[4].

## RESULT AND DISCUSSION

### Available Sulphur status

The available sulphur status (0.15% $CaCl_2$ S) of different soils from different locations indicated 32 per cent deficiency and 68 per cent sufficiency in surface soil (0 to 15 cm), while 36 and 64 per cent of in the lower layer (15-30 cm). The status of available sulphur was found to vary from 2-10 ppm in the lower category, while it ranged from 12-15 ppm and 17-199 ppm respectively under medium and high status. Occurrence of S deficiency has been noticed in the taluks of Mettupalayam, Avinashi and Coimbatore. Attention on S deficiency was focused during 1960's itself in our country when widespread deficiency has been noticed in groundnut growing areas of Ludhiana. After that, intensive research work has been undertaken to fix the extent of S deficiency in Indian soils. Earlier Tandon (1995)[5] has indicated the possible occurrence of S deficiency in Coimbatore district. The work carried out in the All India Coordinated Centre on Micro and Secondary Nutrients at Tamil Nadu Agricultural University, Coimbatore revealed a deficiency of 19.9 per cent S in Tamil Nadu soils based on the analysis of 1555 samples collected all over the state (Anon, 2000)[6]. Evaluation of the sulphur status in 1,164 samples collected from different parts of our country by Singh (1991)[7] indicated 40.7 per cent deficiency. Maragatham *et al.* (2014) [8] reported that the available S status ranged from 5.75 to 50.5 mg $kg^{-1}$ in Salem District of Tamil Nadu with an overall mean value of 31.37 mg $kg^{-1}$. Singh and Mishra (2012)[9] observed about 62 % S deficiency in soils of Chiraigaon block of Varanasi district, Uttar Pradesh.

**Critical level of soil sulphur**

Among the low, medium and high S category soils, the drymatter yield ranged from 11.2 to 23.0 g $pot^{-1}$ and the highest drymatter yield was recorded in the lower S status soils (Table 1). In pot culture experiment, the response was maximum in case of soils having low available sulphur, followed by soils having high available sulphur and medium available sulphur. The Bray per cent yield ranged from 83.0 to 99. The available soil sulphur values were plotted against Bray's per cent yield and the critical level of soil sulphur for sunflower crop was fixed as 6.5 ppm (Fig. 1) by following the graphical method and it was confirmed by statistical method. Earlier Sharan *et al*. (1989)[10] indicated a critical value of 16.0 and 9.4 ppm of S respectively for sunflower and sesame in Alfisol soils of Ananthapur. Variation in critical level in different locations is an expected one because the limit on S concentration is likely to change depending upon the crop, crop water requirement and soil characteristics. Shelke *et al.* (2007) [11] indicated plant critical level of 0.24 % and 0.27 % by graphical and statistical methods, respectively, for soybean suggesting that if soils containing less than 0.24 % sulphur by 15% $CaCl_2$ extractable sulphur method, the crop would respond to the application of sulphur. For acid soils of Manipur, Herojit Singh Athokpam *et al.* (2005)[12] fixed 0.35 per cent as critical S content in mustard plant.

Thus, the present study lays emphasis on S fertilization on sunflower based on critical values in the soils. The threshold value of soil sulphur to assess the responsiveness of sunflower crop was found to be 6.5 ppm. This study indicated the need for critical management of sulphur to obtain maximum productivity of oilseed crops.

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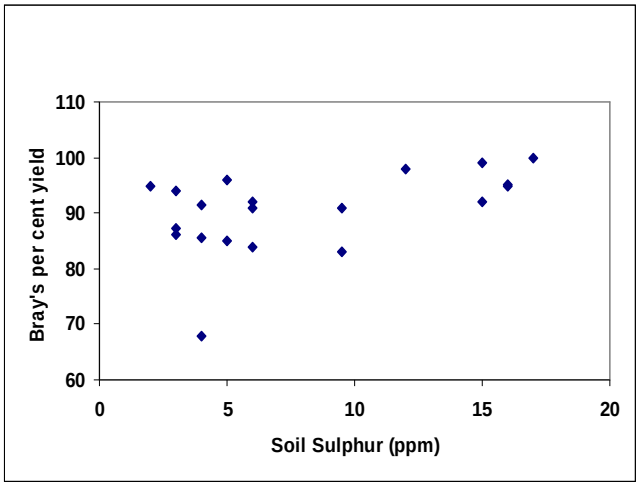


**Fig.1. Critical Level of Soil Sulphur for Sunflower Crop**

**Table 1. Soil sulphur status and bray's per cent yield in sunflower**

| S. No. | Soil sulphur status (0.15% $CaCl_2$- ppm) | Control yield (g) ($y_0$) | Maximum yield at optimum level of nutrient (g) ($y_{max}$) | Bray's per cent yield $y_0/y_{max}*100$ |
|---|---|---|---|---|
| **Low(<10ppm)** | | | | |
| 1 | 2 | 20.8 | 22.0 | 94.7 |
| 2 | 3 | 17.0 | 19.5 | 87.3 |
| 3 | 3 | 17.9 | 19.0 | 94.0 |
| 4 | 3 | 18.1 | 21.0 | 86.0 |
| 5 | 3 | 15.4 | 16.0 | 96.0 |
| 6 | 4 | 13.6 | 20.0 | 68.0 |
| 7 | 4 | 21.1 | 23.0 | 91.5 |
| 8 | 4 | 15.5 | 18.5 | 85.4 |
| 9 | 5 | 12.7 | 15.0 | 85.0 |
| 10 | 6 | 12.5 | 13.8 | 91.0 |
| 11 | 6 | 12.4 | 13.5 | 92.0 |
| 12 | 6 | 11.3 | 13.5 | 84.0 |
| 13 | 9.5 | 13.9 | 16.7 | 83.0 |
| 14 | 9.5 | 12.5 | 13.7 | 91.0 |
| **Medium (10–15 ppm)** | | | | |
| 15 | 12 | 12.1 | 12.4 | 98.0 |
| 16 | 15 | 10.6 | 11.6 | 92.0 |
| 17 | 15 | 12.1 | 12.2 | 99.0 |
| **High(>15ppm)** | | | | |
| 18 | 16 | 10.7 | 11.2 | 95.2 |
| 19 | 16 | 12.0 | 12.7 | 94.9 |
| 20 | 17 | 12.3 | 12.3 | 99.9 |

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RESEARCH ARTICLE

# A Preliminary Microbiological Survey of Bacterial Pathogens from Urban Rats in Ipoh, Perak

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## ABSTRACT

Rats (*Rattus* spp.) have been well documented as sources of several zoonotic pathogens that are known to cause significant human morbidity and mortality.Rats living in close proximity to humans, pose serious harm to human health, welfare and economy. We conducted a bacteriological survey to identify and assess the prevalence of zoonotic bacterial agents carried by rats in wet markets and some restaurants of Ipoh, Perak, Malaysia between February and August 2014.As rats are known to carry a variety of bacterial flora including zoonotic and non-pathogenic organisms, methodologies including direct isolation and indirect methods like PCR were employed.A total of 95 rats belonging to three species namely *Rattus norvegicus, Rattus diardii* and *Rattus exulans* were captured and their bacteriological carriage were examined. About 766 bacterial isolates were cultured from various organs of these rats and identified. Alpha-haemolytic Streptococci were the most prevalent bacteria found in most organs of the rats harvested, followed by Coagulase negative Staphylococci, *Pasteurella pneumotropica*, members of *enterobacteriaceae*, *Staphylococcus aureus*, Beta-haemolytic Streptococci and species of *Pseudomonas*.Though the rates of isolation of zoonotic organisms were not significant in this study, the isolation of bacteria like *E.coli, Klebsiella pneumoniae* and other members of enterobacteriacea pose a serious threat, considering that the rats were trapped from alleys of restaurants and wet markets.This study aimed at a bird's eye view of

bacteriological carriage in urban rats and the results demonstrate that the rats can also carry pathogens commonly associated with humans and continue to be a threat.

**Keywords :** Bacteria, Leptospira, Malaysia,Rats, Streptococci

## INTRODUCTION

Rats (*Rattus spp*.) have been well documented assources of a plethora of zoonotic pathogens that are known to cause significant human morbidity and mortality. With regard to rat-associated-health risks, the urban environmentsare at risk because cities provide an optimal habitat for rats, leading to close contact between rats and people and, potentially, zoonotic disease transmission[1].Given the unprecedented rates of global urbanization, it becomes essential to develop a thorough understanding of rat-associated zoonoses in urban centers[1,2].This understanding is needed to accurately gauge the magnitude of rat-associated health threats, to monitor the risk of emergence of rat-associated zoonotic diseases and to develop effective rat control strategies and awareness among the public.

Rats, being prolific breeders with no seasonal barriers and living in close proximity to humans, pose serious harm to human health, welfare and economy[3].Rats are known to actively explore several different human environments and therefore have the unique opportunity to become exposed to or get colonized with a variety of human pathogens. They might carry bacterial pathogens causing diseases such as bubonic plague, leptospirosis, murine typhus, salmonellosis, tularaemia and rat-bite fever[4].Though the emergence or reemergence of rodent-borne diseases have attracted universal/global attention, there is a dearth of information available in Malaysia on this aspect.A preliminary study on rats captured at Ipoh, Perak, Malaysia had revealed the presence of several bacterial pathogens that can cause zoonotic diseases such as leptospirosis, salmonellosis, to humans [5].Likewise, an investigation of intestinal and blood parasites among wild rats in wet markets in urban areas of Kuala Lumpur, Malaysia, had shown high prevalence of these parasites.[6]High infestation of rats has also been reported in areas close to restaurants.Thus, rats living in close proximity of man could serve as potential for zoonotic infections in man.

Although it has been well established that rats are reported to be hosts for a largenumber of pathogens, a comprehensive bacteriological survey ofpathogens carried by rats in an urban setting hasnot been conducted in Malaysia. It is important to focus on the urban environments, because it is where the humansand rats live in close proximity and the potentialfor spillover of zoonotic agents poses a public healthconcern that has rarely been evaluated.Hence, we conducted a bacteriological survey to identify and assess the prevalence of zoonotic bacterial agents carried by rats in wet markets and some restaurants of Ipoh, Perak, Malaysia betweenFebruary and August 2014. As rats are known to carry a variety of bacterial flora including zoonotic and non-pathogenic organisms, methodologies including direct isolation and indirect methods like PCR were employed.

## MATERIALS AND METHODS

### Trapping of rats

During the period ofsix months, from February 2014 to August 2014, urban ratsfrom alleys behind wet markets and restaurants in Ipoh city (4.5975° N, 101.0901° E), Malaysia were trapped. Steel traps measuring 29 x 22 x 50cm baited with bread, peanut butter and cheese, or dried salted fish were used. Fifteen traps were set alongside drains and alleys behind restaurants and housing estates three times weekly half an hour before sundown.Cages containing the rodents were collected in the morning and placed in ventilated black plastic bags to reduce excitement and stress to the animals during their transportation to the laboratory. For wet markets, the baited traps were placed near chicken cagesand collected three hours thereafter and bought to the laboratory for necropsy the next morning.

### Necropsy and bacterial isolation

Necropsies of rats were carried out the morning after anaesthetizingto moribund stage with chloroform.Thoracic cavity was cut opened and blood was drawn from cardiac puncture using a syringe (23G x 11.4 syringe) and about 5 mL of blood collected. A few drops of the sterile blood was inoculated to a Bujen bottle containing EMJH enriched medium. Necropsies of animals were performed using sterile instruments and organs collected were placed in sterile plastic bags and spastically streaked into appropriate culture media for bacterial cultures.Culture and identification of bacteria were performed at the Veterinary Research Institute, Ipoh.

Kidneys were maceratedin mortar and pestle with 900 µL liquid EMJH medium and 100µL of the suspension was added to a Bujen bottle containingEMJH semi solid medium and kept at 28-30°C for 1-2 months. Bladder samples with reminiscent urine were inoculated into Bujen bottles with EMJH medium. The suspensions were observed for growth of*Leptospira* growth under dark field microscope at weekly intervals for 2 months. Serum obtained from coagulated blood was stored at -20°C until tested by PCR at a later date.

Conventional techniques of bacterial culture were performed on thesamples namely, tongue, heart, liver, lungs, spleen, using 5% sheep bloodagar and MacConkey agar, incubated for 24 hrs at 37°C.Bacterial isolation and identification by biochemical tests were performed according to standard methodologies described elsewhere[7].

### Polymerase Chain Reaction Assay

The inoculated EMJH media were incubated aerobically at 30ºC in the dark, for *Leptospira* culture and were examined under a dark field microscope for the presence of *Leptospira* at weekly intervals for a period of three months. Polymerase chain reaction (PCR)was used to detect the possible isolates using G1/G2 primers for identification of *Leptospira.* The sets of primers used were from 16s rRNA and LipL32 genes.The possible growth in the EMJH medium was harvested and DNA was extracted using Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions.PCR was carried out using Red Mix Master solution (Bioline, UK). The PCR master mix contained 25µl of Bioline Red Mix 2X, 1.25µl of Genus Specific Forward Primer, 1.25µl Genus Specific Reverse Primer and 15µl of RNase free water. The final PCR tube consisted of 45µl of PCR master mix and 5µl of DNA template. For negative control, 5µl of distilled water was used instead of the DNA template and purified DNA from the stock culture of *L. australis* was used as a positive control.The PCR was performed on a Nexus Gradient PCR thermocycler (Eppendorf, Germany). The initial denaturation was at 95˚C for 5 min, followed by 35 cycles of denaturation (94˚C for 60s), amplification (55˚C for 60s), extension (72˚C for 90s) and final extension at 72˚C for 10 min and then held at 4˚C. One µl of each reaction of the amplified PCR products were electrophoresed on a 1.2% agarose gel with 1000bp ladder for 30min at 100V.

## RESULTS

A total of 95 rats belonging to three species namely *Rattus norvegicus, Rattus diardii* and *Rattus exulans* were captured and their bacteriological carriage were examined. All the captured rodents appeared healthy and active. A few male adults were observed to exhibit aggressive and restless behaviour in captivity prior to necropsy.About 766 bacterial isolates were cultured from various organs of these rats and identified. Alpha-haemolytic Streptococci were the most prevalent bacteria found in most organs of the rats harvested, followed by Coagulase negative Staphylococci, *Pasteurella pneumotropica*, members of *enterobacteriaceae*, *Staphylococcus aureus*, Beta-haemolytic Streptococci and species of *Pseudomonas*. The complete list of bacteria isolated from the rats are presented in Table 1.All the kidney, urinary bladder and blood specimens were tested negative for *Leptospira* sp. by PCR method.

## DISCUSSION

The study aimed to investigate and survey thecarriage of bacteriological load by the urban rats and their possible role in impacting the public health scenario. Interestingly, none of the rats were tested positive for *Leptospira* sp. and these negative results corroborate with absence of human leptospirosis cases reported in the General Hospital, Ipoh during the study period. Also, it is to be noted that as reported in previous studies, work or social environments have been associated with lesser risk of leptospirosis than home environment.

Though the rates of isolation of zoonotic organisms were not significant in this study, the isolation of bacteria like *E.coli, Klebsiella pneumoniae* and other members of enterobacteriacea pose a serious threat, considering that the rats were trapped from alleys of restaurants and wet markets. Presence of these bacteria is an indication of exposure to faecal contents and these rats could probably get access to restaurants and could contaminate the food and crockeries leading to several faeco-oral transmitted human diseases[8].

The isolation of bacteria such as species of *Pseudomonas,Aeromonas* and *Acinetobacter* has serious public health implications owing to their inherent resistance to commonly prescribed antibiotics. Moreover, with the rate of isolation of *Staphylococcus aureus* being over 13%, the rates of these strains being methicillin-resistant (MRSA) remains unknown. Studies have shown that rats are well known carriers of MRSA strains and they also act as 'mixing vessels' facilitating transfer of genetic elements between the species of bacteria which open up new doors of emergence of pathogenicity and resistance [9,10].

In an earlier study conducted in the same study group to assess the presence of parasites in liver of rats, about 64% of rats were observed to be infected; 44.9% with *Caladium hepatica* and 39.3% with *Cysticercus fasciolaris*, and 20.4% infected with both parasites [11]. High infection rates suggest that urban rats are common reservoir for such parasites as well, thereby being a potential threat to man. The present study focused mainly on the bacteriological survey and the limitations include the failure to investigate the seroprevalence of zoonotic pathogens such as Hantavirus, Hepatitis E and Seoul virus.Studies have largely focused on disease risk associated with pathogens for which the rats are the primary reservoir such as in case of leptospirosis. This study aimed at a bird's eye view of bacteriological carriage in urban rats and the results demonstrate that the rats can also carry pathogens commonly associated with humans and continue to be a threat.

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**Table 1. Bacteria isolated from the urban rats during February – August 2014, Ipoh**

| S. No. | Isolated bacteria | No. of rats positive | Rate of isolation (%) |
|---|---|---|---|
| 1 | Alpha hemolytic Streptococci | 46 | 48.4 |
| 2 | *Staphylococcus epidermidis* | 43 | 45.3 |
| 3 | *Pasteurella pneumotropica* | 22 | 23.2 |
| 4 | *Klebsiella pneumoniae* | 21 | 22.1 |
| 5 | *Escherichia coli* | 19 | 20 |
| 6 | *Bacillus* sp | 18 | 18.9 |
| 7 | *Acinetobacter iwoffii* | 14 | 14.7 |
| 8 | *Staphylococcus aureus* | 13 | 13.7 |
| 9 | *Aeromonas hydrophila* | 7 | 7.4 |
| 10 | *Enterobacter cloacae* | 5 | 5.3 |
| 11 | Beta-hemolytic Streptococci | 4 | 4.2 |
| 12 | *Pseudomonas* sp. | 4 | 4.2 |
| 13 | *Corynebacterium* sp | 2 | 2.1 |
| 14 | *Enterobacter aerogenes* | 2 | 2.1 |
| 15 | *Alcaligenes* sp | 1 | 1.1 |
| 16 | *Citrobacter* sp. | 1 | 1.1 |

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RESEARCH ARTICLE

# Study the Effect of Micro and Nano Particle Size of $SnO_2$ on the Mechanical Properties of $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ Superconducting System

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## ABSTRACT

The effect of adding $SnO_2$ nano particles (20-40) nm and micro- particles (0.12-0.2)µm respectively on the mechanical properties (Vickers microhardness, Young's modulus, yield strength) of $(SnO_2)_xBi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ superconducting system with x=0,0.1,0.2,0.4 and 0.6wt % has been investigated. The results showed improvement of these properties by nano-particles addition. But, an opposite behavior was found due to the addition of micro-particles .A deterioration of the mechanical properties was noticed.

**Keywords :** Bi-based superconductors ,$SnO_2$ nano-particles , Mechanical properties.

## INTRODUCTION

Chemical doping and the introduction of nano-sized particles in bulk high temperature superconductors have generated great interest because they represent an easily controlled and efficient tool for improving the superconducting and the physical properties of layered structure superconductors (1). The addition of nanoparticles in $Bi_{1.6}Pb_{0.4}Sr_2Ca_2Cu_3O_{10+\delta}$ system is known to enhance its flux pinning ability because their size is comparable to the magnetic flux diameter of high temperature superconductors (2). On the other side the addition of rare earth nanoparticle increases the amount of random orientation platelet grains and decreases its grain size. The rare earth nanoparticles have been homogenously distributed in BSCCO matrix (3). Bismuth substitutes lead easily since $Bi^{3+}$and $Pb^{2+}$ share the same outer shell electronic configuration $6s^26p^0$. In a similar manner, $Sn^{2+}$ has an outer shell

electronic configuration of $5s^2 5p^0$; this means that the substitution of bismuth with tin may occur. Garnier et al.(4) studied the effects of this doping on the Bi-2223 phase formation by studying, in particular, the possibility or not, for the tin to enter to the Bi-2223 matrix .Tin oxide was added at different intervals of the synthesis process. They found that the addition of $SnO_2$ inhibits the Bi-2223 phase formation regardless of when it was introduced to the system. $SnO_2$ partially reacts with calcium to form the $Ca_2SnO_4$ phase. This phase was stable throughout the synthesis process. As a consequence, the formation of the Bi-2223 phase was compromised for the benefit of secondary phases such as Bi-2212,$Ca_2SnO_4$, $Sr_{0.15}Ca_{0.85}CuO_2$ and $Cu_2SrO_2$.Abou-Aly et al. (5) studied the effect of $SnO_2$nano-particles (40nm) addition on the physical properties of $Bi_{1.6}Pb_{0.4}Sr_2Ca_2Cu_3O_{10+\delta}$. superconducting phase which was prepared by a conventional solid state reaction technique. The prepared samples were investigated by X-ray diffraction ,Scanning electron microscope ,electron dispersive spectroscopy ,electrical resistivity and transport critical current density .They found that $SnO_2$nano-particles enhanced the (Bi,Pb)-2223 phase formation up to 0.4 wt%.

Awad et al (6) investigated the Vickers microhardness of $Bi_{1.6}Pb_{0.4}Sr_2Ca_2Cu_3O_{10+\delta}$ superconducting phase with added $SnO_2$ nano-particles. The concentration of $SnO_2$nano-particles varied from 0.0 to 2.0 of the sample's total mass. They noted that Vickers microhardness number $H_v$ increased as $SnO_2$ increased up to 0.4 wt%. The load dependence of $H_v$ exhibited a normal indentation-size effect, $H_v$ increased as the applied load increased. In addition, they calculated Young modulus, yield strength, fracture toughness and brittleness index.Agail and Abd-Shukor (7) investigated the influence of nano-SnO2 particles(~50 nm) addition on the critical current density (Jc) in $Bi_{1.6}Pb_{0.4}Sr_2Ca_2Cu_3Sn_xO_{10}$ superconductor ceramic with *x* ranging from 0 to 0.05. The samples were prepared using the co-precipitation technique with sintering time of 48 h at 850ºC. The critical current density and the transition temperature for sample with 0.02 wt% were found to be the highest with a maximum $J_C$ 1212 mA/cm$^2$ and a maximum TC-onset 112 K. XRD and SEM analysis indicated that nano-SnO up to 0.02% wt enhance the formation of low-*T*C (Bi-2212) phase fraction.

Practical applications of superconducting materials are in the form of wires and tapes, which are subjected to large mechanical stresses in making coils and Lorentz force due to high magnetic fields. Under high stresses, the generation of small cracks at high currents will cause a destruction of the coil. Besides, magneto elastic effects associated with flux pinning induce internal macro-stresses. These effects often cause fatal cracking of Melt-Grown (MG) bulks as they become magnetized (8). Therefore, Mechanical properties such as hardness, elastic modulus, strength, and fracture toughness as well as superconducting properties are very important for industrial application of high temperature oxide superconductors.

In a previous work (9), the $SnO_2$ particle size effect on superconducting properties (structural and the transition temperature) of $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ were studied. The aim of this search is to investigate the mechanical properties of the above system.

# METHODOLOGY

Superconducting samples of nominal composition $(SnO_2)_xBi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ with x equal ( 0, 0.1, 0.2, 0.4 and 0.6) wt % with different particle size of $SnO_2$ ,nano-particles (20-40) nm and micro- particles (0.12-0.2) µm,were prepared using a conventional solid state reaction technique. The samples were prepared using high purity powders (99.9%) of $Bi_2(CO_3)_3$, $Pb_3O_4$, $SrCO_3$, CaO, CuO and $SnO_2$..The powder of precursor was mixed together by using agate mortar. The mixture homogenization was accomplished by adding a sufficient quantity of 2-propanol to form a paste during the process of grinding for about (1 h). After that the materials were grounded to a fine powder and then calcined in air at 800 ºC for 24h , the mixture was then pressed into pellets 1.3 cm in diameter and 0.2 cm thick, using hydraulic type (SPECAC), under pressure of 0.7GPa.. The pellets were sintered in air at 830 ºC for 140 h.The mechanical properties, such as the Vickers micro- hardness (Hv), Young modulus (E) and yield strength (Y) of superconductor samples were calculated as was explained in a previous paper (10).

## RESULTS AND DISCUSSION

Fig.(1) display the variation of Vickers microhardness as a function of applied load for the samples $(SnO_2)_xBi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ with different concentration of $SnO_2$ micro particles . It is observed from the figure that Vickers microhardness values depend on the applied load for all samples; the calculated microhardness value decreases non- linearly with the increase of the applied load. Similar changes in the Vickers hardness were reported by Yilmazlar (11) for $Bi_{1.6}Pb_{0.4}Sr_2Ca_{2-x}Sm_xCu_3O_{10+\delta}$ composition .This behavior can be explained as follows: at larger indention loads, the Vickers hardness registered small values, this may be due to the presence of weak grain boundaries of the superconducting ceramics. While for small indention loads, the Vickers hardness recorded high values, this is ascribed to the fact that the measured hardness values were more indicative of the monocrystalline state which means that there is no interference from grain boundaries (12) .This non- linear behavior has also been observed in many papers for Bi-Pb-Sr-Ca-Cu-O phases and it is known as the indentation size effect (ISE) (12,13).

The variation of Young's modulus (E) and yield strength (y) are plotted as a function of applied load$(SnO_2)_xBi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ with different concentration of $SnO_2$ micro-particles are shown in Figs. (2&3).From these graphs, the E and Y decrease with the increase in the applied load.It should pointed out that the apparent microhardness, Young's modulus and yield strength show a strong dependency on applied load .

Figs.(4-6) show variation of the Vickers microhardness , Young's modulus and yield strength for superconducting samples of $(SnO_2)_xBi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ as a function of micro-particles $SnO_2$ concentration. These figures demonstrate that microhardness Hv,Young's modulus E and yield strength Y decrease gradually with the increase of $SnO_2$ micro-particles.

For the superconducting samples $(SnO_2)_x Bi_{1.7} Pb_{0.3}Sr_2Ca_2Cu_3 O_{10+\delta}$ with $0 \leq x \leq 0.6$ wt% with nano $SnO_2$,the variations of the Vickers microhardness Hv, Young modulus (E)and yield strength (Y) with the applied load are shown in Figs.( 7-9) and listed in Table (1). It is observed that Vickers microhardness Hv decreases rapidly with the increase of the load. Rapid decrease of the microhardness was observed as the load increases from 0.49 to 0.98N. The reason for this behavior is due to the contribution of weak grain boundaries. It is also obvious from the figure the dependence of Vickers microhardness on the load for all samples. Microhardness values decreases non linearly with increasing the applied load up to 1.96 N, then they tend to have approximately close values. This behavior was explained on the basis of the penetration depth of the indenter as reported by Awad (6). At small loads, the indenter have affects only on the surface layers and surface effect dominates, but at higher loads, the penetration depth increases and the effect of inner layers becomes more prominent and ultimately leads to a slight change in Hv values combined with the increase of loads. (14,15)

The variation of Hv, E and Y with nano $SnO_2$ concentrations (x) for different loads are shown in Figs. (10-12). All grahs have the same trend.The H ,E and Y values icrease as the load increases from 0.00to 0.4 wt% , reaching a maximum at this value and decrease for further increase of the load The high value of Hv at x=0.4 is attributed to the increase in grain connectivity and the crack resistance propagation. Similar results were obtained by Liu et al. (16) when a small amount of SiC nano- particles were added to Sn-Ag-Cu alloys. The decrease of Hv for x>0.4 happened because a higher nano $SnO_2$ –concentration may weaken the coupling between the superconducting grains. Also, it is attributed to the presence of few secondary phases and some defects in grain boundaries.These defects may produce micro - crakes which have deleterious effect on the mechanical resistance. This result is in agreement with previous results (9)of T investigation employing XRD and resistivity measurements which recorded high values of both volume fraction of the high phases of Bi-2223 and critical temperatures at x= 0.4.But at x=0.6, the values decreased.
Finally, from our results, it can concluded that nano $SnO_2$ particles improve the mechanical properties of (Bi,Pb)-2223 by enhancing microhardness, Young modulus and yield strength (Y) of superconducting for this phase compared to the micro-particles of $SnO_2$ , as seen in Table (1).

## CONCLUSION

It was revealed that the addition of $SnO_2$ nano- particle to $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ superconducting system might strengthens the coupling of the grains, leading to improvement of the mechanical properties.While $SnO_2$ micro-particles addition, deteriorate the mechanical properties of the superconducting samples.

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**Table (1):Vickers microhardness(Hv) , Young's modulus (E) and Yield strength (Y) with different loads for $(SnO_2)_xBi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ superconducting system.**

| | | Micro-particles of $SnO_2$ (0.12-0.2)micrometer | | | nano-particles of $SnO_2$ (20-40) manometer | | |
|---|---|---|---|---|---|---|---|
| x | F (N) | Hv (GPa.) | E (GPa.) | Y (GPa.) | Hv (GPa.) | E (GPa.) | Y (GPa.) |
| 0.0 | 0.49 | 1.056 | 86.598 | 0.352 | 1.056 | 86.598 | 0.352 |
| 0.1 | | 0.923 | 75.649 | 0.307 | 1.260 | 103.269 | 0.420 |
| 0.2 | | 0.796 | 65.240 | 0.265 | 1.330 | 109.006 | 0.443 |
| 0.4 | | 0.654 | 53.601 | 0.218 | 1.471 | 120.563 | 0.490 |
| 0.6 | | | | | 1.1566 | 94.794 | 0.385 |
| 0.0 | 0.98 | 0.98 | 80.320 | 0.326 | 0.980 | 80.320 | 0.326 |
| 0.1 | | 0.91 | 74.583 | 0.303 | 1.166 | 95.565 | 0.388 |
| 0.2 | | 0.723 | 59.257 | 0.241 | 1.211 | 99.253 | 0.403 |
| 0.4 | | 0.614 | 50.323 | 0.204 | 1.313 | 107.613 | 0.437 |
| 0.6 | | | | | 1.011 | 82.861 | 0.337 |
| 0.0 | 1.96 | 0.930 | 76.222 | 0.310 | 0.930 | 76.222 | 0.310 |
| 0.1 | | 0.886 | 72.616 | 0.295 | 1.036 | 84.910 | 0.345 |
| 0.2 | | 0.679 | 55.650 | 0.226 | 1.130 | 92.614 | 0.376 |
| 0.4 | | 0.575 | 47.127 | 0.191 | 1.196 | 98.024 | 0.398 |
| 0.6 | | | | | 0.930 | 76.222 | 0.31 |
| 0.0 | 2.94 | 0.906 | 74.255 | 0.302 | 0.906 | 74.255 | 0.302 |
| 0.1 | | 0.766 | 62.781 | 0.255 | 0.946 | 77.534 | 0.315 |
| 0.2 | | 0.610 | 49.995 | 0.203 | 1.056 | 86.549 | 0.352 |
| 0.4 | | 0.522 | 42.783 | 0.174 | 1.093 | 89.582 | 0.364 |
| 0.6 | | | | | 0.863 | 70.731 | 0.287 |

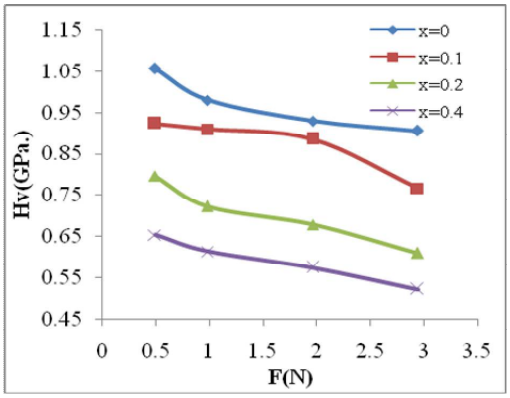


**Fig.(1): The variations of microhardness(Hv) as a function of applied load of $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ for different concentration of $SnO_2$ micro-particles.**

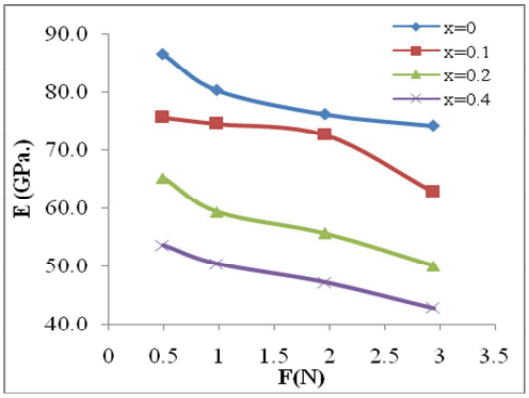


**Fig.(2): The variations of Young's modulus (E) as a function of applied load of $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ for different concentration of $SnO_2$ micro-particles.**

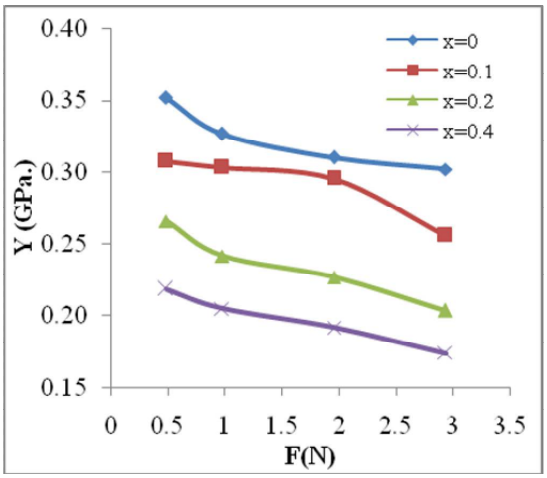


**Fig.(3): The variations of yield strength (Y) as a function of applied load of $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ for different concentration of $SnO_2$ micro-particles**

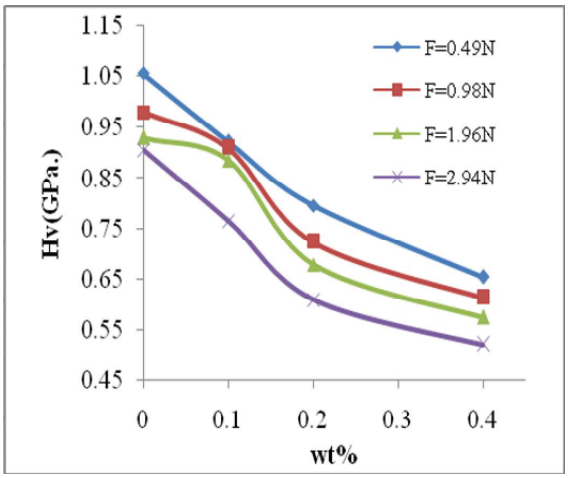


**Fig.(4): The variations of microhardness (Hv) as a function of micro-particles $SnO_2$ concentration for $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ for different loads**

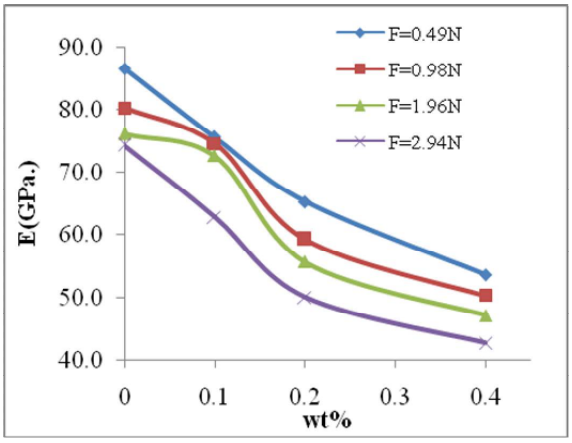


**Fig.(5): The variations of Young's modulus (E) as a function of micro-particles $SnO_2$ concentration for $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ for different loads**

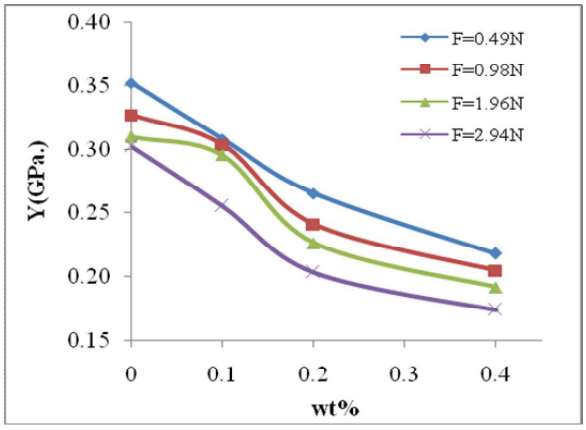


**Fig.(6): The variations of yield strength (Y) as a function of micro-particles SnO concentration for $(SnO_2)_x$ $Bi_{1.7}Pb_{0.3}Sr_2Ca_2Cu_3O_{10+\delta}$ for different loads**

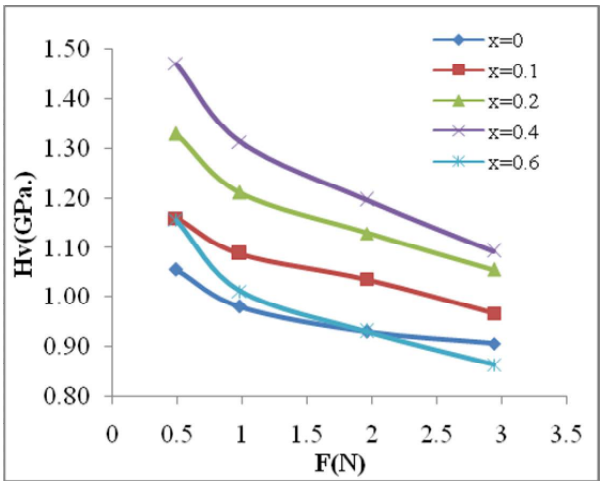


**Fig.(7): The variations of microhardness(Hv) as a function of applied load of $(SnO_2)_x$ $Bi_{1.7}$ $Pb_{0.3}Sr_2$ $Ca_2Cu_3$ $O_{10+\delta}$ for different concentration of $SnO_2$ nano-particles.**

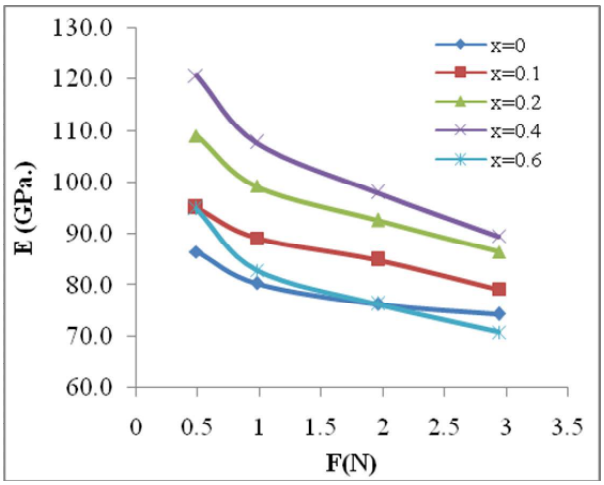


**Fig.(8): The variations of Young's modulus (E) as a function of applied load of $(SnO_2)_x$$Bi_{1.7}$ $Pb_{0.3}Sr_2$ $Ca_2Cu_3$ $O_{10+\delta}$for different concentration of $SnO_2$ nano-particles.**

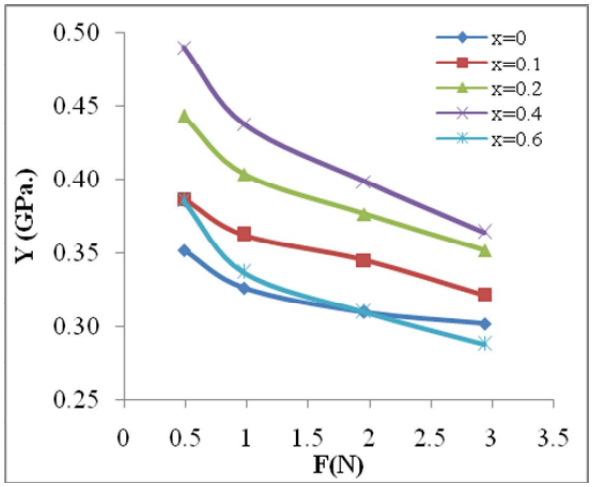


**Fig.(9): The variations of yield strength (Y) as a function of applied load of $(SnO_2)_x$ $Bi_{1.7}$ $Pb_{0.3}Sr_2$ $Ca_2Cu_3$ $O_{10+\delta}$for different concentration of $SnO_2$ nano-particles**

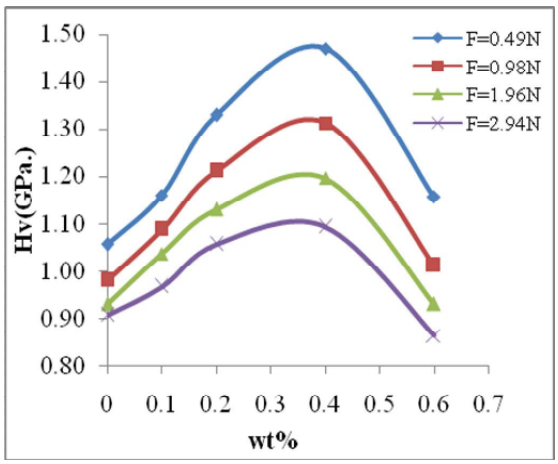


**Fig.(10): The variations of microhardness (Hv) as a function of nano-particles $SnO_2$ concentration for $(SnO_2)_x$ $Bi_{1.7}$ $Pb_{0.3}Sr_2Ca_2Cu_3$ $O_{10+\delta}$ for different loads**

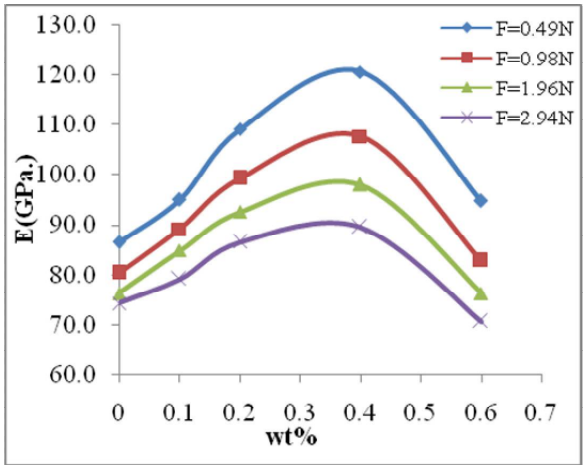


**Fig.(11): The variations of Young's modulus (E) as a function of nano-particles $SnO_2$ concentration for $(SnO_2)_x$ $Bi_{1.7}$ $Pb_{0.3}Sr_2Ca_2Cu_3$ $O_{10+\delta}$ for different loads**

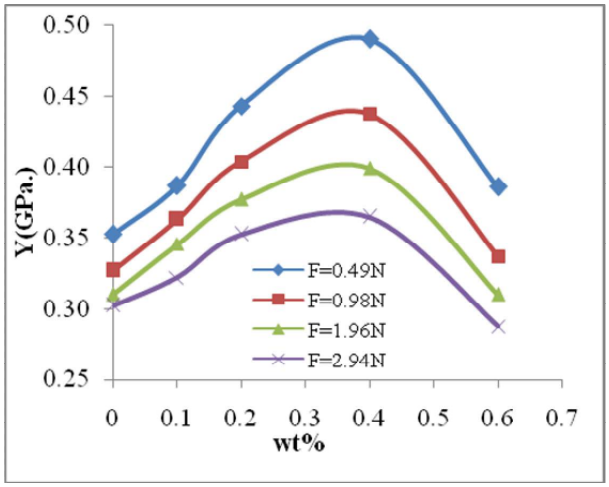


**Fig.(12): The variations of yield strength (Y) as a function of nano-particles $SnO_2$ concentration for $(SnO_2)_x$ $Bi_{1.7}$ $Pb_{0.3}Sr_2Ca_2Cu_3$ $O_{10+\delta}$ for different loads**

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RESEARCH ARTICLE

# Structural Elucidation and Characterisation of Fermentative Compounds Obtained from *kombucha* Culture

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## ABSTRACT

*Kombucha* or Manchurian tea is a popular beverage produced by symbiotic growth of bacteria (*Acetobacter xylinum*, *Acetobacter xylinoides*, *Bacterium gluconicum*) and some yeast strains (*Schizosaccharomyces pombe*, *Saccharomycodes ludwigii*, *Saccharomyces cerevisiae*) on sugared tea. Two important components namely cellulose and flavoured broth are produced during fermentation of substrates by *kombucha* cultures. The present study was carried out to study the antibacterial spectrum of *kombucha* extract and the compounds were identified using GCMS. The extract showed the maximum inhibition zone of 21.5 mm at 5000 ppm for *Staphylococcus aureus* and for *Salmonella typhimurium* maximum inhibition of 18.00 mm was observed at 6000 ppm. The compounds that was essential for antibacterial action was proved to be dodecane, heptadecane, octadecane, hexadecane, octacosane, heneicosane, tricosane and nonane. The typical flavor of the extract was found to be produced by Neopentyl-2-oxobutanoate, Benzaldehyde dioctyl acetal and Santolin diacetylene. The water insoluble pellicle was characterized using FTIR and SEM. The results of Infrared spectral patterns obtained using Fourier Transforming Infrared spectrophotometer revealed the presence of hydroxyl and $CH_2$ stretching behaviour at the absorption wavelength of 3229 $cm^{-1}$ and 2911$cm^{-1}$. The banding patterns of bacterial cellulose closely resembled the structure of pure celluloses. *Gluconacetobacter xylinum* (sju-1) isolate produced bacterial cellulose of type Iα in quantities of commercial interest.

**Keywords :** Fermentation, Bacterial Cellulose, *G. xylinum*, Antibacterial activity, volatile compounds

## INTRODUCTION

Kombucha is a product of symbiotic growth of bacteria (*Acetobacter xylinum, Acetobacter xylinoides, Bacterium gluconicum*) and some yeast strains (*Schizosaccharomyces pombe, Saccharomycodes ludwigii, Saccharomyces cerevisiae*, etc.) cultured on sugared tea. The acetic acid bacteria and the yeast are in symbiotic association in which the yeasts by their invertases decompose sucrose and ferment its hexose units to ethanol and carbondioxide. The Acetobacter genus oxidize ethanol through acetaldehyde to acetic acid. From glucose the Acetobacter synthesize gluconic acid and cellulose which occurs as a membrane on the surface of fermented liquid Kersters et al. (2006). It is a popular beverage among many traditional fermented foods across the world. It was originated in Northeast China (Manchuria) and later spread to Russia and rest of the world. It has sour, sweet and lightly carbonated taste. Kombucha tea or its extract show therapeutic properties, such as the ability to prevent cancers, decrease blood cholesterol level, reduce nephrotoxicity of pharmaceuticals, toxic metals and provide protection against the harmful effect of radiation. Several medical studies has revealed that kombucha has certain therapeutic value possessing antibiotic activity, positive effects on gastrointestinal activity, arthritis, gout, haemorrhoids, cholesterol value, arteriosclerosis and nervous system. Daily consumption of kombucha significantly reduces the risk of cancer. Organic acids produced during fermentation shield the symbiotic colony from contamination with unwanted foreign microorganisms that are not part of the tea fungus. In addition to tea and sugar components the beverage also contains acetic acid, gluconic acids, L-lactic acids, amino acids, biogenic amines, vitamins such as B-complex and vitamin C. One of the most important metabolite from therapeutical point of view is glucuronic acid, a carrier of detoxification activity of kombucha. Recent research on kombucha has proved that its antimicrobial activity against pathogenic microorganisms is largely attributable to acetic acid. Acetic acid is known to inhibit and destroy a number of Gram-positive and Gram negative microorganisms (Pinto et al., 2008). Kulkarni et al. (2011) has evaluated the use of bacterial cellulose from the kombucha culture as pharmaceutical excipient in tablet formulations.

Dutta and Gachhui (2006) has indicated that *kombucha* is frequently called “tea fungus”, although no fungus is actually involved in the fermentation process. Fermentation using *kombucha* colonies is composed of two portions: a floating cellulosic pellicle layer and sour liquid broth below the cellulosic layer formed by *A. xylinum* and yeasts. This fungus-like mixture of microorganisms and cellulose is likely the reason why *kombucha* is also called “tea fungus”. Klemm (2005) has indicated that the cellulosic mat is an organic high dietary fiber rich food product, low in fat and calories and contains no cholesterol and further the cellulose is recognized by the FDA as edible and the *Acetobacter* is a non-pathogenic cellulose-producing food grade bacterium. Trcek (2005) have reported that some species of *Acetobacter*, recently named as *Gluconacetobacter* are known to produce cellulose exhibiting superior features over plant cellulose.

Legaz *et al* (2004) has developed a non-edible biomaterial called Bioskin using *Acetobacter xylinum*. Cellulose formed from the culture medium was mechanically disrupted, filtered through a multi-layered cheese-cloth, pressed and dried. The final dry product was not dissolved in water or organic solvents, but when rewetted in distilled water absorbed about 250 per cent of its dry weight. Ultra structural studies by TEM reveal that Bioskin was an anastomous structure composed by fibers surrounding concrete bodies and was able to fix osmium tetroxide. The bioskin was lipidic or lipoproteic nature. SEM analysis shows that Bioskin is apparently formed by crossed fibers with very large interfibrillar spaces. Fibers showed a very regular structure without superficial adherences. Interfibrillar spaces were the basis of the water-absorbing property of bioskin. Some low molecular weight compounds, such as sucrose, dissolved in water, also enter the void spaces and they crystallize on the surface of fibers to which they remain adhered. Considering all the above, a research was carried to develop the cellulosic membrane and fermented organic extract from *kombucha*. The structure of the cellulose was characterized using FTIR and the antibacterial activity of the crude extracts of *kombucha* was verified by agar well diffusion assay and the structural elucidation of the bioactive compounds was elucidated by GCMS.

## MATERIALS AND METHODS

### Production of *kombucha* cultures

The *Gluconacetobacter* sp. (sju-1) used in this study was previously isolated and identified in our laboratory from fermenting sugarcane juice (Coimbatore, Tamil Nadu, India). The yeast namely *Saccharomyces cerevisieae* was obtained from MTCC 6507.The culture was resuscitated by incubation on YPM (25 g/L mannitol, 5 g/L yeast extract, 3 g/L peptone, and 15 g/L agar) at 30°C for 2 days. Working cultures were routinely prepared on YPM and stored at 4°C until use. The basic growth medium used for the *Acetobacter* strain was Hestrin and Schramm (HS) medium (20 g/L glucose, 5 g/L peptone, 5 g/L yeast extract, 2.7 g/L $Na_2HPO_4$, 1.15 g/L citric acid.$H_2O$) (Hestrin and Schramn, 1954). A traditional tea-based medium (80 g/L sucrose and 3 g/L tea) was also used to culture the organism. Inoculum was prepared by transferring a single colony from the YPM working culture plates into 100 ml of HS medium in 500 ml bottles and incubating the culture without agitation at 30°C for 2 days. The broth was shaken vigorously to release the attached cells from the cellulose pellicle and then aseptically filtered through sterilized gauze. The resulting cell suspension was used for all subsequent experiments. Experiments were performed by adding 10 ml of inoculum into a 500 ml bottle containing 90 ml medium, which was then incubated without agitation (static) or with shaking at 60 oscillations per minute (agitated) at 30°C for 7 days.

### Preparation of crude extract of *kombucha* culture

The fermentation media containing the cellulosic pellicle and the broth was homogenized well in a blender homogenizer and the cellulose was allowed to sediment and settle down. The crude extract of *kombucha* was obtained as per the procedures of Ibrahim *et al* (2011). The supernatant was mixed with equal quantities of chloroform and methanol (2:1) and shaken well vigorously in a separating funnel and incubated overnight (Parliament, 1997). Culture extract obtained was prepared in different concentration from 250 ppm upto 7000 ppm to check the antibacterial activity. The solvent extract were separated and used for testing antibacterial activity and GCMS analysis.

### The targeted bacteria

The micro-organisms used in antimicrobial assays were supplied by Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial species taken for the study are (*Staphylococcus aureus* MTCC-96, *Salmonella typhimurium* MTCC-98). A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these were streaked on to the appropriate culture slants and incubated at 37°C for 24 h.

### Antibacterial testing
### Agar-well diffusion method

About $1\times10^5$ spores/ml of different bacteria was prepared and 0.2 ml spore suspension was spread over the agar surface of the plates. The plates were placed at 27±2°C for 30 min in order to make the agar surface dry. Different concentration of the *kombucha* extract was added into the well with help of sterilized micropipette. The plates were kept in an upright position in an incubator until the extracts diffused in the agar at least for 3 to 4 h. These plates were then inverted and further incubated at 27°C for 3 to 5 days. The plates were observed for zone of inhibition (mm) around the wells.

**Structural elucidation of bioactive compounds by GCMS**

The Gas chromatography Mass Spectrometry (GC-MS) analysis of the samples isolated from the chloroform and methanol (2:1) extract of fermented broth was performed using Thermo GC - Trace Ultra VER: 5.0,Thermo MS DSQ II equipped with DB 5 - MS capillary standard non-polar column of 30 Mts dimension, ID: 0.25 mm, film: 0.25 μm, for GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium was used as carrier gas with a flow rate of 1.0 ml/min and oven temperature 70°C raised to 250°C at 6°C/ min. The chromatographic peak identification was carried out by comparing their mass spectra with those of the bibliography data of unknown compounds from the NIST library mass spectra database on the basis of the criterion similarity (SI)>800 (the highest value is 1000). According to the method of Wanakhachornkrai and Lertsiri (2005) an approximate quantification of volatile compounds was estimated by the integration of peaks on the total ion chromatogram using Xcalibur software (Vienna, VA). The results are presented as the peak area normalized (%). Structural characterization of cellulose by FTIR.

The cellulose samples obtained from HS medium was analysed to study conformational characteristics by FT-IR spectrometer (Perkin-Elmer S2000) using KBr plate method. 1.0 mg of dried bacterial cellulose samples were mixed with KBr powder and pressed into a small tablet. Then FT-IR spectrum was measured in the transmittance mode with the resolution of 1.00 $cm^{-1}$ at wave numbers ranging from 4000 $cm^{-1}$ to 400 $cm^{-1}$. Pure microcrystalline cellulose obtained from Sigma Aldrich was used to compare the structural characteristics of bacterial cellulose obtained from the two medium. The moisture per cent of the cellulosic pellicle was also recorded prior to spectral analysis.
SEM Analysis of cellulose membrane.

The morphological investigations of the bacterial cells on the cellulose and the cellulose fibrils were characterized using Scanning Electron microscope (SEM) (Model: S-3400 HITACH Co., Japan). Thin layers of freeze dried cellulose were gold coated using ion sputter (Fisons Instruments, UK). The gold coated sample was viewed and photographed using SEM.

## RESULTS AND DISCUSSION

**Antibacterial activity**

The results obtained from the present study concerning the antibacterial activity of *kombucha* were recorded. In case of *Staphylococcus aureus*, the extract showed the maximum inhibition zone of 21.5 mm at 5000 ppm but as the concentration increased to 7000 ppm its inhibition decreased to 19.0 mm. The minimum inhibition was observed at 250 ppm of 10.00 mm. The negative control (solvent alone) showed no inhibition whereas the positive control (antibiotic chloramphenicol) showed an inhibition of 24.00 mm at 7000 ppm. In case of *Salmonella typhimurium* maximum inhibition of 18.00 mm was observed at 6000 ppm and at 7000 ppm it was only 16.00 mm, least inhibition was observed at 500 ppm of 12.00 mm.

### GCMS Analysis

The result of GCMS analysis of crude extract of *kombucha* is presented in Table 1. The result revealed the presence of seven volatiles compounds dominated by thiophene-2-carboxylic acid, santolin diacetylene (24.8%), 1,5-dihydroxyl-3,3-dimethyl-7-acetylaminomethyl-tricyclododecane (10.75 %), 3-phenyl-3-m-tolylpropionamide (12.33 %), Nonane, 4- hepatanol (6.53 %) and 3-(4-nitrobenzoyl) cinnoline (5.8 %) (Fig. 1,2,3,4)

**IR activity**

Figure 5, shows the IR spectrum of bacterial cellulose. IR spectrum obtained for BC produced from *kombucha* extract showed strong absorption peak at 3229 $cm^{-1}$ and 2911 $cm^{-1}$ representing OH and $CH_2$ grouping. Broader bands of 3229 $cm^{-1}$ indicates the presence of more hydrogen bonding patterns. The strong absorption peak at 1644 $cm^{-1}$ confirms the presence of carboxylic acid groups (COOH) in cellulose structure. The band at 1428 $cm^{-1}$ attributed to the occurrence of carbonyl group in BC. The band at 1163 $cm^{-1}$ and 1068 $cm^{-1}$ shows the possibilities of C-O-C functionalities present in the BC. The transmittance peak nearing to 3240 $cm^{-1}$ indicated that cellulose Iα was abundantly present in BC produced by the *kombucha* culture *Gluconacetobacter xylinum*. (sju-1).

**SEM analysis**

Figure 6, shows the SEM micrographs of thread like cellulosic microfibrils and the bacterial cells entangled in it. The thickness of the fibrils was from 128 nm to 207 nm at 8000 X magnification. The fibrils were tightly packed and conferred morphological features similar to that of pure microcrystalline cellulose.

The crude extracts of *kombucha* showed a good antibiotic spectrum against both the pathogenic cultures *Staphylococcus aureus* and *Salmonella typhimurium.* The antibacterial activity may be attributed due to the relative abundance of alkane hydrocarbon or fatty acid molecules like dodecane, heptadecane, octadecane, hexadecane, octacosane, heneicosane, tricosane and nonane. Haigh (1973) has illustrated that induction of bifringence (orientation of microfibrils) were due to the liberation of 1:1 mixture of saturated and monounsaturated tetra-hydroxy terpene. Previous publications reported that the compounds such as 1-octadecene, 1-heptadeceane found in algae, bacteria and plants show anticancer, antioxidant and antimicrobial activity (Lee *et al*., 2007; Mishra and Sree. Santos *et al* (2009) has indicated that antimicrobially active lipids and active fatty acids are present in a high concentration in many Gram negative bacteria and kill microorganisms by leading to disruption of the cellular membrane of bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. The GCMS results revealed the presence of seven important volatile compounds essential for antibacterial activity in crude extract of *kombucha*. The typical flavour of the end product was due to the prevalence of thiophene-2-carboxylic acid, santolin diacetylene, acetic acid, neopentyl alcohol, lavanduyl acetate, 1-heptanol and 3-(4-nitrobenzoyl) cinnoline. Neopentyl-2-oxobutanoate is yet another flavour compounds present in crude extract. Benzaldehyde dioctyl acetal being an important ketone derivative is a prominant flavour compound that are normally present in most of the distilled beverages. Santolin diacetylene is an hepatoprotective agent and an important antioxidant compound. Compounds like acetamide and diacetamide are also present in the extract which are essential for stabilisation of cellulose derivatives which are formed as floating pellicle by the bacterium *G. xylinum* in the *kombucha* extract. Some other medically important compounds identified were piperazine, leucinol, crocusatin, sarracine, haliclorensin and quinoxaline.

The IR spectrum of the cellulosic fibres revealed that the material produced was a pure form of type Iα cellulose and the fibres are tightly arranged with microfibrillar interspsace of only 1.6 μm. The results of the present study correlates with the findings of Sluraska *et al* (2008) who has indicated that *G. xylinum* grown in HS medium produced cellulose showing IR spectrum in the region of 3400 $cm^{-1}$. Nguyen *et al* (2008) has explored that *kombucha* had different cellulose producing charecteristics such as high purity and high crystallinity with no contaminating polysachharides like acetan. According to Jung *et al* (2010) IR spectrum of BC produced from molasses medium was in the region of 3240 $cm^{-1}$ attributing to the presence of more quantities of cellulose Iα. The present study indicated an appropriate coincidence with earlier studies relating to IR spectrum of pure cellulose and bacterial cellulose and authentically proved that the component produced by the sugarcane juice isolate *G. xylinum* (sju-1) was cellulose. The SEM micrographs of the membrane formed as a floating pellicle in the media showed the presence of adhering bacterial cells in the cellulosic fibres (Fig. 1). SEM analysis revealed the microfibrillar nature of the cellulose with a

dense mesh like structure. In general, due to the structural charecteristics of BC as an ultra fine and highly pure fibre network, it has unique properties, including high mechanical strength, high water absorption capacity and high crystallinity (Moon *et al*., 2006). In earlier investigatons carried out by Czaja *et al* (2006) it has been indicated that BC obtained from *A. xylinum* can be used for making chronic and acute wound care products. Toda *et al* (1997) has reported that cellulose produced by acetic acid tolerant *A. xylinum* DA strain has an excellent moisture retaining capacity and continous production stability suited for industrial scale production. Because of the unique properties, resulting from the ultrafine reticulated structure several application such as skin grafts, face peeling, collagen, blood vessels and granulation have been proposed for this cellulosic layer by Lynd *et al* (2002). From the present investigation, the cellulose obtained with high moisture retaining capacity can better be applied for developing a wide array of rewettable supra - adsorbent pads.

It is concluded from the present study that *kombucha* extract used in the investigation showed better antibacterial activity against the pathogens used, but further researches should be made to purify various natural product against pathogenic bacteria and fungi. The enhanced antibacterial activity expressed in crude extracts might be due to the occurance of both hydrophobic and hydrophilic bioactive compounds. An improved knowledge of the composition, analysis and properties of *A. xylinum* with respect to antimicrobial compounds and bacterial cellulosic membrane would assist in efforts for the pharmaceutical application of this bacteria.

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**Table -1.Volatile Compounds of Crude Extract of *Kombucha* Analysed By GCMS**

| RT$^{-1}$ | Compounds | Area (%) |
|---|---|---|
| 3.04 | Acetic acid | 1.25 |
| 4.31 | Buyladamantan-1-ol | 1.68 |
| 6.18 | Illimaquinone-epoxide | 0.19 |
| 6.77 | 2-(5-chloromehoxyphenyl)pyrrole | 0.77 |
| 8.98 | 3-ethylaminosulpholane, 2-propenoic acid | 0.59 |
| 9.44 | Dodecane, heptadecane, octadecane, hexadecane, octacosane, tricosane, nonane | 1.86 |
| 9.72 | Heneicosane, 1-pentanol | 1.02 |
| 11.29 | 2,2,6-trimethyl-5-hepten-3-one | 6.68 |
| 13.13 | Benzaldehyde dioctyl acetal | 0.45 |
| 16.07 | 1-piperazine | 0.37 |
| 17.76 | Nonane | 0.17 |
| 18.54 | Crocusatin B | 0.30 |
| 22.56 | Thiophene-2-carboxylic acid, santolin diacetylene | 24.80 |
| 25.59 | 1,5-dihydroxyl-3,3-dimethyl-7acetylaminomethyl-tricyclododecane | 10.75 |
| 30.22 | Lavanduyl acetate, 1-heptanol | 0.13 |
| 31.17 | 3-phenyl-3-m-tolylpropionamide | 12.33 |
| 34.85 | Nonane, 4-hepatanol | 6.53 |
| 37.48 | 3-(4-nitrobenzoyl) cinnoline | 5.80 |
| 41.71 | Methylsilylpropyne | 0.78 |

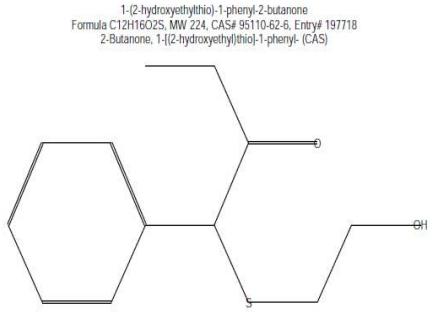


**Fig. 1. Chemical structure of phenyl butanone – a sweet odour producing compound**

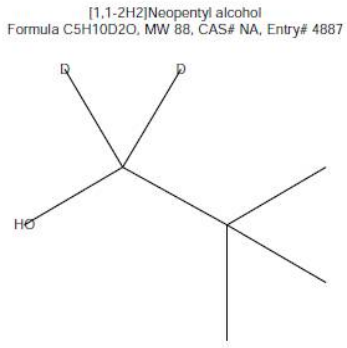


**Fig. 2. Chemical structure of neopentyl alcohol– an alcoholic product**

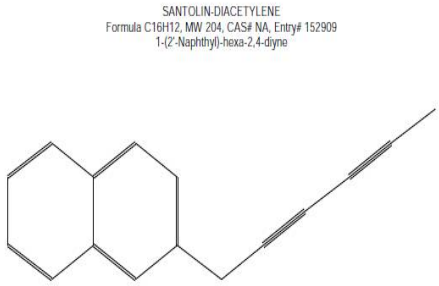


**Fig. 3. Chemical structure of santolin diacetylene – a flavour compound**

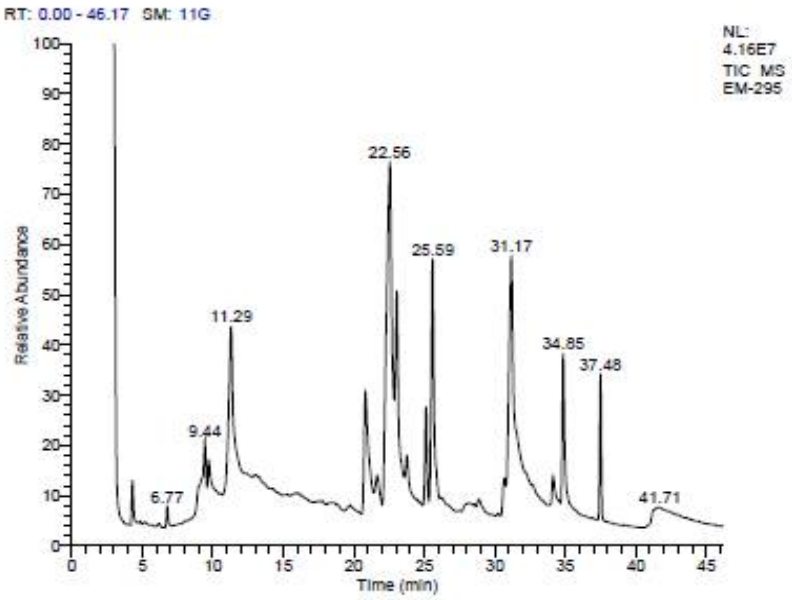


**Fig. 4. GCMS spectrum of different volatile compounds produced by *kombucha* culture**

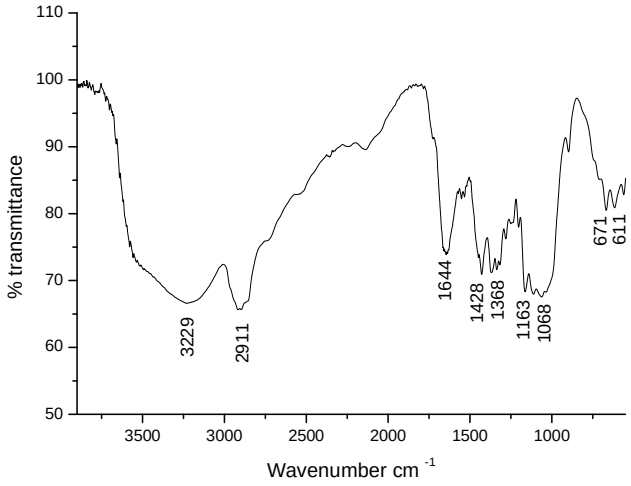


**Fig. 5. FTIR spectrum of bacterial cellulose produced by *kombucha* culture**

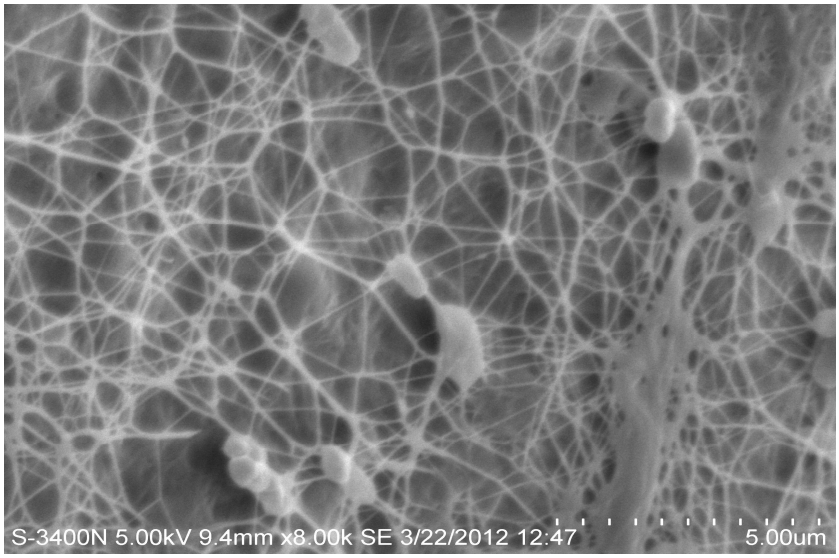


**Fig.6. SEM micrograph of celllulosic fibrils formed by *G. xylinum* (sju-1) of *kombucha* culture**

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REVIEW ARTICLE

# Study on the Production of Ethanol from Waste Biomass- A Sustainable Fuel for Future

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## ABSTRACT

The use of renewable energies addresses to tackle climatechange and to reduce energy relianceon oil.The bio fuels meet the requirement of transportation fuels and energy needs of India's vast rural population by use of non-food stocks. The intent is to provide a higher degree of national energy security in an environmentally friendly, cost effective and sustainable manner. The 2nd-generation biofuels are produced by processing the whole plant – particularly its lignocellulose, the main component of plants. The resource is available in large quantities in a variety of forms: wood, straw, agricultural and forestry waste, etc. Different treatments are used to convert thesevarious types of biomass into biofuels.Its main strengths are thatit does not compete with food uses and the potential resources are much greater. 2nd-generation processesaim to produce fuels that can be usedwith gasoline, diesel and kerosene.Biochemical conversion is used toconvert biomass into ethanol.The various steps related to process the biomass such as pretreatment torelease the complex sugars, enzymatic hydrolysis to convert the complex sugars into simple and readily fermentable were studied. The ultimategoal is to bring to market a process, technologies and products that havebeen optimized in terms of their energy efficiency, for the production of bioethanol in line with sustainable development principles.

**Keywords:** climatechange, renewable energies, bioethanol, sustainable development, enzymatic hydrolysis.

## INTRODUCTION

Energy is the lifeline of global economy. The world energy scene is now in a period of transition. The exhaustion of fossil fuels is inevitable till we adopt a best substitute form of energy. The large dependence on petroleum lends importance to hydrocarbon supplies on a self-sustaining and renewable basis. The bio-energy system can significantly contribute to the world's growing energy needs. The renewable sources would only be able to compete with the fossil fuel resources. The energy producing crops should be cultivated in large. The earth has vast areas of land which are unsuitable for food and fodder crops, and this may be utilized for growing hydrocarbon yielding plants which may yield a substitute for conventional hydrocarbons. There are several compelling reasons for seriously exploring the prospects of hydrocarbon plantations. First, the prospects of increased dependence on oil imports pose a difficult challenge. Secondly, oil prices are likely to go up substantially in the next 10–15 years. Petro farming could therefore provide a welcome solution to some of these problems.

### Biomass – Potential source of energy

The use of biomass energy has the potential to greatly reduce greenhouse gas emissions. Burning biomass releases the same amount of carbon dioxide as burning fossil fuels. But carbon dioxide released is largely balanced by capturing $CO_2$ for its own growth during photosynthesis. The biomass can be grown on under-utilized farm land.The biofuels are the only renewable liquid transportation fuels which can reduce dependence on foreign oil.

### Bio fuels

Biofuels are sustainable sources to satisfy our future energy needs and act to mitigate deleterious impacts of greenhouse gas emissions. Liquid transportation fuels (bio ethanol and bio diesel) now represent key contributors to the bio energy port folios in many countries. At present our Indian government mandates the blending of bio fuels in petrol and diesel and these are acting as great stimuli to the industrial sector. The use of alternative sources like plant biomass as a renewable source for fuel or chemical feed stocks, has received much recent attention now a days.

### Ethanol as a renewable fuel

Ethanol is one of the best tools to fight air pollution from vehicles.Bioethanol is a sustainable and renewabletransportation fuel that is a promising substitute togasoline and represents an environment-friendly fuelbecause it reduces the amount of greenhouse gasemissions, which is a major cause of global warming.The development of alternative fuel and energy frombiomass has a researchpriority in recent years. And there is no fuel available at scale today that matches ethanol's ability to improve overall environmental quality compared to gasoline. At present there is a need to look for renewable and environmentallysustainable energy sources. In this context, ethanolderived from biomass can meet our energyneeds.

### Fuel properties of Ethanol

Ethanol contains 35% oxygen. Addition of oxygen to fuel results in more complete fuel combustion, reducing harmful tailpipe emissions. Ethanol also displaces the use of toxic gasoline components such as benzene, a carcinogen. Ethanol is non-toxic, water soluble and quickly biodegradable. Ethanol is a renewable fuel produced from plants, unlike petroleum-based fossil fuels that have a limited supply and are the major contributor of carbon dioxide ($CO_2$) emissions; greenhouse gases (GHG) .To makes an effort to combat climate change, aid energy independence and counteract diminishing supplies of fossil fuels, there is a need to research on renewable fuel energy sources. Bioethanol is a sustainable and renewable biofuel that is a promising substitute to gasoline and represents an environment-friendly fuel because it reduces the amount of greenhouse gas emissions. The rise in

prices and environmental problems caused by fossil fuels has contributed to this recent interest in biofuel research from economic and ecological perspectives. The biofuel that is expected to be most widely used around the world is bioethanol.

### Current status of demand and supply of ethanol in India

In January 2003, the Government of India launched the Ethanol Blended Petrol Programme (EBPP) promoting the use of ethanol for blending with gasoline and the use of biodiesel derived from non-edible oils for blending with diesel at 5%. Due to ethanol shortage during 2004-05, the blending mandate was made optional in October 2004, and resumed in October 2006 in the second phase of EBPP with a gradual rise to 10% blending. These ad-hoc policy changes continued until December 2009, when the Government came out with a comprehensive National Policy on Biofuels formulated by the Ministry of New and Renewable Energy (MNRE), calling for blending at least 20% biofuels with diesel and petrol by 2017. Given that the mandatory blending requirements will be met in phases, the demand projections for ethanol blending are estimated at 5, 10 and 20% blending mandates. Based on the projections it is estimated that bio-ethanol requirement would be 3.46 billion liters by 2020 at the rate of 10% blending (Basavaraj 2013). It is expected that demand for fuel ethanol will rise from current estimates of 4 billion to 22.7 billion gasoline-equivalent gallons (or 20% market share) by 2020 (BRDI, 2006). The future risks of global warming and shortage of petroleum, as well as the superior environmental characteristics of ethanol as an oxygenated additive to gasoline that improves the knocking resistance of gasoline, promote the production and usage of bioethanol in the fuel market.

With a view to give boost to agriculture sector and to reduce environmental pollution, Government of India has been examiningsupply of ethanol-doped-petrol in the country. In order to ascertain financial and operational aspects of blending 5% ethanol with petrol as allowed in the specifications of Bureau of Indian Standards for petrol.The following table 1 shows the projected demand for petrol and diesel and the amount of ethanol and biodiesel required for 5, 10, and 20 per cent blending.

### Production of Ethanol from Biomass

Bioethanol is ethanol derived from biologicalfeedstocks utilizing microbial fermentation processes. Bioethanol has a low toxicity and is readily biodegradablethan petroleum fuel. Production of ethanol by fermentation from cheap carbohydrate materials for use as analternative liquid fuel is the current focus of research worldwide. As per the statistics published by the Ministry of Petroleum the potential for Bio ethanol in India is 500 million litres per annum.(**Source:** The information is taken from document published by Govt. of India Ministry of Petroleum and Natural Gas.)Using ethanol in place of gasoline helps to reduce carbon dioxide ($CO_2$) emissions by an average of 34% compared to gasoline**.**Because ethanol is made from renewable, plant-based feedstocks, the $CO_2$ released during a vehicle's fuel combustion is "recycled" during the growth of ethanol feedstocks.

### Growth of Bio fuels

The use of renewable energies addresses a dual objective: to tackle climate change resulting from $CO_2$ emissions and to reduce energy reliance on oil, particularly in the transport sector. Plant biomass is a major energy source. A distinction is made between lingo cellulosic biomass (wood, straw, green waste, etc.) biomass with a high sugar and starch content (beetroot, sugar cane, wheat, maize, etc.) and oleaginous biomass (rapeseed, soya, sunflower, etc.). Different treatments are used to convert these various types of biomass into biofuels.As the first generation of biofuels currentlyavailable are being produced using fats derived from oil-containing plants(bio diesel), and ethanol produced by fermentation of sugar extracted from sugar-containing plants or from starch produced by cereals (maize, wheat, etc.). The second generation biofuels produced from lignocellulosic biomass. The second generation biofuels are produced by processing the whole plant – particularlyits lingo cellulose, the main component of plants.

The resource is available in large quantities in a variety of forms: wood, straw, agricultural and forestry waste, etc. Its main strengths are thatit does not compete with food uses and the potential resources are much greater. The second generation processes aim to produce fuels that can be used with gasoline, diesel and kerosene. The bio chemical conversion is used to convert biomass into ethanol, or other alcohols. The researchers focus on the steps related to processes specific to the second generation: pretreatment to release the complex sugars, enzymatic hydrolysis to convert the complex sugars into simple, readily fermentable sugars.

Biofuel produced from lingo cellulosic materials, so-called second generation bioethanol shows energetic, economic and environmental advantages in comparison to bioethanol from starch or sugar. However, physical and chemical barriers caused by the close association of the main components of lingo cellulosic biomass, hinder the hydrolysis of cellulose and hemicellulose to fermentable sugars. The main goal of pretreatment is to increase the enzyme accessibility improving digestibility of cellulose. Each pretreatment has a specific effect on the cellulose, hemicellulose and lignin fraction thus, different pretreatment methods and conditions should be chosen according to the process configuration selected for the subsequent hydrolysis and fermentation steps. This paper reviews the most interesting technologies for ethanol production from lingo cellulose and it points out several key properties that should be targeted for low-cost and advanced pretreatment processes.

Today, ethanol is made from starches and sugars, but our focus is to develop a technology to produce it from cellulose and hemicellulose, the fibrous material that makes up the bulk of most plant matter.Ethanol is mostly used as blending agent with gasoline to increase octane and cut down carbon monoxide and other smog-causing emissions. Some vehicles, called Flexible Fuel Vehicles, are designed to run on E85, an alternative fuel with much higher ethanol content than regular gasoline.During recent years, production of ethanol by fermentation on a large scale has been of considerable interest to meet to increased demand for new sources of energy (Akhir*et al*., 2009; Turhan*et al*., 2010). Ethanol production via yeast fermentation may provide an economically competitive source of energy (Cysewski and Wilke, 1978; Nguyen *et al*., 2009; Zhao and Bai, 2009; Csoma*et al*., 2010; Ding *et al*., 2010; Dutta*et al*., 2010; Ibrahim *et al*., 2010; Jeon and Park, 2010; Oda*et al*., 2010; Tang *et al*., 2010; Ghorbani*et al*., 2011; Razmovski and Vucurovic, 2011). This paper attempts to review the works carried out by many workers on evaluation of some plant materials as source of energy for the production of ethanol.

## Works carried out in India

The plants belonging to the families Euphorbiaceae, Asclepiadaceae, Apocynaceae, Urticaceae, Convolvulaceae, Sapotaceaewere studied for their suitability as petrocrops by various workers. Bio-crudepotential was determined by preservation and coagulation of latex in case of species amendable to latex tapping. In rest of the species, the dried biomass was extracted withhexane–methanol. Bio-crude potential varied from 26–29%, whereas for other species likeEuphorbiaantisyphilitica, 8.46%. This study resulted in the identification of 17 potentialpetrocrops.A new genus Capaifera was also evaluated as a source of fuel oil. CapaiferalingsdorfilandCapaiferamultijuga are trees in which a hole is drilled at a height of 90 cm from theground to tap oil. The wood has a system of canals, which contain oil. It was claimed thatthis oil could be used directly in an engine without further processing or purification.A single tree yields 20–30 lof oil in 2–3 h in a single tapping and could be tapped twice ayear. Capaiba oil, as it is called, consists of 25 different compounds of which each compound is a $C_{15}$sesquiterpene. Because of its mw and volatility, the material could beused directly in diesel engines. Aleuritesmoluccana was identified as a source of commercially produced lumping oil. The prospects are bright for developing a large industry for processing lumping oil. Dipterocarpuslaevis, a species of plant that is comparable to Capaiba, is famous all overeastern India on account of its thin liquid balsam commonly called wood oil. The propertyof Capaiba is similar to that of D. laevis oil.

Marimuthu et al. also studied 29 laticiferous taxa of different families for theirsuitability as alternative sources of renewable energy, rubber and other phytochemicalsand selected the most promising ones for large-scale cultivation. They found that themajority of the species under investigation might be considered for large-scale cultivationas an

alternative source of rubber, intermediate energy and other chemicals.Another plant, *Pedilanthus tithymaloides*, was found to be a potential petrocrop withhigh biomass and hydrocarbon yields In India, it is cultivated as ornamental or hedgeplant or even grown in marginal wastelands. Plant species like P. *tithymaloides varcuculatus*, *P. tithymaloides* var. *verigatus* and *P. tithymaloides* (proper) were found to bethe promising varieties for development as petrocrop.Sharma and Babucarried out a preliminary study at Dehradun, India, on five latexbearingplants. Chlorophyll, terpenes and other polar compounds could be obtained fromthese plants by extraction with acetone. Subsequent extraction of the plant materials withpetroleum ether and benzene yielded hydrocarbon, which could be utilized as liquid fuels.Gravellearobusta and Hakeasaligna contained long chain n-alkyle ($C_{14}$) resorcinol derivatives.

**Production of Ethanol from various indigenous sources**

In India, alcohol is usually produced from molasses, though some distilleries also use cane juice, deteriorated cane juice, gur. Alcohol may however be produced from any sugary cane or beet molasses or sugar cane juice, sorghum or starchy agriculturalcrops ,vegetable crops, crop residues, grain,starch, sago waste, corn, jowar, barley, wheat, rice, millets, tapioca cassava, potatoes, soybeans etc. or even from cellulose- hemicellulosicmaterials.Large amount of lingo cellulosic wastes are generated through forestry and agricultural practices , sugar industry, pulp and paper industries, timber industries and many agro-industries,bagasse, rice straw, wheat straw, cotton straw, corn stover,groundnut shells, wood, grasses, sarkanda, paper pulp and many others.

The average yield from some of the sources is shown in Table 2 below.The biomass requires pretreatment, saccharification of cellulose and hemicelluloses complexes and simultaneous fermentation of reducing sugars(hexose and pentose sugars). Acid hydrolysisof lingo cellulosic biomass in addition to sugars,aliphatic acids (acetic, formic, and levulinic acids), furan derivatives, furfural and 5-hydroxymethylfurfural (HMF) and phenolic compounds are formed. These compounds are known to affect ethanol fermentation efficiency.

**Production of Ethanol from Ligno cellulosic wastes**

Studies were done on the utilization and value addition of Lantana Camera and Pine needle (*Pinus roxburghii*) as lingo cellulosic wastes for the production of ethanol**.** Attempt has been made to convert Lantana Camara and Pine needle into maximum fermentable sugars by acid hydrolysis.The method utilizes biomass, macerated with 72 %$H_2SO_4$ at ambient temperature in bath ratio 1: 1.25 for 18 hrs, then diluted to 5% acid concentration, digesting it at 120$^oC$ for different time periods (20 min. to 90 min.), detoxified following standard procedures and finally fermented with yeast culture of *Sachharomyces cerevisiae*. The results obtained are shown in the Table 3.

**Utilization of bagasse pith and low grade non-recyclable waste paper**

Thakur et. al used thermophilicethanologen, yeast strain at 50$^oC$ to produce alcohol from bagasse pith and found that 80% glucose (30g/l) of enzyme hydrolysate was fermented to ethanol (9.5 g/l) within 40 h and thereafter some oligosachharides also converted to ethanol bringing the final ethanol concentration 11.5 g/l in the broth.

**Bioethanol production from mixed wastes using Fungi**

Archana Mishra et al.( 5) employed enzymatic hydrolysis of biomass , namely rice straw and vegetable wastes by Trichodermaviride followed by fermentation with Sachharomycescerevisiae. Detailed kinetic studies have been conducted at different temperatures (21± 2$^oC$,27± 2$^oC$ and 33± 2$^oC$ during 6 days to 12 days of incubation for formation of TRS and total sugar as well as ethanol production. The yield of TRS was found to be 55.27 mg/g of biomass and ethanol of the order of 17.54 mg/ml of substrate from 3:1 ratio of rice straw and vegetable waste after 9

days of incubation at 27°C and alcohol after 4 days of fermentation at 27°C. Results of fermentation at different temperature are shown in figure 1.

### Bioethanol from agri-residues and groundnut shells

Padmaja et.al.(6) utilized various agri-residues such as bagasse, rice straw, cotton straw, groundnut shells using thermophilic anaerobic bacteria ,Clostridium thermocellum and found 0.20-0.12 g ethanol per g of substrate degraded( compared to the yield of ethanol on pure crystalline cellulose was 0.25 g/g of substrate degraded).The reaction has been carried out at 60oC and pH of 7.5 under continuous sparging of nitrogen to make the system oxygen free. Mild alkali treatment given to the substrate enhanced their utilization and ethanol yields. The extent of conversion in the treated and untreated wastes ranged between 65.95 % and 42.68 % respectively.

### Improved production of ethanol using Zymomonasmobilis from molasses

Using modified strains of zymomonasmobilis for hydrolysed and unhydrolysed molasses, high ethanol production has been obtained over conventional yeast fermentation at an optimum temperature of 30°C and pH 6(7,8) . Ethanol concentration of 47 g/l with 92 % fermentation efficiency was obtained from 20% molasses medium.Cane molasses contains sufficient nutrients for growth of Zymomonasmobilis and no supplements were required for ethanol production.Effects of temperature, initial sugar concentration and pH on ethanol production and fermentation efficiency were studied.

### Production of alcohol from Sago wastes

Subashini et al. conducted a series of experiments on sago waste( both solid as well as liquid phase) using saccharomyces cerevisiae strain Vits-MI isolated from molasses and found that this strain is betterthan the standard strain MTCC 173. The process consists of simultaneous saccharification with acids (0.3 N hydrochloric as well as 0.3 N sulphuric acid) followed by fermentation.Effect of temperature(10°C-40°C), concentration of sugars( 10%48%,glucose),pH(2.5 -5.5),time (60 min-120 min) were studied for saccharification step followed by fermentation with varying time ( 5 day-20 day) with liquid phase as well as solid phase. Maximum yield of 15.8 % alcohol were obtained in 15th day in liquid phase.

### Production of alcohol from mahua flowers

Behera et al. attempted from mahua flowers by submerged fermentation (Smf) using immobilized cells of Saccharomyces cerevisiae and Zymomonasmobilis in calcium alginate beads. Maximum ethanol concentration was 154. 5 and 134. 55 gkg-1 flowers using immobilized cells of S. cerevisiae in calcium alginate beads which were found more effective (14. 83% more yield) for ethanol production than immobilized cells of Z. mobilis.The ethanol yields were found of the order 0.483 g/g and 0.473 g/g for S. cerevisiae and z. mobilis respectively.

### Production of alcohol from corn and tapioca

Lehri and Agarwalutilized both corn and tapioca (which are rich sources of carbohydrates, viz . 77.3 and 82.1 % respectively) employing a fungal glucoamylase at 60 °C for 30 h for hydrolysis and found 85 % hydrolysis of both the raw materials.The concentrated hydro lysates containing 13-15% TRS content when subjected to fermentation with distiller' yeast ,S.cerevisiae NSI-113 , at 30°C for 72 h , resulted alcohol productivity 9 0.496 g/g) with fermentation efficiency of the order of 98%. The alcohol content in wash varied from 8 to 9.1 % (v/v) and the residual sugar content in wash from 0.18 to 0.25 % (w/w). The alcohol yields from corn and tapioca were 362 and 370 litres at 24 h , and 411 and 437 litres per ton , at 72 h , respectively; as compared to about 225 liters per ton of molasses normally obtained in

India distilleries operating on conventional batch fermentation process. In the study it is found that when period of fermentation has been varied from 24 h to 72 h the alcohol productivity g/g FS increases at first significantly, then almost remaining constant after 30h.Results regarding yield of reducing sugar as well as productivity of alcohol from both corn and tapioca are shown in figures 2 to 4.

### Bioethanol from renewable cellulosic wastes and waste newspapers

Ali et al. studied on the renewable agricultural cellulosic wastes , groundnut hulls , rice husks and waste newspapers for production of bioethanol using two locally isolated microorganisms cellulase producing fungus Aspergillusniger during saccharification and ethanol producing Saccharomyces cerevisiaefermentation. Groundnut hulls gave highest yield of ethanol in chemically defined medium around 4.5 g/100 g with 5 days of incubation in stationery fermentation process. Results of ethanol production from groundnut Huls in shaking fermentation are shown in figure 5.

### Bioethanol from Jatropha oilseed cakes

Mishra et al. (13) dealt with the bioconversion of cellulose from press cakes of Jatropha oilseeds into ethanol by using the method of dilute acid pretreatment ( sulphuric acid), hydrolysis and fermentation by Saccharomyces cerevisiae . About 80% ethanol was recovered as a result of the process.

### Alcohol from sweet Sorghum

The National Institute of agriculture research, Hyderabad has advocated the extraction of ethanol from sweet sorghum. About 800 acres is currently cultivated with sweet sorghum in AndraPradesh, ,Maharastra, and Karnataka. The extraction of ethanol from sweet sorghum is cheaper than extraction of alcohol from sugar cane, since the cost of sugar cane is higher than sweet sorghum. The grain from sweet sorghum can be used for ethanol extraction or it can be used as a by-product.

### Ethanol from Sugar beet

The sugar present in sugar beet is normally between 14-16%. One can extract alcohol directly from sugar beet about 75 to 90 litres / tonnes of sugar beet as against to 60 litres alcohol per tonne of sugar cane. The yield per acre is around 40 MT and the average age of the crop is only 4 months as against the 11 months of sugar cane. The water consumption is less by 75 % on cane. That is the reason the Northen states and Tamil nadu are contemplating the production of bioethanol from Jatropha seeds.

### Production of Bioethanol from waste flowers

Bioethanol is fermentation alcohol that refersto ethyl alcohol produced by microbial fermentationprocesses of biomass as opposed to synthetic ethanol produced from petrochemical sources. In generalbioethanol can be extracted from every sort ofcarbohydrate material. The main cost element inbioethanol production is the feedstock. These can bedivided into three main groups: sugary, starchy and lingo cellulosic biomass. First generation feedstocksfor bioethanol production primarily refer to plantbiomass sources that are also sources of human andanimal nutrition, namely cereal starches and sugarcrops. Sucrose based materials are predominantlyderived from sugarcane and sugar beet; while starchbased materials are predominantly derived from cerealcrops such as maize, wheat and other cereals. Secondgeneration raw materials for bioethanol productiontypically refer to non-food biomass sources, mainly lingo cellulosic biomass. Behera et al. (2011) carried out a study of ethanol fermentation of mahula flowersusing free and immobilized bacteria *Zymomonasmobilis*MTCC92. Jadhav et al. (2011) worked onbioethanol

production by four gram-positive bacteriaon substrate Mahua flowers. Tiwari et al. (2011)studied bioethanol production from somecarbohydrate sources by Gram positive bacteria.Bioethanol production from mahula (*Madhucalatifolia*L.) flowers by solid-state fermentation wasreported by Mohanty et al. (2009). Swain et al. (2007) reported Mahula flowers (*M. latifolia*L.) forbioethanol production using free and immobilizedyeast.TriptiAgrawal et al made attempts to produce ethanol from Mahua (*Madhuca indica*) Flowers by Soil Bacteria

PotphodeArati andAgarwaalSeema produced ethanol from Flowers of *Quisqualis indica.*The flowers of *Quisqualis indica*(Rangoon Creeper) contain 13.2 gm % fermentable sugars. Fermentation of fresh Rangoon creeper flowers with *Saccharomyces cerevisiae*was carried out by submerged fermentation method. Estimation of sugar was done by Cole's Ferricyanide method. The initial sugar content (before fermentation) of *Quisqualis indica* flower was 13.2 gm % and was decreased after fermentation to 5.6 gm %**.** The ethanol estimation was done by dichromate method and the ethanol yield was found to be 1.41 gm % with fermentation efficiency was found to be 36.29 %. Presence of ethanol was confirmed by Gas Chromatography Mass spectroscopy (GCMS). GCMS shows that along with ethanol; dimethyl ether, diethylene glycol mononitrate, formaldehyde oximetrimer, oxalic acid are also present in the distillate.

PranavMandal and NirenKathale studied the production of ethanol from mahua flower (*madhuca latifolia* L.) using saccharomyces cerevisiae – 3044 and study of parameters while fermentation.This study presents production of ethanol from mahua flower (*Madhuca latifolia* L.) in submerged fermentation (SmF) using immobilized cells of Saccharomyces cerevisiea–3044. The change in certain parameters during the course of fermentation inproduction of ethanol has been studied. The substrate mahua flower contains 72 – 74% of total sugar. Yeast strain S.cerevisiae–3044 was obtained from NCL laboratories Pune. Maximum production of ethanol is obtained at different optimized parameters such as slurry composition as 1:5, pH at 5 to 5.5, inoculum level at 1.5g/100ml, inoculum age at 48 hours, temperature 30–32°C, nitrogen source 0.05-0.06%, sodium potassium tartarate 1.2 g/l and fermentation period is 2-4 days is 338 ml for 1:5 slurry composition. Maximum production of ethanol is obtained by use of sodium potassium tartarate and urea. The method and design can be used for ethanol yield for large scale production.

D.S.N. Benerji et al., Studies on Physico-Chemical and Nutritional Parameters for the Production of Ethanol from Mahua Flower (Madhucaindica) Using *Saccharomyces Cerevisiae– 3090* through Submerged Fermentation (smf). The Effect of various Physico-Chemical and Nutritionalparameter for the production of ethanol from mahua flowerusing *Saccharomyces cerevisiae-3090* through submergedfermentation has been studied. Sub stratemahua flowercontains 68% Total sugar. Yeast strain *S.cerevisiae-3090*was obtained from National Collection of IndustrialMicroorganisms (NCIM), Pune, South India. Maximumproduction of ethanol obtained at different optimizedparameters such as substrate concentration at 28%, pH at5.0, Inoculum level at 2%, Inoculum age at 48 hours,Temperature 300c, Urea at 0.06 %, Copper sulphate 3 ppm,Sodium Potassium Tartrate 1.0 g/l and fermentation periodis 48 hours is 13.450%(w/v). Effect of Sodium PotassiumTartrate and Urea showed the maximum production ofethanol. It is also confirmed that the designed media issuitable for ethanol yield for large scale production.

ShuvashishBehera et al., did comparative study of bioethanol production from mahula (*Madhucalatifolia*L.)flowers by immobilized cells of *Saccharomyces cerevisiae*and*Zymomonasmobilis*in calcium alginate beads.This study presents ethanol production from mahula flowers in submerged fermentation (SmF) using immobilized cells of*Saccharomycescerevisiae*(CTCRI strain) and *Zymomonasmobilis*(MTCC 92) in calcium alginate as beads. Maximum ethanolconcentrations were 154.5 and 134.55 g kg-1 flowers using immobilized cells of *S. cerevisiae*and*Z. mobilis,* respectively.Immobilized cells of *S. cerevisiae*in calcium alginate beads were more effective (14.83% more yield) for ethanol production thanimmobilized cells of *Z. mobilis*.

Geetha S, Kumar A & DeiveekaSundaram M studied ethanol production from degrained Sunflower head waste by*ZymomonasMobilis*and *Saccharomyces Cerevisiae.* This work was conducted to study the potential of degrained sunflower head waste as substrate for ethanolproduction and to optimize the bioprocess for higher yields of ethanol. The various pretreatment methods viz., the aqueoushydrolysis, heat treatment, acid hydrolysis, alkali hydrolysis,

steaming and fungal enzyme hydrolysis resulted in thesignificant release of fermentable sugars from the degrained sunflower head waste. The combination of acid @ 0.2N +autoclaving at 121°C and 15lb pressure yielded the highest quantity of reducing sugars. The increase in reducing sugarswas significant upto 10hrs of incubation period. Hence, acid + steam pretreatment were followed for further studies onethanol production. The *Zymomonas mobilis*2427 and *Saccharomyces cerevisiae*AU-05 were tested to study theirefficiency to ferment hydrolysed sunflower head waste for alcohol production.The fermentation period of 5 days, pH of6.5, temperature of 30°C and 2.0 % inoculum level were found optimum for production of ethanol. *Zymomonas mobilis*2427 was found to be better than *Saccharomyces cerevisiae*AU-05 to ferment the sugars released from sunflower headwaste. The studies on the supplementation of nitrogen sources showed that urea at 500 $mgL^{-1}$favoured alcohol production(24.40 $gL^{-1}$). Thus, sunflower head waste an agricultural residue on hydrolysis can serve as one of the potential substratefor alcohol fermentation employing the bacterium *Zymomonasmobilis*2427.

Veerabadrappa*et al*. investigated on floral wastes into Bioethanol and its performance evaluation on single cylinder I.C. engine. The Gas Chromatography GC method was used. The untreated flower sample powder yielded 2.74% ethanol.The fuel properties like flash point(65°C), fire point(73°C), viscosity(3016$mm^2$/sec), density (980 $Kg/m^3$) of bioethanol was found.TriptiAgarwal and et al. studied ethanol production fromMahua(*Madhucaindica*L.) flowers by soil bacteria. Quantitative estimation of ethanol was done by specific gravity method and ethanol yield was expressed as percentage volume of ethanol/ volume of fermented liquid. Ethanol production in Mahula sample inoculated with soil bacteria was in the range of 7.67 – 8.94%. Swain *et al., studied about* Ethanol fermentation of *Mahula(Madhucalatifolia* L) flowers using free and immobilized yeast *Saccharomyces cerevisiae*. Ethanol yield from fermentation mash utilizing free yeast cells were 193g/Kg.

### Description of Innovation

Sugars being considered as good source of flower wastes are viable sources for bioethanol production activities. As flower wastes is a renewable source, need of hour is to develop processes which have potential to produce value added items. Anaerobic fermentation has been known to produce bioethanol from sugar compounds like molasses, sweet sorghum, sweet potatoes etc. Since fermentation is microbe assisted process these are highly selective, sensitive, involving number of complex metabolites. Thus there is a need to select right kind ofmicrobe, to develop appropriate technologies and optimize process parameters for the production of bioethanol activitiesfrom flower wastes.In the present investigations modest efforts are made to identify appropriate type of microbes for flower wastes, the various processes and to demonstrate how flower wastes can be converted to value added bioethanol. Optimizations of various process parameters were known.These researches would solve disposal problems of flower wastes. The study also demonstrates how value added product like bioethanol can be produced from these wastes. The study brings awareness about utilization of waste flowers and its conversion in to value added bioethanol amongst public. The study dealt with design and demonstration of technologies associated with characterization and process optimization. The study also paves way to reduce the cost of biofuel and makes them competitive to petroleum fuels.

## CONCLUSION

Production of Ethanol, its demand, and supply projection from Indian perspective have been reviewed. Production of bioethanol from various sources has been discussed. Some research efforts in Indian research institutions as well as from industry are highlighted**.**The above detailed study was done to identify an alternative source and appropriate processes that has to be developed to produce ethanol from waste flower which was identified as a potential biomass source , a non-food crop that can be utilized efficiently to meet the demands of fuel consumption in coming years. Along with molasses, starches, the waste flowers can have potential application for the production of ethanol in India where bio ethanol demands are very high and also the disposal problems of floral wastes can be tackled. Production

of ethanol adds monetary value to flower wastes and creates awareness amongst public how wastes can be converted into wealth. It can offer employment opportunities to rural people, provides clean environment and indirectly helps to increase rural economy, which can improve their standard of living.

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**Table.1 Projected demand for petrol, diesel and ethanol requirements in India**

| | | Ethanol Blending | | | | Bio diesel blending | | |
|---|---|---|---|---|---|---|---|---|
| **Year** | **Petrol demand (Mt)** | **@5%** | **@10 %** | **@20%** | **Diesel demand (Mt)** | **@5%** | **@10 %** | **@20%** |
| 2006-2007 | 10.07 | 0.50 | 1.01 | 2.01 | 52.32 | 2.62 | 5.23 | 10.46 |
| 2011-2012 | 12.85 | 0.64 | 1.29 | 2.57 | 66.91 | 3.35 | 6.69 | 13.38 |
| 2016-2017 | 16.40 | 0.82 | 1.64 | 3.28 | 83.58 | 4.18 | 8.36 | 16.72 |

**Source: Planning Commission of India Report of the Committee on Development of Biofuel, 16 April 2003 & 2008**

The above demands are based on estimated growth rates of 7.3 and 5.6 per cent for petrol and diesel, respectively, in the 10th plan (2001-2002 to 2006-2007), 5.0 and 5.0 per cent in the 11th plan (2006-2007 to 2011-2012) and 5.0 and 4.5 per cent in the 12th plan (2011-2012 to 2016-2017).

**Table2. Yield of Ethanol from various substrates, kg of ethanol/ tonne of substrate.**

| **Substrate** | **Approx.yield kg/ tonne** |
|---|---|
| Mohua Flower | **250** |
| Molasses | **200** |
| Bagasse | **150** |
| Rice Husk | **113** |
| Wheat Straw | **57** |
| Jute Stick | **42** |
| Rice Straw | **42** |

**Table 3.Ethanol from Lantana plant of Indian origin**

| | **EXP 1** | | **EXP 2** | | | |
|---|---|---|---|---|---|---|
| | | | **A** | | **B** | |
| **Biomass** | **Lantana Camara** | **Pine needle** | **Lantana Camara** | **Pine needle** | **Lantana Camara** | **Pine needle** |
| T.R.S(g/l) | 24.8 | 22.2 | 22.18 | 21.49 | 25.9 | 23.13 |
| Phenolics*(%) | 0.005 | 0.007 | 0.005 | 0.005 | 0.003 | 0.002 |
| Ethanol(g/l) | 9.35 (11.76) | 7.06 (8.88%) | 8.43 (7.01) | 8.02 (6.67%) | 10.22 (8.51%) | 8.94 (7.43%) |
| Efficiency (%) | 70.56 | 64.59 | 74.60 | 72.51 | 78.93 | 77.27 |

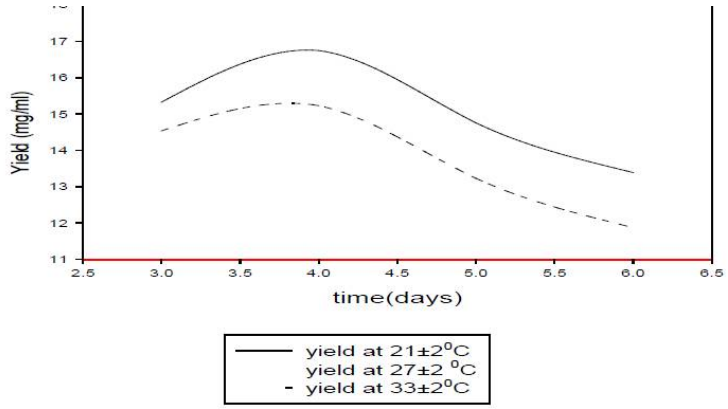


**Figure 1:Ethanol yield at various conditions**

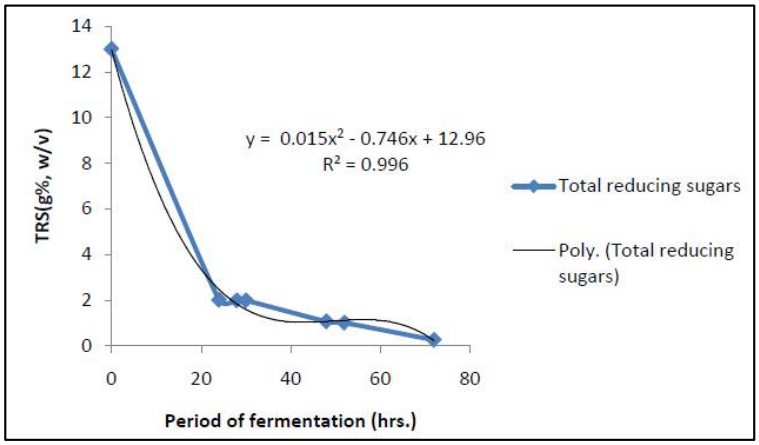


**Figure 2: Total reducing sugars by fermentation of Enzymatichydrolysate of Tapioca powder by C. Cerevisiae (11)**

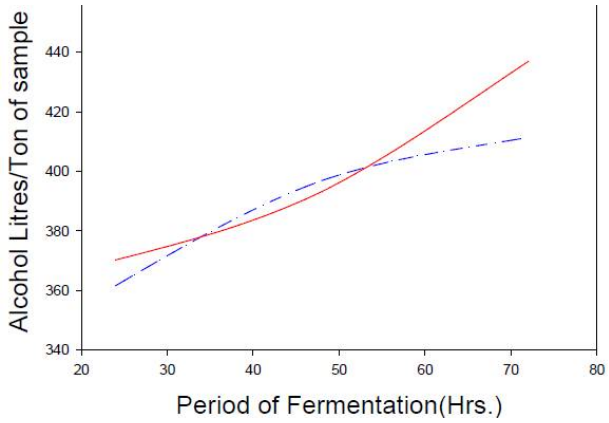


**Figure 3: Comparison between quantities of alcohol produced from enzymatic hydrolysates of Corn and Tapioca powders (11)-** Production by Hydrolysates of Corn powder, Production by Hydrolysates of Tapioca powder.

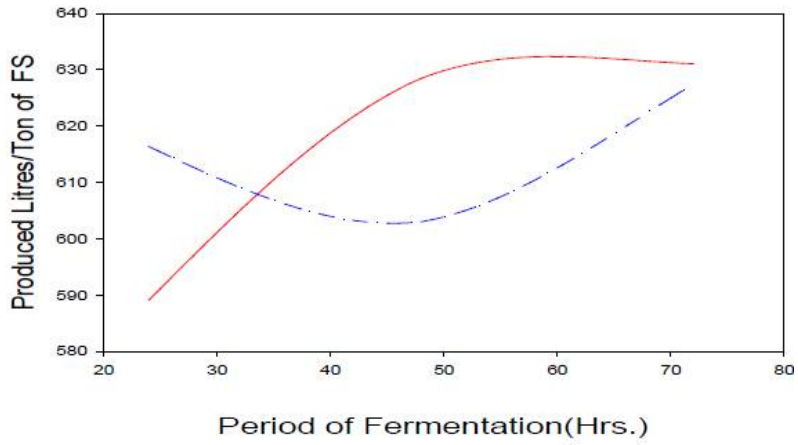


**Figure 4: Comparison between quantities of alcohol produced from enzymatic hydrolysates of Corn and Tapioca powders.**

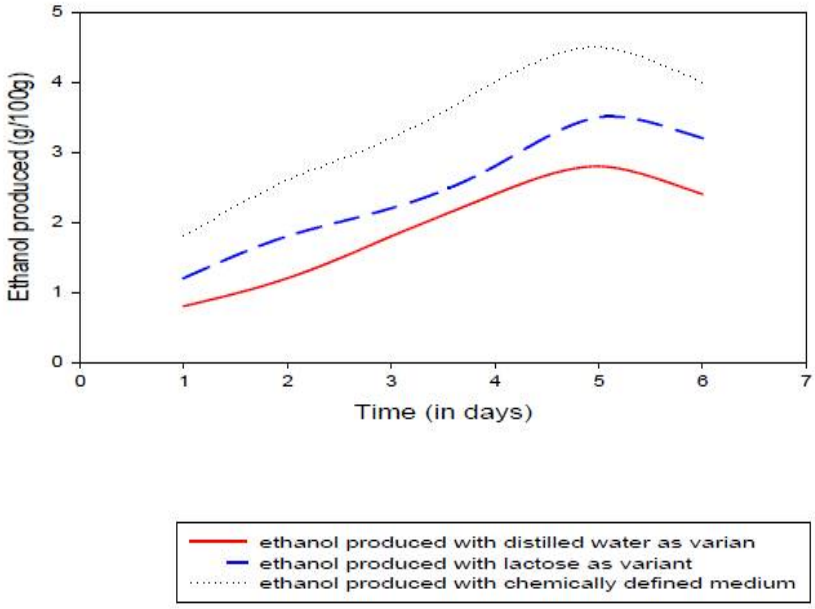


**Figure 5. Ethanol production from groundnut hulls in shaking fermentation**

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RESEARCH ARTICLE

# Green Synthesis of Nanosilver Particles using Leaf Extract of *Baliospermum montanum*

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## ABSTRACT

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles generate a large amount of hazardous byproducts. Thus, there is a need for green chemistry that includes a clean, non toxic and environment friendly method of nanoparticles synthesis. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration. One of them is the synthesis of nanoparticles using plant leaf extracts eliminating the elaborate process of marinating the microbial culture and often found to be kinetically favourable than other bioprocesses. The present study deals with the synthesis of silver nanoparticles using leaf extract of Baliospermum montanum. The extracellular synthesis of silver nanoparticles occurred during the exposure of plant leaf extract to 1 mM aqueous silver nitrate solution. Complete reduction of silver ions was observed after 48 h of reaction at 30$^0$ C under shaking condition. The colour change in reaction mixture (leaf extract and metal solution) was observed during the incubation period, because the formation of silver nanoparticles is able to produce particular colour in the reaction mixture due to their specific properties (Surface Plasmon Resonance). Formation of silver nanoparticles was confirmed by UV-Visible spectroscopy, X-ray diffraction pattern, Scherrer's formula and Scanning Electron Microscopy

(SEM). The synthesized silver nanoparticles were predominately spherical in shape, polydispersed and ranged in size from 30 - 40 nm. Fourier Transform Infra-Red (FT-IR) spectroscopy analysis showed that the synthesized silver nanoparticles are capped with biomolecule compounds which are responsible for reduction of silver ions. The approach of plant-mediated synthesis appears to be cost efficient, eco-friendly and easy alternative to conventional methods of silver nanoparticles synthesis.

**Keywords :** Biological synthesis, Silver nanoparticles, *Baliospermum montanum*, FT-IR.

## INTRODUCTION

Nanoparticles are being viewed as fundamental building blocks of nanotechnology. An important aspect of nanotechnology concerns the development of experimental processes for the synthesis of nanoparticles of different sizes, shape and controlled dispersity. With the development of new chemical or physical methods, the concern for environmental contaminations are also heightened as the chemical procedures involved in the synthesis of nanoparticles generate a large amount of hazardous byproducts. Thus, there is a need for green chemistry that includes a clean, non toxic and environment friendly method of nanoparticles synthesis. As a result, researchers in the field of nanoparticles synthesis and assembly have turned to biological system of inspiration. Among the biological system plants have found application particularly in metal nanoparticles synthesis. Use of plants for synthesis of nanoparticles could be advantageous over other environmentally benign biological processes as this eliminates the elaborate process o maintaining cell culture. Biosynthetic processes for nanoparticles would be more useful if nanoparticles were produced extracelluarly using plants or their extracts and in a controlled manner according to their size, shape and dispersity (Kumar and Yadav, 2008).

Biosynthesis of nanoparticles by plant extracts is currently under exploitation. The aqueous silver nitrate solution, after reacting with geranium (*Pelargonium graveolens*) leaf extract, led to rapid formation of highly stable, crystalline silver nanoparticles (16 to 40 nm) (Shankar *et al.,* 2003). Silver nanoparticles were synthesized by treating silver ions with *Capsicum annuum* L. leaf extract, the crystalline phase of the nanoparticles changed from polycrystalline to single crystalline and their size increased with increasing reaction time. Five hours reaction time led to spherical and polycrystalline shaped nanoparticles (10 ± 2 nm) (Li *et al.,* 2007). In this paper, we report on the biosynthesis of pure metallic nanoparticles of silver by the reduction of aqueous $Ag^+$ ions with the water extract of *Baliospermum montanum* leaf.

## MATERIALS AND METHODS

### Preparation of leaf extract

The fresh and young leaf samples of *Baliospermum montanum* was collected and washed thoroughly with sterile double distilled water (DDW) and finally surface sterilized with 0.1 % $HgCl_2$ for 2 - 3 min under the hood of laminar air flow. Twenty gram of sterilized leaf samples were taken and cut into small pieces. Finely cut leaves were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile DDW. After that the mixture was boiled for 5 min and filtered. The extract was stored in 4 $^0$C.

### Synthesis of silver nanoparticles

Silver nitrate was used as precursor of synthesizing the silver nanoparticles**.** Five ml of leaf extract was added to 100 ml of 1 mM $AgNO_3$ (99.99 %) aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (150 rpm) at 30$^0$ C and reaction was carried out for a period of 48 h.

### UV-visible spectroscopy analysis

The colour change in reaction mixture was recorded through visual observation. The bioreduction of silver ions in aqueous solution was monitored by periodic sampling of aliquot (1 ml) and subsequently measuring UV-vis spectra of the solution. UV-vis spectra of these aliquot was monitored as a function of time of reaction on Elico UV-vis spectrophotometer (model S3-159) operated at a resolution of 1 nm.

### XRD measurement

The sample was drop-coated onto aluminum plate by just dropping a small amount of sample on the plate frequently, allowed to dry and finally thick coat of sample on plate was prepared. The XRD measurement was performed on a Shimazdu, model LabX-XRD-6000 instrument operated at a voltage of 20 to 30 keV and a current of 30 mA with Cu K $\alpha$ radiation with a wavelength of 1.5418 Å.

### Determination of crystalline size

Average crystallite size of silver was calculated using the Scherrer's formula,

$$D = k\lambda / \beta\cos\theta$$

D- Average crystallite size: K- Constant: $\lambda$- X-ray Wavelength: $\beta$- Angular FWHM of the XRD peak at the diffraction angle: $\theta$- Diffraction angle.

### SEM analysis

The thin film of the samples were prepared on a small aluminum plate by just dropping a very small amount of the sample on the plate, extra solution were removed using a blotting paper and then the film on the plate was allowed to dry for overnight.The SEM analysis was performed on a JEOL, model JSM-6390 instrument operated at an accelerating voltage of 20 keV and counting time of 100 s.

### FT-IR measurement

FT-IR measurement of sample was performed using the Nicolet Avatar Model FT-IR spectrophotometer in a diffuse reflectance mode at a resolution of 4 $cm^{-1}$ in KBr pellets.

## RESULTS AND DISCUSSION

The extracellular synthesis of silver nanoparticles occurred during the exposure of *Baliospermum montanum* leaf extract to 1 mM aqueous silver nitrate solution. The complete reduction of silver ions was observed after 48 h of reaction at $30^0$C under shaking condition. The colour change in reaction mixture was observed during the incubation period, because the formation of silver nanoparticles is able to produce particular colour in the reaction mixtures due to their specific properties. The appearance of dark yellowish-brown colour is a clear indication of the formation of silver nanoparticles in the reaction mixture (fig.1). The colour exhibited by metallic nanoparticles is due to the coherent excitation of all the "free" electrons within the conduction band, leading to an in-phase oscillation and is known as Surface Plasmon Resonance-SPR (Akanna *et al.,* 2010)

UV-vis spectroscopy analysis showed that the SPR absorbance band of silver nanoparticles synthesized using *Baliospermum montanum* leaf extract centered at 450 nm (fig 2.) and steadily increases in intensity as a function of time of reaction without any shift in the peak wavelength. The frequency and width of the surface plasmon absorption

depends on the size and shape of the metal nanoparticles as well as on the dielectric constant of the metal itself and the surrounding medium (Mukherjee *et al.,* 2002). XRD pattern obtained for silver nanoparticles showed characteristic peaks near the $2\theta$ value of $38.43^0$ (fig.3). A Bragg reflection corresponding to the (111) sets of lattice planes are observed which may be indexed based on the face-centered cubic (fcc) structure of silver (Dubey *et al.,* 2009). The XRD pattern thus clearly shows that the silver nanoparticles are crystalline in nature. In addition to the Bragg peak representative of fcc silver nanocrystals, additional and yet unassigned peaks were also observed suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Sathyavathi *et al.,* 2010).

Crystallite size of silver nanoparticles as estimated from the Full width at half maximum (FWHM) of the (111) peak of silver using the Scherrer's formula exhibited average particles size of 24 nm.SEM image has shown individual silver particles as well as a number of aggregates. The morphology of the silver nanoparticles was predominately spherical and aggregated into larger irregular structure with no well-defined morphology observed in the micrograph (fig.4). The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent (proteins secreted by plant leaf extracts). The presence of secondary materials capping with the silver nanoparticles and this capping may be assigned to bio-organic compounds from leaf extracts (Rajesh *et al.,* 2009).

The wavenumber or frequency ($cm^{-1}$) of absorption band or peak assigned to the type of vibration, intensity and functional groups of the silver nanoparticles synthesized using *Baliospermum montanum* leaf extract are shown in fig 5. Different functional groups were involved in reduction of silver ions to silver nanoparticles. The peaks in the region of 3400 to 3200 $cm^{-1}$ and 3000 to 2850 $cm^{-1}$ were assigned to O-H stretching of alcohol and phenol compounds and aldehydic -C-H- stretching of alkanes, respectively. The peaks in the region of 1640 to 1550 $cm^{-1}$ and 1450 to 1375 $cm^{-1}$ correspond to N-H (bend) of primary and secondary amides and C-H (-$CH_3$- bend) of alkanes, respectively. The peaks at the region of 1350 to 1000 $cm^{-1}$ correspond to -C-N- stretching vibration of the amine or -C-O- stretching of alcohols, ethers, carboxylic acids, esters and anhydrides. FT-IR analysis reveals that the carbonyl group from amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly form a layer covering the metal nanoparticles (*i.e.,* capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous medium (Sathyavathi *et al.,* 2010).

## CONCLUSION

Leaf extract of *Baliospermum montanum* capable of producing silver nanoparticles extracellularly and the synthesized silver nanoparticles are quite stable in solution. The control of shape and size of silver nanoparticles seems to be easy with the use of plant leaf extracts. The synthetic methods based on naturally occurring biomaterials provide an alternative means for obtaining the nanoparticles. Use of plants in synthesis of nanoparticles is quite novel leading to truly 'green chemistry' route. This green chemistry approach towards the synthesis of nanoparticles has many advantages such as, ease with which the process can be scaled up, economic viability and safe way to produce nanoparticles.

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**Fig 1. Optical photograph of (a) 1 mM $AgNO_3$ solution (b) Leaf extract (c) Leaf extract + $AgNO_3$ after 48 h of reaction**

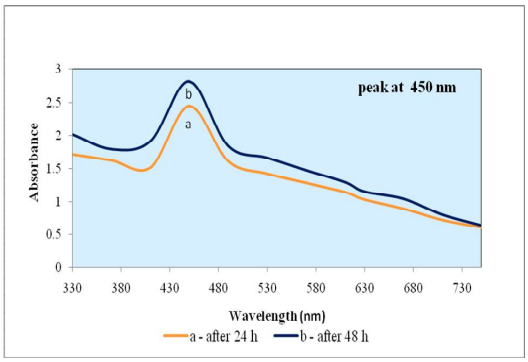


**Fig 2. UV-vis spectra of reduction of Ag ions to Ag nanoparticles**

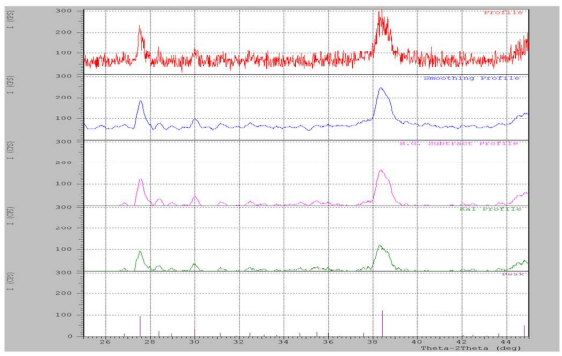

**Fig 3. XRD pattern of Ag nanoparticles**

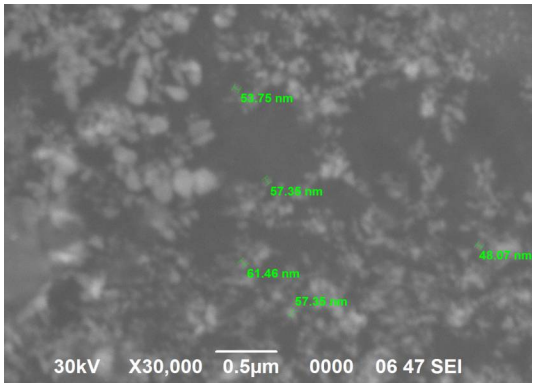


**Fig 4. SEM image of Ag nanoparticles**

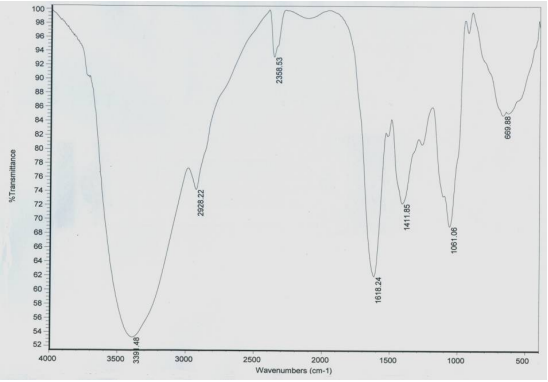


**Fig 5. FT-IR spectrum of Ag nanoparticles**

**Table 1.Crystalline size of synthesized silver nanoparticles**

| Plant extract | θ value [degree] | d- spacing [Å] | FWHM [degree] | Intensity [CPS] | Average Particle size [nm] |
|---|---|---|---|---|---|
| *Baliospermum montanum* | 19.22 | 2.340 | 0.660 | 29.0 | 23.24 |

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RESEARCH ARTICLE

# New Recorded Specimens of the North African Jackal (*Canis aureus lupaster*; Schwarz, 1926), Canidae, from Libya

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## ABSTRACT

The North African Jackal (*Canis aureus lupaster*; Schwarz, 1926) is one of the largest mammals of Libya, known from only one road-killed specimen collected from about 45 years ago. The unique collected animal was recorded in Misurata city with little data about this animal in Libya at all. This work will provide information about morphology, habitat, behavior, food habits and cranial characters of this subspecies.

**Keywords :** *Canis aureus lupaster*, North African Jackal, Canidae, Libya.

## INTRODUCTION

Canids of genus *Canis* form a widely distributed group of mammalian carnivores including wolves, jackals and coyotes. Their ancestor has been found in Africa from about 4 million years ago (Perini *et al*., 2010).The Golden Jackal (*Canis aureus*; Linneaus 1758) is currently considered a monophyletic species among the wolf-like canids (Wayne *et al*., 1997; Bardeleben *et al*., 2005). The Golden Jackal is found throughout north and east Africa, southeastern Europe and central, southern and western Asia and Middle East (Sillero-Zubiri *et al*., 2004; Wozencraft, 2005). This species shows large morphological and ecological intra-species variability (Macdonald, 1979; Krystufek and Tvrtkovic, 1990; Jhala and Moehlman 2008).Schwarz (1926) renamed the subspecies of the Golden Jackal from *Canis aureus* to be *Canis aureus lupaster*. Schwarz's nomenclature has since been widely used to describe this canid (Ellerman and Morrison-Scott, 1951; Setzer, 1957, 1961; Hoogstraal 1964; Hufnagl, 1972; Clutton-Brock *et al*., 1976; Osborn and Helmy, 1980; Ferguson, 1981; Wassif and Soliman, 1993; Wassif, 1995; Wilson and Reeder, 2005). Rueness *et al*. (2011) provided a recent evidence from mitochondrial DNA analysis supporting the last conclusions.

North African Jackal (*Canis aureus lupaster*; Schwarz, 1926) is a medium-sized wild canid and widely distributed in Africa, Asia, Arabian Peninsula and central and south-eastern Europe (Jhala and Moehlman, 2008). Formerly it was known as The Egyptian Jackal (*Canis aureus lupaster*; Hemprich and Ehrenberg, 1828-1833), but now it is considered as a cryptic lineage of African wolf (Huxley, 1880; Ferguson, 1981; Rueness *et al*., 2011).North African Jackal *Canis aureus lupaster* overlaps in size with the Grey Wolf *Canis lupus*, the Grey Wolf is larger and has more long-limbed than the North African Jackal *Canis aureus lupaster* and its cranial features differ markedly from other golden jackals (Ferguson, 1981).

There are two subspecies of *Canis aureus* in Libya, *Canis aureus algirensis* and *Canis aureus lupaster*, which differ in size, color and profile. Hufnagl (1972) gave a brief description for *Canis aureus lupaster* based on one road-killed specimen obtained from Misurata city about 225 km east of Tripoli the capital of Libya. He also provided his description with an illustrated sketch to the skull of the species *Canis aureus* with its lower jaw.Essghaier *et al*. (2015) identified only the tracks of *Canis aureus* in Taraghen and they mentioned that IUCN classified it as "Least Concern". According to their personal observations, they stated that *Canis aureus* were no longer seen in the nature due to more urbanization that destructs the natural habitats of this canid.

New recorded specimens (n = 17) of the North African Jackal *Canis aureus lupaster* were obtained from five sites in south of Libya; Ghadduwah, Om Al-Araneb, Murzuq, Taraghen and Sabha during 2008 to 2015. These collected specimens are considered the first hunted specimens of such subspecies from Libya at all. New obtained data will shed light on morphology, habitat, behavior, food habits and cranial measurements of this subspecies.Identification of recorded specimens was based on descriptions and identification keys available in the literature (Anderson and De Winton, 1902; Ellerman and Morrison-Scott, 1951; Setzer,1957, 1961; Hufnagl, 1972; Clutton-Brock *et al*., 1976; Osborn and Helmy, 1980; Harrison and Bates, 1991; Wassif, 1995).

## MATERIALS AND METHODS

### Study site

Libya is characterized by its arid conditions, except the Libyan coastal strip and the northern hills toward the east and the west. The rest of Libya is geographical located under the exact conditions of desert and semi-desert. These conditions encompassed by distinct environments with distinct characteristics in temperature, humidity and rainfall that reflected on the biota components of the plants and the animals that are able to live together in various ways with those difficult conditions (Hufnagl, 1972).There are a lot of ecosystems in Libya have the range from the coastal salt marshes along the coastline, to green plains along the northeastern region and northwest highlands especially Nafusa Mountains, to desert and semi-desert ecosystem represented by oases and valleys (Toschi, 1969). The Libyan desert include a few biodiversity and abundance of species particularly those that have the capacity to live under the harsh circumstances and some of them are endemic (Harding King, 1925; Bennett, 1970).

South Libya is a part of Sahara, characterized by many habitats that are utilized by wild species as shelters and for feeding (Bundy, 1976). The study area is situated in the southwest of Libya which includes desert wadis, oases, palm plantations and irrigated croplands. Studies and reports about mammals in Libya are relatively scarce since the period of Italian occupation until the present time. One may concluded that the wildlife in this region is vague and number of species and situation of different taxa are either unknown or may be subjected to extinction or already had disappeared from their previous range, all data about wild life will depend upon personal observations and tales of dwellers. (Sbeta *et al*., 2006).There are no permanent rivers in the study area, but many wadis are temporary fill quickly after rains but dry out again rapidly. Many wadis are fed by rainfall from upland areas and feed into depressions in the surrounding desert to form ephemeral pools. In addition, surface water is present in desert oases

in this area where the water-table of the extensive underground aquifers breaks the surface in desert depressions. Capillary action brings water to the surface of few springs (Harding King, 1925; Hufnagl, 1972; Kerambrun, 1986).

Sabha province is considered one of the important regions furnished with oases located in the southwest of Libya. About 75% of the total area of the region is mainly arid. A few permanent small pools are found within the area and vegetated with Saharan plants such as *Calligonum comosum*, *Nitraria retusa*, *Anabasis articulata*, *Acacia* spp., *Euphorbia* spp., *Fagonia* spp., *Zizyphus lotus*, *Tamarix africana*, *Juncus* spp., *Phragmites australis*, *Scirpus holoschoenus* and *Typha capensis*. Submerged species are also found such as *Ceratophyllum demersum*, *Potamogeton* spp. and *Chara* spp. Human cultivations appear obviously in study area such as barley, vegetables, fruits, cotton and, date palms (Keith, 1965; Boulos, 1975; Jafri and El-Gadi, 1986; Sbeta *et al*., 2006).

## Collected specimens

This study was focused on five sites (Ghadduwah, Om Al-Araneb, Murzuq, Traghen and Sabha) at Sabha province in the southwest Libya (Fig. 1). The study was based on 17 skulls of the North African Jackal *Canis aureus lupater* reported for the first time from these localities. Collected specimens were captured and killed from the five locations representing 10 males and 7 females (Table 1). Specimens were collected between 2008 and 2015. Animals were hunted alive by a native dweller in Sabha province at night by using leg traps. Only skulls and lower jaws were obtained from him, bleached, measured by using a sensitive caliper and photographed. Each specimen has taken a specific museum number and deposited in Walid Fathy Mohamed Collection (WFMC). A detailed comparison of these specimens with the published descriptions of specimens from Libya of Hufnagl (1972) and from Egypt of Osborn and Helmy (1980) was also carried out. Notes on the ecological settings at the collection localities were also taken.

30 cranial measurements were taken, 28 to the skulls and 2 to the lower jaws. The 28 measurements that were taken to the skulls and their abbreviations are defined as follow: greatest length of the skull (GLS), condylobasal length (CBL), basal length (BL), basicranial length (BCL), basifacial length (BFL), viscerocranial legth (VCL), facial length (FL), greatest length of nasal (NL), snout length (SL), palatal length (PL), greatest length of the auditory bulla (ABL), greatest breadth across the mastoid processes (GBM), zygomatic width (ZB), least width of the skull at the postorbital constrictions (PCW), frontal width across the postorbital processes (FSW), minimum interorbital width (MnIW), maximum palatal width (MxPW), minimum palatal width (MnPW), width at canine alveoli (CAW), depth of braincase (DP), prosthion (IF), length from foramen magnum to mid of frontal (FM), palatal depth behind toothrow (PDT), depth at interorbital foramen (DIF), maximum width of braincase (MxWB), width across auditory meatus (WAM), width of bulla (WB) and the maximum width of the sagittal crest (WS). The two measurements that were taken to the lower jaws and heir abbreviations are: mandibular toothrow (MT) and mandible length (M) (Walid, 2011). Means and standard deviations were calculated to all the previous measurements (Table 2).

## Morphology

The North African Jackal *Canis aureus lupaster* is considered one of the largest canids in Libya. According to the dwellers there in south Libya they described it much bigger than a dog-like carnivore with hair like a comb on its head and back, it has a short brush tail with a black tip, has a grey color, elongate face and round ears, short snout and robust canines. More accurate descriptions are needed in this section.

## Habitat

The specimens and their tracks were observed in the five sites and in the vicinity of the villages around there. *Canis aureus lupaster* is usually found inland and also in the agricultural areas, wastelands and desert margins, rocky areas

and cliffs (Hoath, 2003). *C. a. lupaster* lives in cliffs and rocky hillocks of the semi-barren deserts. *C. a. lupaster* prefer to live in mesic environments that give it the chance to select its food from various sources (De Winton, 1899; Anderson and De Winton, 1902; Flower, 1932). It prefers caves and self-dug burrows (Galov *et al*., 2015).

## Behavior

North African jackal is a nocturnal animal but also active at dusk.. Individual usually travels and hunts in small packs usually consist of three to five animals which prefer to move freely in open areas. It sometimes interbreeds with feral dogs. Jackals seldom attack man except when cornered or maddened by rabies. Jackal pups can be tamed easily like any pet dog (Harrison, 1968; Galov *et al*., 2015). *C .a. lupaster* has a remarkable ability to successfully co-exist in close proximity of human communities could be interpreted as reflecting behavioral traits developed as a result of some level of domestication or at least commensalism. The same are observed in North America, the Large Eastern Coyote, *Canis latrans*, which is about the same size of *C. a. lupaster* has become common in urban and suburban areas (Way *et al*., 2001; Gompper, 2002; Bozarth *et al*., 2011).According to the dwellers living in the study area, they mentioned that they raised jackals in captivity from a very young age.

## Food habits

*Canis aureus lupaster* in Libya mainly feeds on hares or sheep. Many authors reported that *C. a. lupaster* is an omnivore feeding on insects, snails watermelon and small mammals (Flower, 1932; Setzer, 1957, 1961; Hoogstraal, 1964; Dorst, 1970). North African Jackal are known in the study area to eat dates and other fruits found in the season. It is also attracted to carcasses (Dorst, 1970). Jackals in the study areas feed on various cultivated crops and fruits such as tomato, pepper, cucumber and grapes and prey domestic animals such as chickens and young sheep.

### Cranial measurements

Osborn and Helmy (1980) listed a number of cranial characters for specimens of *C. a. lupaster* collected from Egypt that can be used in comparison with the present data. The characters that will be used in comparison as defined by Osborn and Helmy(1980) are: condyloincisive length (CIL), nasal length (NL), greatest zygomatic width (ZW), postorbital width (POW), rostral width (RW) and braincase width (BCW).No significant variations were be noticed in the skulls of the collected specimens except the variations in size between sexes where female skulls were smaller than male ones. Table (2) showed the means and standard deviations of the cranial measurements of the collected specimens in the present work. Table (3) and Figure (2) show the comparison with the specimens of Osborn and Helmy (1980) where CBL in the present work was expressed as CIL in Osborn and Helmy (1980), NL was expressed as NL, ZB was expressed as ZW, FSW was expressed as POW, CAW was expressed as RW and FM was expressed as BCW.

It is obvious from Table (3) and Figure (2) that the means of the cranial measurements of the collected specimens of Osborn and Helmy (1980) recorded slightly larger measurements from the means of the cranial measurements of the present work where CIL, NL, ZW and RW recorded 18.52, 7.20, 10.14 and 3.38 cm respectively. While the similar measurements CBL, NL, ZB and CAW recorded 17.37, 6.28, 9.71 and 3.03 cm respectively.Only FSW and FM measurements in the present work were larger than those, POW and BCW, in the other study. FSW and FM were measured 4.48 and 5.49 cm against POW and BCW were measured 3.48 and 5.44 cm respectively.

## RESULTS AND DISCUSSION

At the beginning of Tertiary, in the Paleocene, the continent of Africa was greatly similar to its basic features in the present time. In the Miocene, a great migration of Asiatic mammals happened into Africa. There is no material from the Sahara belonging to the Miocene. Nevertheless it seems that what is now Sahara already constituted an important barrier for Asiatic migrants; they had to use two routes to penetrate into Africa: to the west along its northern coast or to the south along its eastern shores. No direct migration of fauna between Europe and North Africa during the Miocene until its final stage has been sufficiently demonstrated (Kowalski and Rzebik-Kowalska, 1991).

Due to the aridity of Saharan climate, which was already the desert climate in the Miocene, North Africa was to a large extent isolated from the rest of the African continent at that time. Some endemic animals of the old African fauna still survived, but their number was decreasing with time. The Sahara has had its present-day desert vegetation since the beginning of the Pliocene and had already become a successful barrier against animal migrations. The ancestors of genus *Canis* has been found in Africa from about 4 million years ago (Perini *et al*., 2010). Periods of increased humidity permitted the penetration of *Canis aureus* into North Africa. The desert barrier was more difficult to negotiate for smaller animals. *Canis aureus* was developed in the Middle Pleistocene and early Holocene (Kowalski and Rzebik-Kowalska, 1991; Roberts, 1998).

Wolf-like canids have colonized Africa from Eurasia at least 5 times throughout the Pliocene and Pleistocene. Fossil evidence suggesting that much of African canid fauna diversity resulted from the immigration of Eurasian ancestors. The ancestors of *Canis aureus* crossed from Asia to Africa through the Ismuth of Suez located in Egypt during one of the wet episodes of the Pleistocene. The ancestors of *Canis aureus* invaded Africa from the northern passage then spread throughout North Africa (Wendorf *et al*., 1977; Roberts, 1998).Hufnagl (1972) recorded *Canis aureus lupaster* from Misurata city, western Libya, giving some external measurements. Occurrence of *Canis aureus lupaster* in Libya reflected the distribution of this canid during the past wet episodes in the Sahara where the abundance of ground water allows a locally rich primary productivity and moderate rich ecosystems that enables the North African Jackal to survive.

It is noteworthy from Table (3) and Figure (2) that the specimens collected during this work are slightly smaller than the specimens collected by Osborn and Helmy (1980) and the present work specimens have wider rostrum than those of Osborn and Helmy (1980).It proves that there is a great similarity between the collected specimens from various localities from Egypt by Osborn and Helmy (1980) and the new recorded specimens of the present work from south Libya. May be little differences are still found according to the statement of (Pocock, 1941) that *Canis aureus lupaster* of Egypt is considered to be the largest and darkest of North African and Southwest Asian subspecies, and this will encourage to get more descriptive comparison future studies between African and Asian subspecies.

## CONCLUSION

The new recorded specimens were agreed with the findings of Osborn and Helmy (1980) of the North African Jackal *Canis aureus lupaster*. It proves the theory of distribution of this canid from Asia through the Ismuth of Suez moving toward North Africa in the ancient decades.Although the present work extended for several years and a few number of specimens of the North African Jackal *Canis aureus lupaster* were collected, nevertheless, it is emphasized to implement a comprehensive study on this subspecies in Libya at all and also make a comparison with such subspecies in Africa and Asia.

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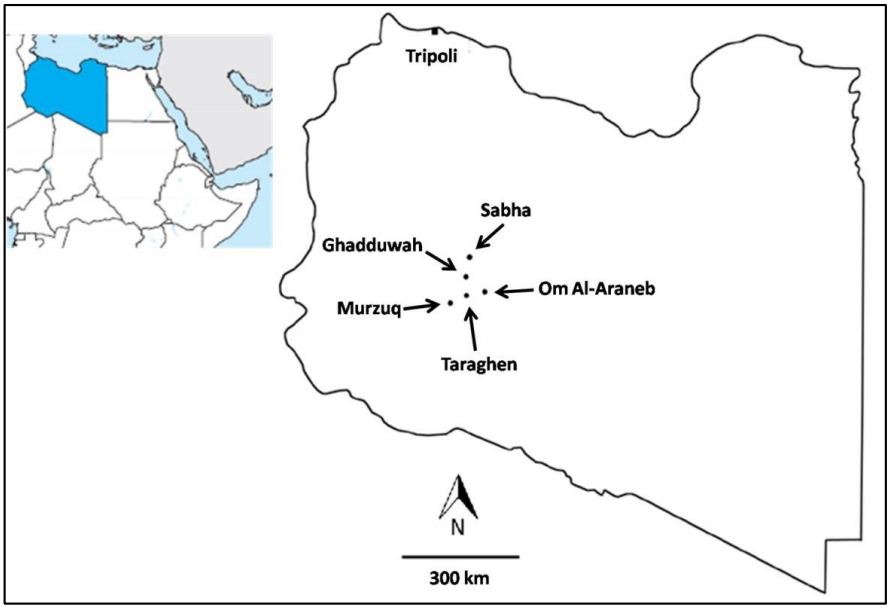


**Fig. 1. Study sites of the present work.**

**Table 1. Collection localities, number of specimens with sexes and coordinates of the North African Jackal *Canis aureus lupaster* collected during the study.**

| Locality | No. of collected specimens | Coordinates of locations |
|---|---|---|
| Ghadduwah | 4 (2 M and 2 F) | N 27º 10′, E 14º 50′ |
| Om Al-Araneb | 3 (2 M and 1 F) | N 26º 15′, E 13º 85′ |
| Murzuq | 3 (1 M and 2 F) | N 26º 90′, E 15º 10′ |
| Taraghen | 3 (2 M and 1 F) | N 27º 20′, E 14º 40′ |
| Sabha | 4 (3 M and 1 F) | N 27º 05′, E 14º 40′ |

**Table 2. Means and standard deviations (SD) of the cranial measurements of the collected specimens of the present work.**

| Character | Mean (cm) Present work | SD |
|---|---|---|
| GLS | 17.12 | 0.55 |
| CBL | 17.37 | 0.69 |
| BL | 15.58 | 0.66 |
| BCL | 5.94 | 0.26 |
| BFL | 9.64 | 0.43 |
| VCL | 7.80 | 0.53 |
| FL | 10.41 | 0.44 |
| NL | 6.28 | 0.72 |
| SL | 7.46 | 0.46 |
| PL | 8.47 | 0.43 |
| ABL | 2.20 | 0.24 |
| GBM | 5.52 | 0.24 |
| ZB | 9.71 | 0.18 |
| PCW | 3.41 | 0.17 |
| FSW | 4.48 | 0.25 |
| MnIW | 3.24 | 0.15 |
| MxPW | 5.19 | 0.23 |
| MnPW | 2.85 | 0.13 |
| CAW | 3.03 | 0.26 |
| DP | 5.03 | 0.28 |
| IF | 5.42 | 0.22 |
| FM | 5.49 | 0.05 |
| PDT | 4.62 | 0.25 |
| DIF | 2.65 | 0.15 |
| MxWB | 5.18 | 0.21 |
| WAM | 5.26 | 0.27 |
| WB | 2.16 | 0.19 |
| WS | 1.11 | 0.56 |
| MT | 8.89 | 0.38 |
| M | 12.62 | 0.62 |

**Table 3. Cranial measurements of the collected specimens of the present work against the same measurements from Osborn and Helmy (1980).**

| Character | Mean (cm) Present work | Mean (cm) Osborn and Helmy(1980) |
|---|---|---|
| CBL/CIL | 17.37 | 18.52 |
| NL/NL | 6.28 | 7.20 |
| ZB/ZW | 9.71 | 10.14 |
| FSW/POW | 4.48 | 3.48 |
| CAW/RW | 3.03 | 3.38 |
| FM/BCW | 5.49 | 5.44 |

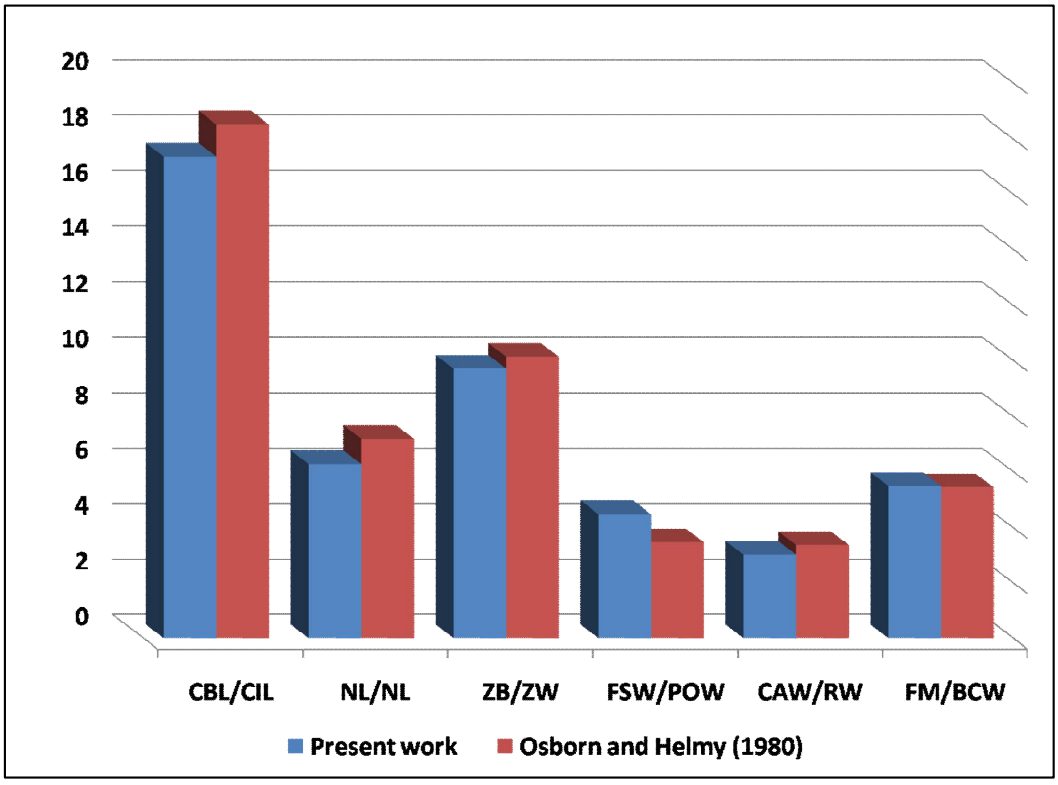


**Fig. 2. Comparison between cranial measurements of the collected specimens of the present work and cranial measurements from Osborn and Helmy (1980).**

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RESEARCH ARTICLE

# Construction of BBTV *rep* gene RNAi Vector and Evaluate the Silencing Mechanism through Injection of *Agrobacterium tumefaciens* Transient Expression System in BBTV Infected Hill Banana Plants *cv.Virupakshi* (AAB)

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## ABSTRACT

One of the most severe viral diseases of banana worldwide is caused by banana bunchy top virus (BBTV). It is a nanovirus transmitted by the aphids *Pentalonia nigronervosa*. Currently, there are no strategies available in order to completely protect bananas against the BBTV virus. The improvement of banana resistance to BBTV disease through conventional breeding has not been successful and therefore an alternative approach such as genetic engineering system requires producing resistance banana cultivar. In this study, gene isolation and construction of RNAi vector for bunchy top disease management in hill banana was determined. The target of the RNAi is the *Rep* protein encoded by the BBTV-DNA1. It can be concluded that the isolated *rep* gene displayed 100% sequence identity to the BBTV *rep* gene of Indian isolate deposited in the NCBI database. This result shows that BBTV *rep* gene is highly conserved among the different isolates of BBTV that infect the banana in India. Hence, an attempt was made to construct the RNAi vector for BBTV *rep* gene and developed method for evaluate the silencing mechanism through injection of *Agrobacterium* transient expression system in BBTV infected hill banana plants *Virupakshi* (AAB) using RNAi technology to silence and suppress the *rep* gene of BBTV.

**Keywords :** RNAi-BBTV *rep*, Hill banana, Pathogen-derived resistance.

## INTRODUCTION

Hill bananas are known for their special flavour and long shelf life which is unique to the state of Tamil Nadu in India. Hill bananas are perennial in nature which are cultivated along with coffee, pepper and through multitier system. In Tamil Nadu (Southern India), hill bananas (two ecotypes, AAB Pome group) namely *Virupakshi* and *Sirumalai* were grown at a height of 2000 to 5000 feet with well distributed annual rainfall of 1250-1500 mm in the lower Palani, Sirumalai and Kolli hills. However, hill bananas were highly susceptible to banana bunchy top virus (BBTV). BBTV has been the sole cause for reduction of hill banana cultivation from 18,000 ha in 1970s to a mere 2,000 ha at present. Currently, there are no strategies available in order to completely protect hill bananas against the virus and improvement of this crop through conventional breeding has not been successful.

BBTV genome consists of at least six circular single-stranded DNA components (BBTV DNA-1 to 6) each of 1.1 Kb (Burns et al., 1995). Each of the six DNA components associated with BBTV encodes at least one gene (Beetham et al., 1997). BBTV DNA component 1 (BBTV DNA-1) contains two transcribed ORFs that include replication initiation protein (Rep) (Harding et al.,1993), while (Wanitchakorn et al.,1997) demonstrated that BBTV DNA-3 encodes the viral coat protein. There are two groups of BBTV, the South Pacific group (isolates from Australia, Burundi, Egypt, Fiji, India, Tonga and Western Samoa) and the Asian group (Vietnam, Philippines and Taiwan) based on sequence analysis of BBTV DNA-1, -3 and -6 (Wanitchakorn et al.,1997).

For the last five years, two research groups namely Queensland University of technology (QUT) and University of Hawaii (UH) were involved in developing transgenic bananas, particularly on Cavendish type with an attempt to produce resistance variety to BBTV disease. John Hu and co-worker of University of Hawaii reported that several putative transgenic lines expressing mutated or anti-sense *Rep* genes with partial resistance to BBTV (Broth et al., 2009). They reported that some of the transgenic banana plants remained virus free symptom after infected for up to a year. Similarly, James Dale and his group of Queensland University of technology developed BBTV resistance in banana by using a novel approach (Njoroge et al., 2009). In this strategy, virus activated cell death which involves integration into the host plant by construct an encoding a split suicide gene, flanked by the target virus intergenic region in turn is embedded in the introns. The suicide gene is only activated upon infection by the target virus and is only expressed in cells by the similar by the target virus. Activation by viral *Rep* mediated replicative release and circularisation which the suicide gene is reconstituted leading to transcription which will processing out the intergenic region embedded in the intron prior translation of the suicide gene in the final stage. However, both the groups did not use the RNA interference (RNAi) technology for imparting the BBTV resistance in banana.

Many reports have demonstrated that RNAi can be engineered to target viral RNA in plants (Smith et al., 2000; Tenllado et al., 2004). As a proof of the concept, RNAi can be engineered to effectively target DNA virus of Mung Bean Yellow Mosaic Virus (MYMV-Vig) (Pooggin et al. 2003). Furthermore, a PTGS-based strategy to control DNA virus replication was demonstrated when plant cells simultaneously transfected with African Cassava Mosaic Virus (ACMV) and with a synthetic siRNA designed to target the AC1 gene of the virus. It was shown that reduction in the accumulation levels of AC1 mRNA was achieved by more than 90% and viral DNA by 70% compared with control treatments (Vanitharani et al., 2003). Similarly, transgenic cassava expressing the full length AC1 gene (which encodes the replication-associated protein) from ACMV imparted resistance against the virus (Chellappan et al., 2004).

It is now well established that both RNA and DNA viruses can be controlled by RNAi approach. The RNA viruses are effectively controlled by silencing the coat protein gene whereas the DNA viruses are effectively controlled by silencing the *rep* gene, which is indispensible for DNA replication of virus. Genetic engineering for viral diseases caused by DNA viruses in several crops resulted in successful viral protection. Hence, engineer resistance to bunchy top disease in hill banana cultivar, *Virupakshi* (AAB*)* was possible by using RNAi technology to silence the *rep* gene of BBTV. Before to generate the transgenic hill banana, to study the mechanism of the RNAi construct in infected hill

banana plants through injection of *Agrobacterium tumefaciens* transient expression system in BBTV infected hill banana plants *Virupakshi* (AAB*)* using RNAi technology.

## MATERIALS AND METHODS

### Cloning of the BBTV *rep* gene with PCR

Fresh young emerging green leaves with midribs were collected from the infected plants and DNA was isolated using the modified CTAB protocol (Sambrook et al., 1989) and stored at -70˚C for further use. Prior to extraction, 100 to 300 mg of midrib of young hill banana leaves were cut into bits and transferred to a zip lock bag (7 cm x 9 cm) and one (1) mL of extraction buffer (0.2 M EDTA, 1.4 M NaCl, 1 M, CTAB 2 %) was added immediately. The samples were kept at room temperature and squeezed by rolling a glass rod over the sample to extract the cell contents. About 500 µL of the cell extract was transferred into an Eppendorf tube, and then 33 µL of 20 % SDS was added into the tube and mixed well. The tube was kept at 65ºC (heating blocks) for 10-12 min and then the tube was centrifuged for 10 minutes at 12,000 rpm and 450 µL of the supernatant was transferred immediately to a new Eppendorf tube. Then 450 µL of ice cold IPA (Isopropyl alcohol) was added to the supernatant and after mixing, the tubes were kept in ice for 20 min. The tubes were centrifuged for 15 min at 12,000 rpm and supernatant was discarded without disturbing the pellet. The pellet was washed with 500 µL of 70 % ice cold ethanol and centrifuged for 10 min at 12,000 rpm. The supernatant was discarded and the pellet was air dried for 5 min and suspended in 40 µl of 0.1X TE buffer (1mM Tris-HCl pH 8.0 and 0.1mM EDTA pH 8.0) and kept at 65$^0$ C for 3 min (to suspend the pellet well) and stored at -20º C. The isolated DNA was checked for its purity by 0.8 % agarose gel electrophoresis and quantified by UV Spectrophotometer.

For BBTV *rep* gene specific primer designing, complete nucleotide sequence of several BBTV *rep* genes deposited in NCBI were retrieved. Forward (BBTV-Rep-F: 5'- ACGACAGAATGGCGCGA-3') and reverse (BBTV-Rep-R: 5'-TCAGCAAGAAACCAACTTTATTC -3') primers were designed for the amplification of the complete ORF (870 bp) of the *rep* gene after multiple alignment of the Indian isolate of BBTV. PCR amplified BBTV *rep* gene into T/A cloning vector (pTZ57R/T (MBI Fermentas) and sequenced.

### Construction of RNAi vectors using particle 5' and 3' end of *rep* gene

For the RNAi construct targeting the BBTV *rep* gene was initiated with the full-length *rep* gene 870bp cloned from BBTV infected hill banana sample by designed gene specific primer. Then, the amplified PCR product was cloned and the presence of *rep* gene confirmed by DNA sequencing. The isolated *rep* gene displayed 100% identity to the previously reported BBTV sequences. In order to construct the RNAi vector, 440 bp of 5' and 440bp of the 3'end were utilized. The partial gene fragment was cloned in sense and anti- sense orientation in intermitted RNAi vector pSTARLING (CSIRO plant industry, Australia Fig 2.A). This vector contains Ubiquitin promoter, Ubi intron, Cre intron, restriction site of sense and anti-sense. The cloned RNAi gene cassette was released by *Not* I enzyme digestion and cloned into the *Not* I site of binary vector pART27 (CSIRO plant industry, Australia Fig 2.B). The pART27 contains *npt11* gene to be used a plant selection marker.

### Plant materials, preparation of agroinjection suspension and injection method

Field grown BBTV infected hill banana suckers were obtained from an orchard in the Lower Pulneys Hills, located at 2000 to 5000 feet sea level in the Western Ghats of Tamil Nadu. *Agrobacterium tumefaciens* strain LBA4404 used in this study. Schematic presentation of the binary vectors used is shown in Fig 2. The *Agrobacterium* strain LBA4404 (pEB1) was grown in YEP medium for 16-24 hrs to obtain 1 O.D (optimal density) culture. The *Agrobacterium* cells pelleted by centrifugation at 4000 rpm for 10 minutes and dissolved in equal volume agroinjection medium +10g/glucose,200

uM acetosyringone pH 5.5(Sallaud et al.,2003) by vortexing to give an absorbance at 600nm between 0.5 and 0.8. Injection of RNAi construct with help of insulin syringe 12.7mm needle (BD Ultra- Fine needle) into BBTV infected hill banana rhizome the junction between the cortex tissue and terminal growing point (apical meristem) of the rhizome. Sucker is planted in autoclaved sterile pot mixer. After 3-4 weeks, the BBTV recovery symptoms were observed based on the typical symptoms of BBTD (Goddard, 1929). Agroinjection work was carried out in Department of Biotechnology (DBT) Government of India approved Tamil Nadu Agriculture University transgenic green-house condition.

## RESULTS

### Cloning and characterization of BBTV *rep* gene

Total genomic DNA isolated from virus infected banana leaves was used as a template for PCR amplification of *rep* gene. By using the designed gene specific primers, full length BBTV *Rep* gene of an expected size of 870 bp was obtained via PCR amplification. The PCR amplified products was then cloned into T/A cloning vector, pTZ57R/T (MBIFermetase, USA). Recombinant colonies were identified by blue/white screening and the presence of the insert confirmed by digestion with *Eco*RI and *Hind*III to release the DNA fragment of 870bp. Five recombinant clones were selected and DNA sequencing was carried to determine the DNA sequence. All the five clones displayed no variation in DNA sequence, indicating very high level of sequence similarity. The isolated BBTV *rep* gene showed 100% sequence similarity to the previously reported sequence from Indian BBTV isolate deposited in NCBI database.

### Construction of RNAi-*Rep* gene constructs

### Cloning of partial BBTV *rep* gene in pSTARLING-A vector

Partial *rep* gene of size 440 bp (1-440 nt region of *rep* gene) was amplified from full length *rep* gene 870bp (Fig. 3a), then cloned into sense and antisense orientation in the pSTARLING-A. Recombinant clones containing both sense and antisense of *rep* gene was identified by the release of 1.58 kb on double digestion with *Bam*HI and *KpnI*. In addition, cloning of either sense (*Bam*HI and *Asc*I) or antisense (*Spe*I and *Kpn*I) of *rep* gene into pSTARLING-A releases 1.14 kb (Fig. 3b, c) fragment. The recombinant clones containing both the sense and antisesense of the *rep* gene was selected and the RNAi-*Rep* gene cassette of size 4.1kb was then released through *NotI* restriction digestion (Fig. 3d). Then, the released fragment was cloned into the *Not*I site of the plant transformation vector (Fig. 3e), pART27 (Fig2.B) and designated as pEB1.The pEB1 binary vector was mobilized into the *Agrobacterium* strain LBA4404 by tri-parental mating method. The presence of pEB1 in *Agrobacterium* strain LBA4404 was confirmed by back transformation into *E.coli* and PCR analysis. Another RNAi vector was constructed utilizing the 440 bp of the 3'end of the BBTV Rep gene (3' of 440bp to 3'of 870bp) and designated as pEB2.

### Evaluation of *Agrobacterium* injected pEB1 RNAi construct into BBTV infected hill banana for recovery to BBTD

*Agrobacterium* injected BBTV infected hill banana plants, the emergence of new leaf the bunchy top virus symptom recovery expression was observed. The leaf number one and two the green streaks were absent and leaf area normally grown as such bunchy top affected leaf and third leaf was the leaf area was expanded and completed green streaks was absent (Fig.4 C). After 35 days the fourth leaf was no bunchy top symptoms and normally grown the plants (Fig.4.D).After 45 days the non-injected hill banana (NIHB) and injected hill banana (IHB**),** the agro injected infected hill banana plant did show the BBTV recovery symptoms through the duration of the experiment 45 days, The control non- injected plantlets developed symptoms were completely stunted and bunchy top symptom (Fig

5.A). The result clearly indicating that the ihpRep gene could provide resistance to BBTV. This result clearly indicated RNAi mechanism was working in transient expression in banana plant.

## DISCUSSION

Banana bunchy top virus consists of at least six component of DNA (DNA1 to DNA6) and is transmitted by the aphid, *Pentalonia nigronorvosa.* Out of six DNA component, DNA1 codes for the replication initiation protein (*rep* gene). The *rep* gene is selected for the constructed of RNAi vector as it is reported to be indispensable for the replication of BBTV. *rep* gene is also found to be important for the replication of ssDNA virus that include Geminivirus and nanovirus (includes BBTV, Faba bean necrotic yellow virus [FBNYV]), Milk vetch dwarf virus [MDV] and Subterranean clover stunt virus [SCSV] that causes disease in French bean, Faba bean, Pea). Unlike RNA viruses, the genomes of plant single-stranded DNA viruses do not encode polymerases. Instead, their replication requires interaction between a viral replication-associated protein (Rep) and host polymerases. Rep protein mediates origin recognition and DNA cleavage/ligation to begin and end rolling circle replication (Fontes et al., 1994; Heyraud et al., 1995). In addition to role in replication, Rep protein also interact with host cellular protein (Morilla et al., 2006) and with Gemini virus Ren protein (Settlage et al., 2005).,which induces the cellular genes required for geminivirus DNA accumulation (Selth et al., 2005). These crucial functions in the replication cycle and its multiple interactions make *Rep* an excellent target for DNA virus control by the expression of mutant Rep protein or to express the dsRNA (RNAi) in order to silence the *rep* gene of virus. Gemini viral Rep proteins have been widely exploited to generate resistance in Mung bean, Bean, Cassava, Tabacco, Tomato and Maize.

The John Hu and co-worker of University of Hawaii had generated several putative transgenic Cavendish banana lines expressing mutated or anti-sense *rep* genes with partial resistance to BBTV (Broth et al., 2009). Even though expression of mutated Rep protein provided resistance to DNA virus in plants, it is often associated with negative effect on plant growth and development (Kong et al., 2000). A potential drawback of *Rep* expression in transgenic plants could be the recovery of phenotypically normal plants due to its interaction with pRBR or with other plant proteins that may alter the cell cycle and differentiation programmes (Kong et al., 2000; Shen, 2002).

It is well documented by several reports that RNAi technology is superior compared to antisense, sense or protein mediated resistance technology. Hence in the present study, RNAi approach was followed to engineer resistance in hill banana. The isolated *rep* gene showed 100% sequence identity to the BBTV *rep* gene which is highly conserved among the different isolates of BBTV that infect the banana in India. In order to target the BBTV Rep gene silencing in banana, two different RNAi constructs were made utilizing the 440 bp of 5' end and 440 bp of the 3' end in the binary vector pART27. First the sense and antisense of the *Rep* gene was cloned into the RNAi intermediate vector, pSTARLING-A containing splicable intron.Linking the sense and anti-sense sequences by an intron which is eventually spliced and resulted in the most efficient silencing in plants (Smith et al., 2000; Wesley et al., 2001). Intron containing constructs (ihpRNA) generally gave 90±100% of independent transgenic plants showing silencing. The degree of silencing with these constructs was much greater than that obtained using either co-suppression or anti-sense constructs. Wesley et al. (2001) reported that use of 98 to 853 nt size gene target produced an efficient silencing in a wide range of plant species.

It is now well established that both RNA and DNA viruses can be controlled by RNAi technology. Unlike the RNA virus where only PTGS is mainly used for virus control, the DNA virus control involves both by TGS and PTGS (reviewed by Vanitharanai et al. 2005). Reports on siRNA accumulation in tomato plants infected with the monopartite geminivirus TYLCV (Lucioli et al., 2003) and in cassava plants infected with the bipartite geminivirus ACMV (Chellappan et al., 2004) clearly reflect the role of the RNAi pathway as a natural defence mechanism against these DNA viruses. Therefore, engineer resistance to bunchy top disease in hill banana cultivar, *Virupakshi* (AAB*)* was possible by using RNAi technology to silence the *Rep* gene of BBTV.

## CONCLUSION

The present study demonstrates that transient expression of the BBTV ihp *rep* gene in BBTV infected hill banana leads to recovery of BBTD. Results obtained shown that the RNAi can be engineered to effectively target DNA virus. As this transgenic approach has shown resistance against several viral pathogens, it may also provide effective control of other viral diseases of banana.

## ACKNOWLEDGMENTS

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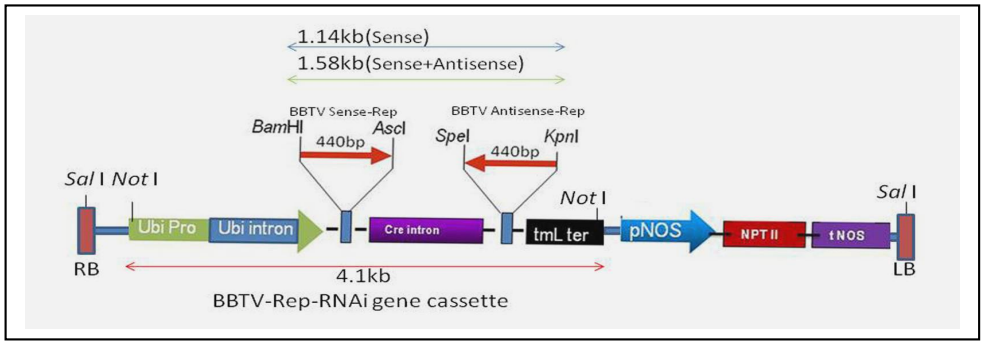


**Figure 1: T-DNA region of pEB 1 showing the BBTV rep hpRNAi gene cassette under the control of Ubiquitin Promoter, *Cre* intron and tmL terminator.**

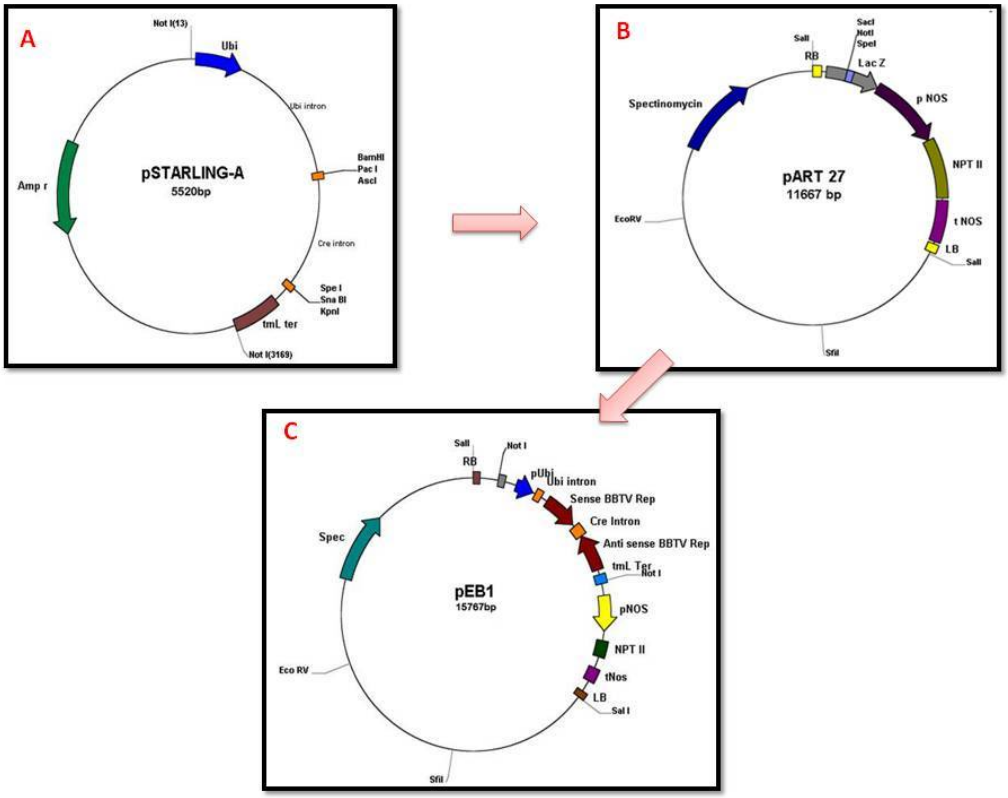


**Figure 2 .Steps involving to construction of RNAi vector, by use of intermediated RNAi vector.**
**(A)** Physical map of intermediated RNAi vector - pSTARLING-A ; **(B)** Plant transformation binary vector pART27; **(C)** Binary vector of RNAi-BBTV *rep*.

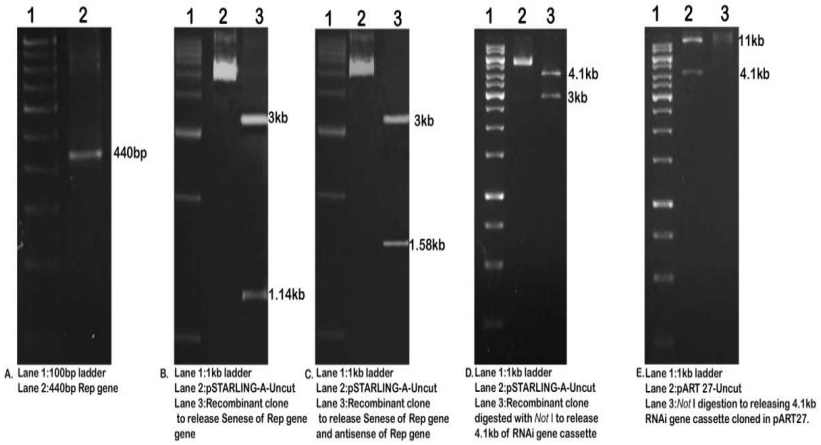


**Figure 3. T-DNA region of BBTV *rep* gene RNAi construction**


**Figure 4 .Transient expression of pEB1 through Agro injection method recovery of BBTD. (A)** BBTV infected hill banana plant with typical symptom as plant source of the experiment ; **(B**) Injection of RNAi *rep* gene vector into cortex tissue and apical region of the rhizome region ; **(C** and **D)** Observation made the leaf recovery of BBTV infected hill banana.

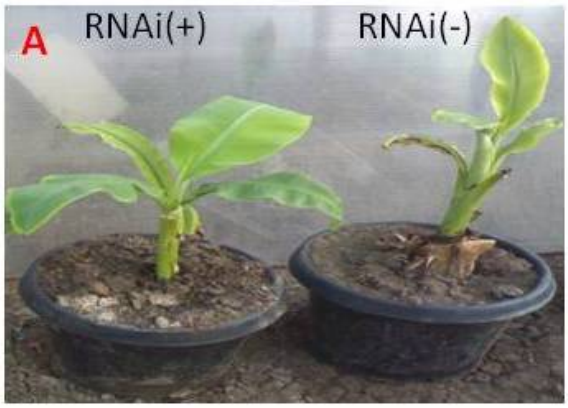


**Figure 5. After 45 days BBTV symptom recovery.** *Agrobacterium* injected hill banana with BBTV recovery symptom (RNAi +) and non-injected hill banana with bunchy top symptom (RNAi-).

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RESEARCH ARTICLE

# Use of Traditional Knowledge among Irular Tribes in the Nilgiris District of Tamil Nadu

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## ABSTRACT

The present study highlights the use of traditional knowledge in agriculture and allied activities among Irulars in the Nilgiris district of Tamil Nadu. They have continuously being used till in this modern technology world. They have the capacity to search for number of use of traditional knowledge. Methods: The list of tribal respondents from the selected village was obtained from the Horticulture department. A sample size of 100 tribal respondents was fixed for the study. The technique proportionate random sampling was followed for the selection of respondents from three habitations from Nilgiris district viz., kunjappanai, mantharai and thuthikarai. The information on traditional knowledge and their utilization was collected by Focus Group Discussion (FGD) and personal interview. Result: Traditional knowledge on different categories has been documented. Discussion: The present study observed that, the Irular tribe of The Nilgiris district having good experience in use of traditional knowledge on different areas. Conclusion: This type of studies may help the policy makers to take efforts on conservation and restoration of agricultural biodiversity by practicing traditional agriculture and traditional knowledge in various dimensions.

**Keywords :** Traditional knowledge, Irular tribes.

## INTRODUCTION

Traditional knowledge refers to the knowledge, innovations and practices of indigenous and local communities around the world. Developed from experience gained over the centuries and adapted to the local culture and

environment, traditional knowledge is transmitted orally from generation to generation.Traditional knowledge is a valuable asset to indigenous and local communities who depend on traditional knowledge for their livelihood as well as to manage exploit their local ecosystem in sustainable manner. The traditional communities are intelligent and have made agriculture sustainable through their different agricultural practices. They create a balance between the environment and requirement.The knowledge of tribal people in traditional agriculture is invaluable. Their farming practices are truly sustainable in many ways. Tribal communities especially Irulas,living in Tamil Nadu have been cultivating the traditional cultivars *viz*. paddy, millets, pulses and vegetable crops. Their subsistence life style, local diet habits and dependence on rain fed irrigation have influenced them to cultivate and conserve the traditional cultivars or land races. Traditional knowledge is vital for sustainability of natural resources including forests, water, and agro-ecosystems across landscape continuum spanning from households through farms, village, commons and wilderness.

## METHODOLOGY

The list of tribal respondents from selected village was obtained from horticulture department. A sample size of 100 respondents was fixed for the study. The technique proportionate random sampling was followed for the selection of respondents from three habitations *viz*., kunjappanai, mantharai, thuthikarai. A separate interview schedule contain open ended questions were prepared for conducting focus group discussion (FGD). Maximum of twenty tribal respondents were selected for a focus group discussion. A total of 5 focus group discussion was conducted among 100 tribal respondents. The respondents were personally interviewed after every focus group discussion, with the help of checklist prepared for each objective. Traditional knowledge on different categories has been documented and presented below.

## RESULTS AND DISCUSSION

The tribal community may possess traditional/ indigenous technical knowledge on agriculture and allied activities associated with agro biodiversity conservation. Such Indigenous Technical Knowledge (ITKs) may influence them in carrying out the different conservation measures of agro biodiversity effectively and efficiently. The ITKs identified are presented with different sub heads are given below.The Irular tribes designed their own structures and methods for storing grains with locally available materials. Their storage structure specially designed to allow aeration protect insect and rodent infestation.These storage structures are all still using even today and the important advantage of these methods and they are eco-friendly and safe method of grain storage.The findings of the present study is in line with Valeria lakra et al.,(2010) and Amuthavalluvan(2011), who had also reported that ‘neem’ leaves were used for storing food grains.Traditional knowledge and beliefs are abundant in tribal community. In many cases traditional beliefs are existing in the form of well-defined technologies which are valued much and followed in their farming system. Traditional knowledge includes both technical and non-technical fields covering various social and religious taboos, beliefs and customs, communication pattern, music, ecology, vegetation, climate and so on. This research finding draws supports from the result of Anandaraja et al., (2008).

## CONCLUSION

It could be observed from the research findings of the study that,tribal’s were enriched with various traditional knowledge’s related to agriculture and allied activities. It could be an evidence for the tribes had more indigenous knowledge. In order to conserve traditional knowledge effectively, more attention is needed on promotion of intellectual property rights of traditional people. Innovation projects may need to be developed that aim at the enhancement of the capacity of tribes and local communities to use, express and develop their traditional knowledge

on the basis of their own cultural and institutional norms. Appropriate documentation of traditional knowledge is needed which helps to transfer the traditional knowledge to next generations.

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**Table 1.Traditional/ indigenousstorage methods and structures**

| Sl.No | Traditional/Indigenous storage methods and structures |
|---|---|
| 1. | Sun drying of pepper seeds for 2-3 days and storing the pepper in gunny bags over the platform made of bamboo sticks to avoid termite attack |
| 2. | Storing of coffee seeds in gunny bags over the mud platform specially constructed for a maximum period of three months |
| 3. | Keeping the best ragi ear head selected from their own field along with ash in earthen pot in dark place for 3-4 years for seed purpose during next season |
| 4. | Storing the paddy grains with "Nochi" (*vitex negundo*) and 'Pungam' (*pongamia pinnata*) leaves in the storage godown called "Vallam" made of mud which is placed in the corner of the house |
| 5. | Using small basket (Bethu Koodai) for storing the grains temporarily |
| 6. | Storing the vegetables in their home by just spreading the vegetable on floor with good aeration |
| 7. | Harvested ear heads of samai, thinai, varagu, ragi and other millet are buried under the soil (one meter depth pit painted with cow dung slurry) with the depth of one meter and can be stored up to one year |
| 8. | Pulses are sun dried and then mixed with chilly seeds or "Neem" (*Azadirachta indica*) leaves |
| 9. | Pulses after drying are smeared with any cooking oil preferably castor oil |
| 10. | Pulses are sun dried and then stored in cotton bags |
| 11. | Sorghum grains mixed with "Neem" *(Azadirachta indica)* leaves and then stored in gunny bags |

**Table 2.Traditional knowledge on climate, weather forecasting and rainfall prediction**

| Sl.No. | Traditional knowledge on climate ,Weather Forecasting & rainfall prediction |
|---|---|
| 1. | Thunder in summer and lightening in rainy season bring heavy rain. |
| 2. | Appearance of rainbow in the east side during evening (or) west during morning being good rain |
| 3 | Rainfall during 'marghali' (one of Tamil month) does not benefit the crop but affects the grains. |
| 4 | Drizzles in July assures rain in September |
| 5. | Farmers also forecasting rains by observing the direction of wind/ clouds. |
| 6. | As soon as the Neem kernels ripens and start falling, it is expected that there will be rains after 10-15 days. |

| | |
|---|---|
| 7. | Fast grazing by sheep's indicates occurrence of rainfall |
| 8. | Ants shifting their eggs to safe place, foretells the occurrence of rain |
| 9. | Dense fog in early morning indicates no rain |
| 10. | Continuous drizzling indicates more pest and disease incidence |
| 11. | Morning cloud and evening thunder indicates the occurrence of rain |
| 12. | Dried appearance of neem tree in summer causes heavy drought |
| 13. | Termite flying in the evening hours is an indication that there will be rain |
| 14. | A ring around the moon is as indication of rain to be followed. |
| 15. | When dragon flies fly low, it may rain. |

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REVIEW ARTICLE

# An Overview on Phytochemical Composition of Banana (*Musa spp.*)

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## ABSTRACT

Banana (*Musa spp.)* the most significant of all the tropical fruits .All the known cultivars of banana are a rich, diverse source of many of the chief dietary health-promoting phytochemicals like carbohydrates, potassium, vitamin C, fibre and provitamin A carotenoids. Beta-carotene is the precursor of vitamin A. Vitamin A deficiency is the most common dietary problem in malnutritioned children, around the globe. Many epidemiological studies have found out that augmented intake of plant-based foods with rich nutritions are interrelated with a reduced threat of several diseases like cancers and cardiovascular diseases. Hence, food based approaches are the most profitable and sustainable strategies for the prevention of Vitamin A deficiency. So, a complete survey of all the genetic diversity available within the sexually compatible species of banana becomes very indispensable at this moment. It is also necessary to understand the many attributes like botanical, agronomic, nutritional and processing quality of the fruits. Due to the very huge number of genetic diversity present in the *Musa* species, regrettably, only modest data are available regarding the nutritional composition of even the wild species and the popular, often traded modern cultivars of banana. The review elaborates the precious bioactive compounds such as

carotenoids and vitamin C and carbohydrates present in the pulp and peels of selected banana cultivars against the traditional cultivars of banana fruits.

**Keywords :** Banana, carotenoid, vitamin C, nutritional levels

## INTRODUCTION

Bananas are edible ripe berries [7] [8] [9] [32] [37] of the herbaceous flowering plants in the genus *Musa*. *Musa* belongs to a genus containing 50 of tropical monocot (pseudo) plants, significant for food, primarily for their delicious fruits, beverages (wine and beer), fiber for industry and as ornamental plants. The genus, of the wet tropical worlds, is the fourth most cultivated food crop in the world.They are different is sizes and fruit colour and are elongated and curved, with soft flesh full of starch covered with a peel which is usually found in a gamut of colours like green, yellow, red, purple, or brown when ripe. All modern cultivars are edible parthenocarpic (seedless) bananas domesticated from two wild species – *Musa acuminata* and *Musa balbisiana* (wild progenitors of the complex hybrids). Musa species are indigenous to the tropical Indomalaya and Australia, and have been first domesticated in Papua New Guinea [39]. They are cultivated in at least 107 countries [37]. But the archaeological and palaeological evidences propose that banana cultivation dates back between 5,000 B.C. and perhaps, to 8,000 B.C. As per these records, *Musa* species is expected to have originated and were domesticated in south east Asia. Bananas are considered to be a vital food source in Southeast Asia and Africa, and as a chief food commodity in export in Central and South America.

The word banana was first used in West Africa, and later spread on to from the English through the Spanish or Portuguese [37]. Bananas and plantains are developed from the same species, but they are different in ratio of sugar to starch. In the Americas and Europe, soft, pliable, sweet, dessert bananas, belonging to the Cavendish group are referred to as “bananas”. These cultivars with high sugar are eaten fresh or cooked when green. But the ones which are firmer and starchier fruits are called "plantains", which are with high starch are eaten only after cooking. They both are high in carbohydrates, fiber, potassium, magnesium, phosphorus, and several vitamins. Bananas are eaten fresh, pureed for baby food, and cooked in assorted dishes typical of tropical cuisines. Fruits, leaves, and stems have plentiful traditional medicinal uses, counting as a drug for dysentery, diarrhea, and many more digestive disorders (Morton, 1987).

The genus *Musa* was fashioned by Carl Linnaeus in 1753[37]. A number of 70 species of Musa were documented by the World Checklist of Selected Plant Families [26]. Linnaeus was the first to place bananas into two species on the basis of their uses as food: *M. sapientum* for dessert bananas and *M. paradisiaca* for plantains. Subsequently further species names were added [9].In 1947, Cheesman, based on the pre-Linnaeuian description by Luigi Aloysius Colla refined that Linnaeus's *M. sapientum* and *M. paradisiaca* were only descendants of two wild seed-producing species, *M. acuminata* and *M. balbisiana* [9,37]. Later, Norman Simmonds and Ken Shepherd projected a genome-based nomenclature system in 1955. According to this system, all discrepancies and inconsistencies of the earlier classification were eliminated. At present, the majority of cultivated bananas are accepted as *M. acuminata Colla* and *M. balbisiana Colla* for the ancestral species, and *M. paradisiaca L.* for their hybrid [32]. The cultivars are positioned in groups based on their number of chromosomes and the species from which they are derived. The AAB Group is a triploid derivative of *M. acuminata* (A) and *M. balbisiana* (B). Figure 3.

### Botanical aspects

Musa species belongs to the Family, Musaceae. It is commonly referred to as Banana, Bananier Nain, Canbur, Curro and Plantain. The common species among the cultivars is *Musa acuminata Colla*, *M. paradisiaca L.* (hybrid). Some of the

few related species are Abyssinian Banana (*Ensete ventricossum Cheesman*), *Musa balbisina Colla*, *M. ornata Roxb.* and *M. textilis Nee.*[32],[37].

The plants emerge like to be trees and raise upto 3.5 to12 meters. But *Musa* species are in principle, perennial herbaceous plants due to their rigid, tough, fibrous pseudo-stems made of overlapping bases of the large, spirally arranged leaves (8 to 20 per plant). The leaves are of 2.4 to3.7 meters long and half a meter wide. The prime stem produces a single huge terminal inflorescence which is a spike. The spike is made up with pistillate or female flowers in the bottom and staminate or male flowers at the top. This spike later on maturity turns beautifully into a cluster of bananas, mostly consisting of 6 to9 clusters of 10 to 25 bananas each. They are placed spirally all around the central stalk of the cluster called as the peduncle. Generally, a bunch of banana weighs around 22–34 kg, but sometimes may reach around 70 kg. A single flowering process leads to the completion of the main stem which dies paving way for new stems to rise up from the rhizome/corm under the soil [32].

These herbaceous perennial plants are very fast in growth from the underground corms. The fibrous and fleshy fresh pseudo stems are placed in concentric circles of sheaths of leaves, facing the sun which comprise of the trunks. Though, the main true stem starts growing from the corm from inside the soil. It is pushed outwards towards the sun from the centre of the stalk after 10 to 15 months from planting. During the time of flowering, the terminal inflorescence emergers and bears into the fruit. Every stalk of the plant bears one heavy flower, matures into a fruit cluster and then dies. The bananas are exceptionally decorative only next to palm trees in the tropics and they are a main element in landscaping(5).Figure 1.

The very big oblong or elliptic leaf blades are extended from the sheaths of the pseudo-stem. They are united by plumpy, deeply ridged, little petioles. As the plant grows, the leaves open out from the whorls at the rate of one per week under humid conditions. They lengthen upward and outward, to as much as 9 feet long and 2 feet wide. They are found to be wholly green, green with mixed reddish purple splotches, or green on the upper side and red-purple underneath. The veins of the leaf run from the mid-rib out straight to the outer rim of the leaf. A very wonderful fact about the veins of these leaves is that even after shredding, they are able to function. Roughly 40 to 45 leaves emerge prior to the bearing of the inflorescence.Figure. 2.

The inflorescence of banana spurts out from the heart of the pseudo-stem. It is, initially, a bulky, long-oval, narrowing, purple-coloured bud. The slender, nectar-rich, tube-like, toothed, white flower slowly opens up later. Double rows of whorls are clustered around the floral stalk and each of the clusters is sheathed into by a chunky, waxy, hood like bract that is purple-clad on the out and dark red inside. The florets present in the first 5 to 15 rows are female (Figure 2). The inflorescence keeps on elongating as the rachis of the flower grows out with sterile flowers with abortive male and female parts. Later, normal staminate ones with abortive ovaries are formed on elongation. The two lately formed flower types finally dry and die back in almost all edible cultivars [6,7,8,9].Parthenocarpic (without pollination) fruits are produced from the ovaries present in the first female flowers of the fruit clusters which are called the hands. The number of hands differs with the species and cultivar/variety. The berry changes colour from deep green to yellow or red. They are from 2 to 12 inches in length and 2 inches in width. The pulp is ivory-white in colour or may be yellow and may be rigid, produce latex when unripe. On ripening they turn into a tender, slippery to a soft and mellow starchy flesh. The aroma is commonly soft and sweet. The pulp is seedless but few vestiges of ovules are visible as brown specks in all common cultivars.

Bananas grow in almost all soil types except salty ones. They are found to be very productive if planted in nutrient rich, well-drained soils. Soils fed with heavy compost heaps with a preferable acidic pH between 5.5 and 6.5 are very suitable. The thick chunky banana plant enjoys great amounts of water. Customary profound watering is a total necessity and drying is definitely detrimental under hot climatic conditions. Standing water causes the root to rot. Thick mulching preserves moisture and the fibrous roots. Bananas and plantains belong to the humid tropical regions. About 10 to 15 months of frost-free weather supports very healthy flower stalk production Almost all

varieties stop growing below 53° F. the plant growth slows down at about 80° F and stops at a temperature of 100° F. Scorching of leaves and fruit happens at higher temperatures. Freezing temperatures certainly kill the plant completely. They are very susceptible to be blown away in strong winds.

**Biochemical composition of banana fruit**

Bananas are consumed as fresh, dried, cooked or baked recipes. The biochemical composition of riped fruits mainly depends on the cultivar/variety, abiotic and environmental factors and the nutrient status and the nature of the soil (2). Bananas are considered as an abundant source of vitamin B6. Vitamin C, manganese and digestible food fibers are present in the fruits in sizeable levels.[1,3,19] About [40] 358 mg of the potassium is present in every 100 g of the fruit, thus making it an easily accessible source of the nutrient to the common man.

As a major staple source of starch to a many of the populations in the tropics, the flesh of bananas can diverge from starchy to sugary in flavor and firm to squashy in texture. This is mainly due to the factors like the cultivar quality and the stage of ripening. The inner pulp and even the skin are edible, raw or cooked. Isoamyl acetate also called as banana oil, is the primary chemical component yielding the peculiar fragrance of fresh fruits. Butyl alcohol and isobutyl alcohol are also role players in the pleasant flavor of the banana cultivars/varieties [20,23,24,25,26]

Ripening induces the production of ethylene gas which is a plant hormone that indirectly affects the flavor of the fruit. Ethylene stimulates the production of the enzyme amylase which breaks down starch into sugar. So the pulp turns very sweet to eat. Yellow bananas are much sweeter because of the higher concentrations of sugar molecules than greener bananas which are only starchier in taste. Ethylene production also initiates the synthesis of the enzyme pectinase to act upon the pectin between the cells in the pulp. This results in the softening of the tissues on ripening.[24,28].Figure 4.

Higher potassium to sodium content present in bananas are helpful in preventing high blood pressure and its other related complications [40). The rich levels of fiber content also contribute to the same effect. Renal calcium losses and so a huge effect in the prevention of bone breakdown is found to be connected with the higher levels of potassium in the fruits of banana.[31] During diarrhea, the fruits help by contributing with the replacement of electrolytes, as well as in the increased absorption of nutrients that are lost.[28] Bananas are found to prevent peptic ulcers due to their antacid effects. [30] A hydrocolloid, namely, pectin, can relieve constipation by stabilizing and lubricating the movement of the intestine. Diabetic patients have sizaeable benefits from unripe bananas because of its low glycemic index . The presence of higher contents of fructooligosaccharide which plays a role as a prebiotic, adds up in the nourishing of the intestinal flora to generate useful vitamins and enzymes. The carotenoid content of the fruit has significant antioxidant effects sufficient enough to protect against vitamin A deficiency which results in night blindness and other diseases. Regular consumption of this fruit decreases the risk of cancers in the kidney since phenolic compounds with antioxidant properties are abundant. The consumption of bananas also generally decreases the risk of age-related macular degeneration [33,34].

Free amino acids pattern is typical to a fruit. So it can be used for the analytical characterization of a particular fruit product (Table 3). Various aliphatic and aromatic amines are present in banana. The common amines found in banana are tryptamine (0.03 mg/kg), melatonin (466 ng/kg), methylamine, ethylamine, isobutylamine, isoamylamine, dimethylamine, putrescine, spermidine, ethanolamine, propanolamine, histamine, 2-phenyl-ethylamine, tyramine, dopamine, noradrenaline and serotonin (11.7 mg/kg). Active amines like dopamine are derived from tyramine and serotonin from tryptophan, whose occurrence in these fruits could directly influence their concentrations in human serum [31,33].

The carbohydrate content in banana had been studied. In addition to glucose (3.5% of the edible portion) and fructose (5.7% of the edible portion), monosaccharides occur only in odd amounts. Apart from Saccharose (sucrose- 2.4% of

the edible portion), being the dominant oligosaccharide, maltose also occurs in small amounts in banana. 6-Kestose has been identified in ripe bananas. Sugar alcohols like D-Sorbitol are absent in banana since it is a berry. Starch is a building unit of polysaccharides of bananas [37]. It is present in chiefly in unripe berries and its level decreases to a insignificant limit as ripening proceeds. Bananas contain 3% or more of starch content in ripe bananas. The level of lipids molecules is listed in the table below

Carotenoids are present naturally in notable quantities in all fruits and they are the prime factors responsible for the determination of fruit colours[2,3,4]. Bananas are classified as fruits with low carotenoid contents. The distribution pattern of carotenoids could be easily analyzed by HPLC. Various carotenoids are classified based on their structures out of which banana contains beta-carotene (VII) and lutein (IX). Among organic acids malic acids are predominant in berries like bananas and other tropical fruits. They are quantified to be present in 4 milli-equivalents per 100 g of fresh weight of the banana pulp. Almost many fruits are important sources of Vitamin C. Banana contains 7-21 mg/100 g of edible portion. [11,12,13].

As far as aromatic compounds are considered, the distinguishing aromatic compound of bananas is isopentyl acetate. Esters of pentanol, like the esters of acetic, propionic and butyric acids, are also found to contribute to the distinctive aroma of bananas. At the same time the esters of butanol and hexanol with acetic and butyric acids generally are fruitier in character. The aroma of banans could change on heating due to the liberation of glycosidic precursors, oxidation, addition of water and cyclization of individual compounds. A very important contributor to the inclusive, [14,15,16]. soft aroma of the bananas is supposed to be provided by the chemical compounds, eugenol (1), O-methyleugenol (II) and elemicin (III) [17,21,22].

There are two known forms of allergic reaction to banana. The first one caused the birch tree and other pollen allergies is the oral allergy syndrome which is characterized by itching and swelling in the mouth or throat within one hour after consuming the banana fruits. The second form is related to latex allergies which causes urticaria with potentially serious upper gastrointestinal symptoms . The banana fruit also contains high notable levels of biogenic amines such as dopamine and serotonin (Foy and Parratt 1960). The production of dopamine due to the intake of banana fruits also has an allergic effect on the tyrosine-deficit population, (tyrosine is a dopamine precursor present in bananas) . There are no toxins or toxic properties reported in any nutritional study of banana. [22,27].

## CONCLUSION

Several studies provided evidence that flesh color can be used to screen for carotenoid-rich banana cultivars.The rich carotenoid content of the identified banana cultivars provides a good case for the introduction and distribution of these cultivars in countries where vitamin A deficiency (VAD) is high. Providing consumer acceptability, this could provide a quick solution to VAD.Additionally, consumption of rich Iron and Zinc banana cultivars could be have potential to alleviating micronutrient malnutrition deficiency in developing countries. Fe'i banana cultivars contained rich riboflavin concentrations that could potentially meet daily estimated riboflavin requirements, according to traditional eating patterns. However, in future the recombinant technology has to help the increase carotenoids and micronutrients in the bananas through biofortification to levels which are higher than the current ones.

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**Table 1: Chemical composition of banana fruits (Nutritional value per 100g)**

| | |
|---|---|
| Energy | 371 kJ (89 kcal) |
| Carbohydrate | 22.84 g |
| Sugars | 12.23 g |
| Dietary fiber | 2.6 g |
| Fat | 0.33 g |
| Protein | 1.09 g |
| Thiamine ($B_1$) | (3%) – 0.031 mg |
| Riboflavin ($B_2$) | (6%) – 0.073 mg |
| Niacin ($B_3$) | (4%) – 0.665 mg |
| Pantothenic acid ($B_5$) | (7%) – 0.334 mg |
| Vitamin ($B_6$) | (31%) – 0.4 mg |
| Folate ($B_9$) | (5%) – 20 μg |
| Choline | (2%) – 9.8 mg |
| Vitamin C | (10%) – 8.7 mg |
| Iron | (2%) – 0.26 mg |

| | |
|---|---|
| Magnesium | (8%) – 27 mg |
| Manganese | (13%) – 0.27 mg |
| Phosphorus | (3%) – 22 mg |
| Potassium | (8%) – 358 mg |
| Sodium | 1 mg |
| Zinc | (2%) - 0.15 mg |
| Flouride | 2.2 µg |

Source: USDA Nutrient Database,Units: µg - micrograms; mg - milligrams; IU – International units

**Table 2. Average chemical composition (as % of fresh edible portion)**

| | |
|---|---|
| Dry matter | 26.4 |
| Total sugar | 20.0 |
| Titratable acidity (citric acid + malic acid + tartaric acid) | 0.6 |
| Dietary fiber | 1.8 |
| Pectin (expressed as calcium pectate) | 0.9 |
| Ash | 0.8 |
| pH | 4.7 |

**Table 3. Free amino acids in banana fruits (as of % of total free amino acids)**

| | |
|---|---|
| Asp | 5-10 |
| Asn | 15 |
| Gln | 10-15 |
| minobutyric acid | 5-10 |
| Histidine | 10-15 |
| Pipecolinic acid | 5-10 |

**Table 4. Fatty acid composition of bananas (as % of the total fatty acids)**

| Fatty acids | Banana |
|---|---|
| 14:0 | 0.6 |
| 16:0 | 58 |
| 16:1 | 8.3 |
| 18:0 | 2.5 |
| 18:1 | 15 |
| 18:2 | 10.6 |
| 18:3 | 3.6 |

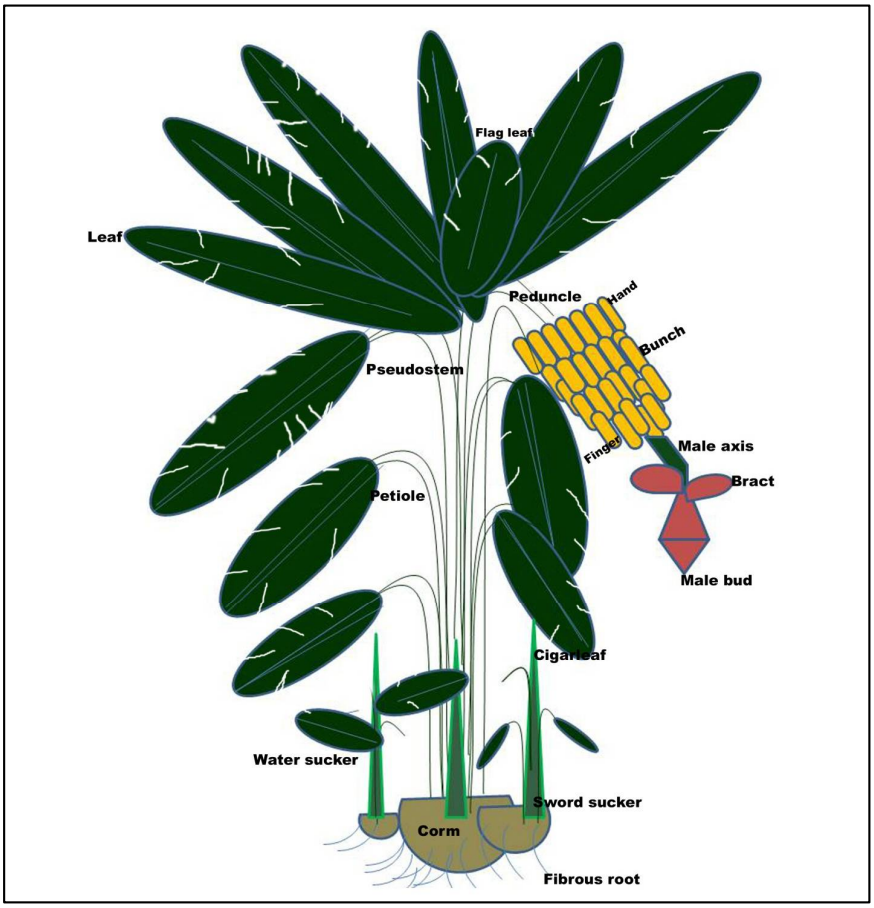


**Figure 1.General morphology of banana plant**

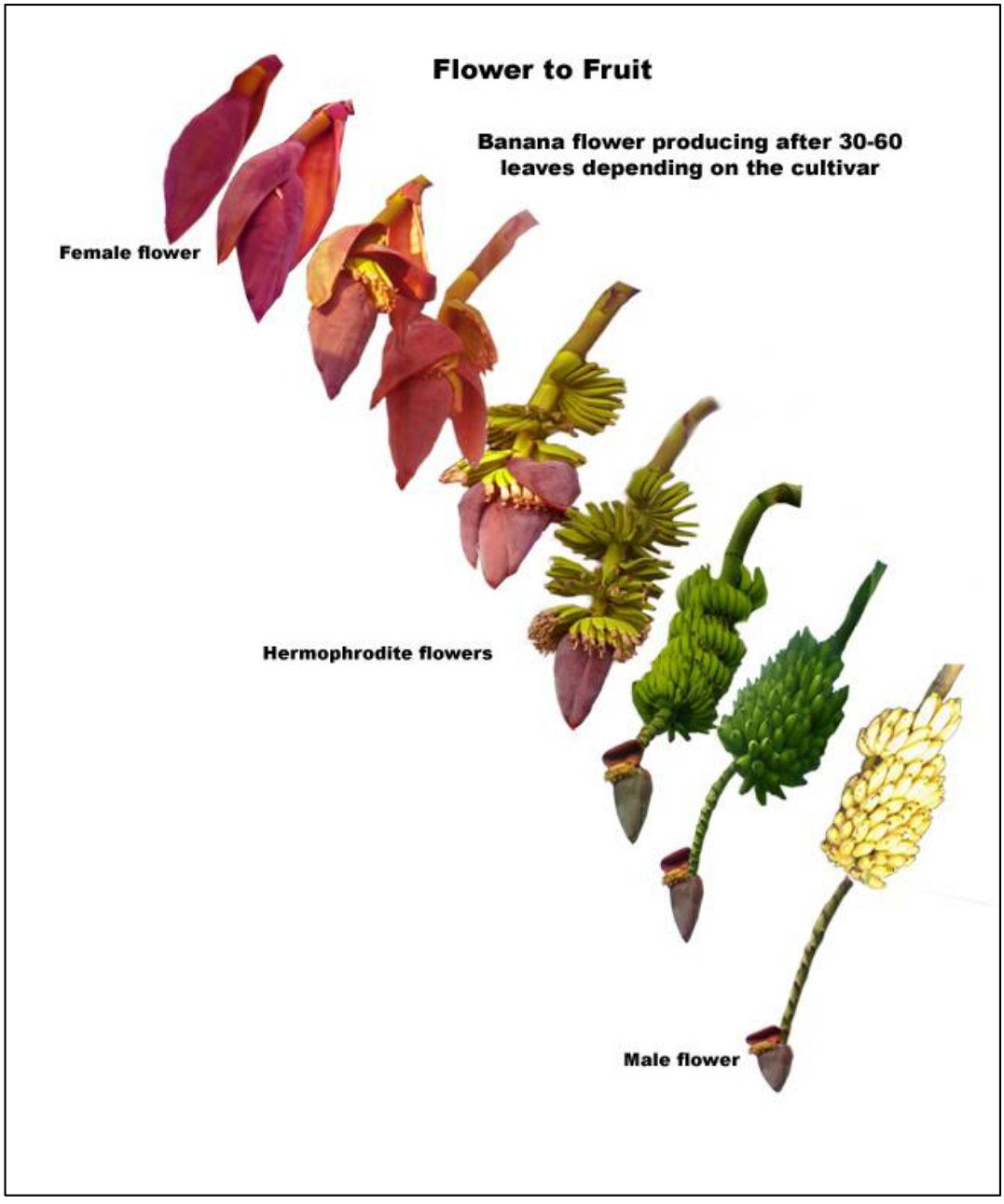


**Figure 2.Banana inflorescence to fruit developmental stage**


**Figure 3.Commercially important banana cultivars in India.**

| SNO | Component | Unit | Banana | | | | Plantain | |
|---|---|---|---|---|---|---|---|---|
| | | | Ripe | Unripe | Dried | Flour | Ribe | Unripe |
| 1 | Energy | Kcal | 89 | 110 | 257 | 340 | 91 | 122 |
| 2 | Water | g | 74 | 69 | 28 | 3.0 | 63 | 65 |
| 3 | Protein | g | 1.1 | 1.4 | 3.0 | 3.9 | 0.8 | 1.3 |
| 4 | Total lipid | g | 0.3 | 0.2 | 1.0 | 1.8 | 0.1 | 0.37 |
| 5 | Carbohydrate | g | 21.8 | 28.7 | 63.0 | 82.1 | 24.3 | 32 |
| 6 | Dietary fibre | g | 2.0 | 0.5 | 5.5 | 7.6 | 5.4 | 2.0-3.4 |
| 7 | Na | mg | 1.0 | | 8.0 | 3.0 | | 4.0 |
| 8 | K | mg | 385.0 | | 1150.0 | 1491.0 | | 500 |
| 9 | Ca | mg | 8.0 | 8 | 20.0 | 22.0 | 7 | 3.0 |
| 10 | Mg | mg | 30 | | 90.0 | 108.0 | 33 | 35.0 |
| 11 | P | mg | 22 | | 75.0 | 74.0 | 35 | 30.0 |
| 12 | Fe | mg | 0.42 | 0.9 | 1.3 | 1.15 | 0.5 | 0.6 |
| 13 | Cu | mg | 0.11 | | 0.4 | 0.39 | 0.16 | |
| 14 | Zn | mg | 0.18 | | 0.5 | 0.61 | 0.1 | |
| 15 | Mn | mg | 0.2 | | | 0.57 | 15 | |
| 16 | Eq. b-carotene | mg | 68.0 | 48.3 | 150.0 | 183.0 | 0.03-1.20 | 390-1035 |
| 17 | Vitamin E | mg | 0.29 | | 0.6 | | | |
| 18 | Vitamin C | mg | 11.7 | 31 | 4.0 | | 20 | 20 |
| 19 | Thiamin | mg | 0.04 | 0.04 | 0.1 | 0.18 | 0.05 | 0.08 |
| 20 | Riboflavin | mg | 0.07 | 0.02 | 0.18 | 0.24 | 0.05 | 0.04 |
| 21 | Niacin | mg | 0.61 | 0.6 | 2.0 | 2.8 | 0.7 | 0.6 |
| 22 | Panthotenic acid | mg | 0.28 | | | | 0.37 | |
| 23 | Vitamin B6 | mg | 0.47 | | | | | |
| 24 | Total Folate | mg | 23.0 | | | | 0.016 | |
| 25 | Biotin | mg | 2.6 | | | | | |
| 26 | Isoleucine | mg | 34.0 | | | 167.0 | | |
| 27 | Leucine | mg | 71.0 | | | 359.0 | | |
| 28 | Lysine | mg | 50.0 | | | 162.0 | | |
| 29 | Methionine | mg | 14.0 | | | 74.0 | | |
| 30 | Cystine | mg | 20.0 | | | 63.0 | | |
| 31 | Phenylalanine | mg | 41.0 | | | 201.0 | | |
| 32 | Tyrosine | mg | 26.0 | | | 121.0 | | |
| 33 | Threonine | mg | 36.0 | | | 171.0 | | |
| 34 | Tryptophan | mg | 13.0 | | | | | |
| 35 | Valine | mg | 49.0 | | | 282.0 | | |
| 36 | Arginine | mg | 57.0 | | | 176.0 | | |
| 37 | Histidine | mg | 86.0 | | | 333.0 | | |
| 38 | Alanine | mg | 43.0 | | | 222.0 | | |
| 39 | Aspartic acid | mg | 120.0 | | | 503.0 | | |
| 40 | Glutamic Acid | mg | 115.0 | | | 399.0 | | |
| 41 | Glycine | mg | 41.0 | | | 190.0 | | |
| 42 | Proline | mg | 43.0 | | | 229.0 | | |
| 43 | Serine | mg | 49.0 | | | 226.0 | | |
| 44 | Dopamine | mg | 65.0 | | | | | |
| 45 | Serotonine | mg | 3.3 | | | | 45 | 76 |
| 46 | Thiamine | mg | 0.7 | | | | | |
| 47 | Malic acid | meq | 6.20 | 1.36 | | | | |
| 48 | Citric acid | meq | 2.17 | 0.68 | | | | |
| 49 | Oxalic acid | meq | 1.37 | 2.33 | | | | |
| 50 | Other acids | meq | 0.17 | 0.19 | | | | |
| | Reference: | | | | | | | |
| 1 | Anonymous (1981). | | | | | | | |
| 2 | Marriott and Lancaster (1983). | | | | | | | |
| 3 | Woolfe (1992). | | | | | | | |
| 4 | Ciqua Cneva (1993). | | | | | | | |
| 5 | Lassoudie`re (2007). | | | | | | | |
| 6 | Guyle`ne Aurore (2009). | | | | | | | |

**Figure 4.Chemical composition and biochemical features of banana and plantain at different physiological stages, and after transformation, per 100 g of fresh weight**

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RESEARCH ARTICLE

# Dr.PDKV Toll Free Helpline – An Effective Tool for Transfer of Technology in Agriculture

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## ABSTRACT

Toll free helpline runned by Agriculture Technology Information Centre, Dr.PDKV, Akola since 2010 to solve the farmers queries and to create awareness about University developed technologies. The data for the year 2013-14, 2014-15, 2015-16 were gathered to analyse the farmers queries and extent of awareness created by helpline among farmers. Results indicated that farmers made enquiry regarding all facets of farming such as fertilizer and irrigation management, information regarding new varieties released, disease and pest management, weed management and herbicide application, package of practices for kharif and Rabi crops, integrated nutrient management of crops soil and water analysis as well as farmers to information regarding entrepreneurship development related to agri-business. Considering the sustainable response of farming community from last three years, it can conclude that this helpline serves (cater) lot for farmers for breeding the gap between farming community to access university technology. Timely availability of farming solutions created awareness and ultimately improved the adoption behavior within farming community; it is another effective side of this helpline. Analysis of this toll free helpline made constructive criticism to improve the efficacy of this service. Which will ultimately beneficial for well being farmers.

**Keywords :** Agriculture Technology, Dr.PDKV, farming community, fertilizer and irrigation, herbicide application.

## INTRODUCTION

The latest advances in information technology (IT), computers, telecommunications and internet have provided condusive environment for adopting new technologies and making the method of instructions more effective & interactive (Karthikeyan 2008) Telephone, a powerful electronic machine, was a farmers dream earlier which now has

become a reality as he can immediately make use of it to address their field problems and other farm difficulties (manhas et al 2005) In 1991 India had about 5.5 million telephones. At the end of August 2001, the total number of telephones lines in India was estimated to have increased to 33.8 million lines and the number of rural telephone lines increased by approximately 357 per cent per annum during 1989-90 to 1999-2000 (Jhunjhunwala, 2005) Keeping in view the efficacy of telecommunication, Dr.Panjabrao Deshmukh Krishi Vidyapeeth,(Dr.PDKV) Akola running a Toll Free Helpline from 2010, for bridging the gap between University technology & farming community as well as, to facilitate timely solution regarding farming. Although the jurisdiction of University is within 11 districts of Vidarbha but due to proper & beneficial suggestion this Toll Free Line received queries from Maharashtra as well as nearby states such as Chattisgarh, Madhya Pradesh & Andhra Pradesh. Taken in to consideration the huge response of farmers, it is decided to evaluate PDKV Toll Free Helpline on selected indicates.

## METHODOLOGY

The overall objective of the study was to evaluate performance of PDKV Toll Free Helpline using logic model approach. Five indicators were considered such as participation, awareness about PDKV toll free line, adoption, gratification & information sharing behavior of users of PDKV toll free line. Logic method PDKV Toll Free Helpline is equipped at Agricultural Technology Information Center, Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola wherein BSNL switch with I hunting lines, computer, telephones, internet connectivity including the toll free number, it works from 10.00 am to 5.45 pm on all working day. The toll free call charges are borne by Dr.PDKV. After receiving a query from farmers, documentation of call details like name, address & cell no. farmers is done on system & then immediately the scientists of University answer the call and solve the queries.

### Selection of respondents

Respondent is the end user of program (farmer) with whom impact was measured. The respondents on the PDKV toll free line are the farmers & Scientist (University officials). The farmers selected for evaluation were from University Jurisdiction namely Vidarbha, outside Vidarbha, whole Maharashtra & nearby states of Maharashtra.

### Outcomes

Outcome are the results of project about PDKV toll free line, in terms of awareness about this number, participation for taking information adoption of given solution, gratification & information sharing behavior. All these were evaluated with the help of data generated from participants & on the PDKV helpline.It is observed from the Table-1 that 70.00 per cent respondents were Young (20-40 years). About 62 per cent of them were educated up to high school level, around 38 % were of them had graduation. Major occupation amongst respondents was farming having about 86.66 per cent of farmers 38%. Most of respondents have land holding of 3 to 10 acres between (40.00 %)

The data given in Table -2 illustrated that, the popularity of advice given by PDKV Toll Free Helpline results in continuos recipt of queries 6156,6428 and 6292 in the year 2013-14,2014-15,2015-16 respectively. The success of scheme is mainly depend on timely, feasible solutions given by University scientists, which were really beneficial to farmers. It is evident from Table 3, that farmers took information regarding farming from various agencies, which deals information such as, Kisan Call Centre, PDKV Toll free line, department of Agriculture(Helpline) and Krishi Vigyan Kendra. Among the respondent 55 per cent farmers took information of farming from Kisan Call Center.Cent per cent farmers took information from PDKV Toll Free Helpline, 5 per cent took information from Department of Agriculture and 10 per cent farmers took information from Krishi Vigyan Kendra's.

Data obtained from Table 4, reveals that, nearly about ¼ th i.e. 24.01 per cent queries obtained for management of pest and diseases. Nearly 23.64 query obtained for crop husbandry i.e. solution for farming such as package of practices followed by 10.07 queries obtained to know about elite germplasm, sources of availability of seed. It is evidenced from the Table that farmers have more orientation towards timely management of pests and disease, and it is obvious that pests, diseases and fertilizers were the key factors directly affecting the yield. In order to get immediate information on these aspects farmers might have preferred to make calls on PDKV Helpline as well as, he wishes to complete all farm operations timely and same time he is interested in high yielding seed(varieties)with desirable traits.It is observed from Table -5 that a larger number of calls 1812 attempted by farmers to know the information on cash crops. Most of the farmers have made calls to seek clarification mostly on pulses (20.43 per cent) may be due to the good prices fetch by pulses in past season followed by oilseed (12.85per cent), cereals (13.15per cent), flower and ornamentals (8.12 per cent) and vegetables(6.42 per cent).In general, it may be inferred that food grains, vegetables and fruits were preferred by significant number of farmers. The possible reason for such results would be inadequate awareness knowledge about other crops and significance of these crops. Above all area under cash crops, pulses oilseed followed by flower, vegetables and fruits.

It is seen from Table – 6, that majority of users have made calls during Kharif (53.98 per cent) and Rabi (26.98 per cent) season as well, while only a lesser proportion of calls were received in (19.00 per cent) summer season.
The reason could be attributed to the fact that Kharif and rabi is the crucial timing of cropping for farmers and so many operations worked out during this period. But during summer limited number of farmers are able to do farming (sowing) because of unavailability of irrigation facility and some other limitations, so this result is justified with the results of Karthikeyan,(2007).The data obtained from Table -7 revealed that one fourth (25.00 per cent) of the users were aware of the PDKV toll free line through agricultural exhibitions followed by radio (23.33 per cent), because during weekly broadcast of some queries received on toll free is done by means of radio and tollfree number is announced. This was followed by newspaper (3.33 per cent),agricultural magazines (5.00 per cent), Friends(13.33 per cent), neighbors(8.33 per cent) and soil testing mobile van(11.33 per cent).

The data given in Table -8 implies that, maximum number of users are aware long back i.e. more than 3 years (56.66 per cent) because since from the establishment of helpline huge publicity made by means of radio etc. followed by number of farmers were aware about Helpline from 1-3 year(38.22 per cent) and very few farmers were unaware about Helpline less than 1year(5.00 per cent) .

## Gender wise participation of Farmers in PDKV toll free line

The data given in Table-9 reveals that the participation about of PDKV toll free line gives an idea about the people belonging to different gender using this service.

The genderwise participation of farmers on PDKV toll free line, reveal to analyze which gender group had maximum involvement to access the service of PDKV toll free line Table-9 Indicated that (99.9 %) of calls made by men followed by 0.01 per calls made by women. Even though female have more involvement in agriculture & allied fields. But still judgment regarding all facets of farming done by mens.

The users of PDKV toll free line categorized areawise and the data given Table 10 illustrated that the highest number of queries made by farmers of Vidarbha. As the jurisdiction of university in Vidarbha, hence results obtained are justifiable followed by queries received from outside Vidarbha (9.99 per cent) and outside Maharashtra (0.41 per cent).The data explained in Table 11 indicated that the highest adoption on solutions gives by PDKV scientist i.e.95 per cent because mere giving advice to farmer is not enough but it is necessary to understand the significance and seriousness of query to promote immediate advice. PDKV Toll free Helpline turned the query to respective scientist untill he don't get satisfactory solution.

In order to know the extent of satisfaction about the advice given by PDKV Toll Free Helpline, the data presented in Table – 12 indicate that highest number of farmers (95 per cent) are satisfied with advice of PDKV Toll Free Helpline advice because the solution given by officials is need based, economic and easy to work out by considering the situation in the farm. If farmers are unable to workout the given solution some option provided to him if available. All these aspects increase the efficacy of PDKV Toll Free Helpline.

After getting information from PDKV Toll Free Helpline, farmers adopted the recommended advice, if farmers get satisfaction, then they share the information about the advice. The results explained in table 13 reveals that the major proportion of respondents (95.00 per cent) shared the information, which they received on PDKV toll free line, while meager (5.00 per cent) proportion not shared the information.

It is observed from Table-14 that higher proportion of respondent (83.33 per cent) shared the information regarding PDKV Toll Free Helpline more than 10 farmers. These higher number of farmers desired to promote awareness about scheme, there might be one reason that no cost needs to pay i.e. free of cost availability of service. The results followed by information about PDKV Toll Free Helpline shared between group of 6-10 farmers (13.33 per cent) and less than 5 farmers (3.33 per cent) respectively.Once the advice received from Pdkv Toll Free Helpline, farmers implement on his farm and after getting satisfied he shared the information amongst the peer group. And in respect of data given in Table 15, it is observed that information is shared in all groups. Highest number of sharing recorded with neighbors(36.66 per cent) followed by friends (26.66 per cent) and friends and neighbors (18.33 per cent), which is a parallel spread of University technology for which PDKV Toll Free Helpline is installed.

During the same study one another aspect was analysed that the peak season and peak hour of calls made which makes more attentive to University officials, so that more effective transfer of technology should happen. And as per data obtained in Table 16 reveals that, high period for calls during Kharif was 28$^{th}$ May to 30$^{th}$ June and average 21 calls received during peak time at 4.30 to 5.30 pm of day (52.5 per cent)followed by high period for Rabi was 19$^{th}$ Oct to 12$^{th}$ Nov. and average 17 calls received during 11.30 to 1.00 pm of the day, and for Summer 15$^{th}$ Jan to 20$^{th}$ Feb. and on average 14 calls received during peak time at peak hours i.e.10.30 to11.30 am of the day, respectively.

## CONCLUSION

It is concluded that Dr.PDKV Toll Free Helpline is an effective medium of transfer of university developed technologies for farming community.It was observed that on an average 52.5 per cent calls received during 4.30 to 5.30 pm during Kharif season of the total calls received during day. Therefore, it is recommended that PDKV scientists should be high alert and attentive during 4.30 to 5.30 pm alongwith scientists from different disciplines.

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**Table 1– Background profile of respondents**

(N=60)

| Demographic characteristics | Frequency | Per cent |
|---|---|---|
| Age | | |
| Age- <21 | 3 | 05.00 |
| 20-40 | 42 | 70.00 |
| More than 40 | 15 | 25.00 |
| Education – Doctorate | 01 | 01.67 |
| Completed High school | 37 | 61.67 |
| Graduate Degree | 22 | 36.66 |
| Major occupation – Agriculture | 52 | 86.67 |
| Business | 07 | 11.67 |
| Labour / Service | 01 | 01.66 |
| Farm size in acres | | |
| 1-3 | 05 | 08.34 |
| 3-10 | 24 | 40.00 |
| 10-15 | 31 | 51.66 |

**Table 2- Number of calls received on PDKV Toll Free line during last three years.**

| Sr.No | Calls received during the year | No.of calls received | No.of Beneficiaries |
|---|---|---|---|
| 1 | 2013-14 | 6156 | 18468 |
| 2 | 2014-15 | 6428 | 19284 |
| 3 | 2015-16 | 6292 | 18876 |
| | Total calls received during last three years | 18876 | 56628 |

Table 3– Distribution of Different sources of information providing information regarding agriculture. (N=60)

| Sr.No. | Coverage areas | Number | Percent |
|---|---|---|---|
| 1 | Kisan call center | 33 | 55.00 |
| 2 | PDKV Toll free line | 60 | 100.00 |
| 3 | Department of Agriculture | 03 | 5.00 |
| 4 | Krishi Vigyan Kendra | 06 | 10.00 |

**Table 4–Distribution of calls attended by the scientists on PDKV Toll Free Helpline on the basis of their query.**

| Type of query | No. of calls | Per cent |
|---|---|---|
| Pest & Diseases | 1511 | 24.01 |
| Crop Husbandry | 1488 | 23.64 |
| Weather Forecasting | 634 | 10.07 |
| Seed inquiry | 629 | 09.99 |
| Enquiry about extension activity | 532 | 08.45 |
| Fertilizer & composting | 511 | 08.12 |
| Enquiry about University Publications | 234 | 03.71 |
| Farm equipment & mechanization | 225 | 03.57 |
| Diversified farming | 186 | 02.95 |
| Seed treatment & biofertilzers | 149 | 02.36 |
| Other | 193 | 03.06 |
| Total no of calls | 6292 | 100.00 |

**Table 5 – Distribution of calls as per queries on various crops**

| Sr.No. | Crop type | No. of calls | Per cent |
|---|---|---|---|
| 1 | Cash crop | 1812 | 28.79 |
| 2 | Pulses | 1286 | 20.43 |
| 3 | Oilseed | 809 | 12.85 |
| 4 | Cereals | 828 | 13.15 |
| 5 | Flower & Ornamental | 511 | 08.12 |
| 6 | Vegetables | 404 | 06.42 |
| 7 | Fruit crops | 336 | 05.34 |
| 8 | Medicinal & aromatics | 129 | 02.05 |
| 9 | Mushroom | 112 | 01.78 |
| 10 | Turmeric | 65 | 01.03 |
| | Total | 6292 | 100.00 |

**Table 6 –Distribution of season-wise calls**

| Season | No. of calls | Per cent |
|---|---|---|
| Kharif | 3397 | 53.98 |
| Rabi | 1698 | 26.98 |
| Summer | 1196 | 19.00 |
| Total | 6292 | 100.00 |

**Table 7 – Sources of awareness of PDKV Toll Free helpline (N=60)**

| Sources of Awareness | Number | Per cent |
|---|---|---|
| Radio | 14 | 23.33 |
| Agricultural Exhibition | 15 | 25.00 |
| Newspaper | 2 | 03.33 |
| Agricultural Magazines | 3 | 05.00 |
| Friends | 8 | 13.33 |
| Neighbours | 5 | 08.33 |
| Relative & Friends | 6 | 10.00 |
| Soil Testing Mobile Van | 7 | 11.33 |
| Total | 60 | 100.00 |

**Table 8–Duration of awareness about PDKV Helpline**

(N=60)

| Sources of Awareness | Number | Per cent |
|---|---|---|
| Less than 1 year | 03 | 05.00 |
| 1 to 3 years | 23 | 38.33 |
| More than 3 years | 34 | 56.66 |
| Total | 60 | 100.00 |

**Table -9 Gender-wise participation of farmers on PDKV Toll Free Helpline**

| Gender | No. of calls made | Per cent |
|---|---|---|
| Male | 6266 | 99.58 |
| Female | 26 | 0.42 |
| Total | 6292 | 100.00 |

**Table 10 – Areawise distribution of calls received on PDKV Toll Free Helpline.**

| Sr.No. | Coverage areas | No. of calls | Per cent |
|---|---|---|---|
| 1 | Vidarbha | 5637 | 89.58 |
| 2 | Outside Vidarbha | 629 | 09.99 |
| 3 | Outside Maharashtra | 26 | 00.41 |
| | Total | 6292 | 100.00 |

**Table 11 – Extent of adoption of the advices recommended by PDKV Toll Free Helpline.**

| Sr.No. | Coverage areas | No. of calls | Per cent |
|---|---|---|---|
| 1 | Adopters | 57 | 95.00 |
| 2 | Non-Adopters | 03 | 05.00 |
| | Total | 60 | 100.00 |

**Table 12 – Gratification with the results of advices recommended by PDKV Toll Free Helpline**

| Sr.No. | Coverage areas | No. of calls | Per cent |
|---|---|---|---|
| 1 | Satisfied | 57 | 95.00 |
| 2 | Not satisfied | 3 | 5.00 |
| | Total | 60 | 100.00 |

**Table 13 – Information sharing behaviour about the advices by PDKV Toll Free Helpline**
(N=60)

| Sr.No. | Coverage areas | No. of calls | Per cent |
|---|---|---|---|
| 1 | Shared | 57 | 95.00 |
| 2 | Not shared | 03 | 05.00 |
| | Total | 60 | 100.00 |

**Table 14–Advices shared to number of farmers recommended by PDKV Toll Free Helpline.**
(N=60)

| Sr.No. | Coverage areas | No. of calls | Per cent |
|---|---|---|---|
| 1 | Less than 5 | 2 | 3.33 |
| 2 | 6 to 10 | 8 | 13.33 |
| 3 | More than 10 | 50 | 83.33 |
| | Total | 60 | 100 |

**Table 15 – Nature of persons shared about the advices recommended by PDKV Toll Free Helpline.**
(N=60)

| Coverage areas | Number | Percent |
|---|---|---|
| Neighbors | 22 | 36.66 |
| Friends | 16 | 26.66 |
| Relatives | 04 | 06.66 |
| Friends & Neighbors | 11 | 18.33 |
| Friends, Neighbors & Relatives | 07 | 11.66 |
| Total | 60 | 100.00 |

**Table 16 – Peak hours of calls received during the year**

| Sr No. | Season | High period of receiving calls | Average calls receive per day during peak time | Peak Time of receiving call | Percent of calls received during peak time |
|---|---|---|---|---|---|
| 1 | Kharif | 28th May to 30th June | 21 | 4.30 to 5.30 | 52.5 |
| 2 | Rabi | 19th Oct to 12th Nov. | 17 | 11.30 to 1.00 | 42.5 |
| 3 | Summer | 15th Jan to 20th Feb | 14 | 10.30 to 11.30 | 35.00 |

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RESEARCH ARTICLE

# Red Blood Cell Morphometry and Sickling in Sambar Deer (*Rusa unicolor*)

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## ABSTRACT

Morphometry of erythrocytes was studied in fifteen healthy adult male Sambar Deer maintained at Zoological Gardens, Thiruvananthapuram, which underwent vasectomy under general anaesthesia. Blood smears were stained with Giemsa method and were observed under 100X oil immersion objective. The mean diameter of erythrocytes observed was 3.6 ± 0.05 µm with the minimum and maximum values 3.13 µm and 4.06 µm respectively. Sickling phenomenon of the erythrocytes was also observed in five deer. Oat seed shaped, crescent shaped and sickle shaped erythrocytes were observed.

**Keywords :** Sambar Deer, *Rusa unicolor*, red blood cell, erythrocyte, sickle cell.

## INTRODUCTION

Morphometry of Red blood cells plays a major role in disease investigation, forensics, identification of pathological alterations and to some extent even species identification in animals.Among the domestic species, bovine erythrocytes are the largest with a diameter of 5-6 µm [1] and ovine erythrocytes are the smallest with a diameter of

2.5-3.9 µm [2].Determination of the morphometric characters like diameter, circumference and surface of erythrocytes helps in the identification of the species[3].The reportedsize of erythrocyte in White-tailed Deer was 3.5 - 4.5 µm in diameter[4].One of the major peculiarities of cervid erythrocytes is the sickling phenomenon, Gulliver, in 1840, first reported sickling of red blood cells in cervids[5]. Unlike sickle cell anaemia in humans, sickling in cervids is not related to any pathological condition. It ismainly correlated to the type of haemoglobin present and the physiological alterations [4].Detailed studies on the haematology and morphometry of Indian deer species are limited. The aim of this study was to determine the diameter of red blood cells of adult male Sambar Deer (*Rusa unicolor*) and record the various forms of erythrocyte sickling.

## MATERIALS AND METHODS

The study was conducted in fifteen adult male Sambar Deer maintained at the Zoological Gardens, Thiruvananthapuram, Kerala, India. The samples were collected from animals which were anaesthetised for vasectomy.Blood was collected from the jugular vein using an 18 G hypodermic needle and blood smears were prepared immediately. The smears were air dried and fixed with methanol for 5 minutes and stained using Giemsa staining method (1 in 10 dilution). The stock solution was diluted to 1 in 10 parts and filtered to remove the stain articles. The fixed blood smears were flooded with freshly prepared stain and were kept for 30 minutes. The smear was washed in running water and air dried. The smears were first observed under 10x followed by 100x oil immersion objective (Fig. 1). Images were captured using an inbuilt camera unit (Leica DM750 microscope and Lecia DFC295 camera unit). Diameter of the erythrocytes were measured using a compatible software (Leica Application Suite, Version 4.2.0., Leica Microsystems Ltd). The entire blood smear was screened for any haemoparasites and presence of sickle shaped erythrocytes. The mean, standard error and range of the recordings were calculated using the statistical software SPSS version 21.

## RESULTS AND DISCUSSION

Kolmorogov–Smirnov statistical test was done to check the observations for normal distribution and was found to be normally distributed. The mean diameter of erythrocytes observed was 3.6 ± 0.05 µm with the minimum and maximum values being 3.13µm and 4.06 µm respectively. The observed size of the erythrocytes was within the range observed in White-tailed [4].The observed size of Sambar Deer erythrocytes was comparatively similar to the size of sheep and goat erythrocytes[2].But, compared to the reported size of Reindeer erythrocyte (5.3 -5.8 µm) the Sambar Deer erythrocyte was reported tohave a smaller diameter [6].Morphometric characters like diameter, circumference and surface of erythrocytes help in the identification of species [2]. Since the diameter of erythrocytes of each species lies in a specific range, morphometry of erythrocytes can be relied in forensic cases for tentative species identification.

Abnormally shaped erythrocytes were observed in five deerduring blood smear examination. Few RBCs may attaint a sickle shapeafter the blood collectionin cervids.Sickling of erythrocytes is usually observed in blood maintained in room temperature, or which is alkaline and oxygenated[7]. Sickling and abnormal shaped RBCs were observed in 75% of Mule Deer and White-tailed Deer and abnormal shapes like oat seed shaped, sickle shaped, crescent shaped and holly leaf shaped have been reported[8]. In the current study, authors observed crescent shaped, oatseed shaped (Fig. 2)and sickle shaped erythrocytes (Fig. 3) in Sambar Deer. Sickled erythrocytes have been reported in Spotted Deer and Barking Deer [9]. The sickling phenomenon does not cause any pathological effect in deer and occurs due to the formation of insoluble tactoids of haemoglobin in oxygenated state[7].

It is already known that morphometry of bovine erythrocytes significantly varies with age, gender and altitude [10]. In the current study,only adult male captive Sambar Deer were included and the sample size was small. Further studies on diverse populations of Sambar Deer with larger sample size would be required to understand better the

morphometric details of erythrocytes in these animals and to evaluate the effect of variables like gender, age and altitude.

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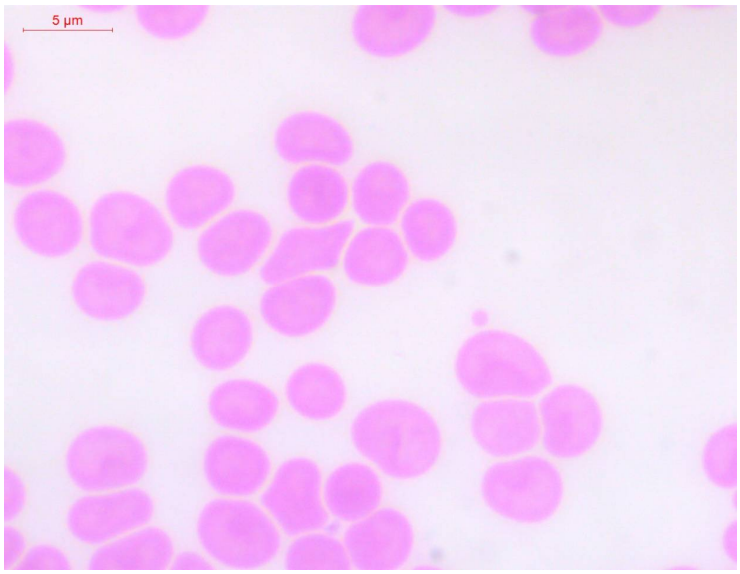


**Figure 1. Sambar Deer erythrocytes--; Giemsa stain (100x objective)**

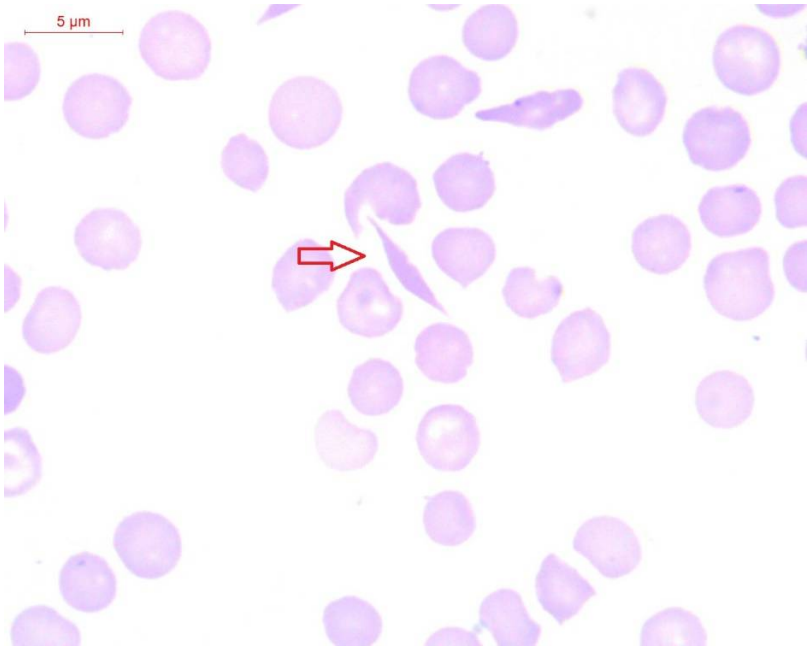


**Figure 2. Oat seed shaped erythrocyte (100x objective)**

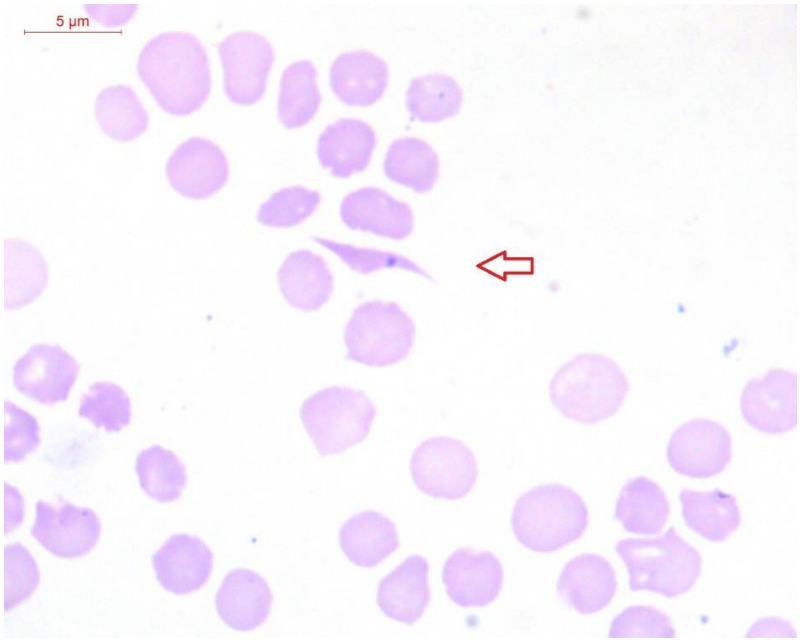


**Figure 3. Sickle shaped erythrocyte (100x objective)**

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RESEARCH ARTICLE

# Promoting Entrepreneurship through PDKV Helpline

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## ABSTRACT

The study was undertaken with the farmers who have attended PDKV helpline for different queries related to entrepreneurship development. A purposive sample of 50 farmers, who had made inquiry about PDKV dall mill on PDKV helpline were selected for collection of data with an objective to assess the extent of adoption of PDKV dall mill as an enterprise by the caller farmers, as well as to assess the constraints faced by them is non adoption of technology. Farmers made enquiry about working hours, cost, feasibility, output of product, ease in handling etc. The findings revealed that 1 farmer (2 per cent) adopted the PDKV dall mill as an effective enterprise after getting the information from PDKV helpline and 6 farmers (12 per cent) expressed that they have planned to start the enterprise in near future. While 86 per cent farmers drop the idea of establishment of PKV mini dall mill due to various constraints as expressed by them viz. lack of sub sidy (48.00 per cent), marketability of product (24 per cent), space constrains (8 per cent), and labour problem.The findings of the study will be useful for the concerned department to modify the policies for overcoming the constraints expressed by the respondent farmers.

**Keywords :** PDKV helpline ,entrepreneurship development, constraints, respondent farmers.

## INTRODUCTION

The term entrepreneurship is used to describe a dynamic process of creating incremental wealth (Shailesh et.al.,2013). This wealth is created by individuals who take the major risks in terms of equity ,time and career, commitment of providing value to some product or services, the product or services itself may or may not be new or unique but value must somehow be infused by the entrepreneur by securing and allocating then necessary skill and resources. In

other words entrepreneurship is the application of energy for initiating and building an enterprise (Mishra et al, 2010). Therefore entrepreneurship is a charismatic concept, widely used and widely defined: for example, as creative & innovative response to the environment (Chandramouli, et. al, 2007). Onobuogu & Esiobu (2014) opined that sustainable development of agribusiness requires the development of entrepreneurial and organizational skills of farmers can take two tracks. The first to amend the social, economic, political and cultural frameworks that hinders, and foster those that stimulate their development. The second is encouragement of farmers, via their personalities & capacities, to kindle the development of entrepreneurship. If farming competitiveness is to be improved by nurturing entrepreneurial behavior, both tracks have to be considered. The improvement of entrepreneurial skills in agriculture is an important condition to generate sustainable rural development (De wolf & Schoorlemmer, 2007) PDKV mini dall mill is an agribased enterprise, provide an opportunity to farmers for value addition of pulses by means of processing as well as to become entrepreneur with raw available with them and another benefit of enterprise is long shelf life of product reduces the risk of enterprise.

## METHODOLOGY

The study was conducted at Agricultural Technology Information centre (ATIC),Dr PDKV, Akola during 2015-2016 with farmers who made enquiry about PDKV dall mill on PDKV toll free line employed at ATIC.A purposive sample of 50 farmers were selected for collection of data as per telephonic interview with them. Primary data was collected through the use of questionnaire and it was supplemented with telephonic interview. The was collected for socio-economic characteristics of farmers such as how they know about PDKV dall mill, how many farmers accept or reject the idea of installation of this enterprise and what constrains faced by them.

## RESULTS AND DISCUSSION

### Socio-economic characteristics of farmers

The data expressed in Table 1 for distribution of farmers age. It reveals that majority (50 per cent) of farmers age fell within the age range of 20-40 years, about 48.00 fell within the range of more than 40 years. While the proportion 2.00 per cent fell within the age less than 20 years . This finding corroborate with the findings of Nwibo and Okorie (2013) who opined an average 43 years for the agribusiness investors. The another demographic character of respondents education, it is observed that (40 per cent) farmer's wishes to start enterprise were graduate followed by 34.00 per cent farmers completed junior college while, meager (26) per cent of farmers having education up to high school level. Majority of respondents are engaged as agriculture (84 per cent) as occupation while 12 per cent farmers have business and only 4 per cent farmers are engaged in service.This finding supports the study of OKoli et al.(2014) who asserted that farmers with more knowledge of farming can make efficient allocation of resources and market situation and are thus, expected to run a more efficient and profitable enterprise.

According to the data given in Table 1 The land holding of respondents were reported farmers have medium land holding 42.00 per cent followed by 34.00 per cent farmers having small land holding.While only 24.00 per cent farmers have land holding more than 10 acres. All together 66.00 per cent i.e. 42 per cent farmers have 3-10 acres of land and 24 per cent farmers have more than 10 – 15 acres of land. This findings corroborate with the findings of Esiobu and Onubuogu (2014) asserted large farm size increases agricultural productivity and improves farmers implicative and technical resource use efficiency. Hence large farm size is a positive variable for entrepreneurship development in agribusiness in the study area. Simultaneously, majority of farmers have (66.00 per cent) farmers have irrigated land holding followed by Dryland (34.00 per cent) respondents. The data obtained from Table 1 indicates that the size of family of respondents who want to start enterprise ( less than 4 person) belong to small family and only meager farmers (more than 4 persons) belongs to big family.

### Sources of information providing information regarding PDKV dall mill

The data in Table 2 analyzed the distribution of sources of information to the respondents farmer from which, they knew about this enterprise are news paper agri-exhibition, TV & radio. Amongst the different sources highest number of farmers ( 48 per cent) read the information of PDKV dall mill from news paper, while the other sources of information followed by news paper were agri exhibition ( 32.00 per cent), radio (12.00 per cent) & TV (8.00 per cent) respectively.

### Status of respondents to choose PDKV dall mill as an enterprise

The data obtained from Table 3 illustrate that the amongst the sample of respondents i.e. 1 farmer (2.00 per cent) started the PDKV dall mill as an enterprise. While 12.00 per cent farmers planned to start the enterprise in near future & highest respondents (86.00 per cent) drop the idea of installation of PDKV dall mill because of some constrains faced by them.

### Constraints for installation of PDKV dall mill

The data addressed in Table 4 reveals that the highest number of farmers ( 48.00 per cent) could not established PDKV, dall mill for the sake of unavailability of market ( 24.00 per cent) respondents couldn't run the enterprise. While some other constraints followed were space (12.00 per cent) & ease in handling (16.00 per cent) respectively. This finding tallies with the result of European Commission (2004) who reported that poor knowledge of appropriate entrepreneurship skills and development of agri-business left most of the farmers unaware of better skills to choose in entrepreneurship for agribusiness.Inability to withstand competition, poor government policies on entrepreneurial development.

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**Table – 1 Demographic Characteristics**

| Demographic characteristics | Frequency | Per cent |
|---|---|---|
| Gender | | |
| Age- <21 | 1 | 2.00 |
| 20-40 | 25 | 50.00 |
| More than 40 | 24 | 48.00 |
| Education – | | |
| Graduate | 20 | 40.00 |
| Completed junior college | 17 | 34.00 |
| High School | 13 | 26.00 |
| Major occupation – Agriculture | 42 | 84.00 |
| Business | 6 | 12.00 |
| Labour / Service | 2 | 4.00 |
| Farm size in acres | | |
| 1-3 | 17 | 34.00 |
| 3-10 | 21 | 42.00 |
| 10-15 | 12 | 24.00 |
| Farm (Irrigated/Dryland) | | |
| Irrigated | 33 | 66.00 |
| Dryland | 17 | 34.00 |
| Size of family– | | |
| Small Family(<4) | 36 | 72.00 |
| Big Family (>4) | 14 | 28.00 |

**Table -2 Sources of Information providing information regarding PDKV dall mill.**

(N=50)

| Sr No. | Coverage Areas | No of Farmers | Per cent |
|---|---|---|---|
| 1 | News Paper | 24 | 48.00 |
| 2 | Agri-Exhibition | 16 | 32.00 |
| 3 | TV | 4 | 8.00 |
| 4 | Radio | 6 | 12.00 |
| | | | 100.00 |

**Table -3 Status of respondent to choose PDKV dall mill as an enterprise**

(N=50)

| Sr No. | Status | No.of Farmers | Per cent |
|---|---|---|---|
| 1 | Not established | 43 | 86.00 |
| 2 | Established | 1 | 2.00 |
| 3 | Planned to establish in near future | 6 | 12.00 |
| | | | 100.00 |

**Table -4 Constraints for installation of PDKV dall mill**

(N=50)

| Sr.No | Constraints | No. of Farmer | Per cent |
|---|---|---|---|
| 1 | Subsidy | 24 | 48.00 |
| 2 | Marketability | 12 | 24.00 |
| 3 | Space Constraints | 6 | 12.00 |
| 4 | Ease in handling | 8 | 16.00 |
| 5 | Labour Problem | 0 | 0.0 |
| | | | 100 |

**(* The percentage is more than 100 due to multiple responses.)**

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RESEARCH ARTICLE

# A Study on the Effects of Compounds from *Clinacanthus nutans* on Dengue Virus Type2 Infection

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## ABSTRACT

Dengue viruses classified into 4 serotypes (DV1, 2, 3 and 4) are etiological agents of dengue fever (DF) and dengue hemorrhagic fever (DHF) that are arthropod-borne diseases and cause a serious global health problem. There are no specific approved drugs or vaccines for the treatment or prevention of infectious DV and there are very few compounds known to inhibit the replication of this virus. *Clinacanthus nutans* Lindau has long been used in Thailand as a traditional medicine for the treatment of herpes simplex virus (HSV), and varicella-zoster virus (VZV). Four chlorophyll derivatives were isolated from the chloroform extract of *Clinacanthus nutans* Lindau leaves by means of chromatographic techniques to give four pure compounds. Structure elucidation of the isolated compounds was carried out on the basis of spectral analyses. These compounds were investigated for anti-DV2 activity in A459 infected cell. Thus, this study aimed to investigate anti-DV2 activity from 4 compounds of C. nutans inhibitory effects of these compounds on $PGE_2$ production in DV2 infected cells by detection of $PGE_2$ level and COX-2 expression in cell culture.It can be concluded that chlorophyll relative compound from C. nutans exhibited immunostimulating property in A549 cells at subcytotoxic level and decreased viral load bt inhibiting DV adsorption in pre-entry step of replication and suppressed $PGE_2$ production as well as DV replication in the tested cells. This finding may be the starting point for further study in more detail of virus compound interaction to understand the inhibitory effect of these compounds on DV infection.

**Keywords :** Dengue virus, dengue hemorrhagic fever, *Clinacanthus nutans (Burm. f.)* Lindau, anti-viral activity

## INTRODUCTION

Dengue viruses classified into 4 serotypes (DV1, 2, 3 and 4) are etiological agents of dengue fever (DF) and dengue hemorrhagic fever (DHF) that are arthropod-borne diseases and cause a serious global health problem. There are no specific approved drugs or vaccines for the treatment or prevention of infectious DV and there are very few compounds known to inhibit the replication of this virus[1].

Medicinal plants have been used since ancient time for the treatment of some diseases as well as several compounds from Thai medicinal plant are used for protection and treatment of viral diseases. *Clinacanthus nutans* (Burm. f.) Lindau (Thai name: Phaya Yo or Phaya Plong Thong) is a small shrub, native to tropical Asia, and is often cultivated *C. nutans* has long been used in Thailand as a traditional medicine for the treatment of skin rashes, insect and snake-bite, herpes simplex virus (HSV), and varicella-zoster virus (VZV) lesions[2].

The present communication reports a preliminary study initiated by the Medicinal Plant Reserch Institute, Department of Medical Science, Ministry of Public Health on antiviral compounds from C. nutans using bioassay-guided fractionation. The most antivirally active fractions were selected for further antiviral-guided fractionation by means of chromatographic techniques. This led to the isolation of four pure compounds, which were identified as chlorophyll a and chlorophyll b related compounds by spectroscopic methods, and the determination of their anti-DV2 activity in A459 infected cell.

## MATERIAL AND METHODS

### Preparation of compounds

Fresh aerial parts of *C. nutans* were collected. The leaves were separated from the stems, washed thoroughly and dried in an oven at 50 °C. The dried sample was ground to powder. The powder was sequentially extracted with hexane and chloroform, respectively. The compounds were isolated from these crude extracts as described previously[3].

### Cell lines

C6/36 cell line, a mosquito cell line from *A. albopictus*, was cultured in Leibovitz (L-15) medium supplemented with 1% tryptose phosphate broth, and 10% fetal bovine serum (FBS) at 28 °C without $CO_2$.A549 cell line (type II human lung alveolar epithelial cell carcinoma) was cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium with 10% FBS, penicillin, streptomycin, gentamicin and fungizone at 37°C with 5% $CO_2$.

### Virus preparation

The stocks of DV2 strain 16681 were prepared in C6/36 cell line and titrated by immunofluorescence technique and confirmed by reverse transcriptase polymerase chain reaction (RT-PCR)

### Cytotoxicity study of the compounds from *C. nutans*

The cell viability was evaluated by the percentage of the mean value of the optical density resulting from the cell control that set 100%. The 50% cytotoxic concentrations ($CC_{50}$) of compounds were calculated from the mean dose-response of three independent assays.

A549 confluents cells in a 96-well tissue culture plate were exposed to different concentrations of the compounds in RPMI medium and maintenance medium without compounds (as cell control) then incubated at 37˚C, 5% $CO_2$ for 72 hours. MTT assay was used to measure cell viability by adding a 20 µl of MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide; Sigma-Aldrich) into each well of the tested cells (final concentration of MTT = 5 mg/ml). After incubation 3 h at 37°C, the supernatant was removed and 100 µl of DMSO (dimethyl sulfoxide) was added into each well to dissolve the formazan crystal. After shaking 10 min, absorbance was measured by an ELISA reader at 595 nm.

### Screening for anti-viral activity

DV2 was treated by incubation in the absence or presence of the compounds in the subcytotoxic concentration at 37°C. After incubation 1 h, A549 cell monolayers grown in 24-well tissue culture plates were adsorbed with the 0.01 MOI of treated DV2 for 1 h at 37°C. After adsorption, the unadsorbed DV2 was removed by washing the cells with PBS twice. Then the cells were cultured in 2% FBS-medium with the compound in the subcytotoxic concentration. After 2 days of incubation at 37 °C, supernatant was collected for RNA extraction and the virus replication was evaluated by RT-PCR. After 5 days of incubation at 37 °C, the infected cells were collected for confirmation by immunofluorescence technique.

### Study of anti-viral activity in pre-incubation

The procedure was the same as the screening for anti-viral activity except after removing the unadsorbed viruses, the tested cells were cultured in medium without the compounds. Dextran sulfate sodium salt in RPMI was used as a positive control.

### Study of anti-viral activity in post-incubation

The procedure was the same as the screening for anti-viral activity except in the first step, the virus was adsorbed on the cells without incubation with the compounds. Ribavirin in RPMI was used as a positive control.

## RESULTS AND DISCUSSION

The structures of compounds 1-4 were identified as new compounds related to chlorophyll a and chlorophyll b related compounds as follow (Fig. 1)

**Compound 1** was obtained as a bright green powder. The MS (ESI-TOF) mass spectrum of 1 showed a molecular ion peak at m/z 923.6 [M+1]+; $C_{55}H_{70}N_4O_7Mg$ requires 922.5. The $^1$H-NMR and $^{13}$C-NMR data of compound 1 were found to be closely similar to those of compounds 3. It was therefore proved to have a chlorin ring system like compounds 3, except for the lack of two NH protons for chlorin (dihydroporphine) ring. Direct comparison of the $^1$H-NMR and $^{13}$C-NMR data of compound 1 with those of the known compound13$^2$-hydroxy-(13$^2$-S)-chlorophyll b[3] they were closely equivalent indicating that compound is 13$^2$-hydroxy-(13$^2$-S)-chlorophyll b (Fig. 1).

**Compound 2** was isolated as dark green powder. The $^1$H-NMR and $^{13}$C-NMR spectra of compound 2 showed similarity to that of the known compound phaeophorbide a methyl ester[3]. The $^1$H-NMR and $^{13}$C-NMR spectrum of compound 2 however, lacked the methyl ester signal at δ 3.57. Showed the interaction via multiple bonds between C and H giving the support to the assignments. Thus compound 2 was identified as phaeophorbide a (Fig. 1).

**Compound 3** was obtained as a green powder. The IR spectrum present of amine,hydroxyl, and ester functional groups. Direct comparison of the $^1$H-NMR and $^{13}$C-NMR data of compound 3 with those of the known compound

$13^2$-hydroxy-($13^2$-S)-chlorophyll b**1**[3] showed that they were closely equivalent indicated that compound **3** is $13^2$-hydroxy-($13^2$-S)-chlorophyll b (Fig. **1**).

**Compound 4** was isolated as a grayish green solid. The $^1$H-NMR and $^{13}$C-NMR spectra of compound 4 closely matched with those of compound 3 and purpurin 18.[3] The $^1$H-NMR and $^{13}$C-NMR data comparison showed similarity to purpurin 18 with an extra phytyl ester proton side chain. Thus compound 4 was identified as purpurin 18 phytyl ester (Fig. 1).

These compounds were investigated for anti-DV2 activity in A459 infected cell.The CC50 of the compound 1, 2, 3 and 4 were determined and showed 43, 25, 50, 50 µg/ml, respectively. The sub-toxic concentration used for testing of anti-viral activity were 34, 5, 20, 25 µg/ml, respectively.

In the screening step, it was observed that compound 2 suppressed DV2 replication in A549 cell but other compounds did not inhibit DV2 replication when the viral replication was detected by RT-PCR (Figure 2a) and immunofluorescence assay (figure not shown). The compounds were further evaluated anti-viral activity in pre-incubation and post-incubation in A549 cells. The result showed that the compound 2 could inhibit the DV2 replication in post-incubation whereas the other compounds could not (Figure 2c) and all of them could not inhibit DV2 replication in pre-incubation (Figure 2b).

Figure 2 DV2 RNA synthesis was analysed by RT-PCR. A549 cells were infected with DV2. Supernatant of infected cell were collected at 48 h post infection. (a) Screening for anti-viral activity, (b) Anti-viral activity in pre-incubation, (c) Anti-viral activity in post-incubation. Lane P = positive marker (511 nt); B = blank; 1 = compound 1; 2 = compound 2; 3 = compound 3; 4 = compound 4.

## CONCLUSION

Four compounds isolated from leave of *C. nutans* were evaluated for anti-DV2 activity. The results showed that compound 2 could inhibit the production of viral RNA as well as viral protein when the DV2 infected cells were cultured in the compound. This novel property should be further investigated for action on DV infection in the molecular level. This compound may be developed for treatment of DV infection.

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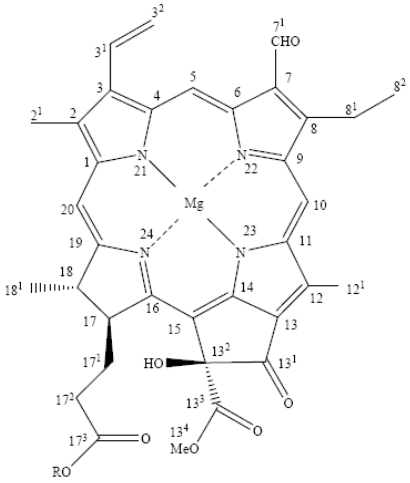

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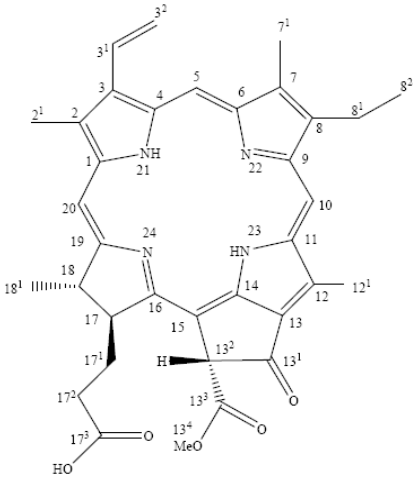

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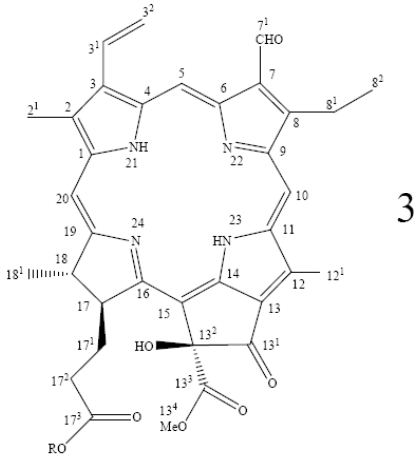

**3**

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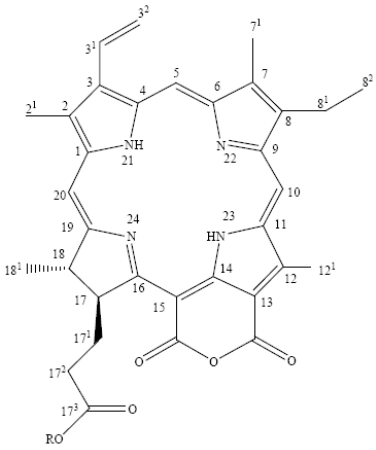

**4**

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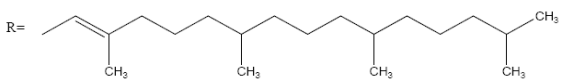

**Figure 1- Structures of compounds 1- 4.**

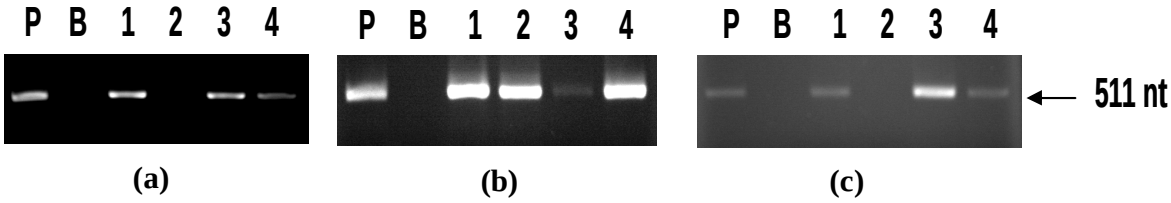


**Figure 2- DV2 RNA synthesis was analysed by RT-PCR.**

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RESEARCH ARTICLE

# Genetic Variations between Some Olive Varieties by AFLP and Protein Banding Pattern and their Impact to Breeding Programs

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## ABSTRACT

The present study aimed to quantifying the genetic diversity of some olive varieties from Al Jouf region, northwestern Saudi Arabia by using AFLP and protein banding pattern techniques and also some morphological features were mentioned. Morphological characters showed similarities between varieties under study such as shape of leaves, shape of fruits and flowering period.The AFLP analysis of six olive varieties generated a total of 220 fragments of which 168 were polymorphic, corresponding to 75.34% level of polymorphism.The protein banding pattern extracted from the leaves of six olive varieties showed clear differences in the number of variety's specific bands and their molecular weights that reflect the variations between studied varieties.

**Keywords :** *Olea europaea* , Genetic variations, AFLP, protein banding pattern, breeding programs.

## INTRODUCTION

Saudi Arabia is one of the largest countries in consuming of olives and olive oil in the world, but over the past 15 years it had become one of the world's new olive oil producing countries especially in Al Jouf region represented the study area located between 32.29° latitude and 42.37° longitude in the northwestern part of Saudi Arabia.Olive oil is

an important product due to its nutritional and health advantages in comparison to other vegetable oils (Rallo et al., 2000). Olive tree can be used as a bio indicator for pollutions (Abd El Hamid and Kamel, 2013 and Al-Ruqaie, et al., 2016). In the last years, expanded plantations of olive have been founded in the northern parts of Saudi Arabia using varieties gained from the neighboring countries (Al-Khalifah et al., 2012) such as Syria and Jordan, where many strange and domestic cultivars of olives are extensively cultivated. Al Jouf olive oil has a good quality due to the suitable climatic conditions in Al Jouf region are closely resembled to the conditions of Mediterranean basin in which olive plant grow effectively.Identification of olive varieties based only on morphological and biochemical is not sufficient because they are highly affected by environmental conditions (Contento et al., 2002). So, Morphological, biochemical and genetical markers were used to compare between six olive varieties (Zalmati, Chemlali, Chétoui, Oueslatia, Jerboui, Picual). DNA markers are used as a powerful genetic tool for comparison between different varieties due to its accuracy (Kojima et al., 1998).

AFLP protocol is based on the complete endonuclease restriction digestion of total DNA, followed by selective PCR amplification resulting in solitary, numerous fingerprint for each individual and a large number of informative markers (Pompanin *et al*., 2005 ; Cinzia et al. 2008 and Arrigo et al., 2012). AFLP is an environment-independent and efficient to identify varieties (Besnard et al., 2001 and Sensi et al., 2003). It is a very useful technique that does not require prior information of DNA sequence. AFLP is able to detect high levels of polymorphism and detect genetic relationships among varieties that were previously difficult to distinguish with morphological characters (Obae *et al*., 2013; Abd El Hamid, 2016 and Abdel Azim et al., 2016).

The present study aimed to quantifying the genetic variations of six olive varieties (Zalmati, Chemlali, Chétoui, Oueslatia, Jerboui, Picual) from Al Jouf region, by using AFLP and protein banding pattern. Combined morphological and molecular data was considered to be a useful tool for characterization and distinguishing between the different varieties of olive and giving a clear view about the genetic relationships between them that could be helpful in olive breeding programs.

## MATERIALS AND METHODS

Leaves of six varieties of *Olea europaea* L. were kindly obtained from Olive Research Unit, Camel and Research Center, Al Jouf, Ministry of Agriculture, Kingdom of Saudi Arabia.

### AFLP protocol

Vos et al., 1995 protocol was used, while modifications suggested by Kamisugi et al., 2008; Huang and He, 2010; Song *et al*,. 2011 and Mikulaskova et al. 2012 were followed. Primers/adaptors sets were all synthesized by Eurofins (Germany) (Table 1).Schluter and Harris, 2006 method for band scoring and assortment by Peak scanner (Applied bio systems and Microsoft Excel 2013 FAMD software) was used to perform the phylogenetic analysis and Treegraph2 programs to visualize the produced tree (Stover and Muller, 2010).The analysis of the AFLP data was performed in two known forms. The first is based on the allele frequency (number of a band presence relatively to the number of all individuals) and the second is based on the band binary criterion (codifying the detected bands to 0 when absence and 1 when presence). Both forms were combined to obtain the maximum number of valuable indices according to Bonin et al., 2007.

### Protein banding pattern

Protein was extracted from leaves of each variety and analyzed using SDS-PAGE according to the method of Weber and Osborn (1969). Half gram of each leaf sample was extracted with 1 ml extraction buffer. Samples were centrifuged for 15 min at 14.000 rpm at 4°C. Supernatants containing proteins were transferred to fresh tubes. Electrophoresis was carried

out in 12% gel concentration and in Tris-Glycine running buffer (pH=8.3). Standard protein marker was loaded with molecular weights 100, 80, 55, 33, 22, and 18 kDa. Gels were run at 45 volts for 15 min, then, the voltage was raised to 80 volts till the samples reached 2 cm from the bottom of the gel. Gels were stained by Coomassie brilliant blue-R 250 (0.5 g/l) and destained by 5% MeOH/acetic acid mixture. Destained gels were photographed and analyzed by Gel Doc 2000. Bio-RadTM diversity database V.2.1.1.

### Statistical analysis

The pairwise genetic similarity coefficient (GS) was calculated using Jaccard coefficient (Jaccard, 1908) by program package for personal computer NTSYS-pc software version 2.02 (Rohlf, 1998).

## RESULTS

### Morphological characters

The differences in some morphological characters among the six olive varieties are shown in table (2).

### Variety's molecular markers for different olive varieties by AFLP analysis

The AFLP results of the six olive varieties with the three primer combinations generated a total of 220 fragments (Table 3) of which 168 were polymorphic, corresponding to 75.34% level of polymorphism (Table 3). The number of total bands produced by each primer combination ranged from 51 to 91 with an average of 74.33 bands. The percentage of polymorphism varied extremely among the primer combinations. The lowest ratio of polymorphism generated by Eco + AGG / Mse + CTC (70.37%), while Eco + ATA / Mse + CTC produced the highest polymorphism (80.00%).

### Variety's molecular markers for different olive varieties by protein banding pattern

The protein extracted from the leaves of six olive varieties showed clear differences in the number of variety's specific bands and their molecular weights (Figure 1).The electropherogram of Zalmati showed the presence of 8 variety's specific protein, their molecular weights ranged between 18.066 and 91.147kDa. On the other hand, the results of Chemlali showed the presence of 7 variety's specific protein with molecular weights ranged between 17.632 and 90.637kDa. Chétoui electropherogram showed the presence of 11 variety's specific protein, their molecular weight ranged between 16.452 and 97.316 kDa, while the results of Oueslatia revealed the appearance of 11 variety's specific protein, their molecular weight ranged between 16.269 and 88.459 kDa, Jarboui results demonstrated the presence of 4 variety's specific protein, their molecular weight ranged between 16.269 and 81.012 kDa and electropherogram of Picual showed the presence of 13 variety's specific protein, their molecular weights ranged between 16.452 and 100.086 kDa. (Figure 1).

### Genetic relationships

The genetic similarities among the studied six olive varieties based on Nei's method (Nei, 1978) and (Saitou and Nei 1987). The highest pairwise similarity indices resulted from AFLP were between Chemlali and Chétoui, while the lowest similarity indices were between Zalmati and Jarboui. (Table 4).The dendogram produced by Jaccard's coefficient and the UPGMA tree produced by the AFLP and protein banding pattern data divided the six olive varieties into two distinct groups at a distance of 0.50. Group 1 included Zalmati and group 2 subdivided into two clusters at a distance of 0.55. The cluster 1 included Chemlali and Chétoui. The cluster 2 subdivided into two sub clusters at a distance of 0.62, sub cluster1 included Oueslatia and Picual while the other sub cluster include Jarboui

only (Figure 2). However, the analysis of molecular data revealed more diversity among the six olive varieties compared to morphological criteria. Distance scale bar is shown FAMD software (Schluter and Harris, 2006) was used to perform the phylogenetic analysis and Treegraph2 programs to visualize the produced tree (Figure 2) (Stover & Muller, 2010).

## DISCUSSION

Characterization of olive varieties based only on morphological and biochemical characters is not sufficient because they are highly affected by environmental factors that affect the chemical composition and phenotype. So, Morphological, biochemical and genetical markers were used to compare between six olive varieties (Zalmati, Chemlali, Chétoui, Oueslatia, Jerboui, Picual). DNA markers are used as a powerful genetic tool for comparison between different varieties due to its accuracy (Alessandri et al., 1997 and Contento et al., 2002).The results from AFLP and protein banding pattern (Table 4) based on Nei's method (Nei, 1978) and (Saitou and Nei 1987) showed highest similarity indices between Chemlali and Chétoui, this may indicate that the two varieties have the same ancestor (Ben Ayed *et al*., 2015 ), while the lowest similarity indices were between Zalmati and Jarboui that demonstrated a high levels of diversity either among cultivars that originated from the same country (Muzzalupo et al. 2009) and consistent with similar studies and reflect the high dimensionality of the data (Leigh et al. 2005). These results were concomitant with the dendogram that grouped Chemlali and Chétoui in one cluster while Zalmati and Jarboui in different clusters.

The high number of unique markers observed in this study represents a very useful tool for certification of olive varieties (Kaya et al., 2013 and Belaj, et al., 2015). AFLP marker used in this study showed a high level of polymorphism in all of the olive varieties examined in the present work and this with agreement with Angiolillo, et al., 1999; De la Rosa, et al., 2003; Bandelj et al., 2004; Grati-Kamoun, et al., 2006; Baldoni et al., 2006 and Taamalli et al., (2007) obtained similar results regarding the number of bands per primer pair.Genetic identity seems to be the way for characterization of the varieties (Busconia et al., 2003). DNA markers could improve the management of plant genetic resources in crop improvement and plant breeding programs. (Michael Lee, 1995 and Peter et al. 1996). So, it is obvious that AFLP markers combined with protein banding pattern could be a useful tools for identification and differentiation of Al Jouf olive varieties and cleared the relationship and the genetic variations between them and could be helpful in olive breeding programs in which varieties with a wide genetic distance may be used as parents to crosses for taking advantage of heterosis and for making mapping populations in the QTL mapping studies and could help the breeders to register new varieties and development of modern olive culture toward typical olive oil (Zitoun et al 2008; Rao, et al, 2009; Koehmstedt, *et al*., 2010 and Hegazi et al., 2012).

## CONCLUSION

Results revealed that AFLP technique combined with protein banding pattern generated a large number of polymorphisms so, it is considered to be a useful tool for characterization and distinguishing between the different varieties of olive and giving a clear view about the genetic relationships between them that could be helpful in olive breeding programs.

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**Table 1 : Primers/adaptors names and sequence of primers at 5´→3´ used to establish the AFLP technique.**

| Primer/Adaptor | sequence 5´ →3´ |
|---|---|
| *EcoRI*– A1 | CTCGTAGACTGCGTACC |
| *EcoRI*– A2 | AATTGGTACGCAGTC |
| *Mse I*– A1 | GACGATGAGTCCTGAG |
| *Mse I*– A2 | TACTCAGGACTCAT |
| *Eco* + A | GACTGCGTACCAATTCA |
| *Mse* + C | GATGAGTCCTGAGTAAC |
| *Eco* + ACA | FAM-GACTGCGTACCAATTCACA |
| *Eco* + AGG | HEX-GACTGCGTACCAATTCAGG |
| *Eco* + ATA | CY3-GACTGCGTACCAATTCATA |
| *Mse* + CTC | GATGAGTCCTGAGTAACTC |

**Table 2 : The differences of some morphological characters among the six olive varieties.**

| Morphological characters | Zalmati | Chemlali | Chétoui | Oueslati | Jerboui | Picual |
|---|---|---|---|---|---|---|
| **Vigor** | medium | high | weak | weak | medium | medium |
| **Shape of Leaves** | elliptical, lanceolate | elliptical, lanceolate | elliptical, lanceolate | elliptical, lanceolate | elliptical, lanceolate | elliptical, lanceolate |
| **Shape of fruits** | Ovoid | Ovoid | Ovoid | Ovoid | Ovoid | Ovoid |
| **Average weight of Fruit** | 1.5g | 1.2g | 2.8g | 2.5 g | 2.8g | 2-4g |
| **Flowering period** | Early | Early | Early | Early | Early | Medium |
| **Production** | High | Very high | Average | Medium | low | High |
| **Utilization** | Oil | Oil | Oil | Oil and Table | Oil | Oil |

**Table 3 : Number of total bands, polymorphic bands and polymorphism ratio of six olive (*Olea europeae* L.) varieties generated by three AFLP primer combinations.**

| Enzyme – primer combinations | Polymorphic bands | | Total No. of bands | Polymorphism % |
|---|---|---|---|---|
| | Variety's specific bands | Non DNA markers | | |
| **Eco + ATA / Mse + CTC** | **72** | **19** | **91** | **80.00** |
| **Eco + ACA / Mse + CTC** | **39** | **12** | **51** | **76.47** |
| **Eco + AGG / Mse + CTC** | **57** | **24** | **81** | **70.37** |
| **Total** | **168** | **55** | **223** | **-** |
| **Mean** | **56** | **18.33** | **74.33** | **75.34** |

**Table 4 : Similarity matrices based on AFLP and protein banding patttern for the studied six olive varieties.**

| | Zalmati | Chemlali | Chétoui | Oueslatia | Jarboui | Picual |
|---|---|---|---|---|---|---|
| **Zalmati** | 1.00 | | | | | |
| **Chemlali** | 0.55 | 1.00 | | | | |
| **Chétoui** | 0.50 | 0.65 | 1.00 | | | |
| **Oueslatia** | 0.49 | 0.56 | 0.63 | 1.00 | | |
| **Jarboui** | 0.47 | 0.53 | 0.58 | 0.63 | 1.00 | |
| **Picual** | 0.51 | 0.55 | 0.53 | 0.64 | 0.60 | 1.00 |

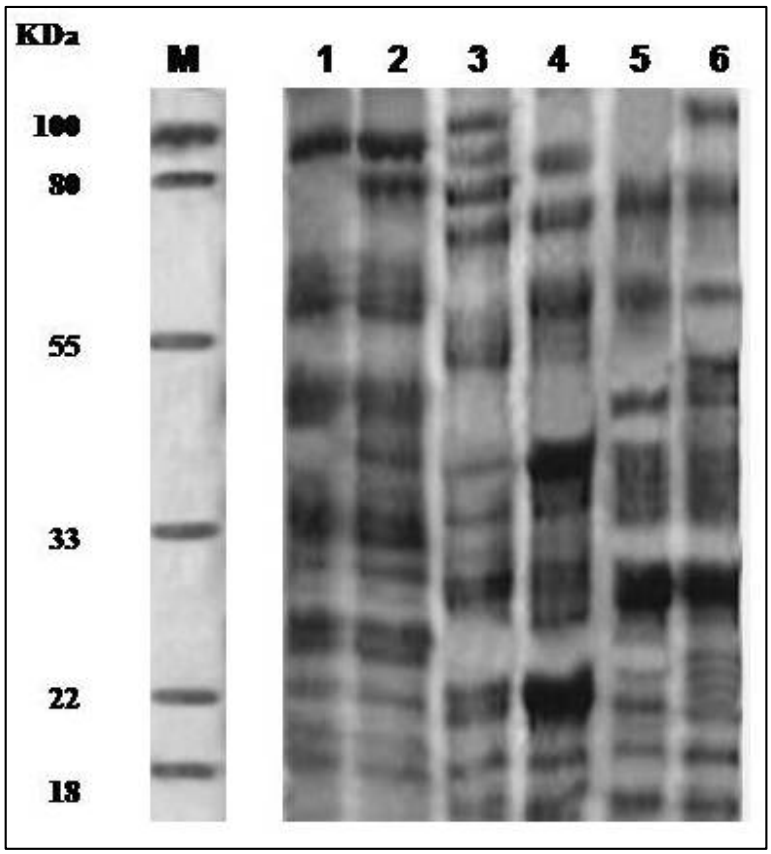


**Figure (1): Electrophoretic banding profiles of protein extracted from the leaves of six olive (*Olea europeae* L.) varieties. Lane (M): Protein Marker**

| | |
|---|---|
| **1- Zalmati** | **2- Chemlali** |
| **3- Chétoui** | **4- Oueslatia** |
| **5- Jarboui** | **6- Picual** |

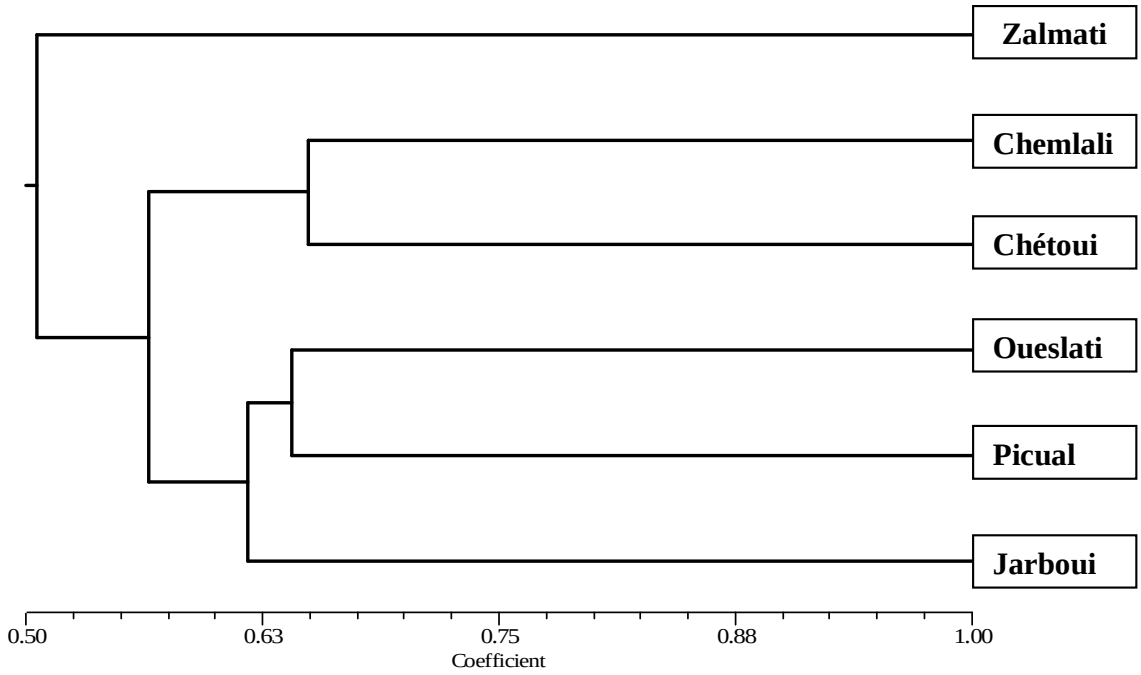


**Figure (2): Neighbor-joining (NJ) tree (Jaccard similarity coefficient ) based on AFLP and protein banding patttern for the six olive (*Olea europeae* L.) varieties.**

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RESEARCH ARTICLE

# Electronic Properties of *Al* $As_x$ $P_{1-x}$ Alloying Composition Nanocrystal, Using Density Functional Theory

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## ABSTRACT

The structural and the electronic properties of III-V zinc-blende AlP , AlAs semiconductors nanostructure and their alloying composition $AlAs_xP_{1-x}$ have been studied in details using ab-initio density functional theory (Ab-initio DFT) at the generalized gradient approximation(GGA) level coupled with large unit cell (LUC) approximation and STO-3G basis set. These calculations for 8 core atoms with concentration of (x=0, 0.25, 0.5, 0.75 and 1) with 3D periodic boundary condition (PBC) effect on electronic properties such as energy gap valance and conduction band width and density of states were included.Gaussian 03 program are prepared to perform computation. Position and properties of atoms which compose these crystals has been used as input data .The final modified LUC-DFT equations are embodied in these computer routines and solved by iterative methods. Results show that the lattice constant and energy gap are increased with increasing the arsenide alloy concentration. The total energy, cohesive energy, electron affinity, ionization potential and iconicity have been reported for these concentrations.

**Keywords :** Ab-initio, DFT, $Al\ As_xP_{1-x}$ alloying composition.

## INTRODUCTION

Recently, III–V zinc-blende semiconductors compounds have become an area of great technological activity. The reason for this is the possibility of producing novel materials with adjustable electronic and magnetic properties. Among them, the aluminum compounds AlAs and their alloy $\boldsymbol{AlAs_xP_{1-x}}$ are concerned in this paper. AlAs is one of the most important electronic and optoelectronic materials because of its frequent incorporation into GaAs-based

hetero structures. Aluminum arsenide, with the largest direct gap of the III–V compound semiconductors, is undoubtedly the most ''exotic'' and least studied [1,2]. However, in recent years, it is attracted special attention to its incorporation in the AlAs/AlP and GaP/AlP based hetero structures. AlAs/AlP super lattices are attractive due to their potential applications in optoelectronic devices because they are expected to become direct band gap materials [3]. GaP/AlP-based hetero structures are attractive in their characteristics for the development of optoelectronic devices operating in the yellow-green spectral region [4,5] and are considered as an alternative to a GaN/AlGaN system for the development of infrared semiconductor lasers and detectors. Many research groups were used FP-LAPW method within local density approximation (LDA) of the exchange – correlation energy to calculate the electronic and optical properties respectively [6-9]. Briki et al, were studied the effects of relativistic on the structural and transport properties of III–V compounds utilizing LDA and PBE-GGA for the exchange– correlation energy [10, 11].

The objective of this work is to combine AlP and AlAs compounds which having different structural and electronic properties to obtain new materials. This could open a new and promising field if it transforms the material's properties by making them similar to the ternary $AlAs_xP_{1-x}$ alloys with intermediate properties.

## METHODOLOGY

### Theory and calculations

The basic idea of large unit cell (LUC) is in computing the electronic structure of the unit cell extended in a special manner at k=0 in the reduced Brillion Zone. This equivalent to a band structure calculation at those k-point, which transform to Brillion Zone center on extending the unit cell. Using linear combination of atomic orbitals (LCAO), the crystal wave function uses the density functional theory at the generalized gradient approximation method level [11, 12].Kohn-Sham density theory [11]is widely used for self consistent – field electronic structure calculations of the ground state properties of atoms, molecules, and solids. In this theory, only exchange – correlation energy $E_{XC} = E_X + E_C$ as a functional of the electron spin densities $n_\uparrow(r)$ *and* $n_\downarrow(r)$ must be approximated. The local spin density (LSD) approximation:

$$E_{XC}^{LSD}[n_\uparrow, n_\downarrow] = \int d^3r \;\; n \in_{XC}^{unif} (n_\uparrow, n_\downarrow) \qquad (1)$$

Where $n = n_\uparrow + n_\downarrow$, and the generalized gradient approximation (GGA) [13,14].

$$E_{XC}^{.GGA}[n_\uparrow, n_\downarrow] = \int d^3r \; f(n_\uparrow, n_\downarrow, \nabla n_\uparrow, \nabla n_\downarrow) \qquad (2)$$

In comparison with LSD and GGA's tend to improve total energy, atomization energies, energy barriers and structural energy differences.

To simplify particle calculations, $\in_{XC}^{unif}$ and f must be parameterized analytic functions. The exchange-correlation energy per particle of a uniform electron gas, $E_{XC}^{LSD}(n_\uparrow, n_\downarrow)$, is well established [15]but the best choice for $f(n_\uparrow, n_\downarrow, \nabla n_\uparrow, \nabla n_\downarrow)$ is still a matter of debate.The geometrical optimization calculations are performed with simultaneous optimization and complete convergence of maximum displacements, root mean square(RMS) displacements, maximum forces and RMS forces of all atoms in the nanocrystal. For example, RMS forces are optimized to less than 0.0003 Hartree/Bohr which is the standard convergence limit of Gaussian03program [16].

## RESULTS AND DISCUSSION

Figs.1 and 3 show the total energy as a function of the lattice constant optimization of 8 core atoms per LUC for AlP and AlAs respectively, and Figs. 2 and 4 show The optimized geometrical structure of AlP and AlAs nanocrystal alloy for 8 core atoms per LUC which calculated by Gaussian 03w and Gaussview3.0 as a complementary program, has a cubic structure shape (Bravais cell) with a translation vectors a (1, 0, 0), a (0, 1, 0) &a (0, 0, 1), while for $AlAs_{0.5}P_{0.5}$ is shown in Figs.5 and 6. It is clear from these figures that the stability of the nanocrystal is at equilibrium when lattice constant is equal to (0.53, 0.56)nm of AlP and AlAs, while it is equal 0.54 nm for $AlAs_{0.5}P_{0.5}$ , and these indicate that the attraction and repulsion forces between atoms are equal [17-19].

Fig.7 shows last HOMO (Ionization potential (I.P)) and first LUMO(electron affinity (E.A))of$AlAs_xP_{1-x}$alloyingcomposition as a function of concentration of As for 8 core atoms per LUC which indicate that the energy value of (E.A) is greater than (I.P) ,while the energy of both AlP and AlAs are greater than other alloying composition .The relation between the valence and conduction bands width as a function of concentrations of As atoms were calculated it's shown in Fig .8. It is appear that the bands width decreased with increasing the concentrations of arsenide atoms. While, the valence band generally higher than conduction band and this are in a good agreement from the obtained results by[17,20,21,22].

Fig. 9 shows increment of lattice constant with fraction of arsenide. It is found that when x = 0, the lattice constant is about 0.53nm corresponds to AlP whereas, it is equal 0.56nm for AlAs when x = 1. These results are corresponding to experimental bulk value of 0.545 and 0.565 nm for AlP and AlAs respectively [23,24].The obtained value of lattice constant is acceptable taking into consideration the usual systematic error inhibited in molecular orbital calculations [25, 26]that underestimates this property especially for high atomic number elements such as germanium [27].Fig.10 shows the Density of states as a function of energy level of $AlAs_{0.5}P_{0.5}$of 8 Core atoms. The density of states has maximum of 7withconcentration for x=0.5 for considered nanocrystals. Valence band and conduction band are shown in figure. The energy gap is shown between the two bands (Eg=4.37 eV).Fig. 11 shows increment of maximum density of states with fraction of arsenide. It is clear from this figure that the density of state increases with increasing Arsenide atoms.

## CONCLUSION

The above results show that many properties of $AlAs_xP_{1-x}$nanocrystals change abruptly at the nanoscale. The obtained results show that the lattice constant and density of state increase with increasing the arsenide concentration in $AlAs_xP_{1-x}$alloy. The energy gap varies a fluctuated value with respect to increasing the arsenide concentration. It is found that the equilibrium lattice constant and cohesive energy are in reasonable agreement with experimental result. The valence and conduction bands decrease with increasing the *As* concentration in $AlAs_xP_{1-x}$alloy Composition.

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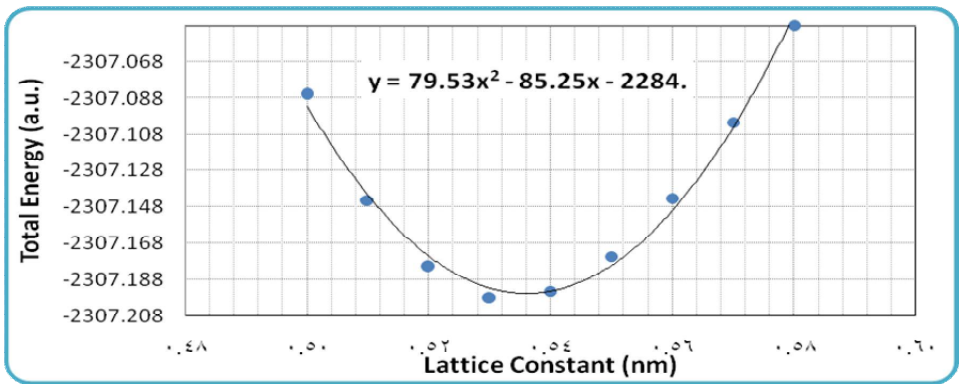


**Fig.1.Total energy as a function of lattice constant of AlP for 8 core atoms per LUC.**

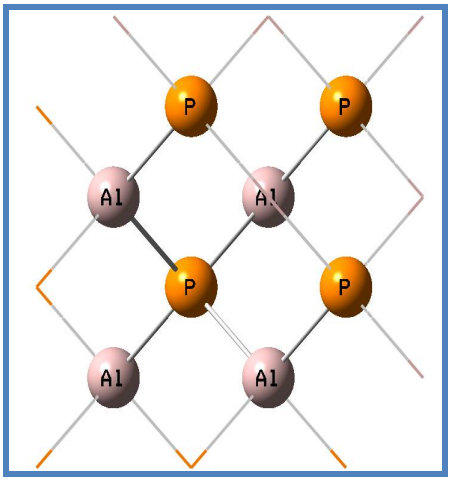


**Fig. 2.(color online) AlP 8 atoms core LUC (cubic shape Bravais cell multiple).**

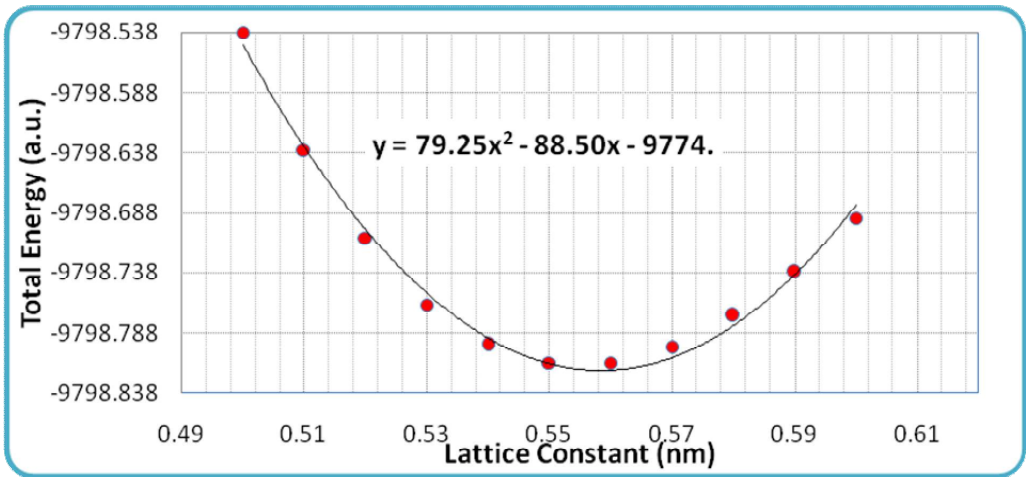


**Fig . 3 Total energy as a function of lattice constant of AlAs for 8 core atoms per LUC.**

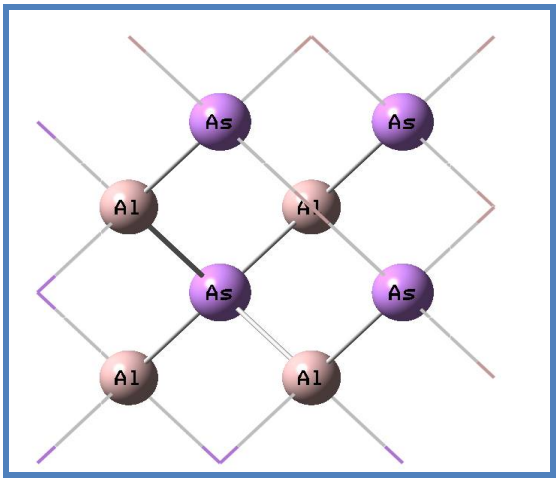


**Fig.4.(color online) AlAs 8 atoms core LUC (cubic shape Bravais cell multiple).**

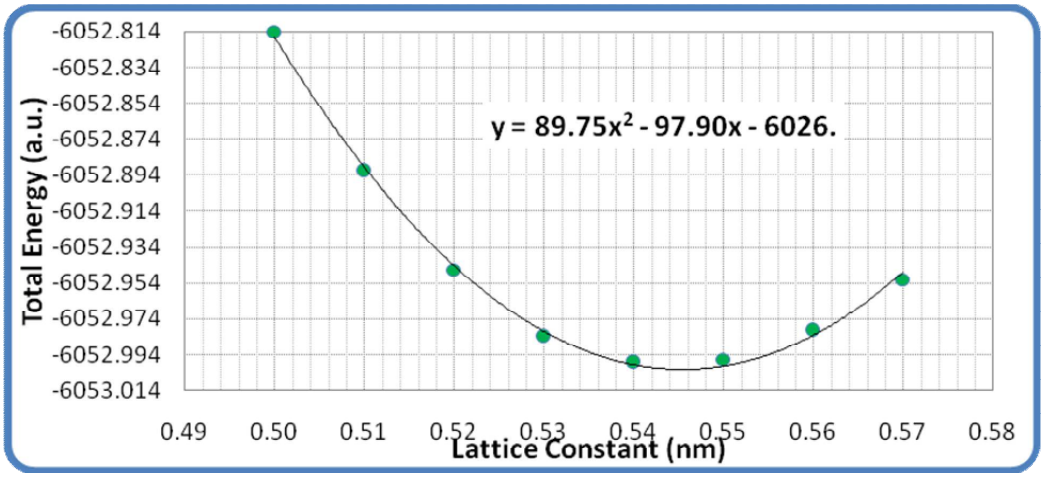


**Fig. 5.Total energy as a function of lattice constant of $AlAs_{0.5}P_{0.5}$ for 8 core atoms per LUC.**

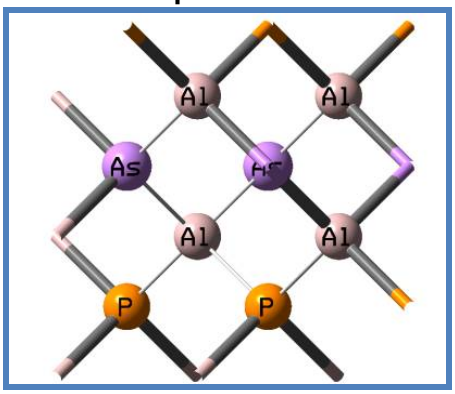


**Fig.6.(color online) $AlAs_{0.5}P_{0.5}$ 8 atoms core LUC (cubic shape Bravais cell multiple).**

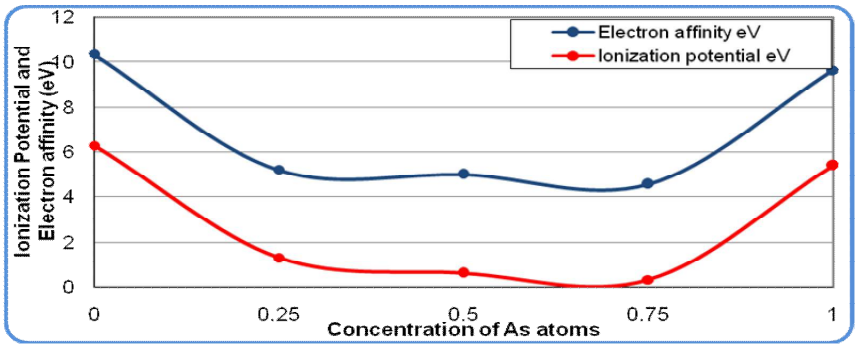


**Fig . 7. Ionization potential and electron affinity of $AlAs_xP_{1-x}$alloying composition as a function of concentration of As for 8 core atoms per LUC.**

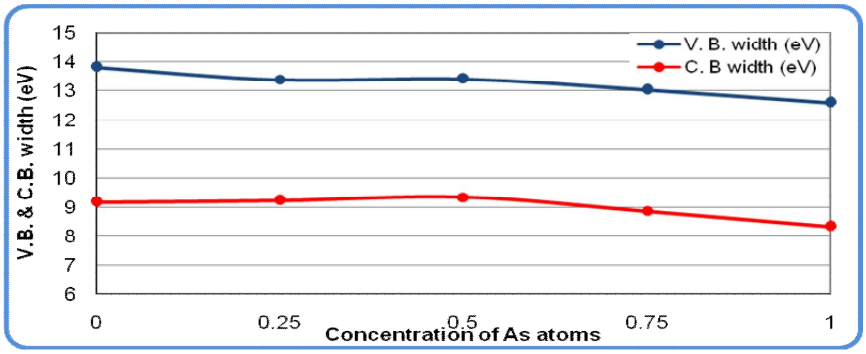


**Fig . 8.Valence and conduction bands width of $AlAs_xP_{1-x}$alloying composition as a function of concentration of As for 8 core atoms per LUC.**

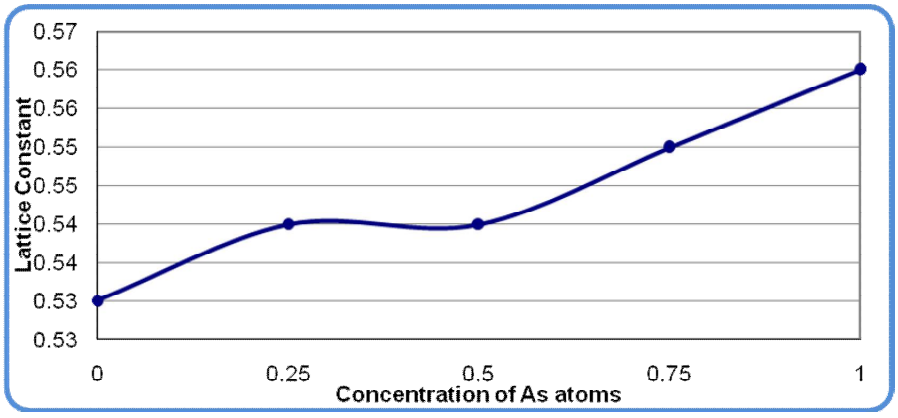


**Fig . 9.Lattice constant of $AlAs_xP_{1-x}$alloying composition as a function of concentration of As for 8 core atoms per LUC.**

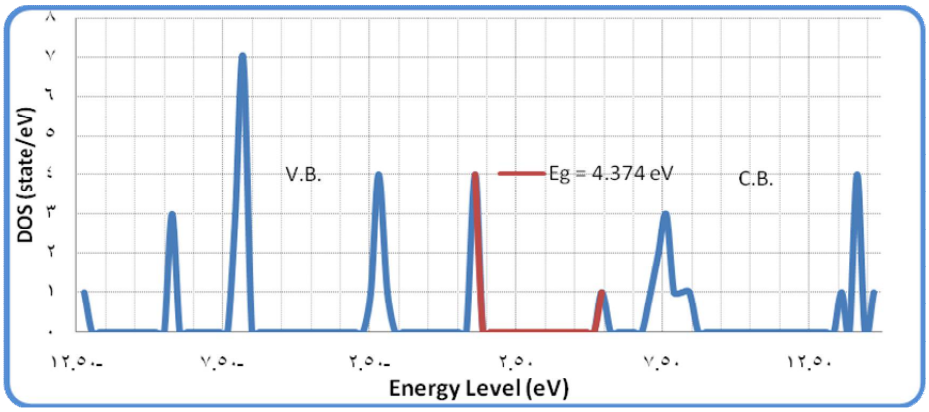


**Fig. 10.Density of states of $AlAs_{0.5}P_{0.5}$ alloying composition as a function of energy level for 8 core atoms per LUC.**

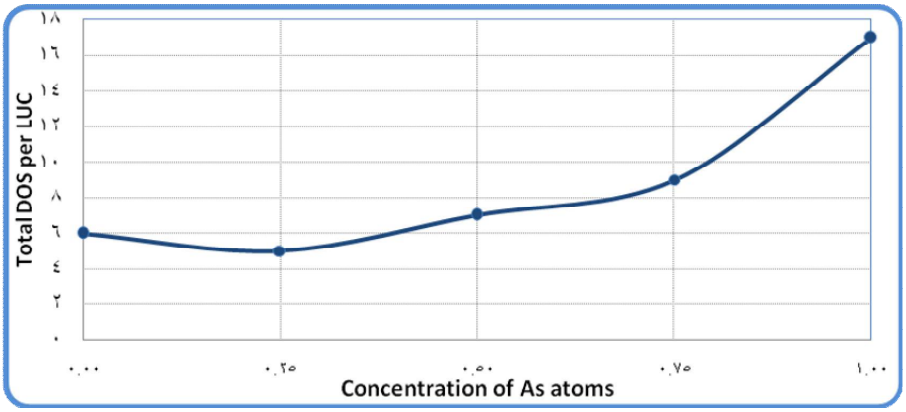


**Fig . 11.Total DOS of $AlAs_xP_{1-x}$ alloying composition as a function of concentration of As for 8 core atoms per LUC.**

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RESEARCH ARTICLE

# Effect of Annealing Temperature on the Structural and Morphological Properties of Ge–Sb Thin films

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## ABSTRACT

The structural characteristic of the films prepared on glass substrates have been studied by using X-ray diffraction, which show that the films have amorphous structure for sample annealed at $T_a \leq 573K$. The samples were annealed at 673 K showed a polycrystalline with Face-Center cubic system preferred orientation along (111).The surface morphological characteristics by atomic force microscope (AFM), showed decrease in roughness with increasing annealing temperature for amorphous film but start upward when the films crystallized .When the film annealed up to temperatures (673,773) K the grain size increases with increasing annealing temperature.

**Keywords :** Thin films, Ge:Sb thin film, structural properties, x-ray diffiraction, morphological properties.

## INTRODUCTION

Ge was the choice of material used in the first bipolar invented transistor in 1949 by Bardeen, Brattain, and Shockley [1].It has diamond structure; this structure belongs to the cubic class, with a face-centered cubic (F.c.c) lattice. The characteristic of a-Ge thin film depend sensitively on the many deposition parameters such as deposition method, nature and kind of substrate, substrate temperature, annealing treatments, rate of deposition, impurities in the starting material , etc. [2,3].Edelman et al., [4] have prepared amorphous germanium thin films with different thicknesses by electron gun-evaporation and annealed at temperature range between (523 - 773)K .They concluded that a-Ge films crystallize at annealing temperature equal 573K for 1hr.

Li et al., [5] prepared polycrystalline and amorphous Ge thin film by low-pressure chemical vapor deposition (LPCVD) system at respective temperatures of 537K and 598K. They found that Ge possesses unique characteristics that are complementary to those possessed by Si.Sakaike et al. [6] deposited germanium films on quartz substrate by inductively-coupled plasma enhanced chemical vapor deposition. They found that germanium crystallized in direction (111),(220),(311).Ge thin films with a thickness of about 110 nm was prepared by electron beam and annealed in air at 100–500 C for 2 h. Their structural properties were studied as a function of annealing temperature. The films annealed at 400 and 450C exhibit x-ray diffraction pattern of Ge with cubic-face structure. The AFM show that the surface roughness decrease with increase $T_a$ in amorphous phase but increases in crystalline phase[7].Capellini G. et al. [8] found that , during the growth of thin films, or *ex-situ* where the ion implantation is the only non-equilibrium technique allowing to introduce impurities into semiconductors significantly above the solid solubility limit. To this day, the highest reported value for the density of electrically active donors in P doped Ge was $1.74 \times 10^{20} cm^{-3}$ with 44% of the electrically active dopants.Prucnal et al.[9] reported that the fast annealing method using flash lamp annealing (FLA) to recrystallize Ge layers amorphized during ion implantation and to activate P dopants. It is shown that P implanted Ge with an amorphous layer thickness above 200 nm can be completely recrystallized without a significant diffusion of P.

Peng et al.[10] investigated Low-temperature Al-induced crystallization of hydrogenated amorphous Ge films by x-ray diffraction,. By investigation of the influence of the annealing temperature on the microstructures and electrical properties of the Ge thin films. It can be seen that the Al-induced layer exchange significantly promotes the crystallization of the amorphous Ge thin films at 250°C and there is an enhancement in film crystallinity and grain size with the increasing of the annealing temperature. Also, the sheet resistance was decreased significantly with the increasing of the grain size.In this paper we investigated the effect of annealing temperature on the structural and morphological properties of Ge–Sb thin films.

## MATERIAL AND METHODS

Ge:Sb thin films were prepared by thermal evaporation technique in vacuum system supplied by Blazers Model (BL 510). All samples were prepared under constant condition (presser, substrate temperature and rat of deposition).The structure of the Ge:Sb films grown on glass substrates and treated at different annealing temperature have been examined by x-ray diffractions using a Philips x-ray diffractometer system which records the intensity as a function of Bragg's angle. The source of radiation was cu($k_\alpha$) with wavelength $\lambda$=1.5406Å, the current was 30mA and the voltage was 40 kv. The scanning angle 2θ was varied in the range of (20 – 60) degree with speed of (4) deg/min.Atomic Force Microscopy studies were recorded by using (Scanning probe Microscope type AA3000), supplied by Angstrom Advanced Inc. to determine the Nano spikes dimensions range of the prepared Ge:Sb on glass substrate and their statistical distribution .

## RESULTS AND DISCUSSION

### Structural properties (x-ray diffraction analysis)

It is possible to find the crystallinity structure of the film and its growth nature through the study of x-ray diffraction (XRD). The XRD results of Ge doped with 1%Sb (Ge:Sb) films prepared on glass substrate at room temperature with thickness 0.5 µm at different annealing temperatures (373, 473, 573,673 and 773)K are shown in Fig.( 1).This figure shows a non-crystalline structure of the as-deposited films. Upon annealing at temperatures of (373, 473 and 573) K, the films appear almost in an amorphous form. Further raise of the annealing temperature up to 673K, the crystallinity is improved. At $T_a \geq$ 673K, the peaks become more sharp and the oriented in (111) increase with $T_a$. The films are fully transformed into a crystalline phase of germanium, which has the face-centered cubic structure, this behavior agreement with the results of Abdul F. et al.[7] and Tsuji et al.[11].

Also we found that the crystal growth which effected by doping the a-Ge by Sb and this behavior may be reduce the annealing temperature for recrystallization of a-Ge films. Farther results have been pointed by Akl. et al. [12] that recrystallization of Ge thin films at 773 K. In addition, The different peaks in the figure(1) are indexed in Table (1) as well as the corresponding values of the inter planar spacing $d_{(hkl)}$ which were compared with the standard values of ASTM data. Moreover, the appearance of multi-reflection peaks is characterized by the single phase of germanium having the lattice parameter $a_o$= 5.656 Å, which is very close to the value given in this card ($a_o$ =5.657 Å).

The variation of the intensity ratio of the (220), (311) and (111) planes as a function of the annealing temperature are recorded in Table (2). It is obvious that the change in intensity is very pronounced in the annealing range. One may conclude that, for any annealing temperature, the preferred orientation along (111) orientation is observed.

We observe from the Table (2) that the crystallinity of the film increases when the annealing temperature increases. The increase in the intensity of the peaks may be attributed to grain growth associated with higher temperatures. It is also clear that the films grow with preferred orientation along the <111> direction as the annealing temperature increases.

### Line broadening analysis

The microstructural parameters of the prepared Ge:Sb thin films were determined using profile analysis. The three planes (111), (220) and (311) were used for the calculation and the average values were taken for the crystallite size and macrostrain

### Crystallite size

Crystallite sizes for all films were calculated by using Scherrer equation . The variation of the crystallites size for the (111),(220)and (311) planes as a function of annealing temperature are shown in Table (3).It is seen from this table that when the annealing temperature increased from 673 to773 K the FWHM value exhibited a tendency to decrease. The trend of FWHM values implied that the crystallinity of the Ge:Sb thin films was improved as the annealing temperature was increased[13]. Another important feature is observed in Table (3) that average grain size was increased with increasing annealing temperature.These results indicate that the thermal annealing induced coalescence of small grains by grain boundary diffusion which caused major grain growth.

### Macrostrain

The macrostrain |e| of the investigated samples was determined using Voigt method formula [12] :

$$|e| = \frac{\Delta d}{d_o} = 1/2 \cos\theta \, \Delta\theta$$

Where $\Delta d$ is (d-$d_o$) ,$d_o$ is the standard value of the interplanar spacing taken from JCPDS data file and d is experimental value of the interplanar. The macrostrain was calculated as an average of fraction al change, $\Delta d/d_o$, in the interplanar spacing, d of the three diffraction planes (111), (220) and (311). The calculated macrostrain of the investigated samples is given in Table (4).From this Table we can see that the values of macrostrain have the same order at different annealing temperatures. It is clear that, the thermal annealing is lightly affected on the macrostrain values, this is due to the power density of ions was nearly remain unchanged when the annealing temperature increases[12].

**Morphological properties of Ge:Sb thin film**

The surface morphology of the Ge:Sb films as observed from the AFM micrograph confirms that the grains are uniformly distributed. Fig (2) shows the structure of Ge:Sb thin films have been deposited on glass substrates and annealed at temperature (473, 573, 673,773) K. We can notice that Ge:Sb/glass films deposited at room temperature substrate and annealed to 573K are amorphous, while the films annealed to (673,773)K are crystalline in nature and the grains are packed very closely.Table (5) show the value of average roughness and average grain, it is observed from this Table that the average roughness value decreasing with increase the annealing temperature for amorphous film that due to the rearrangement of atom in film and reduce the vacancy defect. While the average roughness for films annealed at annealing temperature (673,773)K increasing with increase $T_a$, this behavior is agreement with Abdul F.K. et al[7]. This indicates that the growth of larger grains with increasing temperature leads to an increase in the surface roughness. Also it is observed that the average grain size increases with increasing of annealing temperature. This results are nearly in agreement with Peng et al[10].

## CONCLUSION

1. Structure of the prepared Ge:Sb on different substrate is amorphous that a change to Polycrystalline after annealing process at high $T_a > 573K$ and the crystal lattice is fcc.
2. The average roughness value decreasing with increase the annealing temperature for amorphous film While increased for films annealed at temperature (673,773)K

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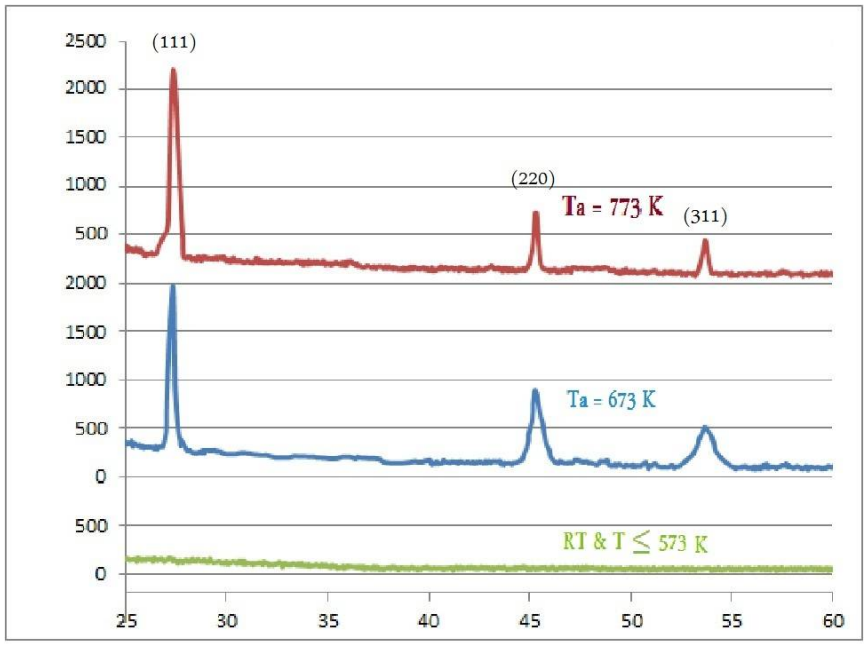


**2Θ(degree)**

**Figure (1): XRD patterns of Ge:Sb films prepared at different annealing temperature**

**Table 1. X-ray diffraction data for Ge:Sb compound as thin film**

| $T_a$ (K) | Hkl | $d_{stand.}$ (Å) | $2\theta_{exp.}$ | $d_{exp.}$ (Å) |
|---|---|---|---|---|
| 673 | (111) | 3.266 | 27.315 | 3.2622 |
| | (220) | 2.00 | 45.285 | 2.0008 |
| | (311) | 1.706 | 53.671 | 1.7063 |
| 773 | (111) | 3.266 | 27.334 | 3.2601 |
| | (220) | 2.00 | 45.316 | 1.9995 |
| | (311) | 1.706 | 53.694 | 1.7056 |

**Table 2. The variation of the intensity ratio of the (220), (311) and (111) planes**

| $T_a$ (K) | I(2 20)/I(111) | I(311)/I(111) |
|---|---|---|
| 673 | 0.452 | 0.256 |
| 773 | 0.333 | 0.202 |

**Table (3) the variation of FWHM and the Crystallite sizes for the (111),(220)and (311) planes with different $T_a$**

| $T_a$ (K) | FWHM | | | Grain size D(n) | | | |
|---|---|---|---|---|---|---|---|
| | 111)( | 220)( | 311)( | 111)( | (220) | (311) | Average Size |
| 673 | 0.47 | 0.68 | 0.945 | 19.32 | 14.06 | 10.46 | 14.62 |
| 773 | 0.45 | 0.3 | 0.3 | 20.18 | 31.88 | 32.97 | 28.35 |

**Table (4) The calculated macrostrain for all investigated samples of Ge:Sb films as revealed from the three planes (111), (2 2 0) and (311)**

| $T_a$ (K) | Macrostrain x $10^{-3}$ | | |
|---|---|---|---|
| | (111) | (220) | (311) |
| 673 | 1.14 | 0.42 | 0.18 |
| 773 | 1.821 | 0.23 | 0.215 |

**Table (5) the value of average roughness, RMS and average grain size**

| $T_a$ K | average roughness (nm) | RMS (nm) | average grain size (nm) |
|---|---|---|---|
| RT | 6.02 | 9.29 | - |
| 473 | 2.89 | 4.48 | - |
| 573 | 0.706 | 0.955 | - |
| 673 | 0.588 | 0.774 | 31.4 |
| 753 | 1.13 | 1.45 | 49.2 |

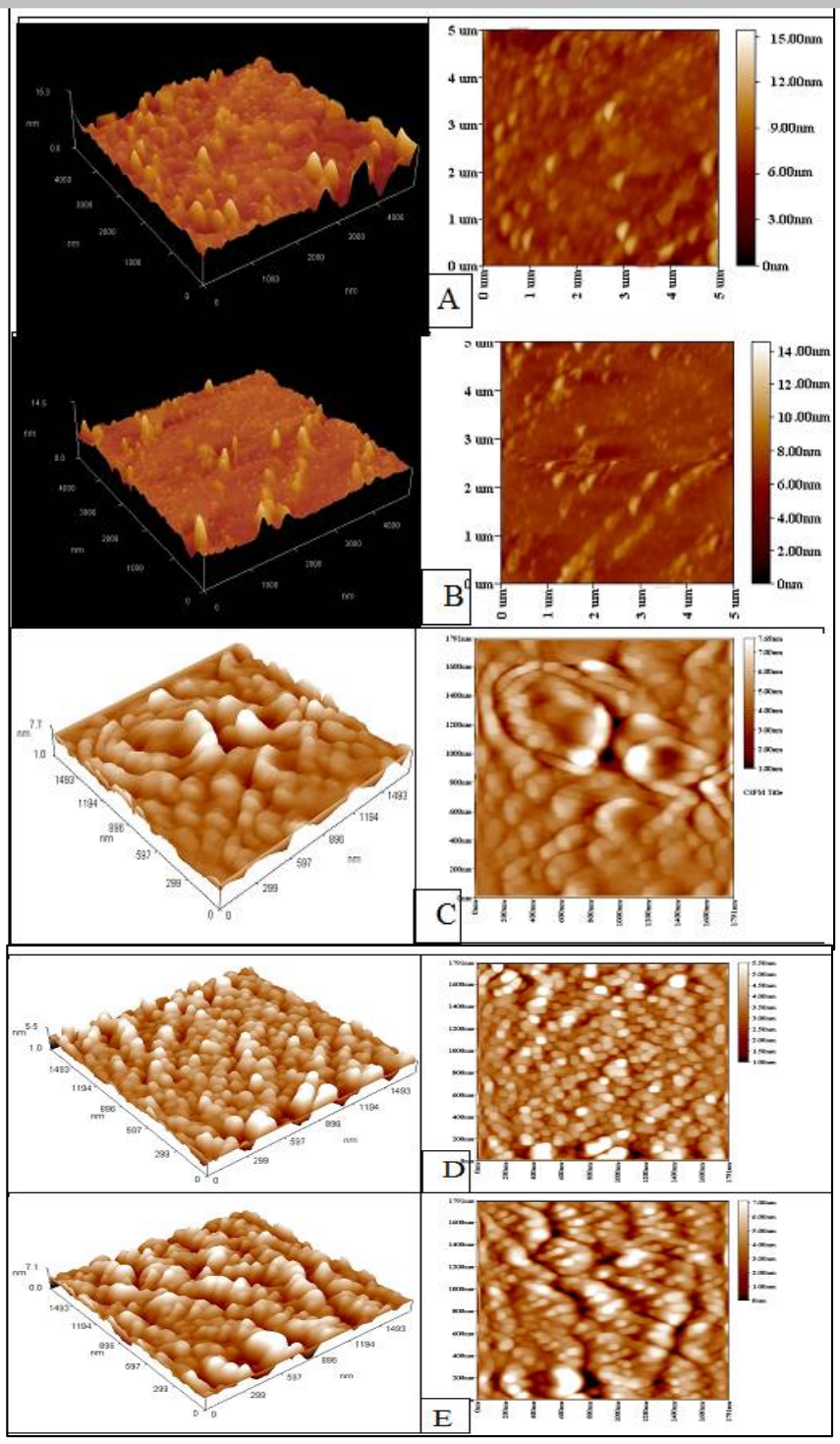


**Figure 2.AFM image for Ge:Sb thin films at(0.5µm) : (A) as deposited and difference $T_a$ (B) 473 K, (C) 573 K ,(D) 673 K , (E) 773 K**

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RESEARCH ARTICLE

# Nutraceutical Retention and Frying Stability of Blended and Interesterified oil of Refined Palm Olein oil with Sesame oil

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## ABSTRACT

Deep fat frying is a straightforward and quick method of preparing tasty and crispy foods. Deep fat fried products give more unwanted changes which impact on human health. Primarily, it causes hydrolysis, oxidation and polymerization of the oil. Moreover, Tocopherols and synthetic antioxidants are less efficient in repeated frying cycles. Hence, there is a need to selectappropriatevegetable oil which retains antioxidants even in deep fat frying. In natural, no native oils have balanced fatty acid and high stable antioxidants.Keeping in mind, we had chosen refined palm olein oil (RPOO) and sesame oil(SESO) to make a good stability blend. Refined Palm Olein Oil has high saturated fatty acid (Palmitic acid) and high in monounsaturated fatty acid (oleic acid) and fewer nutraceuticals. Sesame oil has rich nutraceuticals but high polyunsaturated fatty acid (PUFA).In this study, we used a standardised blend ratio of 40:60 and interesterified of RPOO with SESO for frying studies.Moreover, we assessed the nutraceutical retention and frying stability of blended and interesterified of RPOO with SESO wasstudied in fried oils of papad at 180°C. The colour,viscosity,peroxide value (PV), Free fatty acid value ( FFA), polar compounds, fatty acid composition and nutraceuticals were measured in deep fried oils. In every frying cycle, viscosity had gradually increased in deep fried oils. The PV and FFA showed a significant reduction in blended and interesterified oil of RPOO+SESO as compared to RPOO and suitable for frying upto20$^{th}$ frying cycles.The frying stability of the blended and interesterified oil was increased due to the nutraceutical retention of

sesame lignans and balanced fatty acid as compared to native oil. This study will helpful to the society and food industry economically for efficient use oils to make product development.

**Keywords :** Nutraceuticals, sesame lignans, blended, interesterified, refined palmoleinoil, sesameoil.

## INTRODUCTION

Deep fat frying is one of the conventional method used to prepare food products in India (Suresh *et al.,* 2015;Santos et al, 2017). Deep fat frying, the fat is continuously being exposed to elevated temperature (150 - 180°C) in the presence of the substrates air and water (Alizera*et al.,*2010;BorjianBorojeni et al,2016). Fried foods have a desirable flavour, colour and crispy texture which make deep fat fried food very popular with consumers (Tiwari *et al.,*2014). Deep fat fried products give the reactions such as oxidation, isomerization, polymerization, hydrolysis and cyclization. The decomposition products of these reactions produce off flavours in the oil (Radwan,2010). Since, *Trans* fatty acids are widely reported to be a causative factor in health problems (Kim *et al.,* 2010). Deep fat fried products, contain 35 – 40 % of oil, deep fat fried products regularly intake consumer it easily promote diabetes, obesity and cardiovascular diseases (Sukumar *et al*., 2009). Hence, Researcher has focused to develop reducing fat content of fried products. The instability of oils can also lead to undesirable taste and flavour of oil. Each oil has unique characteristics such as some oils have high saturated fatty acid, unsaturated fatty acid, monounsaturated fatty acid.

Each native oil, have unmodified forms imposed by their triacylglycerol and fatty acid compositions. The physical and chemical properties of oils are an important function of the triacylglycerol and fatty acid composition (Abdulkarim et al.,2010). In modification of oils and fats hydrogenation, fractionation, blending and interesterification. Blending of oils improved the physico chemical characteristics and better functional properties (Anitha, 2009; (Serjouie *et al.,* 2010)). Interesterification of oils exhibit to decrease the viscosity and more resistant to thermal degradation during deep fat frying (Sukumar *et al.,* 2011;(Tiwari *et al.,* 2014)).The liquid portion of palm oil is referred to as palmolein and was separated by partial crystallization and has less saturated fatty acids. Blending and interesterification of Palmolein oil with other vegetable oil as such does not contain any *trans*-fatty acids (Shadi *et al.,*2010;Kumar and Krishna,2014).Sesame oil is known to be significantly resistant to oxidative rancidity although it contains nearly 85% unsaturated fatty acids. (Zoe *et al.,* 2010).Palmolein oil cost very low and sesame oil cost very high. The aim of this present investigation was to make the blend and interesterified withr efined palmolein oil (RPOO)and sesame oil(SESO) in appropriate proportion to reduce the cost as well as improve the frying stability and deteriorating tendency. Hence, we had studied the physicochemical properties to know whether blended oil or interesterified oil helping to improve the frying stability and nutraceutical retention during deep fat frying.

## MATERIALS AND METHODS

### Materials

Refined palmolein oil was procured from the local super supermarket, Madurai; Crude sesame oil was purchased from local market of Kankeyam, Tamil Nadu. Lipozyme RM IM was obtained as gift from Novozymes A/S, Bangalore, India. All chemicals and solvents used were analytical research grade. Reference standard FAME mix (Supelco Inc.), sodium hydroxide, potassium hydroxide, potassium iodide, potassium thalate, acetic acid, starch, chloroform, petroleum ether, ethyl alcohol, phenolphthalein, sodium thio sulphate are procured from Sisco Research Laboratory Mumbai, India, HPLC grade hexane and methanol were procured from Sigma – Aldrich Co., St. Louis, MO, USA.

## Methods

### Preparation of palm olein oil based vegetable oil blend and interesterified oil

Blended oils were prepared by mixing of one oil with another oil. A 200g mixture of two oils were placed in 500 ml beaker in duplicate for each blend and were mixed by using a mechanical stirrer at 180 rpm for 1hr in 40:60 ratio. The temperature was maintained at 40 °C during mixing (Kumar and Krishna, 2014). Interesterified oils were prepared using lipozyme IM RM *Rhizomucor Miehei*at 5% level. The reaction was carried out in a shaking water bath (BS-31) at a speed of 160 rpm for 12 h at 37$^{o}$C. After the interesterification reaction, the oil sample was decanted to separate enzyme; and washed with hexane and dried for reuse (Kumar and Krishna, 2014).

### Frying study

The frying studies were conducted as described by Rangaswamy and Nasirullah, 2014.Three papads were used for frying in oil at a time. The circumference of the frying pan was 121 cm$^{2}$with depth of 12.5 cm and oil holding capacity of 1 litre. The frying temperature of oilwas maintained at 180$^{o}$C and the papad was fried in oil (Fig 1). The oil sample was collected at the end of the repeated frying of papad, approximately 4$^{th}$, 8$^{th}$, 12$^{th}$, 16$^{th}$, 20$^{th}$consecutive end of frying to study the properties of oil (Fig 2).The fried oil samples were collected and stored under refrigerated condition and used for analyzing the properties of oil.

#### Physico-chemical Analyses

The colour of oils was evaluated usingHunter Lab scan XE spectrophotometer (Hunter Associates Laboratory Inc,Restone Virginia and USA). The 15 ml of sample were placed in a sample cuvette was used for transmittance colour measurements in liquid media. The color of samples was obtained by using a 2/°C (2° observer/ illuminant C). The results are expressed as L*, a*, b* respectively indicating observer/ illuminant C)(Prasanth,2015).Apparent viscosities of the different frying oils were carried out using a controlled shear-stress viscometer (Model # RT 10, HaakeGmbh, Karlsruhe, Germany) consisting of coaxial cylinder at a shear rate of 102 s–1. For initial section of oils, the apparent viscosities were measured at 25±1 °C for blended and enzyme interesterified oils (Sukumar et al.,2012).The peroxide value of samples is determined in terms of milliequivalents of peroxide per kilogram of sample that oxidizes KI under the test conditions. The test samples were dissolved in acetic acid/chloroform mixture (3:2) followed by the addition of saturated KI and kept closed for 1 min, and the reaction was terminated by the addition of distilled water.

Starch indicator was added and titrated against sodiumthiosulfate until the blue colour disappeared(*AOCS 2004*).The free fatty acid value (FFA) was determined by AOCS O.M.No. Ca 5a-40 (*AOCS 2004*). Oil was titrated against 0.1 N NaOH solution in neutralized alcohol medium using phenolphthalein as indicator. Fatty acid composition of blended oils was analyzed by GC (Fisons GC fitted with a flame ionization detector, Smith and Morrison, 1964). Total polar compounds were measured by micro method. Total antioxidant activity measured by DPPH method and CUPRAC method (Krishna et al,2010; Dhavamani et al,2014).The fatty acid methyl esters were separated using a fused silica capillary column 25m x 0.25mm (parma bond FFAP – DF – 0.25, Machery – NegalGmbhco., Duren, Germany). The operating conditions were as follows: initial column temperature 120°C, raised by 15°C/min to 220°C, Injection temperature 230°C and detector temperature 240°C,Nitrogen was used as the carrier gas. Individual fatty acid was identified by comparing with retention times of standards (Nuchgek prep, Elysin, MN, U.S.A) and was quantified by an online chromatopac CR- 6A integrator as described by Sugasini and Lokesh(2012).

**Statistical Analysis**

All statistical analysis was performed using SPSS statistical software package version 17.0. All the determinations were carried out in triplicates.

## RESULTS AND DISCUSSION

### Changes in Colour

The change of colour of the palmolein oil, sesame oil, blended and interesterified oil initial raw oil and after used for frying up to 20 cycle is shown Table 1. The a value green to redness in all the oils were found to increase in colour after repeated frying cycles as compared to initial oil. The b value yellow to blueness in all the oils were found to increase in colour after repeated frying cycles as compared to initial oil. The L value for lightness in all the oils were found to increase in colour brightness after repeated frying cycles as compared to initial oil. The colour value it may be partially due to it formation of degraded compounds from the fried product. Colour of oil it depends upon the nature of colouring material like chlorophyll and carotene present in oil (Abdulkarim *et al.,* 2010). Palm olein oil have pale yellow colour and sesame oil dark yellow colour it indicating the presence of colour pigments. Change in colour indicates the deterioration of oil due to oxidation. It is because of accumulation of non-volatile decomposition products (Shaziatabasum *et al.,* 2012). The colour of oils was darkened during each frying. Up to $12^{th}$ frying it darkened very fastest start but then the change in colour became slow and finally the colour persistent.

### Changes in viscosity

Fig 3 was studied about refined palmolein oil, sesame oil, and blended and interesterified oil viscosity content for each initial value and each frying $4^{th}$,$8^{th}$ $12^{th}$,$16^{th}$,$20^{th}$ cycles are gradually increased. Refined palm olein oil(RPOO) showed the highest apparent viscosity.viscosity of palm olein oil (Sukumar *et al.,*2012). Viscosity of oil depends upon the density. When density of oil increases its viscosity would increase.

### Changes in peroxide value and free fatty acid value

The changes in peroxide value of RPOO, SESO,RPOO+SESO(B),RPOO+SESO(I) oil was shown in Fig 4. PV value was present in fresh oils such as refined palm olein oil 2.2 meq$O_2$/kg, sesame oil 5.2meq$O_2$/kg, blended oil 3.9 meq$O_2$/kg and 2.1 meq$O_2$/kg. Initially, peroxide values of all fresh oils were suitable for frying. The frying cycles was done as $4^{th}$ frying, $8^{th}$ frying, $12^{th}$ frying, $16^{th}$ frying and $20^{th}$ frying. The $4^{th}$ frying present in peroxide value was increasing trends such as 9.1 meq$O_2$/kg in RPOO, 5.5meq$O_2$/kg in SESO,6.7 meq$O_2$/kg in blended oil and 6.4meq$O_2$/kg in interesterified oil. It was observed that $4^{th}$ frying the peroxide values were acceptable level upto $4^{th}$ frying. The $8^{th}$ frying cycle increasing amount of peroxide values were 12.6 meq$O_2$/kg in RPOO, 8.4meq$O_2$/kg in SESO, 9.4 meq$O_2$/kg in blended oil and 9.4 meq$O_2$/kg interesterified oil. The result showed that the fried oils are suitable for further frying. The $12^{th}$ frying present in peroxide values were 18.5 meq$O_2$/kg in RPOO, 26.5meq$O_2$/kg in SESO, 13.8 meq$O_2$/kg in blended oil and 13.6meq$O_2$/kg in interesterified oil. The $12^{th}$ frying cycle it was observed suitable for further frying in the oils such as RPOO, RPOO+SESO(B) and RPOO+SESO(I). The sesame oil was not suitable for further frying. The $16^{th}$ frying cycle present in peroxide values were21.5 meq$O_2$/kg in RPOO,31.8 meq$O_2$/kg in SESO,23.7 meq$O_2$/kg in blended oil,24.2 meq$O_2$/kg in interesterified oil. The $20^{th}$ frying present in peroxide value 21.7meq$O_2$/kg in RPOO,44.7 in SESO, 27.5 meq$O_2$/kg in blended oil and 28.8 meq$O_2$/kg in interesterified oil. The $16^{th}$ and $20^{th}$ frying it was observed that the frying was not suitable for upto frying. The primary oxidation of oil is analysed for PV value. Peroxide value is one of the quality indexes of edible oils and indicates oxidation level in oils(Prasanth,2015).PV increased it indicating least oxidative stability respectively for palmolein oil and sesame oil it obviously appropriate to more tocopherol and tocotrienol content in palmolein oil(Tiwari *et al*., 2014).Peroxide value is a measure of

oxidation during storage and the freshness of lipid matrix. In addition, it is a useful indicator of the early stages of rancidity occurring under mild condition and it is a measure of the primary lipid oxidation products. So, greater the PV, the more will be the rate of oxidation of the oil. The most common cause for the deterioration of oil is rancidity which is due to oxidation. The first product formed by oxidation is hydro peroxide; PV determines the amount of hydro peroxides formed. Unsaturated oils are more prone to rancidity compared to saturated ones like palmolein oil in this study every frying cycle peroxide value increased. Peroxide value initial to every frying cycle it increased (Gopalakrishna, Khatoon and Babylatha, 2005).

Free fatty acid contents of oil blend and intersterified (Fig 5) every frying cycle gradually increased. The effect of the higher FFA content on the high quality of oil it means higher diacylglycerol and monoacylglycerol contents. Higher proportion of these additional oil types will affect rate of crystallization and cause cloudiness in oil at low temperature storage condition. This study was experimented that blending and interesterification of palm olein and sesame oil with higher degrees of unsaturation, it showed in blends and interesterified that are more stable at low temperatures. The blends and interesterified stay clear for a longer period of time (Abdulkarim *et al.,* 2010).

## Fatty acid composition of native and interesterified blends of palm olein oil and sesame oil during frying

The fatty acid compositions of the oils collected from papad are shown in Table 2. For all fresh frying oils, the most predominant fatty acids (FAs) were the RPOO had the highest amounts of saturated fatty acid palmitic acid (41.7), monounsaturated fatty acid oleic acid (41.6) and smallest amount of poly unsaturated fatty acid linoleic acid (10.9) and SESO had the highest amount of mono unsaturated fatty acid, oleic acid (42.1), poly unsaturated fatty acid, linoleic acid (41.4) and smallest amount of saturated fatty acid, palmitic acid (10.6). RPOO + SESO (B) had double the amount of mono unsaturated fatty acid, oleic acid (42.5), the amount of saturated fatty acid, palmitic acid (25.8) and polyunsaturated fatty acid, linoleic acid (25.0).RPOO + SESO (I) had double the amount of mono unsaturated fatty acid, oleic acid (43.5), the amount of saturated fatty acid, palmitic acid (25.9) and poly unsaturated fatty acid, linoleic acid (25.2). High amounts of mono unsaturated fatty acids (MUFAs) in all oils are associated with a decreased risk of coronary heart disease. Thereby, oil with high amount of MUFA induces a desirable effect on the health benefits (Alizera et al**.,** 2010).

The changes in the Fatty acid composition of different frying cycle during the $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$, $20^{th}$of the frying process are given in Table 2. RPOO every frying cycles present in the palmitic acid such as 42.3, 40.2, 42.8, 41.8, 43.9, oleicacid such as 42, 43, 42.1, 43.9, 41.3 and linoleic acid such as 9.9, 10.9, 9.5, 9.5,8.6. SESO it present every frying cycles palmitic acid 11.7, 12.0, 12.4, 11.2, 10.9, oleic acid present in amount of 40.42, 41.27, 40.73, 41.3, 40.3 and linoleic acid such as 40.53, 39.13, 38.6, 39.23, 40.4, blended oil it every frying cycle such as palmitic acid 27.0,26.1,25.9,26.2,27.3, oleic acid value such as 43.5, 43.1, 43.8, 44, 43.7 and linoleic acid value such as 23.6, 23.8, 23.1, 22.6, 22.2 and intersterified oil it every frying cycle such as palmitic acid 27.2, 26.4, 26.0, 26.2, 27.3, oleic acid value such as 43.7, 43.1, 43.8, 44.0, 43.7 and linoleic acid values such as 23.8, 24, 23.6, 22.4, 22.4. The result showed that blended and intersterified oils weremono unsaturated fatty acid maintained the same value and balance the saturated and poly unsaturated fatty acid compared to the native oils.

## Total Polar compounds

Polar compounds was analysed for the assessment for the extent of deterioration in frying oils of native, blended and interesterified oil in Fig 6. The total polar contents during the frying process increased gradually when in repeated frying. The blended and interesterified of RPOO with SESO showed an increased stability than native oil of RPOO.

**Total antioxidant activity of native, blend and interesterified oils of RPOO with SESO during frying**

In addition to proportionated fatty acids and triacylglycerols, the vegetable oils also contain some nutraceuitcals which may possess antioxidant activity. This in turn may determine stability of oil. The total antioxidant value of native, blended and interesterified oil of RPOO with SESO were evaluated and given in Fig 7.The total antioxidant value of native oil of RPOO showed 50% lower antioxidant value as compared to blended and interesterified oil of RPOO with SESO. This indicated that compounds present in unsaponifiable fractions of nutraceuticals are responsible for the antioxidant value to vegetable oils. The antioxidant activity has maintained prolonged more in blended and interesterified oil of RPOO with SESO as compared to native oil of RPOO.

**Nutraceutical retention and total antioxidant activity of native, blend and interesterified oils of RPOO with SESO during frying**

When blended and interesterified oil of RPOO with SESO were subjected to high temperature(180°C) for different time periods, it was found that some of the minor constituents are retained to some extent (Table 3).These include lignans in sesame oil. However the minor constituents such as tocopherols and tocotrienols are destroyed within 8h of frying. This reflected on the residual antioxidant value of oils which were subjected to frying (Table 3).The above cited studies clearly indicated that some of the selected minor constituents present in oils possess antioxidant properties and have health benefits which is independent of the effects mediated by fatty acids present in triacylglcerols of the oil. Some of the natural antioxidants such as tocopherols were also highly labile to thermal degradation.More than 80% of oils used in Indian cooking is utilised for frying purposes where the oils are subjected to high temperatures in open pans. When fried oils were tested for their antioxidant activity, it was found that oils such as sesame oil retained their antioxidant value which coincided with retention of the minor constituents such as sesamin which showed greater thermal stability as compared to other minor constituents present in the oils. Therefore blended and interesterified oils RPOO with sesame oil can make better frying oil.

## CONCLUSION

This study showed that the frying stability of the blended and interesterified oil was increased due to the nutraceutical retention of sesame lignans and balanced fatty acid as compared to native oil. This study will helpful to the society and food industry economically for efficient use oils to make product development. This blended and interesterified oil would helpful to satisfying both the consumer demands and processor needs.

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**Table1.Physical characteristics of native and intersterified oil blends of refined palm olein oil and sesame oil.**

| Oils | Colour | | |
|---|---|---|---|
| | L* | a* | b* |
| RPOO initial | 91.63±0.08 | -4.32±0.17 | 32.40±0.16 |
| RPOO 4th | 91.33±0.21 | -2.83±0.09 | 31.75±0.09 |
| RPOO 8th | 91.50±0.17 | -5.43± 0.35 | 38.21±0.22 |
| RPOO 12th | 91.80±0.06 | -6.16±0.03 | 38.94±0.28 |
| RPOO 16th | 91.95±0.07 | -6.29±0.13 | 43.57±0.08 |
| RPOO 20th | 91.48±0.27 | -6.60±0.06 | 46.79±0.14 |
| SESO initial | 91.63±0.08 | -3.50±0.36 | 43.25±0.39 |
| SESO 4th | 91.33±0.47 | -2.44±0.06 | 42.45±0.06 |
| SESO 8th | 91.50±0.05 | -2.87±0.04 | 43.07±0.09 |
| SESO 12th | 91.80±0.06 | -3.33±0.42 | 43.40±0.11 |
| SESO 16th | 91.95±0.19 | -3.89±0.04 | 44.69±0.16 |
| SESO 20th | 91.48±0.23 | -4.39±0.03 | 44.83±0.11 |
| RPOO + SESO (B) initial | 91.63±0.13 | -2.14±0.04 | 52.63±0.04 |
| RPOO + SESO (B) 4th | 91.33±0.04 | -1.84±0.03 | 49.60±0.20 |
| RPOO + SESO (B) 8th | 91.50±0.20 | -2.12±0.08 | 49.84±0.09 |
| RPOO + SESO (B) 12th | 91.80±0.03 | -2.24±0.06 | 50.05±0.26 |
| RPOO + SESO (B) 20th | 91.45±0.07 | -2.70±0.14 | 50.53±0.78 |
| RPOO + SESO (I) initial | 91.48±0.10 | -2.67±0.09 | 52.70±0.15 |
| RPOO + SESO (I) 4th | 91.43±0.04 | -2.21±0.08 | 52.55±0.08 |
| RPOO + SESO (I) 8th | 91.53±0.11 | -2.10±0.05 | 48.31±0.20 |
| RPOO + SESO (I) 12th | 91.56±0.02 | -2.12±0.05 | 50.67±1.15 |
| RPOO + SESO (I) 16th | 91.80±0.14 | -2.22±0.06 | 50.65±0.10 |
| RPOO + SESO (I) 20th | 91.95±0.06 | -2.72±0.09 | 50.54±0.08 |

*Values are mean ± SD, n = 4;nd: not detected, L*- Lightness, a*- indicates redness when positive, greenness when negative, b*- indicates yellowness when positive, blueness when negative, RPOO – palm olein oil, SESO– sesame oil, B – blended, I- intersterified.*

**Table 2. Fatty acid composition of native and interesterified blends of refined palm olein oil and sesame oil during frying**

| Oils | Frying cycle | Fatty acid composition | | | | | | | | | SFA | MUFA | PUFA | Total unsaturation | P/S | S:M:P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C 12:0 | C 14:0 | C 16:0 | C 16:1 | C 18:0 | C 18:1 | C 18:2 | C 18:3 | C 20:0 | | | | | | |
| RPOO | Initial | 0.09±0.06 | nd | 41.7±0.15 | 0.3±0.10 | 4±0.20 | 41.6±0.56 | 10.9±0.21 | 0.4±0.10 | nd | 46.6 | 41.9 | 11.3 | 53.2 | 0.2 | 1.0:0.9:0.2 |
| | 4 | 0.8±0.06 | nd | 42.3±0.15 | 0.3±0.10 | 4±0.25 | 42±0.92 | 9.9±0.53 | 0.6±0.25 | nd | 47.1 | 42.3 | 10.5 | 52.8 | 0.2 | 1.0:0.9:0.2 |
| | 8 | 0.6±0.06 | nd | 40.2±0.10 | nd | 4.5±0.55 | 43.6±0.06 | 10.9±0.36 | 0.2±0.36 | nd | 45.3 | 43.6 | 11.8 | 65.4 | 0.4 | 1.0:1.0:0.5 |
| | 12 | 0.5±0.10 | nd | 42.8±0.15 | nd | 4.3±0.36 | 42.1±0.84 | 9.5±0.20 | 0.5±0.20 | nd | 47.6 | 42.1 | 10 | 52.1 | 0.2 | 1.0:0.9:0.2 |
| | 16 | 0.5±0 | nd | 41.8±0.21 | nd | 4.2±0.80 | 43.9±1.03 | 9.5±0.06 | 0.1±0.06 | nd | 46.5 | 43.9 | 9.6 | 53.5 | 0.2 | 1.0:0.9:0.2 |
| | 20 | 0.5±0 | nd | 43.9±0.15 | 1.2±0.31 | 4.5±0.35 | 41.3±0.17 | 8.6±0.10 | nd | nd | 48.9 | 42.5 | 9.6 | 62.1 | 0.4 | 1.0:0.9:0.4 |
| SESO | Initial | 0.1±0.10 | nd | 10±0.61 | 0.4±0.17 | 5.5±0.45 | 42.1±0.12 | 41.4±0.26 | 0.1±0.10 | 0.4±0.17 | 16.00 | 42.50 | 41.50 | 84.00 | 2.5 | 1:2.6:2.5 |
| | 4 | 0.1±0.06 | nd | 11.7±0.2 | 1.36±0.21 | 5.48±0.36 | 40.42±0.30 | 40.53±0.15 | nd | 0.36±0.0 | 17.68 | 41.79 | 40.53 | 82.32 | 2.2 | 1:2.3:2.2 |
| | 8 | 0.1±0 | nd | 12.0±0.4 | 1.06±0.12 | 5.6±0.26 | 41.27±0.32 | 39.13±0.78 | 0.2±0.10 | 0.57±0.4 | 18.34 | 42.33 | 39.33 | 81.66 | 0.4 | 1:2.3:2.1 |
| | 12 | Nd | nd | 12.4±0.4 | 1.86±0.44 | 5.13±0.15 | 40.73±0.21 | 38.6±0.40 | 0.2±0 | 1.03±0.1 | 18.60 | 42.60 | 38.80 | 81.40 | 2.0 | 1:2.2:2.0 |
| | 16 | Nd | nd | 11.2±0.7 | 1.46±0.45 | 5.93±0.78 | 41.43±0.25 | 39.23±0.21 | 0.13±0.06 | 0.6±0.35 | 17.73 | 42.90 | 39.37 | 82.27 | 2.2 | 1:2.4:2.2 |
| | 20 | Nd | nd | 10.96±0.3 | 1.6±0.10 | 6±0.26 | 40.3±0.70 | 40.4±0.53 | 0.33±0.12 | 0.4±0.26 | 17.37 | 41.90 | 40.73 | 82.63 | 2.3 | 1:2.4:2.3 |
| RPOO+ SESO(B) | Initial | 0.6±0.1 | nd | 25.8±0.1 | nd | 5.1±0.2 | 42.5±0.5 | 25.0±0.7 | 0.3±0.2 | 0.5±0.3 | 26.7 | 30.9 | 25.0 | 55.9 | 2.0 | 1:1.1:0.9 |
| | 4 | 0.7±0.1 | nd | 27.0±1.8 | nd | 5.5±0.2 | 43.5±1.0 | 23.6±0.6 | nd | nd | 27.7 | 32.5 | 23.6 | 56.1 | 2.0 | 1:1.1:0.8 |
| | 8 | 0.6±0.0 | nd | 26.1±0.4 | nd | 5.7±0.1 | 43.1±0.1 | 23.8±0.1 | nd | nd | 26.7 | 31.8 | 23.8 | 55.6 | 2.0 | 1:1.1:0.8 |
| | 12 | 0.6±0.0 | nd | 25.9±0.2 | nd | 5.7±0.0 | 43.8±0.1 | 23.1±0.0 | nd | nd | 26.5 | 31.6 | 23.1 | 54.7 | 2.0 | 1:1.1:0.8 |
| | 16 | 0.6±0.0 | nd | 26.2±0.4 | nd | 5.9±0.1 | 44.0±0.3 | 22.6±0.1 | nd | nd | 26.8 | 32.1 | 22.6 | 54.7 | 2.0 | 1:1.2:0.8 |
| | 20 | 0.6±0.0 | nd | 27.3±0.1 | nd | 5.6±0.1 | 43.7±0.1 | 22.2±0.1 | nd | nd | 27.9 | 32.9 | 22.2 | 55.1 | 1.9 | 1:1.1:0.8 |
| RPOO+ SESO(I) | Initial | 0.8±0.1 | nd | 25.9±0.1 | nd | 5.6±0.2 | 43.5±0.5 | 25.2±0.7 | nd | nd | 26.7 | 31 | 25.0 | 56 | 2.1 | 1:1.1:0.9 |
| | 4 | 0.7±0.1 | nd | 27.2±1.8 | nd | 5.8±0.2 | 43.7±1.0 | 23.8±0.6 | nd | nd | 27.9 | 32.7 | 23.6 | 56.3 | 2.0 | 1:1.1:0.8 |
| | 8 | 0.6±0.2 | nd | 26.4±0.4 | nd | 5.7±0.1 | 43.1±0.1 | 24±0.1 | nd | nd | 27 | 32.1 | 23.8 | 55.9 | 2.0 | 1:1.1:0.8 |
| | 12 | 0.6±0.1 | nd | 26.0±0.2 | nd | 5.7±0.0 | 43.9±0.1 | 23.6±0.0 | nd | nd | 26.6 | 31.7 | 23.1 | 54.8 | 2.0 | 1:1.1:0.8 |
| | 16 | 0.6±0.1 | nd | 26.2±0.4 | nd | 5.9±0.1 | 44.1±0.3 | 22.4±0.1 | nd | nd | 26.8 | 32.1 | 22.6 | 54.7 | 2.0 | 1:1.2:0.8 |
| | 20 | 0.6±0.2 | nd | 27.3±0.1 | nd | 5.6±0.1 | 43.7±0.1 | 22.4±0.1 | nd | nd | 27.9 | 32.9 | 22.2 | 55.1 | 2.0 | 1:1.1:0.8 |

*Values are mean± SD, n = 4;nd: not detected, RPOO– palm oleinoil, SESO– sesame oil, B – blended, I- interesterified, C12:0 - lauric acid,C14:0-myristic acid,C16:0-palmitic acid, C16:1-palmitioleic acid, C18:0- stearic acid,C18:1- oleic acid,C18:2-linoleic acid,C18:3- linolenic acid, C20:0– arachidic acid, SFA: saturated fatty acid, MUFA- monounsaturated fatty acid, PUFA- Poly unsaturated fatty acid, P – poly unsaturated fatty acid, S- saturated fatty acid*

**Table3.Nutraceutical retention of nativeand interesterifiedblends of refined palm olein oil and sesame oil during frying.**

| Frying cycles | Total Tocopherols(mg/100g) | | | | Sesame Lignans (mg/100g) | | | |
|---|---|---|---|---|---|---|---|---|
| | RPOO | SESO | RPOO+SESO(B) | RPOO+SESO(I) | RPOO | SESO | RPOO+SESO(B) | RPOO+SESO(I) |
| 0 | 64±3.5 | 71.5±4.2 | 68.9±3.8 | 70.1±4.2 | nd | 236±12 | 168±14.2 | 160±14.6 |
| 4 | 26±2.1 | 29.6±2.6 | 44.6±1.9 | 38.3±2.1 | nd | 198±10.3 | 141±12.1 | 153±13.2 |
| 8 | nd | 4.2±0.3 | 15.1±0.3 | 14.6±0.3 | nd | 123±13.2 | 112±10.3 | 122±12.6 |
| 12 | nd | nd | nd | nd | nd | 67.5±5.6 | 89.5±5.6 | 79.2±6.2 |
| 16 | nd | nd | nd | nd | nd | 39.5±3.2 | 58.6±7.2 | 60.5±5.3 |
| 20 | nd | nd | nd | nd | nd | 11.5±0.3 | 24.5±2.3 | 28.3±2.5 |

*Values are mean ± SD, n = 4;nd : not detected, RPOO – palm olein oil, SESO – sesame oil, B – blended, I- interesterified*

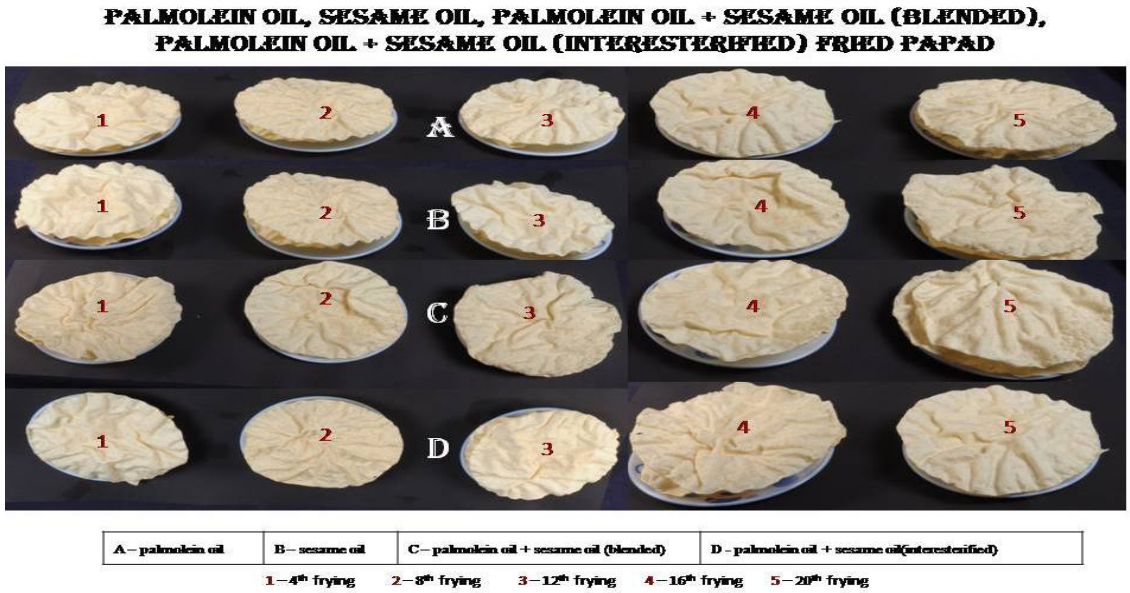


**Fig 1.Papad fried in a)native oil RPOO b)native oil SESO ,c) blended oil of RPOO with SESO(B) and d) Interesterified oil of RPOO + SESO(I)**

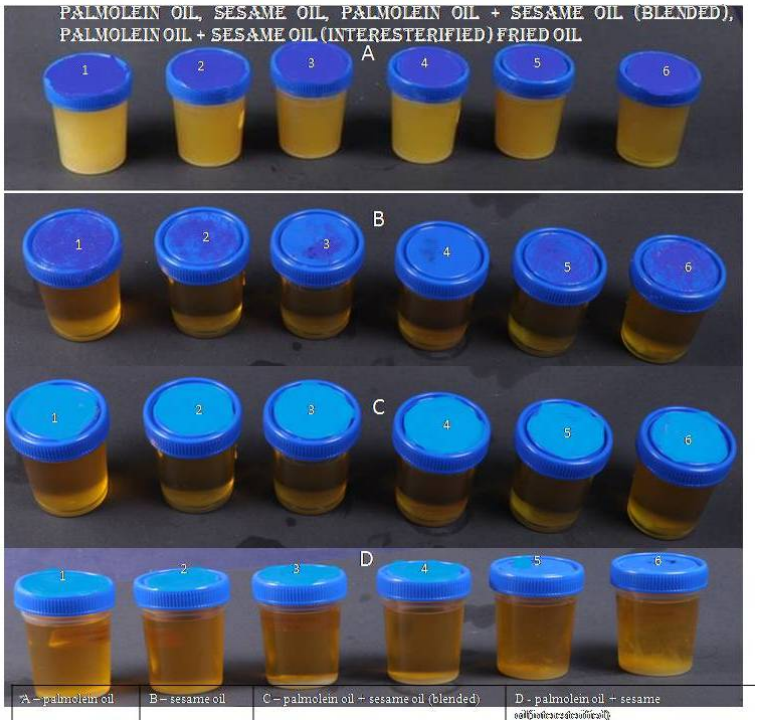


**Fig 2. Repeated frying cycles of 1)native RPOO,2) native SESO, 3)belnded of RPOO+SESO(B) and 4) interesterified oil of RPOO+SESO (I).**

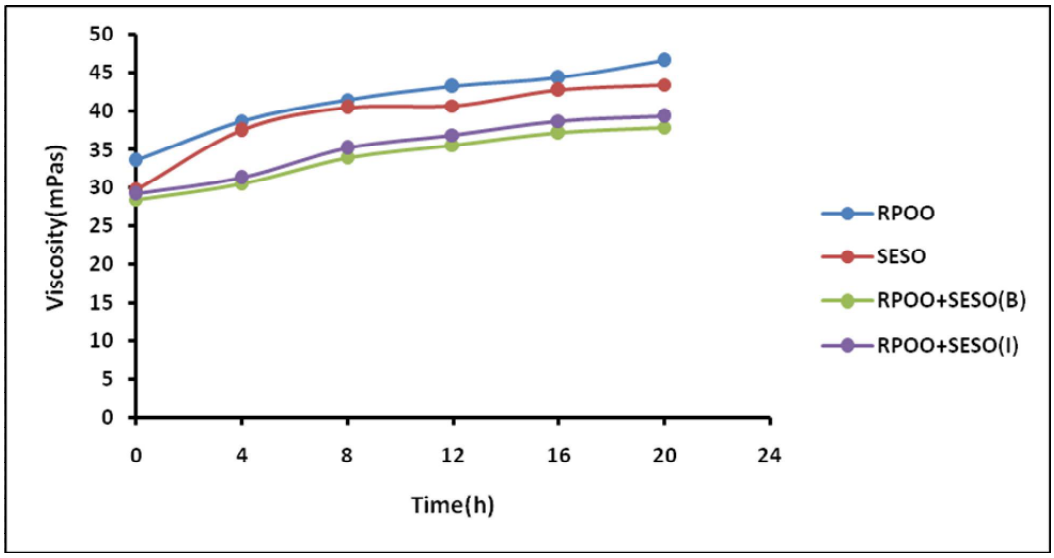


**Fig 3. Viscosity of the native,blended and interesterified oil of refined palm olein oil(RPOO) with sesame oil(SESO) during the frying process at 180°C**

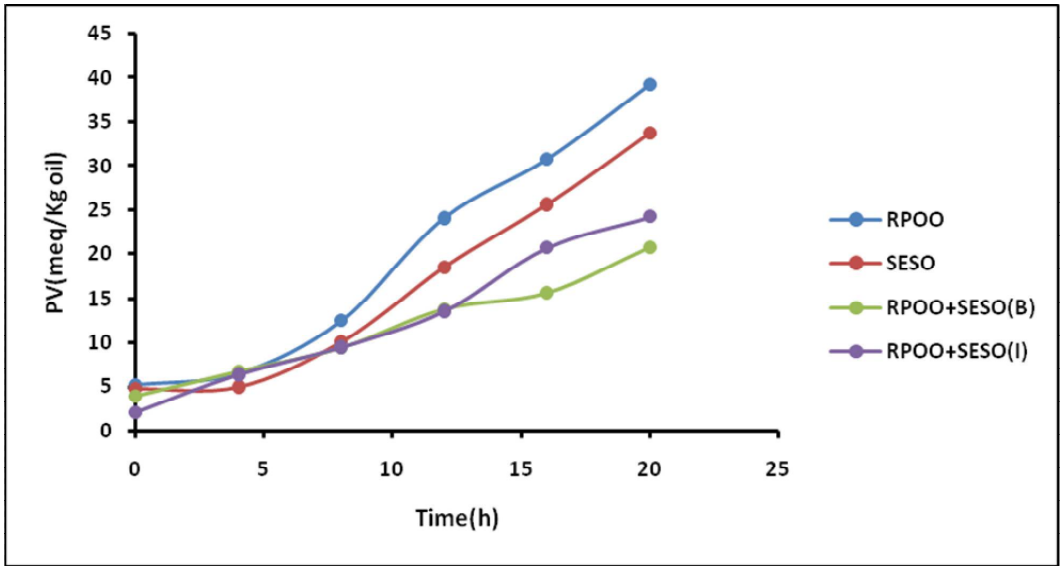


**Fig 4.Peroxide value of the native,blended and interesterified oil of refined palm olein oil(RPOO) with sesame oil(SESO) during the frying process at 180°C**

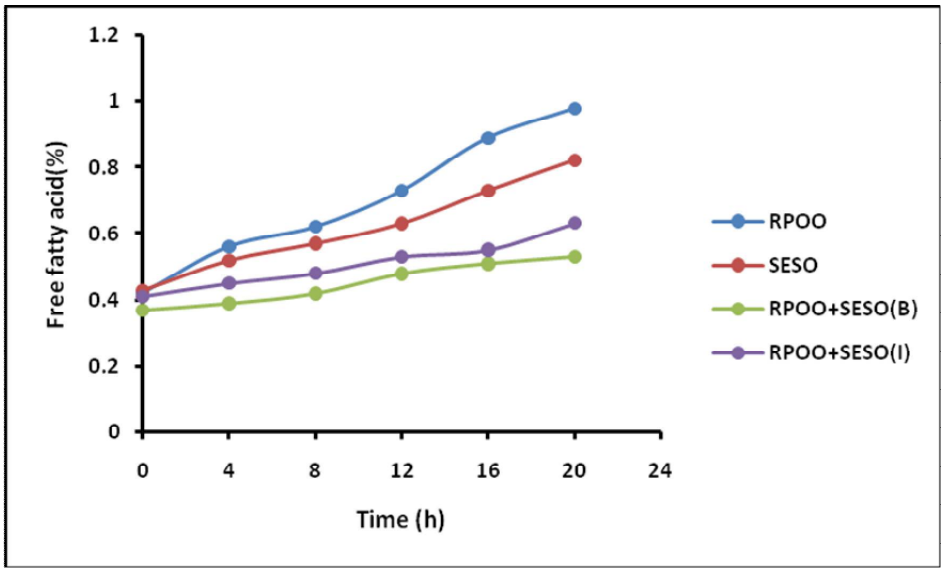


**Fig 5.Free fatty acid value of the native,blended and interesterified oil of refined palm olein oil(RPOO) with sesame oil(SESO) during the frying process at 180°C**

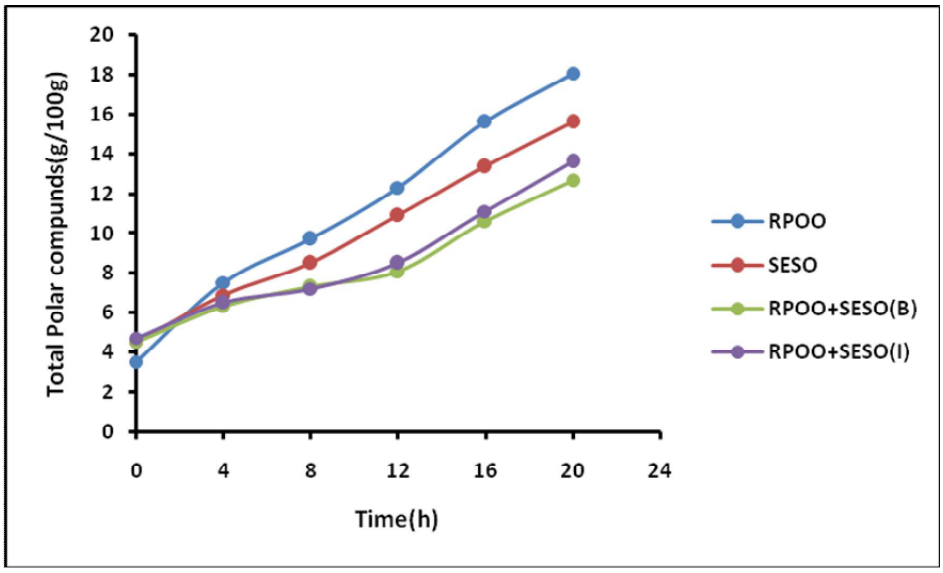


**Fig 6.Total Polar compounds of the native,blended and interesterified oil of refined palm Olein oil(RPOO) with sesame oil(SESO) during the frying process at 180°C**

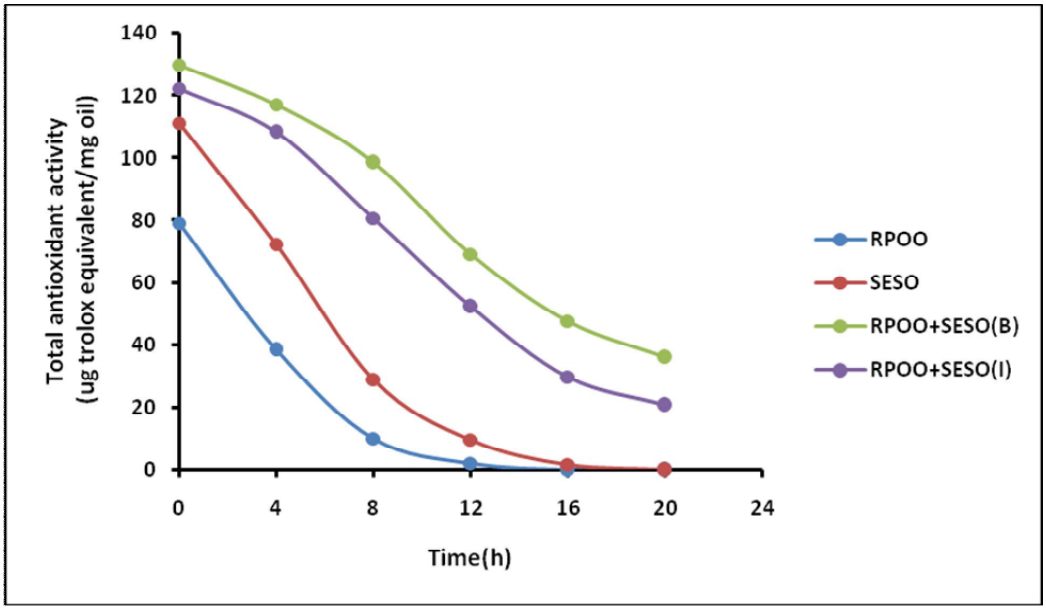


**Fig 7. Total antioxidant activity of oils of native,blended and interesterified oil of refined palm Olein oil (RPOO) with sesame oil (SESO) during the frying process at 180°C**

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RESEARCH ARTICLE

# Biochemical Changes in Open Cervix Pyometra following PGF2α Therapy in Canines

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## ABSTRACT

Caninepyometra is a common reproductive disorder of intact diestrual bitch, which warrants early recognition, diagnosis and appropriate treatment to avoid any disastrous consequences.Despite the latest treatment strategies the mortality rate due to pyometra is about 4 %. So a study was conducted to assess the efficacy of two different PGF2α protocols vis a vis antibiotic therapy.A total of 36 bitches of different breeds and age were divided into three groups consisting of twelve bitches in each groups. Group I were treated with PGF2α at the dose rate of 250 µg/kg body weight once daily for five days subcutaneously. Group II were treated with PGF2α at the dose rate of 30 µg/kg body weight twice daily for eight days subcutaneously. Both the above groups were treated with selected antibiotics based on antibiogram results.Group III were treated with parenteral antibiotics alone for seven days based on antibiogram. Blood samples were collected before and at the end of treatment in the respective groups. It has been concluded that fibrinogen level increase could be used as an indicator of treatment response in open type pyometra of bitches.

**Keywords :** Open cervix pyometra, PGF2α,bitches, biochemical parameters.

## INTRODUCTION

Pyometra is a hormonally mediated acute (or) chronic polysystemicdiestrual disorder which induce high mortality in bitches if not treated. It is one of the life-threatening reproductive disorders in bitches and the clinical manifestations are attributed to haemato-biochemical alterations resulting in toxaemia and toxic bone marrow depression.Physiological, haematological and biochemical changes in pyometra are considered significant to assess the severity of the disease condition (Singh *et al*., 2010).Restoration of fertility may be done with medical treatment(Baithalu*et al.,* 2010). $PGF_{2\alpha}$ has been used to increasemyometrial contractions which might enhance cervical relaxation. It also has luteolytic effect(Gobello*et al.,* 2008). The aim of this study was to compare biochemical profile of pyometric bitches in three different treatment groups and to study response to different doses of $PGF_{2\alpha}$ treatment.

## MATERIALS AND METHODS

Thirty six bitches of different breeds aged between one to ten years, brought to the small animal obstetrics and genecology unit, Madras Veterinary College Hospital with open type pyometra were included for experimental study. Theconfirmative diagnosis of pyometrawas arrived based on clinical signs, abdominal palpation, radiography and ultrasonography. These 36 bitches were divided into three groups consisting of 12 bitches in each group (Group I, II and III).Bitches in Group I were treated with $PGF_{2\alpha}$(InjLutalyse – Up John Company) at the dose rate of 250 µg/kg body weight once daily for five days subcutaneously along with selected antibiotics based on antibiogram.Bitches in Group II were treated with $PGF_{2\alpha}$ at the dose rate of 30 µg/ kg body weight twice daily for eight days subcutaneously along with selected antibiotics based on antibiogram.Bitches in Group III were treated with selected antibiotics alone based on antibiogramparenterally for seven days.Blood samples were collected from all the bitches by cephalic vein puncture in a 5ml vial containing EDTA as an anticoagulant before and at the end of treatment in the respective groups. The blood samples were subjected to blood urea nitrogen (BUN), creatinine, Aspartate amino transferase (AST), Alanine amino transferase (ALT), serum alkaline phosphatase (SAP), total protein, albumin, and globulin and plasma fibrinogen. Statistical analysis of the data was carried out as per the standard procedure outlined by Snedecor and Cochran (1994).

## RESULTS AND DISCUSSION

### Blood Urea Nitrogen (BUN)

The mean BUN values before treatment were 24.60 ± 5.48, 25.58 ± 5.90 and 31.35 ± 7.20 mg/dl in group I, II and III, respectively. Five out of 36 bitches showed abnormally high BUN values ranging from 64.4 to 88.4 mg/dl. The mean post treatment BUN values were 16.80 ± 4.02, 17.62 ± 6.60 and 22.69 ± 4.90 mg/dl in group I, II and III, respectively. Although there was reduction in BUN values in all three group at the end of treatment there was no significant difference observed between pre and post treatment values in all the three groups.

The mean values of BUN (mg/dl) in responded and not responded bitches between the groups at the end of treatment were 13.18 ± 1.89and 11.40 ± 0.90 in group I, 13.32 ± 0.80 and 10.20 in group II, and 14.10 ± 2.42 and 13.18 ± 1.53 in group III, respectively. Statistical analysis revealed significant decline ($P<0.05$) in BUN values from the pre-treatment levels in both responded and not responded bitches of group I, II, and III.This is in agreement with Jena *et al*. (2013). However, no significant difference was observed between the responded and not responded bitches in all three groups.

The present study revealed that the mean BUN and creatinine values before treatment were found to be within the normal range in all the three groups. This was in accordance with the findings of Seveliu*set al.* (1990). However five out of 36 bitches showed abnormally high values ranging from 64.44 to 88.40 mg/dl. In the same bitches creatinine levels were also found to be abnormally elevated and ranging from 4.8 to 7.7 mg/dl. These bitches subsequently died during the course of therapy, which may be due to the elevated levels of BUN and creatinine.

### Creatinine

The mean pretreatmentcreatinine level was observed to be 2.08 ± 0.43, 2.19 ±0.50 and 2.54 ± 0.52 mg/dl in group I, II and III respectively. In five out of 36 bitches the creatinine levels were found to be abnormally elevated and ranged from 4.8 to 7.7 mg/dl. The mean creatinine level at the end of treatment was 1.63 ±0.51, 1.82 ± 0.57 and 2.39 ± 0.76 in group I, II and III respectively. But statistical analysis revealed no significant difference was noticed before and at the end of treatment in all three groups, also between the groups.

The mean values of creatinine (mg/dl) in responded and not responded bitches were 1.08 ± 0.12 and 1.30± 0.10 in group I, 1.20 ± 0.20 and 2.80 ± 0.00 in group II, 0.95 ± 0.24 and 0.82 ± 0.16 in group III respectively. Statistical analysis revealed significant difference ($P<0.05$) between pre-treatment and responded and also between responded and not responded in group I and III, whereas in group III there is significance ($P<0.05$) between pre-treatment and responded, not responded but no significant difference were observed between responded and not responded.

### Aspartate Amino Transferase (AST) & Alanine Amino Transferase (ALT)

The mean pre treatment AST values were 22.31 ± 3.24, 28.46 ± 1.58 and 21.30 ± 2.64 IU/L and the mean AST values at the end of treatment were 22.71 ± 3.08, 24.47+1.37 and 22.35 ± 2.57 IU/Lin group I, II, and III, respectively. Statistical analysis revealed no significant difference between before and at the end of treatment in all three groups and also between the groups at the end of treatment. This is in agreement with Jena *et al*. (2013)who reported that significant increase in ALT, whereas AST did not differ significantly following treatment compared to control group.

The mean values of ALT (IU/L) in responded and not responded were 25.90 ± 1.19 and 28.35 ± 4.05 in group I, 30.29 ± 0.95 and 26.00 ± 0 in group II, 39.50 ± 2.90 and 28.33 ± 1.61 in group III, respectively. There is no significant difference between pre-treatment and responded, not responded, also between responded and not responded in all the three groups. This is in contrast to the observation of Jena *et al*.(2013) who reported significant increase in ALT in post treatment value following treatment with $PGF_{2\alpha}$.

The mean pre treatment ALT values were 29.95 ± 1.76, 30.27 + 2.81, 27.94 ± 1.32 IU/L while the mean ALT values at the end of treatment was observed to be 26.39 ±1.56, 30.26 ± 1.48 and29.79 ±1.17 IU/L in group I, II and III, respectively. Statistically no significant difference in ALT levels was observed before and at the end of treatment in all three groups. This is in contrast to the observation of Jena *et al*.(2013) who reported significant increase in ALT in post treatmentvalue following treatment with $PGF_{2\alpha}$. There was no significant difference in the post treatment values of ALT between the groups.

The mean values of AST (IU/L) in responded and not responded were 23.42 ± 1.50 and 22.00 ± 2.00 in group I, 24.52 ±1.49 and 24.30 ± 0 in group II, 25.20 ± 6.8 and 28.57 ±1.85 in group III, respectively. There is no significant difference between pretreatment and responded, not responded and also between responded in all the three groups.The pretreatment AST and ALT mean values in group I, II and III bitches were 22.31± 3.24 and 28.46 ± 1.5, 21.30 ± 2.64 and 29.25 ± 1.76, 30.27 ± 2.81 and 27.94 ± 1.32 IU/L respectively. The mean AST and ALT values in bitches with pyometra were within the normal range. However Wheaton *et al.* (1989) reported 22 per cent of bitches with pyometra had ALT levels of greater than 60 IU/L.

### Serum Alkaline Phosphatase (SAP)

The mean pre treatment SAP values were found to be 108.25 ± 17.15, 98.01 ± 15.56 and 115.36 ± 14.57 IU/L in group I, II and III, respectively. In 27 per cent of the cases the SAP levels were above normal which ranged from 162.8 to 196.6 IU/L. The mean SAP levels at the end of treatment were found to be 88.98 ± 15.00, 84.64 ± 15.72 and 96.73 ± 13.85 IU/L in group, III and III, respectively. Although a decline in SAP levels following treatment were observed in all three groups and there was no significant difference with regard to pre and post treatment values. This is in agreement with the observations of Jena*et.al.*(2013). No significant difference was observed in the mean SAP levels at the end of treatment between groups.

The mean pre treatment SAP levels were 108.25±17.55, 98.01 ± 15.50 and115.36 ± 14.57 IU/L in group I, II and III, respectively. In 27 per cent of the cases SAP levels were above normal and ranged from 162.82 to 196.6 IU/L. These findings are in agreement with Nelson and Feldman (1986), Wheaton *et al.* (1989), Seveliu*set al.*(1990) and Feldman and Nelson (1996). These mild to moderately increased SAP from hepatocellular damage caused by septicemia and/or diminished hepatic circulation and cellular hypoxia in the dehydrated bitch (Nelson and Feldman, 1982).

### Total Protein

In the present study 42 per cent of the bitches showed hyperproteinemia ranging from 8.2to 11.2gm/dl. This is in contrast to Jena *et al.* (2013) who reported that there was no significant difference in the values of total protein following treatment with $PGF_{2\alpha}$ and control group. Increase in total protein may be a result of either dehydration and/or chronic antigenic stimulation, which is in concurrence with the findings of Nelson and Feldman (1986), Wheaton *et al.* (1989) and Shaw and Ihle (1997).

### Albumin and Globulin

The mean pre treatment albumin and globulin levels were 3.74 ± 0.53 and 3.07 ± 0.57, 3.32± 0.54 g/dl and 4.70 ± 0.35, 5.16 ± 0.41, and 5.47 ± 0.50 g/dl in group I, II and III respectively. Forty two per cent of the bitches showed hyperglobulinemiaranging from 5.8 to 8.8 mg/dl.This is in contrast to Jena *et al.* (2013) who reported that there was no significant difference in the values of total protein following treatment with $PGF_{2\alpha}$ and control group which may have resulted from either dehydration and/ or chronic antigenic stimulation of the immune system, But the present results are in agreement withthe results of Nelson and Feldman (1986), Wheaton *et al.* (1989) and Grooters (1994).

### Plasma fibrinogen

The mean pretreatment and post treatment plasma fibrinogen levels in bitches with pyometra were found to be 567.91 ± 12.71, 588.33 ± 10.97 and 548.33 ± 17.20 mg/dl and431.11 ± 16.11, 423.50 ± 9.55 and 495.00 ± 17.20 in group I, II and III respectively. Increased plasma fibrinogen level observed in the present study is due to inflammatory conditions (McSherry*et al.* 1970). Highly significant decrease in the plasma fibrinogen is in response to treatment.It was concluded thatthough there was variations in the values of serum BUN,creatinine, ALT, AST, total protein and SAP,these valuescannot be used as indicator for assessing treatment response. Fibrinogen level increase could be used as an indicator of treatment response followingtreatment of open type pyometra in bitches.

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RESEARCH ARTICLE

# Impact of Tocopherols and Thermal Stability of MUFA Rich Blended and Interesterified of Refined Palm Olein oil with Canola oil during Repeated Deep Frying

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## ABSTRACT

Refined Palm olein oil (RPOO) blended and interesterified with canola oil (CAO) as ratio of 50:50. The blended and interesterified oil of RPOO+CAO had higher stability. The physicochemical parameters such as colour, viscosity, peroxide value and free fatty acid value were analysed during frying process. In eachfryingcycle, viscosity hadprogressivelyaugmented in deep fried oils. The tocopherols were maintained in first two frying cycles.The PV and FFA showed a noteworthy reduction in blended and interesterified oil of RPOO+CAO as compared to RPOO and suitable for frying upto 20$^{th}$ frying cycles.The frying stability of the blended and interesterified oil was improved due as compared to native oil. This study will helpful to the consumers and food industry economically for efficient use oils to make testable product development.

**Keywords :** Canola oil, refined palm olein oil, blended oil, interesterified oil.

## INTRODUCTION

India is the world's largest importer and the third largest consumer of edible oils (1).India's edible oil manufacturers promote fortified refined palm olein, safflower, olive oil, and rice bran oil as more healthful cooking oils. Cottonseed

oil finds increasing acceptability due to its light color, Oil, Sunflower seed neutral odour, and blending characteristics with other oils. Coconut, peanut and sunflower oils continue to be widely consumed in south India, while peanut and cottonseed oils are more prevalent in Gujarat and Maharashtra. Rapeseed oil is preferred in northeast, eastern and northwest India, while soybean oil prevails in central India, and rice bran oil is gaining popularity in eastern India (2). The most common application of edible oils in deep fat frying, salad dressings, and food emulsions (3). Deep fat frying is to seal the food by immersing it in hot oil so that all the flavours and juices are retained within the crispy crust (4). Deep fat frying it will be changes such as physical, chemical and organoleptical properties were occurred in oil that have a direct effect on quality and health of food (5).The process is based on the oil-food interaction at high temperatures, which cooks and dehydrates the food, leading to physical and chemical changes, such as starch gelatinization, protein denaturation, flavoring and color production via Maillard reaction. Some food and oil compounds are lost in the frying process, and potentially toxic compounds are developed in the oxidized oil (6).

Canola oil is most favourable in terms of the health benefits. Canola oil high in monounsaturated fatty acid particularly oleic acid is 61%. Oleic acid reduces serum cholesterol levels and bad cholesterol (LDL) levels, while it does not affect levels of good cholesterol (HDL) levels (4). Fatty acid composition in canola oil can be modified to reduce the content of Erucic acid and to increase the level of oleic acid. Canola oil contains both omega – 6 and omega – 3 fatty acids in a ratio of 2:1 and is second only to flax oil in omega – 3 fatty acid.Canola oil can have a number of health risks associated with it, as much of the omega – 3 fatty acids are converted to *trans* fats during modern refining processes and using it to cut out saturated fats can leave consumers vulnerable to other heart related problems through a lack of saturated fats in the diet (7). Canola oil have high amounts of unsaturated compounds are not suitable for frying.

Palm oil consists at mixtures of 95 % of triacylglycerols,that is glycerol molecules each esterified with three fatty acids. Palm oil are the metabolites in the biosynthesis of TGs and products from lipolytic activity (8).Blending and interesterification are efficient means to modify the physical and chemical properties of oils and their blends. The blend of canola oil with high oleic acid levels and palmolein oil was reported to show higher oxidative stability, less free fatty acid and polar compound formation during frying conditions as compared to palmolein oil alone. Palm based MLCT (Medium Long Chain Triacylglycerols) oil with the aid of different antioxidants showed significantly better thermal resistance and oxidative strength than its control oil(9).The aim of this present investigation was to make the blend and interesterified with refined palmolein oil (RPOO) and canola oil(CAO) in appropriateproportionto reduce the cost as well as improve the frying stability and deteriorating tendency. Hence, we had studied the physicochemical properties to know whether blended oil or interesterified oil helping to improve the frying stability and nutraceutical retention during deep fat frying.

## MATERIALS AND METHODS

### Materials

Refined palmolein oil was procured from the local super supermarket, Madurai,Tamil Nadu. Canola oil was purchased from Mysore,Karnataka. Lipozyme RM IM was obtained as gift from Novozymes A/S, Bangalore, India. All chemicals and solvents used were analytical research grade.Reference standard FAME mix (Supelco Inc.), sodium hydroxide, potassium hydroxide, potassium iodide, potassium thalate, acetic acid, starch, chloroform, petroleum ether, ethyl alcohol, phenolphthalein, sodium thio sulphate are procured from Sisco Research Laboratory Mumbai, India, HPLC grade hexane and methanol were procured from Sigma – Aldrich Co., St. Louis, MO, USA.

**Methods**

**Process of blending oil**

Two hundred gram of the 50:50 combinations of the oil was taken in a beaker separately. Magnetic beads were put into the solutions and closed with an aluminium foil. The beakers were kept in a magnetic stirrer and allowed to run at a temperature of 45°C for one hour.

**Interesterification of oil**

Interesterified oil was processed with blended oil 50:50 combinations. The mixed oil was taken in a 250 ml conical flask and 5 % immobilizedlipozyme (IM RM, *Rhizomucormiehei*) was added. The conical flask was closed with a stopper and kept in the shaking cum water bath (Julabo, Germany, SW 22) with 1600 rpm speed for 12 hrs at 37ºC. Then the oil was filtered and enzyme was separated using hexane.

**Frying study**

Three murukku were used for frying in oil at a time. The circumference of the frying pan was 121 $cm^2$with depth of 12.5 cm and oil holding capacity of 1 litre. The frying temperature of oilwas maintained at 180ºC and the murukku was fried in oil. The oil sample was collected at the end of the repeated frying of murukku, approximately $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$, $20^{th}$consecutive end of frying to study the properties of theoil. The fried oil samples were collected and stored under therefrigerated condition and used for analyzing the properties of theoil. (Rangaswamy and Nasirullah, 2014).In the fried oil, the parameters viz, colour, viscosity, peroxide value, free fatty acid were analysed.

**Physicochemical characteristics of fried oils**

**Colour**

Colour value of the initial, $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$, $20^{th}$ consecutive end of every frying oil samples were determined in Hunter Labscan XE spectrophotometer.Fifteen ml of sample were placed in a sample cup was used for transmittance color measurements in liquid media. The color of samples was obtained by using a 2/°C (2° observer/ illuminant C). The results are expressed as *L**, *a**, *b** respectively indicating lightness (0 to 100), green to red components and blue - yellow components.

**Viscosity**

Viscosity value of initial, $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$, $20^{th}$ consecutive end of every frying oil samples was determined in viscometer.Apparent viscosities of the different frying oils were carried out using a controlled shear-stress viscometer (Model # RT 10, HaakeGmbh, Karlsruhe, Germany) consisting of coaxial cylinder at a shear rate of 102 $s^{-1}$. For initial section of oils, the apparent viscosities were measured at 25±1 °C for vegetable oils.

**Peroxide Value**

Initial, $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$, $20^{th}$ consecutive end of every frying oil samples were used for peroxide value (PV) determination by AOCS O.M. No. Ca 8-53). Peroxide value (PV) of the samples was determined by titrated against 0.1 N sodium thiosulphate solutions in the presence of potassium iodide solution using starch as indicator.

**Free Fatty Acid Value**

The FFAwas determined in the initial, $4^{th}$, $8^{th}$, $12^{th}$, $16^{th}$, $20^{th}$ consecutive end of every frying oil by AOCS O.M.No. Ca 5a-40. Oil was titrated against 0.1 N NaOHsolution in neutralized alcohol medium using phenolphthalein as indicator.

## RESULTS AND DISCUSSION

### Changes in Colour

The change of colour of the refined palmolein oil, canola oil, blended and interesterified oil initial raw oil and after used for frying up to 20 cycleis shown Table 1. The avalue green to redness in all the oils were found to increase in colour after repeated frying cycles as compared to initial oil. The b value of yellow to blueness in all the oils were found to increase in colour after repeated frying cycles as compared to initial oil. The L value of lightness in all the oils were found to increase in colour brightness after repeated frying cycles as compared to initial oil. The colour value it may be partially due to it formation of degraded compounds from the fried product (10). Refined palm olein oil have pale yellow colour and canola oil pale yellow colour. colour it indicating the presence of colour pigments.The colour of oils was darkened during each frying. Up to 16$^{th}$frying it darkened very fastest start but then the change in colour became slow and finally the colour persistent.This may be attributed to the fact that food when fried at a high temperature can introduce various components into the oil such as carbohydrates, phosphates, sulphur compounds, trace metals etc. The formation of nonvolatile decomposition products is due primarily to thermal oxidation and polymerization of the unsaturated fatty acids in fat. Many of these compounds contribute to color formation along with other changes.

### Changes in Viscosity

Fig 3.Viscosity of the native, blended and interesterified oil of refined palm olein oil with canola oil during the frying at 180$^{o}$c.Viscosity values were increased in initial to every frying such as 4$^{th}$ frying, 8$^{th}$ frying,12$^{th}$ frying,16$^{th}$ frying and 20th frying. This study was observed viscosity of native refined palm olein oil, Canola oil, blended oil and interesterified oil was tended to increase because of their oxidation and polymerization.

### Changes in Peroxide Value

The changes in peroxide value of native RPOO and native CAO blended and interesterified oil was shown Fig 4.Native RRPOO, initially 2.2 present after 4th frying it value increase 9.1meq$O_2$/kg, the each frying cycle increased trends such as 12.6meq$O_2$/kg was 8$^{th}$ frying, 18.5 meq$O_2$/kg was 12$^{th}$ frying, 21.5meq$O_2$/kg was 16$^{th}$frying and 21.7 meq$O_2$/kg was20$^{th}$ frying.CAO, initially 2.4meq$O_2$/kg was13.61meq$O_2$/kg was4thfrying, 12.43meq$O_2$/kg was8thfrying,8.96meq$O_2$/kg was 12$^{th}$ frying,8.73meq$O_2$/kg was16th frying and 10.32meq$O_2$/kg was 20$^{th}$ frying. Blended oils such as initially 2.3meq$O_2$/kg,, 6.5 (meq$O_2$/kg,meq$O_2$/kg was 4$^{th}$ , 10.49meq$O_2$/kg, meq$O_2$/kg was8thfrying, 8.65meq$O_2$/kg, was 12$^{th}$ frying, 9.02meq$O_2$/kgmeq$O_2$/kg was 16$^{th}$ fryingand 13.41meq$O_2$/kg was 20$^{th}$ frying. Intersterified oils such as initially 1.7meq$O_2$/kg,4.2meq$O_2$/kgwas 4$^{th}$ frying, 6.8 meq$O_2$/kgwas 8$^{th}$ frying, 12.6meq$O_2$/kgwas12thfrying, 8.54 meq$O_2$/kgwas16$^{th}$frying and 8.08 meq$O_2$/kg was20thfrying. The primary oxidation of oil is analysed for PV value. Peroxide value is one of the quality indexes of edible oils and indicates oxidation level in oils (10).PV increased it indicating leastoxidative stabilityrespectively for refined palm olein oil and canola oil it obviously appropriate to more tocopherol and tocotrienol content in refined palm olein oil (11).

### Changes in free fatty acid value

Free fatty acid contents of oil blend and intersterifiedFig 5 every frying cycle gradually increased. The effect of the higher FFA content on the high quality of oil it means higher diacylglycerol and monoacylglycerol contents. Higher proportion of these additional oil types will affect rate of crystallization and cause cloudiness in oil at low temperature storage condition. Similar resulted that,was blending and interesterification of palm olein and canola oil with higher degrees of unsaturation,it showed in blends and interesterified that are more stable at low temperatures. The blends and interesterified stay clear for a longer period of time (13).

**Changes in total polar compounds and total tocopherols retention**

The changes in total polar compounds of native RPOO and native CAO blended and interesterified oil was shown Fig 6.The total polar contents during the frying process increased gradually when in repeated frying. The tocopherols were maintained 1 cycle in native oils and degraded fast in other cycles. The blended and interesterified oil of RPOO+CAO showedtocopherolretention uptothreecycles and degraded gradually in other cycles.

## CONCLUSION

This study showed that deep fat frying medium of blended and interesterified oil of RPOO+CAO was higher oxidative stability. The blended and interesterified oil can improve the stability of frying oil against lipid oxidation. The blends and interesterified of RPOO with CAO in 50:50 are more stable to oxidative deterioration due to heating as compared to canola oil. Addition of RPOO to the CAO means longer frying times for the final, and RPOO acceptable to the consumers who prefer foods with aroma and flavour imparted by canola oil.

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**Table 1. Changes in colour value of native, blended and interesterified oils.**

| Parameters | Colour | | |
|---|---|---|---|
| | L* value | a* value | b* value |
| RPOO 0 | 91.63±0.08 | -4.32±0.17 | 32.40±0.16 |
| RPOO 4th | 91.33±0.21 | -2.83±0.09 | 31.75±0.09 |
| RPOO 8th | 91.50±0.17 | -5.43± 0.35 | 38.21±0.22 |
| RPOO 12th | 91.80±0.06 | -6.16±0.03 | 38.94±0.28 |
| RPOO 16th | 91.95±0.07 | -6.29±0.13 | 43.57±0.08 |
| RPOO 20th | 91.48±0.27 | -6.60±0.06 | 46.79±0.14 |
| CAO 0 | 99.9±0.5 | -2.29±0.6 | 8.29±0.38 |
| CAO 4th | 99.59±0.4 | -3.04±1.5 | 11.78±0.20 |
| CAO8th | 99.35±0.5 | -3.79±0.5 | 15.05±0.21 |
| CAO 12th | 99.13±0.3 | -5.3±1.2 | 19.24±0.34 |
| CAO 16th | 98.9±0.2 | -5.85±2.5 | 21.47±0.52 |
| CAO 20th | 98.87±0.5 | -6.05±3.4 | 21.96±0.65 |
| RPOO + CAO (B) 0 | 98.13±0.1 | -5.27±0.3 | 21.22±0.70 |
| RPOO + CAO (B) 4th | 97.27±0.6 | -5.84±0.8 | 25.47±0.91 |
| RPOO + CAO (B) 8th | 97.14±0.5 | -5.99±1.4 | 25.96±0.96 |
| RPOO + CAO (B) 12th | 96.57±0.3 | -6.45±0.9 | 28.76±1.5 |
| RPOO + CAO B) 16th | 96.86±0.2 | -6.99±0.6 | 33.39±0.9 |
| RPOO + CAO (B) 20th | 96.55±0.1 | -6.57±0.4 | 31.76±1.7 |
| RPOO + CAO (I) 0 | 97.04±0.3 | -5.04±0.3 | 19.86±0.5 |
| RPOO + CAO (I) 4th | 97.74±0.2 | -5.18±0.1 | 21.08±0.4 |
| RPOO + CAO (I) 8th | 96.73±0.1 | -5.79±1.6 | 23.07±0.5 |
| RPOO + CAO (I) 12th | 96.56±0.2 | -6.23±1.5 | 23.65±0.8 |
| RPOO + CAO (I) 16th | 95.02±0.1 | -6.89±2.0 | 29.04±1.2 |
| RPOO + CAO (I) 20th | 95.00±0.8 | -7.02±0.2 | 34.29±1.1 |

*Values are mean ± SD, n = 4;nd: not detected, L*- Lightness, a*- indicates redness when positive, greenness when negative, b*- indicates yellowness when positive, blueness when negative, RPOO – refined palm olein oil, CO– canola oil, B – blended, I- interesterified*

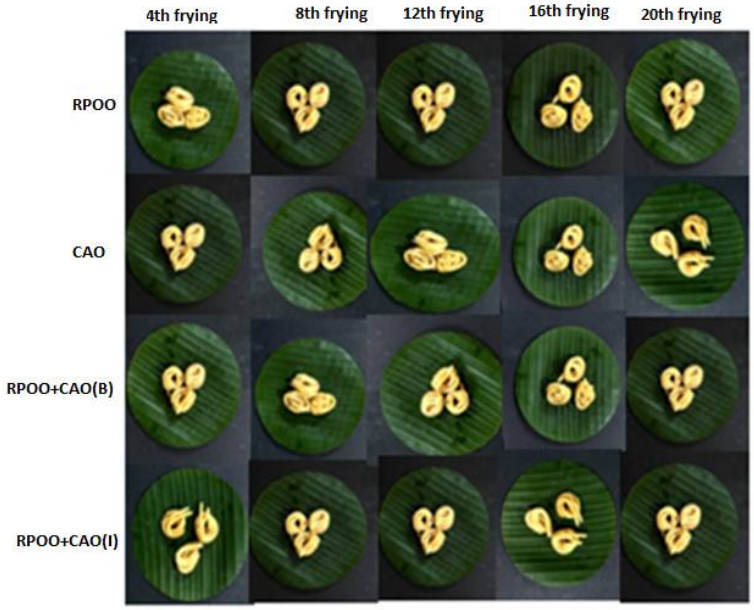


**Fig 1. Product photo of Murukku fried in 1)native oil Refined palm olein oil (RPOO) 2) Canola oil (CAO) 3) blended oil of RPOO +CAO(B) and 4) Interesterified oil of RPOO + CAO(I)**

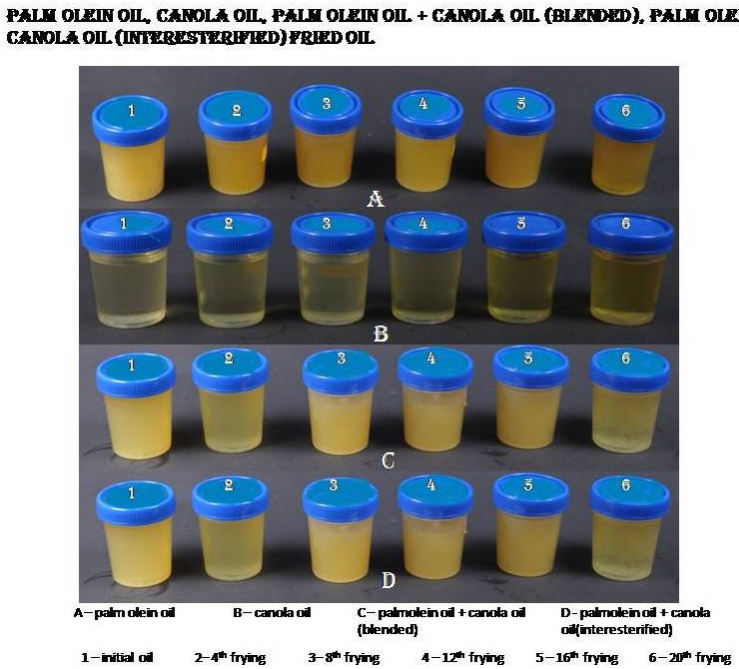


**Fig 2. Repeated frying cycles of 1)native RPOO,2) native CAO, 3)belnded of**

**RPOO+CAO(B) and 4) interesterified oil of RPOO+CAO (I).**

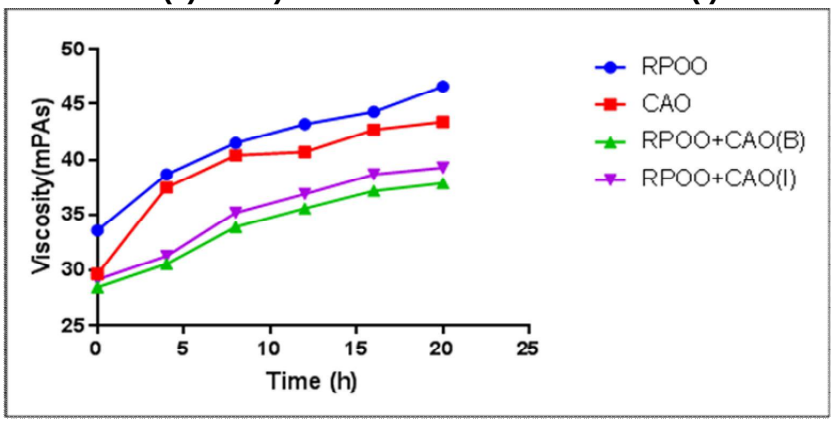


Fig 3. Viscosity of the native, blended and interesterified oil of refined palm olein oil(RPOO) with canola oil(CAO) during the frying process at 180°C

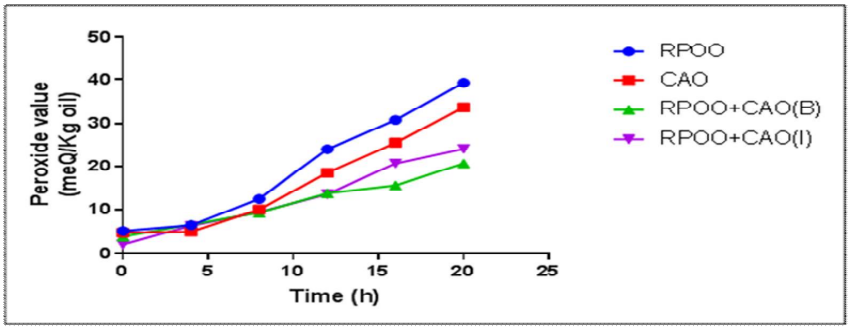


Fig 4.Peroxide value of the native,blended and interesterified oil of refined palm olein oil(RPOO) with canola oil(CAO) during the frying process at 180°C

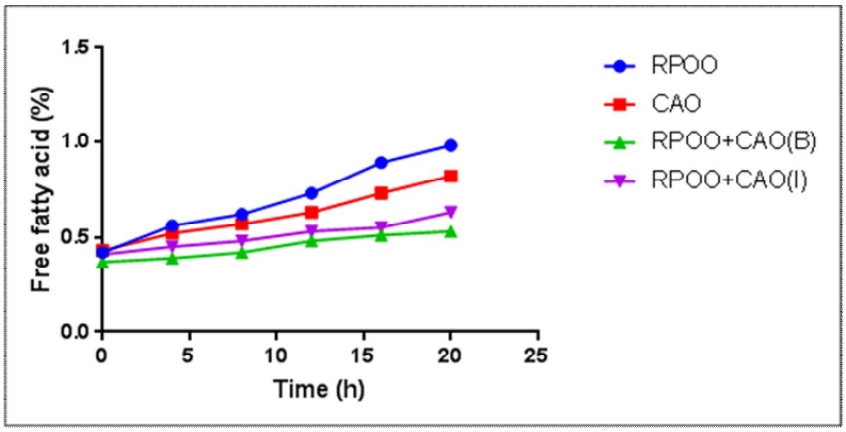


Fig 5.Free fatty acid value of the native, blended and interesterified oil of refined palm olein oil(RPOO) with canola oil(CAO) during the frying process at 180°C

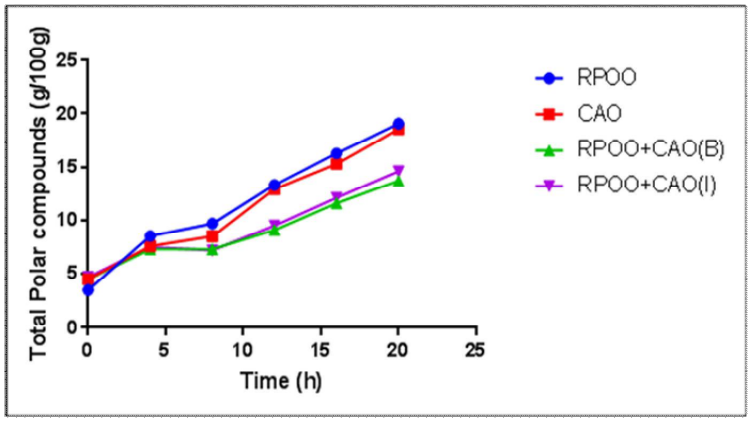


Fig 6.Total Polar compounds of the native, blended and interesterified oil of refined palm oleinoil (RPOO) with canola oil(CAO) during the frying process at 180°C

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**RESEARCH ARTICLE**

# Comparative Gross Anatomical Studies of the Renal Arteries in Indian Goat (*Capra hircus*) and Pig (*Sus scrofa domesticus*)

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## ABSTRACT

Comparative gross anatomical studies of the renal arteries in Indian goat (*Capra hircus*) and Pig (*Sus scrofa domesticus*)" was carried out in twelve each renal artery samples of adult goats and pigs. Morphological features and morphometrical measurements of both right and left renal arteries were studied and recorded. Morphological features of renal arteries of goat and pig showed that they originated from ventral or ventro-lateral surface of abdominal aorta. The left one was caudal in position to that of the right artery in both species. Renal arteries of pig were shorter in length than those of the goat. Majority of goat renal arteries terminated into two primary branches *viz.,* cranial and caudal, whereas in pigs there were three primary branches *viz.,* cranial, middle and caudal close to the hilus of respective kidney. Variations in branching of renal arteries were noticed such as a branch from renal arteries of goats to adrenal gland, cranial pole of left and right kidneys and one additional renal artery from aorta to cranial pole of left kidney. Multiple branches were seen at pre-hilar end of left and right renal arteries in few goat specimens. Morphometrical studies revealed that the Mean length of left renal artery in goats was higher (4.54 ± 0.21 cm) than the right one (4.15 ± 0.21 cm). Mean width was similar *i.e.,* 0.41 ± 0.06 cm on the right and 0.43 ± 0.05 cm on left side. There was no significant difference in length and width of renal artery primary branches within and across the species. Mean length and width of pig renal arteries was similar *i.e.,* 3.54 ± 0.37 cm and 0.35 ± 0.02 mm on right and 3.34 ± 0.29 cm and 0.34 ± 0.02 mm on left side

respectively.Significant difference existed in the left caudal primary branches of goat and pig renal arteries. Mean width of right caudal primary branch of renal arteries was slightly higher (2.46 ± 0.13 mm) than left side (2.26 ± 0.21 mm) in goats which is significantly comparable within species but differed from that of the pig specimens.

**Keywords :** Renal artery, goat, pig,

## INTRODUCTION

Medium sized renal arteries are extremely important blood vessels in the animal body because of their closer location to the heart. They receive 16-30% of the cardiac output, which is why they are well-developed (Leeson *et al.,* 1988, Banks *et al.,* 1993, Dellmann and Eurell, 1998 in domestic animals and Guyton and Hall, 2000 in humans). In domestic animals and humans renal arteries are classified as muscular type arteries. Right and left renal arteries originated from the related side of the abdominal aorta in domestic animals and bovine calves (Nickel *et al.,* 1981 and Jain and Singh 1987 respectively). They usually arise from antero-lateral or lateral aspect of the abdominal aorta just below the origin of the superior mesenteric artery in humans (William *et al.,*1989). In ruminants and other domestic animals they may also arise from ventral aspect of the abdominal aorta (Getty, 1975 and Dyce *et.al.,* 2010). Renal arteries give rise to dorsal and ventral branches before entering the hilus of the kidney after which they respectively divide into interlobar, arcuate and interlobular arteries (Aslan and Nazli, 2001 in goat and sheep; Aksoy and Ozudogru, 2003 in Van Cat and Aksoy *et al.,* 2004 in sheep). These arteries are the largest collateral branches of the abdominal aorta in humans. From their point of origin they run in an oblique inferio-lateral direction towards the hilus of kidneys where they represent the main element of the renal pedicle around which other elements are grouped i.e., the renal vein, lymph vessels and nerves (Ecaterina *et al.,* 2012).

## MATERIALS AND METHODS

Renal artery samples were collected from twelve adult apparently healthy goats and pigs from local slaughter house immediately after slaughter along with kidneys, part of aorta, venacava and ureter. Gross observations of right and left renal arteries and their branching pattern in goats and pigs were studied after gentle dissection regarding the point of origin from aorta and number of primary or any accessory branches arising from either the main trunk of renal artery or directly from aorta to kidneys were recorded.Morphometrical observations of renal arteries such as the length and width of the main arterial trunk and its primary branches in goat and pig were recorded with a thread, scale and digital Vernier caliper's (Mitutoyo) and tabulated (Tables. 1 and 2 respectively).

## RESULTS AND DISCUSSION

In all goat specimens, renal arteries in fresh state were observed in dense mass of retroperitoneal fat and connective tissue (Fig. 1) along with kidneys and adjacent adrenal glands. Careful dissection revealed that both right and left renal arteries originated from the ventral surface of the abdominal aorta at almost right angles to it (Figs. 2 and 3). In one specimen an arterial branch from right renal artery to right adrenal gland was noticed (Fig. 4). The origin of left renal artery was caudal in position to that of right renal artery (Figs. 2 and 3) and it was slightly longer than the right in most specimens. However, in one specimen the right renal artery was longer than the left artery (Fig. 5) and (Table. 1). Renal arteries in pigs arose from the ventral face of the abdominal aorta with the left artery originating slighty behind the right one. Their main trunk was relatively shorter and showed variations in branching pattern (Fig. 15). Similar reports were made by several authors such as Evans *et al*. (1996) in pigs, Aksoy *et al*. (2004) in Tuj sheep, Ozudogru *et al*. (2005) in wolf, Asadi (2006) in sheep, Marques-Sampaio *et al*. (2007) and Ozdemir *et al*. (2009) in dogs, Dyce *et al*. (2010) in ruminants and other domestic animals and Gahlot *et al*. (2014) in their comparative studies of

renal arteries in humans, goat and buffalo. They also opined that all animal kidneys are supplied by right and left renal arteries which originated from related side of abdominal aorta wherein the left artery arose slightly caudal to right one. In present study in both species the left renal artery originated caudal to the right artery which is akin to the findings in Van cats where the right renal artery emerged from abdominal aorta slightly cranial to comparatively longer left renal artery (Aksoy and Ozudogru, 2003). Similarly in Tuj sheep Aksoy *et al*. (2004) stated that right renal artery was longer and arose cranial to the origin of left one but in case of rats the renal arteries originated on the lateral aspect of abdominal aorta (Yoldas *et al*., 2014).Branching pattern of renal arteries of goat and pig was pre-hilar in position wherein the primary branches traversed to some distance before entering into the kidney.

Variations in branching pattern of renal arteries were observed in the goat specimens. Majority of the specimens revealed that each renal artery divided into two primary branches viz., cranial and caudal close to the hilus of respective kidney (Figs. 5 and 6). In three specimens both renal arteries terminated into three primary branches closer to the hilus viz., cranial, caudal and ventral branches (Figs. 8 and 9). In eight pig specimens simple termination of left and right renal arteries into three primary branches (cranial, middle and caudal) was observed at pre-hilar end (Fig. 13), except for one specimen where the right renal artery divided first into cranial and caudal branches after which the former gave an additional (middle) branch (Fig. 14). Similar reports were made by several authors who reported that a single renal artery emerged on either side of the abdominal aorta to supply both kidneys in most domestic animals. It traversed towards the hilus of the kidney and divided into dorsal and ventral divisions which supplied mostly four or five segmental arteries to renal parenchyma (Aksoy and Ozudogru, 2003 in Van cat, Aksoy *et al*., 2004 in Tuj sheep, Asadi, 2006 in sheep, Ozudogru and Ozdemir, 2005 in wolf, Ozdemir *et al*., 2009 in Kangal dog, Shalgum *et al*., 2012 in rabbit and Yoldas *et al*., 2014 in adult mole rats).In two goat specimens the main trunk of left renal artery gave a separate branch to cranial pole of left kidney (Figs. 10 and 11) and then divided into primary branches at its pre hilar end (Fig. 12). In one of the above two specimens a separate branch from right renal artery arose in its mid region which distributed branches to the right adrenal gland and to the cranial pole of right kidney (Figs. 4 and 11) beyond which the main trunk before its division into primary branches at the pre-hilar region further gave an additional collateral branch which entered the cranial pole of right kidney (Fig. 11), similar reports appreciated in different animals and humans by Satyapal *et al*. (2000) in humans, James *et al*. (2010) in Nigerian goat and Mohamed RA (2014) in Baladi rabbit who all mentioned an additional renal artery arising directly from abdominal aorta to the left kidney

In one pig specimen an additional renal branch on left side arose directly from the abdominal aorta and entered the cranial pole of left kidney (Fig. 15), while the left renal artery branched into cranial and caudal divisions wherein the former further gave an additional (middle) branch. Horacek *et al*. (1987) cited that renal arteries in macaque monkey may give rise to supra renal branches before its primary division and in humans Ecaterina *et al*. (2012) stated that the main trunk of renal artery showed variations like two, three and four in 70%, 23.3% and 6.67% cases respectively. The right renal artery in this specimen terminated midway into a dorsal and ventral of which the dorsal branch divided into cranial and caudal branches at the pre-hilar end (Figs. 15 and 16). In two pig specimens the left renal artery behaved similar to other specimens such as Fig. 15, by dividing into two primary branches of which the former gave an additional branch (Fig. 17). However the right renal artery branched primarily into cranial and caudal branches. The latter in turn divided into an additional (middle) branch and then continued as caudal artery into the hilus of kidney (Fig. 18).Multiple branches arose at the terminal end of left (Fig. 19) and right renal arteries (Fig. 20) in two pig specimens which entered the hilus of respective kidney, are in agreement with the findings of Gupta *et al.* (2011) who in their comparative studies found multiple renal arteries in experimental animals like frog and lizard. They also cited that mammalian kidneys get accessory renal arteries such as 17 human kidneys i.e., 28.3% out of 60.

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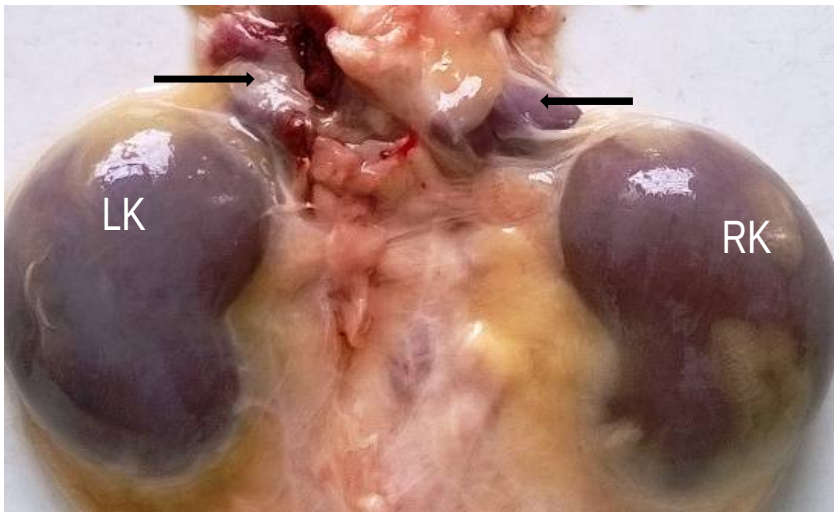


**Fig.1.Photograph of ventral view of renal arteries of goat in situ in retroperitoneal fat and connective tissue. ⚜ Adrenal glands LK - Left kidney RK – Right kidney**

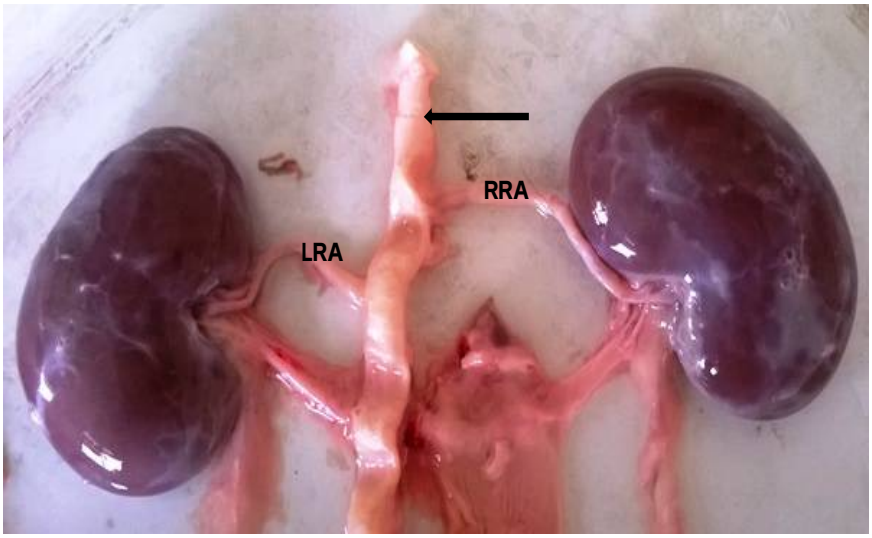


**Fig.2.Photograph of dorsal view of freshly dissected goat renal arteries showing their point of origin. ⚜ Abdominal aorta LRA – Left Renal Artery RRA – Right Renal Artery**

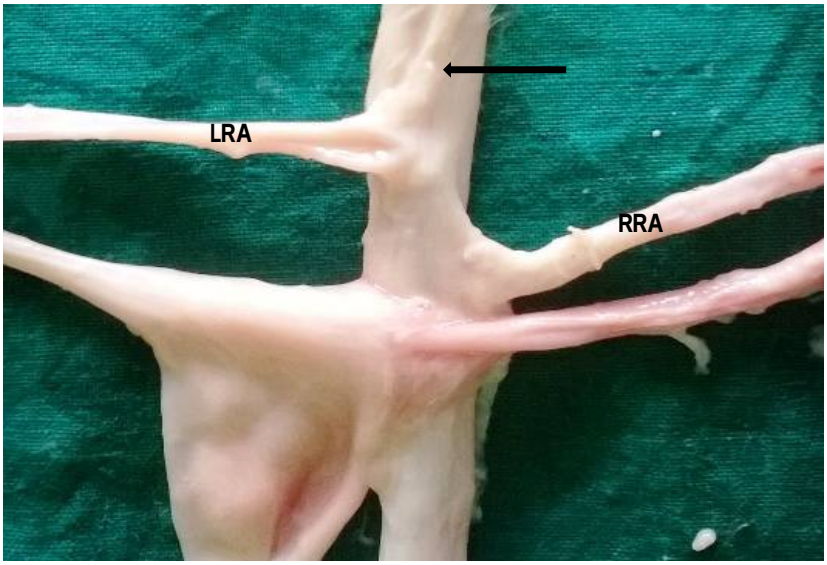


**Fig.3.Photograph of ventral view of goat renal arteries showing their point of origin from ventro-lateral surface of the abdominal aorta. ⚜ Abdominal aorta LRA – Left Renal Artery RRA – Right Renal Artery**

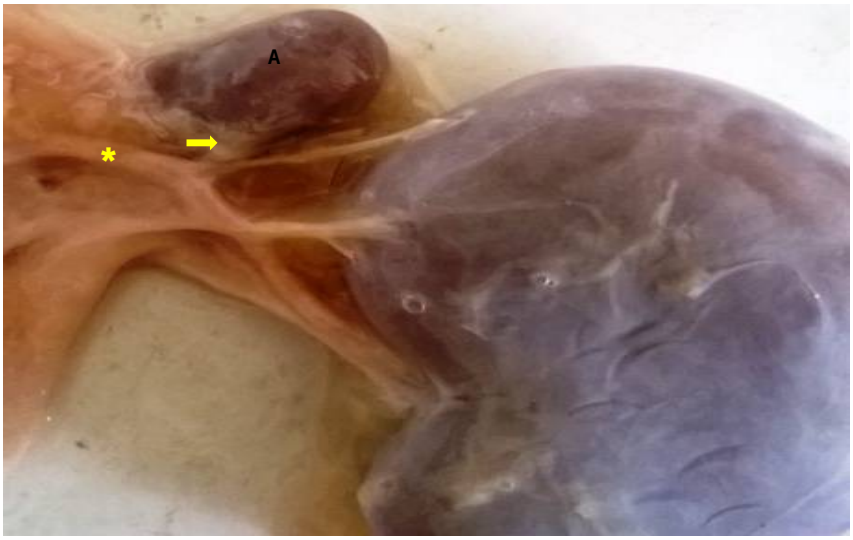


**Fig.4.Photograph showing an arterial branch from main trunk of right renal artery of goat to the cranial pole of the right kidney and adrenal gland. Ad – Adrenal gland**
**⚜ Right Renal Artery ⚜ Blood vessel to right adrenal gland & cranial pole of right kidney**

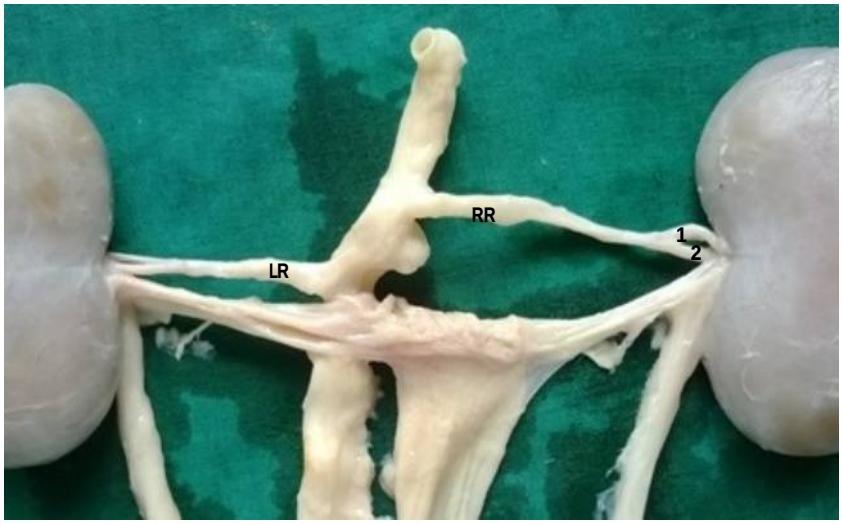


**Fig.5.Photograph of dorsal view of renal arteries of goat showing slightly longer right renal artery. LRA – Left Renal Artery RRA – Right Renal Artery 1 – Cranial branch 2 – Caudal branch**

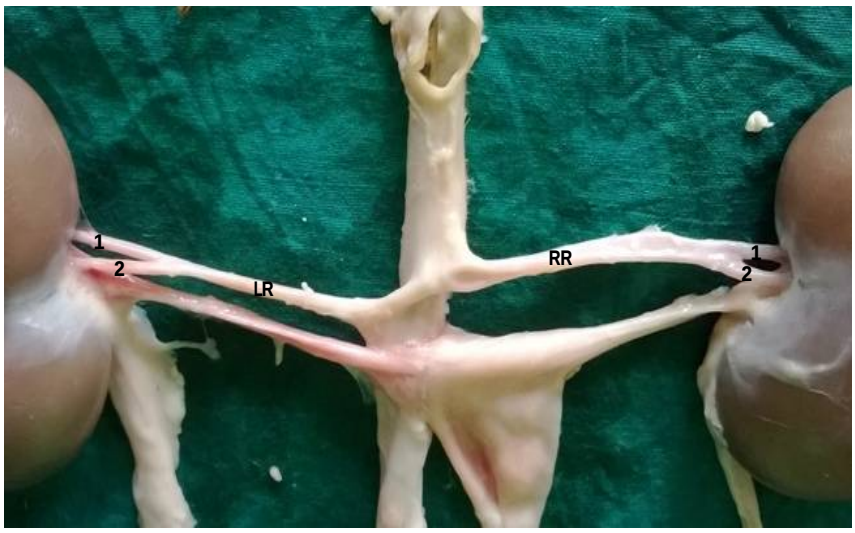


**Fig.6.Photograph showing the prehilar branching pattern of main trunk and itprimary branches of renal arteries in goat. LRA – Left Renal Artery RRA – Right Renal Artery 1 – Cranial branch 2 – Caudal branch**

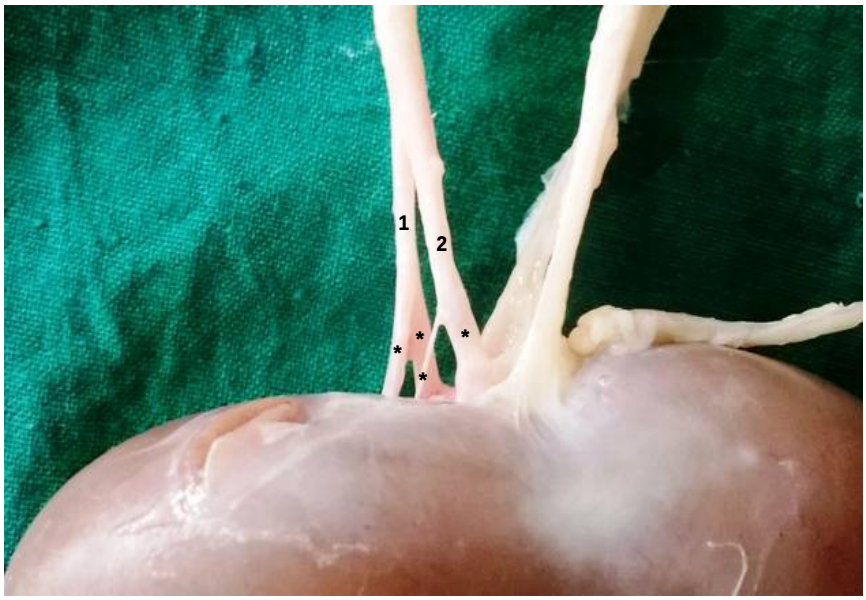


**Fig.7.Photograph showing the prehilar branching pattern of renal arteries deviating from the common branching pattern in goat. ⚜ Secondary branches , 1 – Cranial branch 2 – Caudal branch**

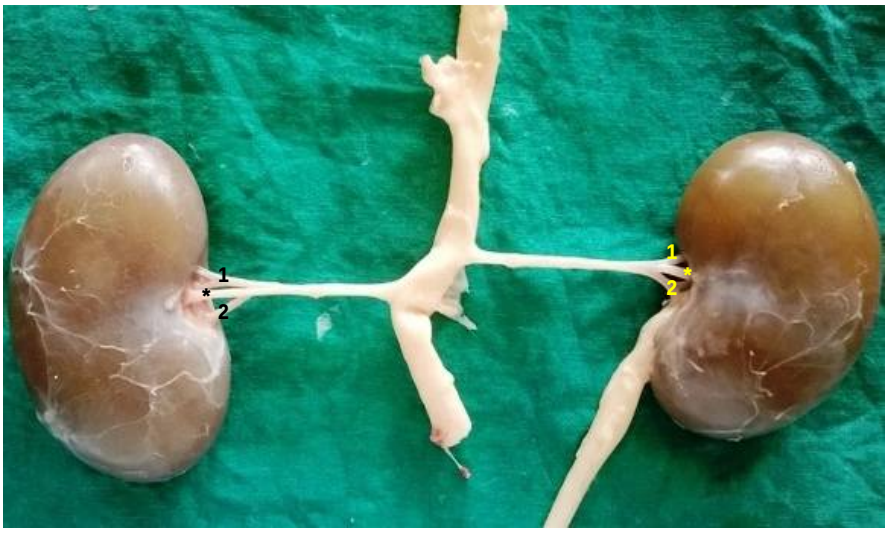


**Fig.8.Photograph showing prehilar branching pattern of renal artery in goat.**
**1 – Cranial branch 2 – Caudal branch ⚜ – Ventral branch**

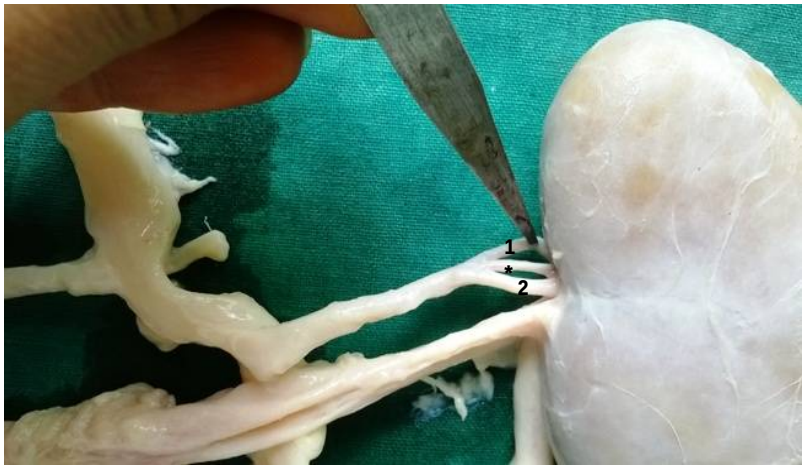


**Fig.9.Photograph of ventral view of prehilar branching pattern of goat renal arteries showing dorsal branch. 1 – Cranial branch 2 – Caudal branch ⚜ - Dorsal branch**

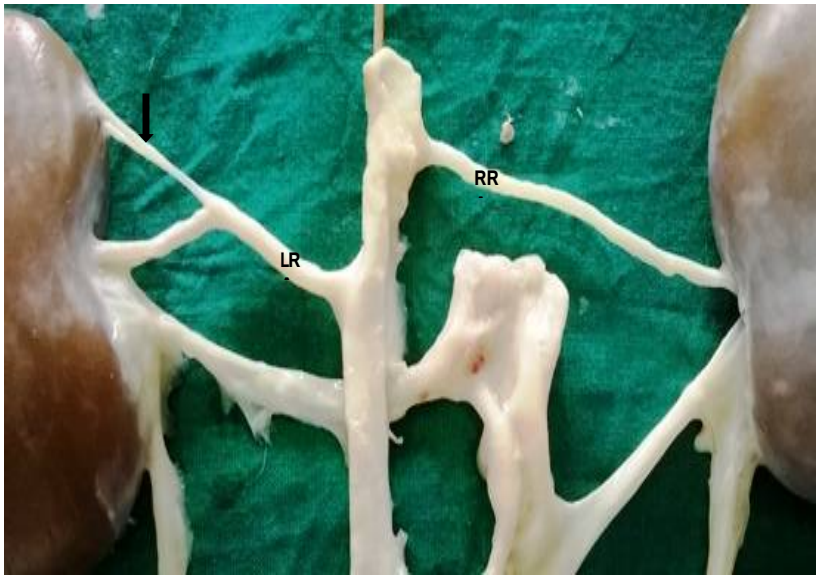


**Fig.10 Photograph showing an accessory branch from left renal artery to cranial pole of left kidney of goat. ⚜ accessory renal artery LRA – left renal artery RRA – right renal artery**

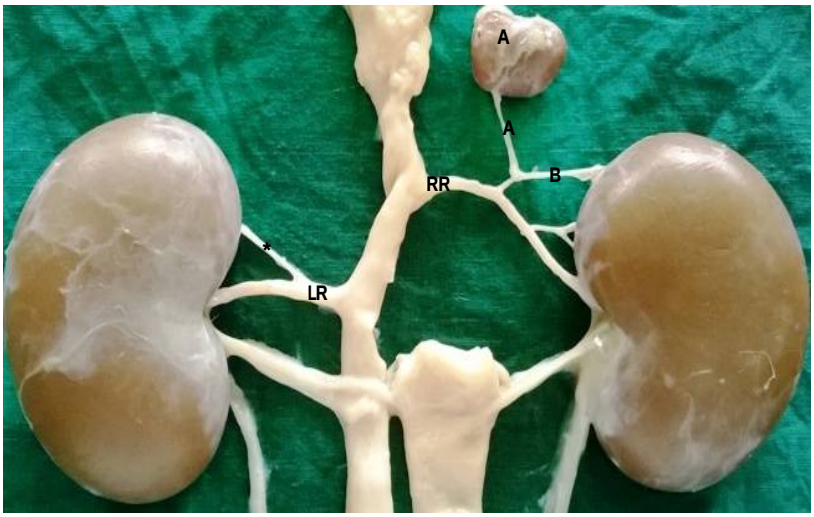


**Fig. 11.Photograph showing the presene of accessory branches form both right and left arteriesof goat to cranial poles of respective kidneys and right adrenal gland.**
**LRA – left renal artery RRA – right renal artery Ad – adrenal gland ⚜ - accessory renal artery**
**A – branch to adrenal B – additional collateral branch**

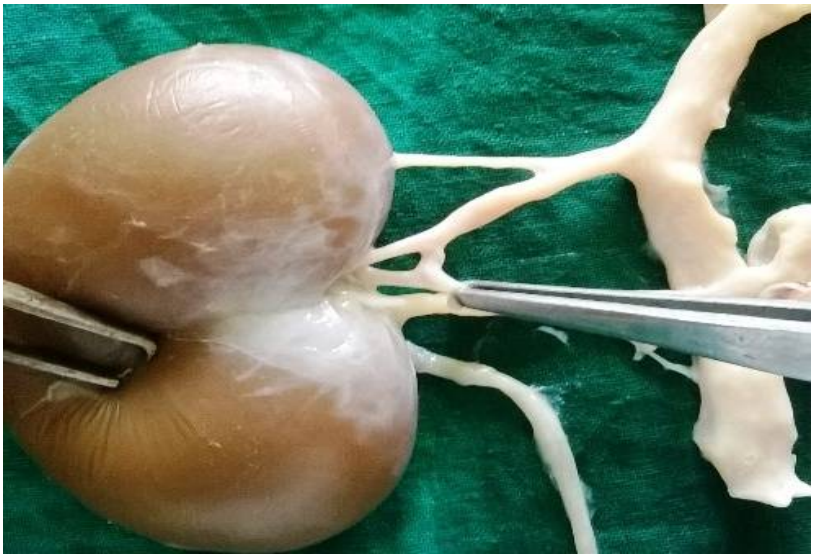

**Fig.12.Photograph showing the accessory renal artery from left renal artery immediately after origin from aorta to cranial pole of left kidney. ⚜ Accessory renal artery ⚜ - prehilar branches**
**LRA – left renal artery**

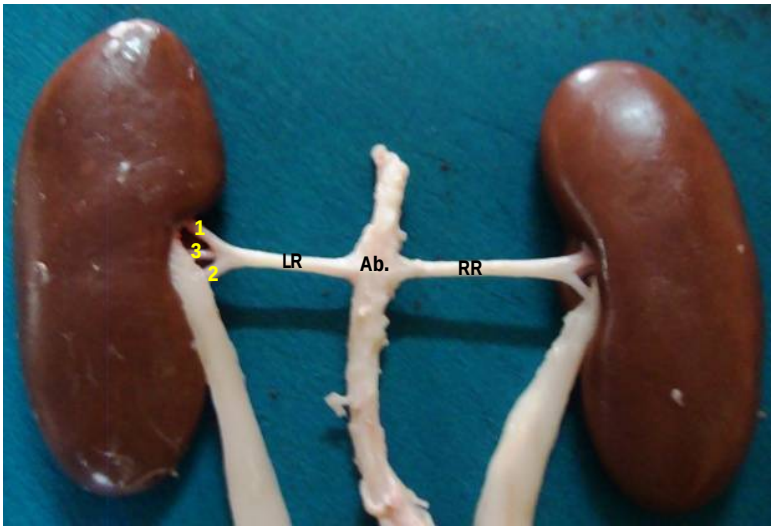


**Fig.13.Photograph showing the origin point of both right and left renal arteries in pig.**
**1 – cranial branch 2 – caudal branch 3 – middle branch LRA – left renal artery**
**RRA – right renal artery Ab.A – Abdominal aorta**

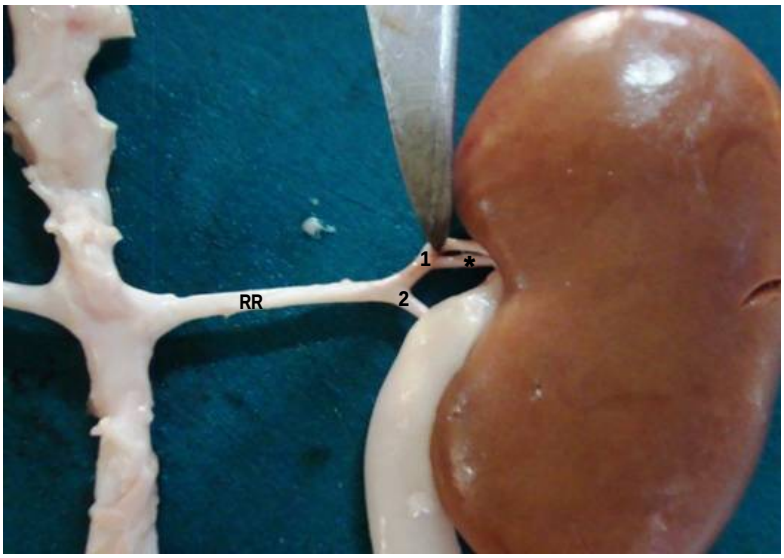


**Fig. 14. Photograph showing the branching pattern of primary branches from right renal artery in pig.**
**RRA – right renal artery 1 – cranial primary branch 2 – caudalprimary branch ⚜ - middle branch**

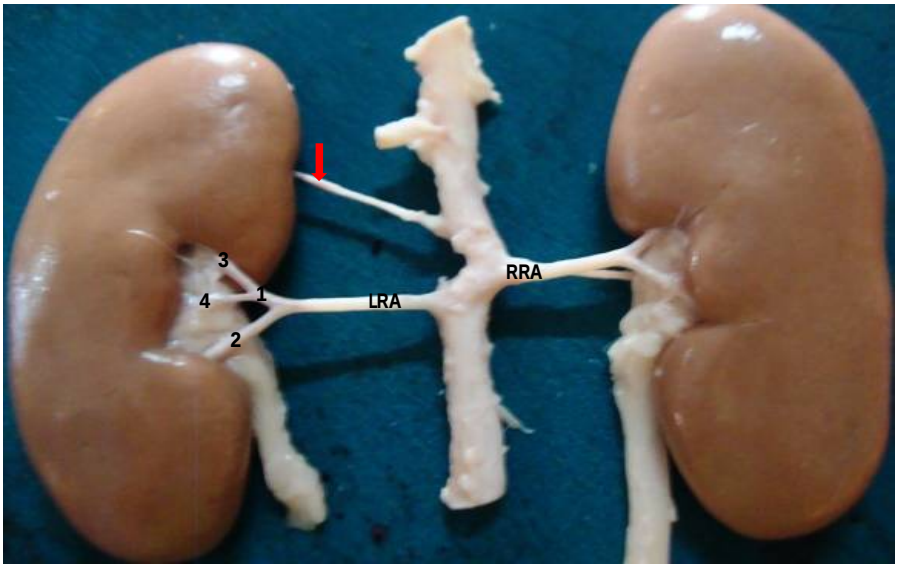


**Fig.15.Photograph showing branching pattern & presence of additional renal artery from the aorta to cranial pole of the left kidney in pig. (⚜) Additional renal artery RRA – right renal artery LRA – left renal artery 1 – Dorsal branch 2 – Ventral branch 3 – Cranial branch 4 – Caudal branch**

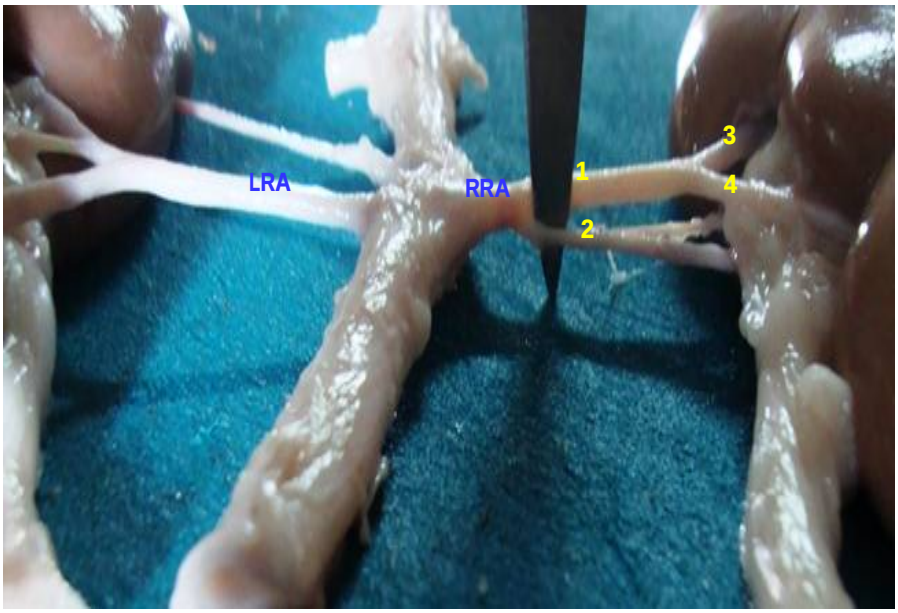


**Fig.16.Photograph showing the deviation of right renal artery in pig into dorsal and ventral branches from the main trunk. RRA – right renal artery LRA – left renal artery 1 – Dorsal branch 2 – Ventral branch 3 – Cranial branch 4 – Caudal branch**

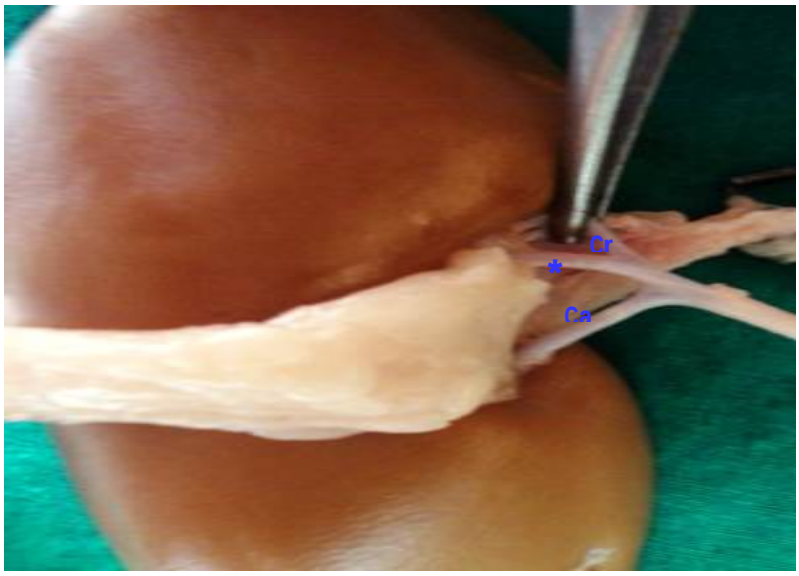


**Fig. 17.Photograph showing the branching pattern of left renal artery in pig.**
**Cr – cranial branch Ca – caudal branch ⚜ - Middle branch**

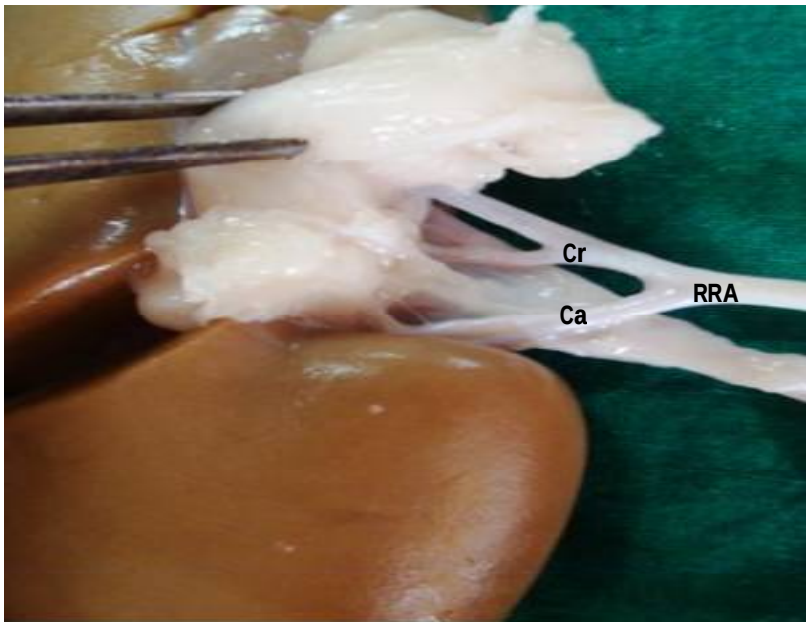


**Fig.18 Photograph right renal artery of pig showing middle branch (⚜) from the primary caudal branch. RRA – right renal artery Cr – cranial branch Ca – caudal branch**

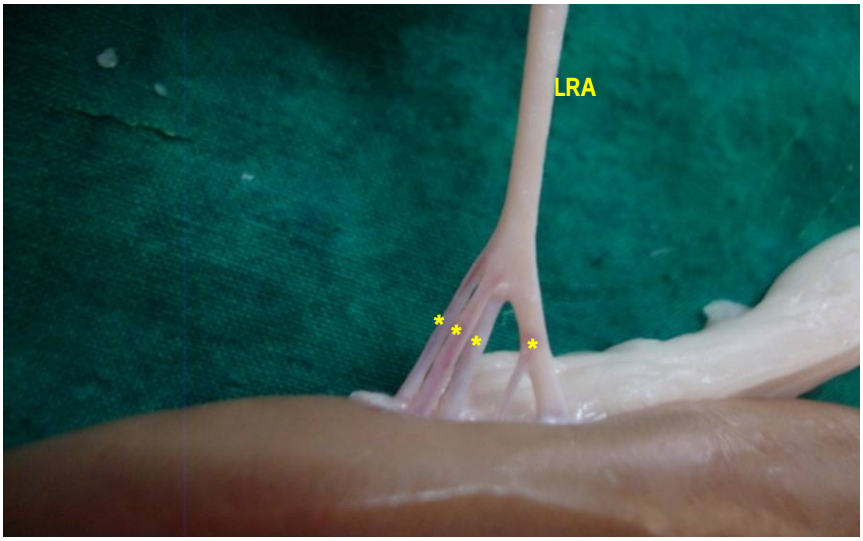


**Fig. 19.Photograph showing prehilar multiple branches from left renal artery in pig.**
**LRA – left renal artery ⚜ - multiple branches**

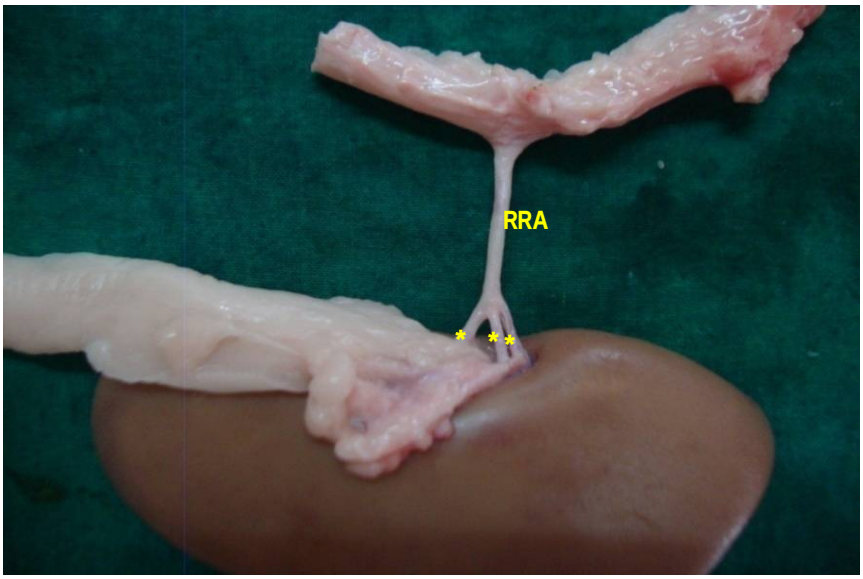


**Fig. 20.Photograph showing prehilar multiple branches from right renal artery in pig.**
**RRA – right renal artery ⚜ - multiple branches**

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RESEARCH ARTICLE

# Haematological and Biochemical Study of Hepatic Disorders in Dogs

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## ABSTRACT

A detailed study was done to evaluate strategies for diagnosis of liver disorders in dogs. Haematologically mean TEC, PCV, Hb and platelets were 5.17±0.03x $10^6$/ul, 32.93±10.54%, 10.31± 0.3g/dl and 177.8±10.53, respectively. The mean ALT, AST, ALP and GGT values were 175.07±16.69, 32.43±4.67, 709.54±84.12and 20.52±2.24, respectively and total protein, albumin, glucose, cholesterol, creatinine, BUN, Direct bilirubin, total bilirubin and calcium were 5.68±0.10, 2.29±0.07,104.21±2.51, 196.82±8.90, 1.02±0.07, 16.22±0.63, 196.82±8.90, 0.95±0.15, 1.50±0.22 and 8.21±0.18 respectively. The pre-prandial and post-prandial total bile acids were23.07±1.64 and 55.74±3.77 respectively. Conclusive diagnosis of liver disorders should be based on a combination of hematobiochemical parameters, followed by ultrasonographic confirmation.

**Keywords :** liver disorders, Haematology, serum biochemistry, pre-prandial and post-prandial total bile acids.

## INTRODUCTION

Liver is a one of the vital organs of the body because it performs different kinds of biochemical, metabolic, synthetic and excretory functions. Nearly all the blood circulated in the body flows back through the portal vein to the liver where it comes in contact with the liver cells, ensuring the products of digestion are presented to the hepatic cells before entering the general circulation. Hence any disorder of this organ affects physiology of the body. Liver disease is the fifth leading cause of death in dogs, and it's estimated that 3% of all diseases in dogs are connected to the liver

(Ruthie 2010).Pets with liver disease can present with array of clinical conditions, from severely ill to asymptomatic (Puja *et al*. 2010). Some vague signs can be depression, weight loss, anorexia, vomiting, lethargy, small body stature and poor or unkempt hair coat (Bunch, 2003). Besides these clinical signs, clients may notice alcoholic feces or other abnormal fecal coloration. More specific signs of liver disease include icterus, ascites, hepatomegaly, microhepatica, coagulopathies and hepatic encephalopathy. Polyuria and polydipsia can also be observed.Hepatic disease is one of the commonly encountered clinical entities in small animal veterinary practice. And in dogs liver disease is difficult to diagnose just based on clinical symptoms and hence to treat. An early and appropriate diagnosis is required for effective therapy as it is concern for both clinician and owner. Estimation of haematological parameters and biochemical profile form the minimum laboratory tests for the diagnosis of liver disorders. The present research work was undertaken to evaluate various haematological diagnostic techniques and serum biochemistry tests for the early diagnosis of liver disorders in dogs.

## MATERIALS AND METHODS

**Source of animals**

Dogs presented/referred to Veterinary College Hospital with clinical signs suggestive of liver disorders such as anorexia, inappetance, lethargy, anaemia, vomition, diarrhoea, icterus, abdominal pain, weight loss, ascites, nervous signs, bleeding tendency, poor hair coat, polyuria and polydipsia were identified while selecting the cases and subjected for thorough history collection and haematological and biochemical test procedures.

**Clinical samples**

Blood collected from the clinical cases in clean sterile vials containing Ethylene Diamine Tetra acetic Acid (EDTA) for haematology and in another vial with clot activator for serum

**Reagents for serum Biochemistry**

ALT, AST, ALP, GGT, BUN, creatinine, total bilirubin, direct bilirubin, total protein, albumin, cholesterol and glucose were estimated using kits manufactured by Transasia Bio-medicals Ltd.

**Haematology Methods**

Haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count(TEC), total leukocyte count (TLC), differential leucocyte count (DLC) and thrombocyte counts were estimated using Auto analyzer/Cell Counter by Mindray (BC - 2800vet) or Erma Automatic Cell counter.

**Biochemical study**

Preparation of glassware: All glassware (Borosil make) were washed with soap water and then rinsed underrunning tap water. Later they were rinsed with single distilled water, air dried and sterilized in a hot air oven at 1600C for one and a half hour. Pipettes, micropipettes were cleaned, washed and sterilized as per the standard procedure.

**Serum biochemistry**

Biochemical parameters– the following biochemical parameters were estimated using Biochemical analyzer (Trivitron Healthcare) and reagents manufactured by TransasiaBio-Medicals Ltd, Solan, HP (George and Kingsley, 1939).

- Blood urea nitrogen
- Creatinine
- Alanine amino transferase
- Aspartate transaminase
- Alkaline phosphatase
- Gama glutamyl transferase
- Total bilirubin
- Total protein
- Serum albumin
- Serum glucose
- Cholesterol

**Electrolyte analyser**

Serum calcium, serum sodium and serum potassium were estimated using electrolyte analyzer (Trivitron, LABMYTE).The above haematology and biochemical parameters were estimated in 06 healthy dogs (which presented for vaccination). In addition, these parameters were estimated in affected dogs and compared with the values of healthy dogs.

**Serum Bile acids**

Blood samples collected after 12 hr of fasting (preprandial) and 2 hours after food (post prandial) in clot activator vials were analysed by DIAZYME Total Bile Acid (TBA) kit (Enzymatic cycling method).

**Statistical analysis**

The results obtained were subjected to statistical analysis as per Snedecor and Cochran, (1994) using Graph pad prism and SPSS 17 software. Data were recorded as percentage, Mean ± SE. Fisher's exact test was used to test statistical significance in the analysis of contingency table.

## RESULTS

The changes in Haematological parameters could be an aid in the diagnosis of liver disorders. The decrease in TEC, PCV, Hb and platelets observed in this study could be because of the fact that the physiological functioning of erythrocytes and the platelets is dependent on the physiological functioning of liver. The increase in TLC is indicative of presence of infection. Increased platelet sequestration in the spleen could be because of congestive splenomegaly that was observed in the patients.Reduced production of thrombopoietin by the liver and increased platelet breakdown due to autoantibodies could be the reason for decrease in platelet count. The values obtained are tabulated and statistical analysis was done as per standard protocol.

## DISCUSSION

### Haematological studies on hepatic disorders

When the haematological parameters were estimated, the average values of Total Erythrocyte count (TEC) (x 106/ul) 5.17±0, leucocyte count (TLC) (x 103/ul) 21.31±1.4,Packed cell volume (PCV) (%)32.93± 10.54, haemoglobin (Hb) 10.31± 0.3 (g/dl) and Platelets 177.8±10.53 estimated in the cases of dogs presented with hepatic disorders, When compared to normal values (TEC-7.12± 0.27, TLC-12.6± .83, PCV-49.1± 1.53, Hb14.4 ±0.40 and platelets-270.50± 25.06)

it is appreciable that there was a significant decrease in TEC, PCV, Hb and platelets and there was a significant increase in TLC. The decrease in TEC, PCV, Hb and platelets is in tandem with the symptoms like anorexia, anaemia, lethargy and ascites because the physiological functioning of erythrocytes and the platelets is dependent on the physiological functioning of liver. The increase in TLC is indicative of presence of infection. Increased platelet sequestration in the spleen as a result of congestive splenomegaly, reduced production of thrombopoietin by the liver and increased platelet breakdown due to autoantibodies could be the reason for decrease in platelet count (Prins *et al*., 2010). These changes in haematological parameters could be an aid in the diagnosis of liver disorders. Similar to the present findings earlier researchers have reported anaemia, lymohocytosis and thrombocytopenia in dogs with liver disorders (Shaker and Khalifa, 2012). However, these changes could also be observed in many other disorders and infections affecting visceral organs and vascular system.

**Biochemical studies on hepatic disorders**

All liver enzymes in hepatic disorders were increased significantly the average values for ALT, AST, ALP and GGT were 175.07±16.69, 32.43±4.67, 709.54±84.12 and 20.52±2.24, respectively. When compared to values of these enzymes in normal dogs (22.5± 3.37, 12.17±1.55, 55.66±7.14, 3±0.36 respectively), there was significant increase in the concentration in the dogs affected with liver disorders. Similar findings were reported by Strombeck and Gribble, (1978), Rutgers and Haywood, (1988), Center (2007), Rothuizen and Brovinda, (2010) and Sumathi (2012). Alanine transferase (ALT) and aspartate transferase (AST) were “leakage” enzymes which become elevated when hepatocytes are damaged. Alkaline phosphatase (ALKP) and gamma-glutamyl transpeptidase (GGT) were “cholestatic” or “inducible” enzymes. Bilestasis causes increased production of these enzymes which were located in the cell membranes of bile canaliculi (ALKP) and bile duct epithelium (GGT). Increases in ALKP alone have been associated with hepatic neoplasia and benign hepatic nodular hyperplasia (Bill, 2012). High ALP could indicate primary hepatic disease in dogs. However in dogs ALP is not liver specific and its elevation could be due to extrahepatic origin (Chapman and Hostutler, 2013). Font *et al*., (1989) reported normal hepatic enzyme levels in liver disorders which is again contradictory to the findings in this study indicating that the estimation of these enzymes is not specific for the diagnosis of liver disorders.

The mean serum total protein, serum albumin, glucose, cholesterol, creatinine, BUN, Direct bilirubin, total bilirubin and serum calcium, values in hepatic disorders were5.68±0.10, 2.29±0.07, 104.21±2.51, 196.82±8.90, 1.02±0.07, 16.22±0.63, 196.82±8.90, 0.95±0.15, 1.50±0.22 and 8.21±0.18 respectively in comparison to normal 6.43±0.19,3.2±0.08, 86.66±4.14, 102.50±11.70, 0.73±0.13, 24.33±1.96, 0.28±0.03, 0.42±0.05 and10.26 ±0.12 respectively. It could be evidenced that there is a statistically significant decrease in total protein, serum albumin and serum calcium and there was statistically significant increase in cholesterol, direct bilirubin and total bilirubin values in hepatic disorders. Liver is the organ which synthesises proteins involved in physiological functions and major one is albumin. Albumin synthesis occurs exclusively in the liver. Approximately 75per cent to85per cent of normal plasma colloid oncotic pressure is provided by albumin (Weil *et al*., 1979). Also liver is involved in absorption and assimilation of calcium and metabolism and elimination of bilirubin from the body. Hence the decrease in total protein, serum albumin and serum calcium and increase in direct bilirubin and total bilirubin in liver disorders obtained in this study is justified.

**Electrolytes**

The mean serum sodium, potassium and chloride values in dogs with hepatic disorders were 4.07±.07, 141.00±0.95 and 116.89±1.35 in comparison to normal4.35±0.08, 144±1.59 and 99.5±2.68 respectively. It could be delineated that there was decrease in sodium and potassium concentration though not significant and there was significant increase in chloride concentration in hepatic disease when compared to healthy dogs. With liver disease, electrolyte imbalances can occur due to vomiting, diarrhoea and anorexia secondary to hepatobiliary disease (Bunch, 2003). And therefore depending upon the clinical symptoms experienced by the hepatic disease, uniform results on electrolyte

concentration cannot be obtained and hence this parameter could be a rare criterion for the diagnosis of liver disorders. However it could be used in addition to other diagnostic tests and for therapeutic purpose.

## CONCLUSION

The changes in Haematological parameters could be an aid in the diagnosis of liver disorders. The decrease in TEC, PCV, Hb and platelets observed in this study could be because of the fact that the physiological functioning of erythrocytes and the platelets is dependent on the physiological functioning of liver. The increase in TLC is indicative of presence of infection. Increased platelet sequestration in the spleen could be because of congestive splenomegaly that was observed in the patients. Reduced production of thrombopoietin by the liver and increased platelet breakdown due to autoantibodies could be the reason for decrease in platelet count.

All liver enzymes in hepatic disorders were increased significantly (the average values for ALT, AST, ALP and GGT were 175.07±16.69, 32.43±4.67, 709.54±84.12 and20.52±2.24, respectively). Alanine transferase (ALT) and aspartate transferase (AST) were "leakage" enzymes which become elevated when hepatocytes are damaged. Alkalinephosphatase (ALKP) and gamma-glutamyl transpeptidase (GGT) were "cholestatic" or "inducible" enzymes. Bile stasis causes increased production of these enzymes which are located in the cell membranes of bile canaliculi (ALKP) and bile duct epithelium (GGT).It could be evidenced from the results of this study that there is a statistically significant decrease in total protein (5.68±0.10), serum albumin (2.29±0.07) and serum calcium (8.21±0.18) and statistically significant increase in cholesterol (196.82±8.90),direct bilirubin (0.95±0.15) and total bilirubin values (1.50±0.22) in hepatic disorders.

Liver is the organ which synthesises albumin and is involved in absorption, assimilation of calcium and metabolism and elimination of bilirubin from the body. Hence the decrease in total protein, serum albumin and serum calcium and increase in direct bilirubin and total bilirubin in liver disorders obtained in this study is justified. Hypercholesterolemia observed could be due to decreased biliary excretion of cholesterol as substantiated by the increase in bilirubin levels in this study (Center, 1996).The statistically significant increase in pre-prandial (23.07±1.64) and post-prandial bile acids (55.74±3.77) obtained in this study exemplifies the bile acid level in liver disorders which is said to be as one of the criterion for the confirmative diagnosis of liver disorders in dogs. There was a significant ($P \leq 0.05$) decrease in BUN (196.82±8.90) in liver disorders in the present study. The glucose and creatinine values in hepatic disorders were104.21±2.51 and 1.02±0.07, respectively.

In the present study, there was decrease in sodium (41.00±0.95mmol/dl) and potassium (4.07±0.07mmol/dl) concentration though not significant and there was significant increase in chloride (116.89±1.35mmol/dl) concentration in hepatic disease when compared to healthy dogs. Electrolyte imbalances can occur due to vomiting diarrhoea and anorexia secondary to hepatobiliary disease (Bunch, 2003). Electrolyte estimation could be used in addition to other diagnostic tests and for therapeutic purpose. The various laboratory tests used in this study for the diagnosis of liver disorders show variable results. It could be concluded that a definitive diagnosis of liver disorder should always be based on a combination of tests as haematology and serum biochemistry parameters along with ultrasound examination and histopathology results in definitive diagnosis.

## ACKNOWLEDGEMENTS

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**Table 1 - Mean values of haematological parameters in normal dogs and in dogs with hepatic disorders.**

| Parameters | Normal | Hepatic disorders |
|---|---|---|
| TEC (x $10^{6}$) | 7.12± 0.27$^{a}$ | 5.17±.03$^{b}$ |
| PCV (%) | 49.10± 1.53$^{a}$ | 32.93± 10.54$^{b}$ |
| Hb(g/dl) | 14.40 ±0.40$^{a}$ | 10.31± 0.30$^{b}$ |
| Platelets(x $10^{3}$) | 270.50± 25.06$^{a}$ | 177.8±10.53$^{b}$ |

Mean values in a row with different Superscripts differ significantly ( $P \leq 0.05$)

**Table 2 - Mean values of TLC(x $10^3$) and differential leukocyte counts in percent in normal dogs and in dogs with hepatic disorders.**

| | TLC | Neutrophil | Monocyte | Lymphocyte | Eosinophil |
|---|---|---|---|---|---|
| Normal | 12.60± .830[a] | 66.10±0.96 | 8.58±1.14 | 23.80±1.27 | 1.48±0.20 |
| Hepatic disorders | 21.31±1.40[b] | 78.18± 0.81 | 5.54±0.21 | 13.92±0.72 | 1.97±0.18 |

Mean values in a row with different Superscripts differ significantly (P ≤0.05)

**Table 3 - Mean values of hepatic enzymes in normal dogs and dogs with hepatic disorders**

| Parameters | ALT (U/L) | AST (U/L) | ALP (U/L) | GGT (U/L) |
|---|---|---|---|---|
| Normal | 22.5± 3.37[a] | 12.17±1.55[a] | 55.66±7.14 [a] | 3.00 ±0.36 [a] |
| Hepatic disorders | 175.07±16.69[b] | 32.43±4.67[b] | 709.54±84.12[b] | 20.52±2.24[b] |

Mean values in a row with different Superscripts differ significantly (P ≤0.05)

**Table 4 - Mean Biochemical values in normal dogs and dogs with hepatic disorders**

| Parameters | Normal Dogs | Dogs with hepatic disorders |
|---|---|---|
| Total protein | 6.43±0.19[a] | 5.68±0.10[b] |
| Serum albumin | 3.20±0.08[a] | 2.29±0.07[b] |
| Glucose | 86.66±4.14[a] | 104.21±2.51[a] |
| Creatinine | 0.73±0.13[a] | 1.02±0.07[b] |
| Blood urea nitrogen | 24.33±1.96[a] | 16.22±0.63[b] |
| Cholesterol | 102.50±11.70[a] | 196.82±8.90[b] |
| Direct Bilirubin | 0.28±0.03[a] | 0.95±0.15[b] |
| Total Bilirubin | 0.42±0.05[a] | 1.50±0.22[b] |
| Serum Calcium | 10.26 ±0.12[a] | 8.28±0.18[b] |

Mean values in a row with different Superscripts differ significantly (P ≤0.05)

**Table 5 - Pre-prandial and post prandial total bile acids in dogs with hepatic disorders.**

| | Pre-prandial TBA (µmol/L) | Post prandialTBA (µmol/L) |
|---|---|---|
| Min | 2.42 | 2.71 |
| Max | 112 | 190.16 |
| Mean ±SE | 23.07±1.64[a] | 55.74 ±3.77[b] |

Mean values in a row with different Superscripts differ significantly (P ≤0.05)

**Table .6- Mean values of serum electrolytes in normal and hepatic disorder dogs**

| Electrolytes | Normal | Hepatic disorders |
|---|---|---|
| Sodium (mmol/dl) | 4.35±0.08[a] | 4.07±0.07[a] |
| Potassium(mmol/dl) | 144±1.59[a] | 141.00±0.95[a] |
| Chloride (mmol/dl) | 99.5±2.68[a] | 116.89±1.35[a] |

Mean values in a row with different Superscripts differ significantly ($P \leq 0.05$)

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RESEARCH ARTICLE

# Intravenous Bacterial Collagenase Therapy for the Treatment of Retained Fetal Membrane to Improvere Productive Efficiency in Cattle

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## ABSTRACT

The collagenase administration through umbilical artery is the effective treatment for Retained Fetal Membrane (RFM) in dairy cows. RFM was treated with collagenase enzyme through jugular vein as it is easy route than previous study of experimenting on umbilical arteries which is very difficult in a delayed case in field conditions in bovines. The study was conducted in bovines with RFM and presented within 12 to 24 hours after parturition to Obstetrics Unit of Madras Veterinary College, Chennai. The post treatment reproductive parameters viz., time to onset of first postpartum oestrus, days open, number of services required per conception and conception rate were studied. It reavealed that the time taken for the onset of first postpartum oestrus, days open and number of services required per conception was significantly (P<0.01) shorter and conception rate was significantly higher (P< 0.01) in control and 2,

00,000 CDU collagenase intravenously administered cows than the cows treated with intrauterine proteolytic bolus. Further, the collagenase administered cows with normal parturition followed by RFM had effectively expelled the fetal membrane within 38 h and achieved better conception rate.

**Keywords :** Retained fetal membrane, Bacterial collagenase therapy, Bovine, reproductive performances, Intravenous route.

## INTRODUCTION

Retained foetal membrane (RFM) is one of the most important postparturient disease (Stephen, 2008), leading to reproductive problems and economic losses in dairy industry (Pathak *et al.,* 1991). The incidence of RFM ranges from 3 to 15 per cent following normal parturition in dairy cows ( Sheetal *et al*., 2015). A variety of methods have been used in the treatment of RFM, which includes manual removal and / or administration of oxytocin, $PGF_{2\alpha}$, antibiotics, immune modulators *etc.,* (Amin *et al.,* 2013), although the efficacy of these treatments are questionable (Eiler, 1997). Hence, bacterial collagenase from *Clostridium histolyticum* was used for the tratment of RFM as it could degrade several types of collagen (Azawi, 2013). However, collagenase administration after 48 h postpartum is ineffective due to residual blood clots in the placental vessels which causes anastomosis of umbilical arteries and tend to close (Eiler, 1997).

The alternate route for the collagenase administration instead of umbilical arteries was reported by Eiler and Hopkins (1993) that the injection of collagenase ($2.2 \times 10^6$ U in 1000 ml of physiological saline solution over a period of 30 mts) through jugular vein caused release of foetal membrane within 36 h in experimentally induced retained foetal membrane. Based on these, the study was formulated to determine the effect of collagenase through intravenous route instead of umbilical arteries on expulsion of foetal membranes and to study the post treatment responses like time of onset of first postpartum oestrus, days open, number of services required per conception and conception rate.

## MATERIALS AND METHODS

Fifty two healthy and parous cows less than 10 years of age, presented to the Large Animal Obstetrics Unit, Teaching Veterinary Clinical Complex, Madras Veterinary College, Chennai-7 were utilized for the study. Seven healthy cows with normal calving and shedding of placenta were served as group I (control). Thirty cows and buffaloes with unassisted calving followed by retained foetal membranes between 12 and 24 h interval were selected and randomly allotted into groups II and III of fifteen each.

Group I received placebo treatment with one litre of normal saline intravenously. Group II cows, treated with intrauterine proteolytic bolus containing nitrofurazone, metronidazole and urea and antibiotic therapy (Inj. Streptopenicillin @ 20,000 units/kg body weight) without manual removal for 7 days. Groups III cows, received single dose of 2, 00,000 CDU of collagenase plus 40 mg of calcium chloride and 40 mg of sodium bicarbonate dissolved in one litre of normal saline at a pH of 7.5 intravenously through jugular vein (Eiler and Hopkins ,1993). Statistical analysis of the data was carried out as per the standard procedure outlined by Snedecor and Cochran (1994)

## RESULTS AND DISCUSSION

The mean (± SE) time taken for the onset of first postpartum oestrus in groups I (48 ± 6.91 days) and III (62 ± 7.91 days) were significantly (P<0.01) shorter than groupII (122 ± 11.06 days) (Table). The early onset of postpartum oestrus in group I was in agreement with the observations of Patel *et al.* (2013) that the onset of first postpartumoestrus was 43.50 ± 1.06 days in normal puerperium cows. Similar result was recorded by Srinivas *et al.* (1998) and Brodzki *et al.* (2014) that the onset of first postpartum oestrus following normal puerperium was 41.73 ± 8.11 and 36.50 ± 0.97 days, respectively in dairy cows without RFM. However, the present results were in contrast to the observations of Kumari *et al.* (2014) that the onset of first postpartum oestrus in normal calving was 85 ± 4 days in cows and these variations might be due to the breed, parity, nutritional status, milk production, season and managerial practices adopted ( Hussain *et al.,* 2013). The early shedding of placenta without endometrial damage, lower bacterial load and early regeneration of the uterine epithelium (Noakes *et al.,* 2001; Sheldon and Dobson, 2004), favours earlier involution and resumption of ovarian activity, results in increased level of oestradiol leading to the development of dominant follicles of more than 10 mm diameter (Hussain *et al.,* 2013) might be the reasons for the early onset of first postpartum oestrus in group I and III. The delayed expulsion of placenta, increased bacterial load with higher concentration of endotoxin release causes delayed uterine involution in group II which leads to delayed resumption of ovarianactivity (Matius*et al.,* 2003) and onset of first postpartum oestrus.

The mean days open in groups I (71.85 ± 5.37 days) and III (81 ± 4.70 days) were significantly (P<0.01) shorter than the group II (155.73 ± 6.27 days) cows (Table ). The shorter days open in group I were in agreement with the observations of Borsberry and Dobson (1989) Zobel and Tkalcic (2013) that the days open was 70.9 and 78.7 days of postpartum in normal puerperium cows, respectively. Furter, Brodzki *et al.* (2014) recorded that the calving to conception interval following normal and inflamed uterus were 90.96 ± 22.88 and 140.96 ± 31.79 days, respectively in dairy cows. The highest bacterial load and absorption of bacterial toxins from the uterus might prevent the follicular phase LH surge, resulting in slower growth rate of first postpartum dominant follicle interfering with the release of oestradiol leading to failure of ovulation and increasing the calving to conceptioninterval (Sheldon *et al.,* 2006). Further, the first postpartum ovulation resumes in cows that developed uterine disease resulting in prolonged luteal phase (Matius *et al.,* 2002), might be the reason for the prolonged days open in group II. The administration of collagenase might facilitate the easy release of placenta (Eilerand Hopkins, 1993), hasten the uterineregression (Youngquist and Threlfall, 2007), improve the follicle size and growth and the ability to secrete oestradiol, result in greater value in eliminating delayed ovulation (Williams *et al.,* 2008), which favours the reduced days open in group III, similar to that of group I cows.

The mean (±SE) number of services required per conception in groups I (1.80 ± 0.22) and III (2.10 ± 0.15) were significantly (P<0.01) lower than group II (3.60 ± 0.33) cows (Table).These results were in agreement with the observations of Biner *et al.,* (2015) that the number of services required per conception in normally calved cows followed by normal shedding of placenta ranged from1.00 to 2.16. Similarly, Zobel and Tkalcic (2013) stated that the number of services required per conception was 1.8 and 3.3 with normal calving followed by normal shedding of placenta and normal calving followed by RFM treated with intrauterine bolus, respectively in dairy cows. The negative effects in the uterus and ovary due to irritation and lesions in the endometrium (Bonnett *et al.,* 1991) results in disturbed endometrial function (Sheldon and Dobson, 2004) that in turn decreases the luteinizing hormone secretion, impairment of first dominant follicle size and growth and ability to secrete oestradiol, thereby affecting the ovulatory capacity (Williams *et al.,* 2008) and failure of fertilization (Hill and Gilbert, 2008) might be the reasons for the more number of inseminations required per conception in group II cows. The increased myometrial contraction, elimination of bacterial infection, regeneration of endometrium and restoration of uterus to non-pregnant size and function within 4 to 5 weeks of postpartum have been achieved by the administration of collagenase in groups III cows, leads to create conducive environment and results in reduced number of insemination requiredper conception (Hafeez, 1990).

The mean (± SE) conception rate in groups I, II, III and IV were 86.00, 33.00.00, 73.00 and 54.00 per cent, respectively (Table).The conception rate observed in group I (86%), and III (73%) were significantly (P<0.01) higher than the group II (33%). These findings were in agreement with the observations of Waheeb *et al.* (2014) who reported that the conception rate in dairy cows ranged from 70 to 91.7 per cent in normal calving. The conception rate in group II findings were concurred with the observations of Butler *et al.* (2002) that the first service conception rate was 32.7 and 42.2 per cent in normal calving with and without RFM, respectively in cows. The severe irritation, necrotic lochia and bacterialgrowth (Sheldon *et al.,* 2009) lead to inflammatory cell infiltration and fibrotic changes in the endometrium, involved in prolonged mucus secretion (Causey *et al.,* 2000) causing the reduced conception rate ingroup II. Further, the uterine bacterial infection associated with uterine inflammation might reduce pituitary FSH and LH release, results in perturbed postpartum ovarian follicular growth and function, which delays the ovulation (Dolozel *et al.,* 2008; Ahmed *et al.,* 2013) thereby reduced fertility in group II. The complete digestion, liquefaction and expulsion of placenta occurs within 12 to 24 h after administration of collagenase (Fecteau and Eiler, 2001; Frazer, 2005), results in reduced bacterial load and improve deficiency of uterine defense mechanism (Sheldon *et al.,* 2006), hasten the uterine involution and provides the conducive environment for complete cellular repair leading to higher conception rate in group III cows.

It can be inferred from the present study that 2, 00,000 CDU of collagenase administered intravenously in group III cows with normal parturition followed by RFM had effectively expelled the fetal membrane within 38 h and achieved better conception rate than group II with normal parturition followed by RFM treated with intrauterine proteolytic drugs.

## CONCLUSION

Based on these study, it is recommended that the administration of 2,00,000 CDU collagenase through intravenous route for cows with normal parturition followed by RFM to achive better conception rate.

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**Table: Mean (± SE) post treatment response with different treatment regimens of RFM cows.**

| Group | Onset of first postpartum oestrus (Days) | Days open (Days) | No. of services required per conception | Conception rate (per cent) |
|---|---|---|---|---|
| I (n=7) | 48 ± 6.90$^{a}$ | 71.85 ± 5.37$^{a}$ | 1.80 ± 0.22$^{a}$ | 86.00 |
| II (n=15) | 122 ± 11.06$^{c}$ | 155.73 ± 6.27$^{c}$ | 3.60 ± 0.33$^{c}$ | 33.00 |
| III (n=15) | 62 ± 7.91$^{a}$ | 81.00 ± 4.70$^{a}$ | 2.10 ± 0.15$^{a}$ | 73.00 |
| IV (n=15) | 93 ± 9.64$^{b}$ | 135.66 ± 5.78$^{b}$ | 2.50 ± 0.29$^{b}$ | 54.00 |

Means bearing different superscripts (A-B) in each row differ significantly (P < 0.01)
Means bearing different superscripts (a-b) in each column differ significantly (P < 0.01)

**(i) Collagenase stock powder**


**(ii) Filter sterilization of collagenase**


**(iii) Collagenase solution and normal saline kept ready for administration**

**(iv) Collagenase administration through intravenous route**

**Plate : Preparation and Administration of Collagenase**

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REVIEW ARTICLE

# Adaptation of Dairy Cattle and Buffalo to Environmental Challenges

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## ABSTRACT

Livestock undergoes various environmental challenges. Thermal stress is the most intriguing factor affecting livestock production in the ever changing climatic scenario. Adaptation is defined as the morphological, anatomical, physiological and biochemical characteristics of the animal which promote welfare and favor survival in specific environment. Environmental challenges negatively affect the growth, production and reproduction of livestock. Combined effect of temperature and humidity proved to be extremely fatal to the entire livestock population. Animal cope up with environmental challenges with various kinds of responses. These include physiological response, blood biochemical response, neuro-endocrine response, molecular and cellular response, metabolic response and behavioral response. The animals try to adapt to the stress condition by altering their behavior. In addition, through evolutionary adaptive mechanisms animals developed few specialized structures and by which they avoid theinfluence of adverse environmental condition. All these responses play a major role in maintaining the homeostasis in livestock and make them survive in adverse environment. A deep understanding of these adaptive mechanisms are required, if one attempts to develop suitable ameliorative strategies to improve livestock production in changing climatic scenario.

**Keywords :** Adaptation; Respiration; Sweating; Behavior.

## INTRODUCTION

Livestock undergo various kinds of stress which includes chemical, physical, nutritional, and thermal stress. Various factors affect livestock productivity which includes photoperiod, geographical location, age, breed, nutrient availability, water availability, management practices, and environmental conditions [1,2].Adaptation is the

morphological, anatomical, physiological and biochemical characteristics of an animal which promote welfare and favor survival in a specific environment. Beyond thermo-neutral zone, animal has to use a portion of the metabolizable energy typically used for production to assure thermal balance. Combined effect of temperature and humidity will be extremely fatal to the livestock population across the globe [3].

## Environmental Stresses Affecting Livestock Production and Reproduction

Major environmental stresses affecting production and reproduction of livestock are heat and nutrition stress [4]. Increased ambient temperature leads to heat stress in livestock which has adverse effects on animal production and reproduction. Effects of heat stress in livestock are reduced feed intake, growth performance, milk yield, increased sweating rate, panting, rectal temperature, respiratory rate, and water intake [2]. Apart from these there are also changes in hematological parameters, electrolytes, metabolites, increased mortality and morbidity,and reduced immune function [5]. The evident effects of heat stress on growth performance are due to decrease in anabolic activity caused by decline in voluntary feed intake, and increase in tissue catabolism [6]. Milk production appears to be particularly sensitive to the effects of heat stress. Heat stress significantly reduce milk protein, fat, somatic cell count (SCC) and solid not fat (SNF) in dairy cattle [8]. Heat stress negatively affects reproduction in livestock. Increase in testicular temperature results in reduced sperm output, decreased sperm motility and increase in proportion of morphologically abnormal spermatozoa in the ejaculate [12]. Further, heat stress also results in reduced fertility, libido and testicular degeneration. Nutrition has a major role in the production performance oflivestock. Nutritional stress affects reproduction, growth and milk production. Poor nutrition delayspuberty reduces conception rate and increases pregnancy losses incattle [14]. Young animals are more sensitive to nutritional stressas the adaptive mechanisms will be poorly developed in the younganimals [15]. Considering the keyrole of the nutritional status on reproductive efficiency, nutritionaland management strategies are essential for optimizing reproductiveperformance in livestock

## Genetic Control of Livestock Adaptation

Fitness and adaptation are influenced by genetic make-up and itdetermines an animal's tolerance to adverse conditions such as hightemperature, drought, pests and diseases. Adaptation in terms ofgenetics is the heritable animal characteristics which favor survivalof a population. Many breeds of the harshenvironment have developed many adaptive traits that increasetheir survivability [16]. Indianbreeds of cattle like Bos indicus perform well in the hot climate ascompared to exotic cattle Bos taurus, primarily due to their ability tosurvive in unfavorable environments. The inherent genetic variationis the major factor that determines adaptability as measured throughsurvival and reproduction which interacts with constraints ofenvironment and creating phenotypic variation [16] .Use of genetic tests like the Bovine SNP50 BeadChip may be used toidentify genetic markers that predict thermo-tolerance [18]. Anotherapproach for improving resistance to heat stress in dairy breeds isto introduce thermo-tolerant genes from other breeds like slickhair genes [18]. The slick hair gene has been introduced naturallyinto some Holstein cows in Puerto Rico and into a dairy breed inVenezuela called the Carora [19] indicating Slick Holsteins are betterable to regulate body temperature during heat stress than cows withnormal hair.

## Physiological Mechanisms to Livestock Adaptation

Physiological mechanisms set in gesture once animal weresubjected to stress, which helps to maintain homeostasis andphysical equilibrium within them [20]. Physiological responses canbe classified into short term changes and long term changes. Shorttermchanges are often caused by acute stressors, while long-term changes may be due to chronic stressors. These changes can be used as a measure of dairy cowcomfort and adaptability to a harsh environment or as a sensitivephysiological measure of environmental modification [22]. On the alterations in thephysiological responses like respiration rate and rectal temperature[27], panting, drooling, reduced heart rates and profuse

sweating,decreased feed intake [7], and reduced milk production beingexhibited by cows, when they were placed beyond their comfort zone.

### Respiratory rate

The animals exhibited increasedrespiration rate under high ambient temperature [24].The increase in skin temperature of heat stressed animals was dueto the fact that the high ambient air temperature alters the bloodflow and its redistribution thereby increasing the blood flow to thesurfaces.

### Rectal temperature

Rectal temperature wasconsidered to be more sensitive indicator of body temperature inheat stressed animals[29]. The rectal temperature is found to be increasedwhen the animal is subjected to hot climate [30].The increase in rectal temperatureoccurs, when the animal's body fails to maintain its heat balance[33].

### Pulse rate

Pulsation was reported to be increased by the effect ofenvironmental temperature [24].Theincrease in the pulse rate will increase blood flow from the core to theperiphery of the body, resulting in higher heat loss by both sensiblemeans (loss by conduction, convection and radiation) and insensiblemeans (loss of water by diffusion through the skin) [31].

## Morphological Adaptation of Livestock

Livestock has been proved to adapt to a variety of environmentalextremities [21].The adaptation process can be classified into sixcategories such as anatomical, morphological, physiological, feedingbehavior, metabolism, and performance [36].Morphological adaptation of the animal includesexternal insulation (coat and fur depth, hair type, hair density andsubcutaneous fat), fat storage in hump or tail especially under desertconditions, skin colour and body size.

Animals having light and sleek coats found to absorb less heatin comparison with those with dark colored and woolly coats. Fora cattle maintained beyond the thermo neutral zone, evaporationis the solitary way of heat dissipation. Therefore, the possibility ofdeveloping heat tolerance in cattle lies in improving sweat glandfunction. Short hair and thin hair, pigmented skin, short ears withtiny hair, movable and slender tail were found to be the morphologicaladaptations of Bos taurus cattle which resulted in enhanced heat loss[21].Buffaloes had poor heat tolerant capacity comparedto other domestic ruminants and were susceptible to heat stress dueto scarce and unevenly spread sweat glands, dark skin and sparse hairon their body surface[22].

Sweating rates werefound to be more in tropically adapted Bos indicus compared totemperate zone. This is because the transfer of metabolic heat to theskin occurs at a lower rate in Bos taurus than in Bos indicus cattle. Crossbred (Bos taurus. Bos indicus)cattle had larger and more sweat glands per unit area of the skin aswell as greater sweat production than pure-bred Bos taurus. The hair coataffects the heat transfer from the skin to the adjacent environmentand thereby regulation of the body temperature[26].Coat depth to be an important factorand suggested that an increase in coat depth from 3 to 10 mm reducedthe sensible heat loss in cattle by 17% at 20°C. The coat layer thickness was found to be greater for animals bred intemperate regions, more than 15 mm, while the same breed adapted to tropical climates present very thin coats, less than 8 mm deep.

**Blood Biochemical Changes in Livestock Adaptation**

Blood composition of animal are influenced by certain factorssuch as nutrition, management, sex, age, diseases and stress factorsthat might affect blood values . The blood biochemical profilesare considered important in evaluating the health status of animals.Heat stress alters significantly the levels of Hb, PCV, plasma glucose,total protein and albumin in sheep. High temperature increases oxygenconsumption of the animals by increasing respiration rate. The higheroxygen consumption increases the partial pressure of oxygen in blood and decreases erythropoiesis, reducing the number of circulatingerythrocytes and thus PCV and Hb values [24,35,]. Thermal stress apartfrom causing lower blood insulin also decreases tissue sensitivity toinsulin thereby increases the insulin response [10,11]. A significantincrease in the levels of plasma albumin was reported in cows and buffalo calves [58] during heat stress. Prolonged exposure to solar radiation increased the concentration of plasma total protein,albumin, and globulin in goats and the reason for this is believed tobe due to vasoconstriction and decreased plasma volume during heatstress. Thermalstress causes reduction in blood glucose and non esterified fatty acid(NEFA) level due to reduction in hepatic glucose synthesis [10].

**Cellular and Molecular Responses of LivestockAdaptation**

The cellular heat stress response is one component of the acutesystemic response to heat stress. Gene networks within and acrosscells and tissues respond to temperatures above the thermo-neutralzone with both intra- and extracellular signals that bring togethercellular and whole-animal metabolism. Activation of these systemsappears to be initiated at the skin surface temperatures exceeding 35°C as the animals begins to rapidly increase evaporative heat loss mechanisms. Gene expression changes include 1) activation of heat shock transcription factor 1 (HSF1) 2) increased expression of heat shock proteins (HSP) and decreased expression and synthesis of other proteins 3) increased glucose and amino acid oxidation and reduced fatty acid metabolism 4) endocrine system activation of the stress response and 5) immune system activation via extracellular secretion of HSP [38]. General response of cells to heat stress includes: inhibitionof DNA synthesis, transcription, RNA processing, translation,progression of cell cycle, disruption of cytoskeletal elements,protein denaturation and aggregation and changes in membranepermeability. It is a well-established fact that the changes in gene expression are an integral part of the cellular response to thermalstress. Heat shock proteins (HSP) are activated by heat shock factorsand their expression is increased when cells are exposed to highambient temperatures. Heat shock proteins play a crucial role in cell survival under heat stress and hence it is considered to be the confirmatory stress marker in livestock. During hypothermic stress the expression of many HSPs including HSP32, HSP40, HSP60, HSP70, HSP90, and HSP110 were found to be increased [24]. The mRNA expression of HSP60, HSP90 and UBQ were significantly higher during peak summer season as compared with peak winter season in both tropical and temperate region goats. HSP70 mRNA expression was significantly higher during summer season as compared with winter season in tropical region goats.

## CONCLUSION

Environmental factors are the primary factors influencing livestock production in the changing climatic condition. Environmental stresses reduce production parameters like growth, milk yield, and reproduction in livestock leading to severe economic constraints. Livestock possess a wide range of adaptive mechanisms such as physiological, morphological, biochemical, cellular and molecular responses to cope up with environmental challenges. To reduce the economic burden on farmers as a result of environmental stresses, strategies need to be developed with multidisciplinary approach to reduce the adverse effects of environmental stresses negatively impacting livestock production.

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REVIEW ARTICLE

# Applications of Molecular Markers in Livestock Breeding

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## ABSTRACT

The applications of the new generations of molecular markers represent amazing tools for the genetic improvement of farm animals. These markers provide more accurate genetic information and better knowledge of the animal genetic resources. Scientists, who are unfamiliar with the different molecular techniques, need to know more about these techniques concerning applications, types, advantages and disadvantages.This review represents an attempt to highlight the different types of molecular markers by introducing a brief summary on the development of genetic markers including both the classical genetic markers and more advanced DNA-based molecular markers.

**Keywords :** Microsatellite, Molecular Markers, Genome.

## INTRODUCTION

A genetic marker is a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or trait. It can be described as a variation, which may arise due to mutation or alteration in the genomic loci that can be observed. A genetic marker may be a short DNA sequence, such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, SNP), or a long one, like mini & micro satellites. The main aim of breeder is to select animal with superior genetic potential as a parents for the next generation. The first attempt to improve animals used the phenotyp of animal for a specific trait as a tool for selection. This application used external animal characteristics as a marker that called morphological markers (i.e. udder shape, coat color, body shape, skin structure, and anatomical characteristics) (Van Wezel and Rodgers, 1996). These markers depend on visual observation and measurement to identify, classify, and characterize the genetic evolution of different species or populations.

Another type of markers represent by using of cytological markers that were included several criteria such as chromosome karyotypes, bandings, repeats, translocations, deletions, and inversions to investigate the genetic resources of animals (Yang et al., 2013).The chromosome mutations lead to genetic variation (Bitgood and Shoffner, 1990).These mutations were used as markers to identify a certain location of the gene on a specific chromosome. In the domestic animals, cytological markers allow to investigate their genetic diversity by comparing chromosome number and structure between domesticated animals and their wild ancestors (Beack et al., 1973).Cytological markers still widely used in elucidating the origin and classification of species (Jonker et al., 1982) because of its good properties; rapid, economic, and straightforward technique.

The third type of markers is biochemical markers such as blood type and isozymes. These markers represent biochemical traits that could be analyzed by protein electrophoresis.The differences in the amino acid composition of isozymes and soluble proteins were used to investigate the genetic variation within species and phylogenetic relationships between species (Buvanendran and Finney, 1967).The application of these markers was limited because the proteins and isozymes are not genetic materials. They are products of gene expression, so they could affect by environmental factors (Drinkwater and Hetzel, 1991). The molecular markers are based on the nucleotide sequence mutations within the individual's genome; they are the most reliable markers available (Yang et al., 2013).

## Marker Assisted Selection (MAS)

Selection is one of the most important tools to improve the performance of animals. It can accomplish based on two types of data – pedigrees and phenotypes to estimate Best Linear Unbiased Prediction (BLUP) that combines these to generate estimated breeding values (EBVs). A third type of data is based on DNA markers to get a new approach named Marker assisted selection (MAS). MAS can be based on DNA in linkage equilibrium with a quantitative trait locus (QTL) (LE-MAS)–LE refers to genotype frequencies at one locus are independent of genotype frequencies at the second locus - , molecular markers in linkage disequilibrium with a QTL (LD-MAS) - LD refers to the non-random association of alleles between two loci-, or based on selection of the actual mutation causing the QTL effect (Gene-MAS). All three types of MAS are being used in the livestock industries (Dekkers, 2004)

## Restriction Fragment Length Polymorphism (RFLP)

The molecular basis of RFLP is that nucleotide base substitutions, insertions, deletions, duplications, and inversions within the whole genome can remove or create new restriction sites (Yang et al., 2013).

Despite the fact that it is less widely used now, there have been numerous benefits to RFLP analysis. It plays an important role in allowing scientists to map the human genome as well as provide information on genetic diseases (Emadi et al., 2010). RFLP analysis is useful to find where a specific gene for a disease lies on a chromosome. RFLP was also one of the first methods used for genetic typing - also known as genetic fingerprinting, profiling or testing.Despite that RFLP have many benefits but it is still a slow and more tedious process compared to some of the newer DNA analysis techniques. Ii is also requires substantially larger sample sizes than other forms of analysis.

## Random Amplification of Polymorphic DNA (RAPD)

In the last decade, the RAPD technique based on the polymerase chain reaction (PCR) has been one of the most commonly used molecular techniques to develop DNA markers (Kumar and Gurusubramanian, 2011).

RAPD technology provides a quick and efficient screen for DNA sequence based polymorphism at a very large number of loci. The major advantage of RAPD includes that, it does not require pre-sequencing of DNA (Nandani and Thakur, 2014).

The principle of RAPD is that, a single, short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template. This means that the amplified fragment generated by PCR depends on the length and size of both the primer and the target genome (Nandani and Thakur, 2014). Since the advantages of RAPDs are the technical simplicity and the independence of any prior DNA sequence information, (Weising et al., 2005) it is viewed as having several advantages compared to RFLP and fingerprint (Lynch and Milligan, 1994).

A disadvantage of RAPD markers is the fact that the polymorphisms are detected only as the presence or absence of a band of a certain molecular weight, with no information on heterozygosity besides being dominantly inherited, and also show some problems with reproducibility of data (Brumlop and Finckh, 2010).

### Amplified Fragment Length Polymorphism (AFLP)

AFLP markers have found the widest application in analyses of genetic variation below the species level, particularly in investigations of population structure and differentiation (Hedrick, 1992).

AFLP methods rapidly generate hundreds of highly replicable markers from DNA; thus, they allow high-resolution genotyping of fingerprinting quality. The time and cost efficiency, reproducibility and resolution of AFLPs are superior or equal to those of other markers (RAPD, RFLP and microsatellites) (Brumlop and Finckh, 2010).The AFLP method is an ideal molecular approach for population genetics and genome typing, it is consequently widely applied to detect genetic polymorphisms, evaluate, and characterize animal genetic resources (Ajmone-Marsan et al., 2002).

### Microsatellites

Microsatellites or simple sequence repeat (SSR) loci, which have been referred to in the literature as variable number of tandem repeats (VNTRs) and simple sequence length polymorphisms (SSLPs), are found throughout the nuclear genomes of most eukaryotes and to a lesser extent in prokaryotes (Varshney et al., 2005).

Microsatellites range from one to six nucleotides in length (Van Oppen et al., 2000) and are classified as mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats. The sequences of di-, tri- and tetranucleotide repeats are the most common choices for molecular genetic studies(Selkoe and Toonen, 2006). They are repeated (usually 5-20 times) in the genome with a minimum repeat length of 12 base-pairs (Goodfellow, 1992)

### Single-Nucleotide Polymorphism (SNP)

In 1996, Lander proposed a new molecular marker technology named SNP. When a single nucleotide (A, T, C, or G) in the genome sequence is altered this will represent the SNP. In other words, it refers to a sequence polymorphism caused by a single nucleotide mutation at a specific locus in the DNA sequence (Yang et al., 2013).

This sort of polymorphism includes single base transitions, transversions, insertions and deletions (Goodfellow, 1992), and the least frequent allele should have a frequency of 1% or greater (Lander, 1996). Transitions are the most common (approx.2/3) among all the SNP mutation types (Zhao and Boerwinkle, 2002). SNP markers are one of the popular approach, despite they can be considered as a step backwards (simple bi-allelic co-dominant markers) when compared to the highly informative multi-allelic microsatellites. The more recent SNP concept has basically arisen from the recent need for very high densities of genetic markers for the studies of multifactorial diseases (Vignal et al., 2002).The fundamental principle of SNPs is to hybridize detected DNA

fragments with high-density DNA probe arrays (also called SNP chips); the SNP allele is then named according to the hybridization results (Yang et al., 2013).

SNPs are third generation molecular marker technology coming after RFLPs and SSRs (Peter, 2001); it was successfully performed to investigate genetic variation among different species and breeds.The role of SNPs in farm animals was very important concerning the population structure, genetic differentiation, origin, and evolution research (Yang et al., 2013). On the other hand, the most important disadvantage of SNPs is the low level information obtained as compared with that of a highly polymorphic microsatellite but this can be solved by using a higher numbers of markers (SNP chips) and whole-genome sequencing (Werner et al., 2002).

## CONCLUSIONS

The accurate genetic evaluation of animals is the primary target for their conservation and utilization.Different methods have been developed and tested at the DNA sequence level. These methods provide a large number of markers and opening up new opportunities for evaluating diversity in farm animal genetic resources. Among all these methods, microsatellites (SSR) remained the marker of choice for the past 15 years (Morin et al., 2004).

Some researchers reported that SNP markers will replace microsatellites for some applications as SNP markers have good genome coverage. However, the results of recent studies revealed that SNP markers can only be transferred to different mapping populations within the same species, but not across species. This will limit the applications of SNP markers on related minor species. In contrast, due to multiple alleles, cost-effectiveness, and transferability, SSR markers will continue to play an important role in different genetic studies in the future.

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REVIEW ARTICLE

# Marker Assisted Selection and its Application in Livestock Improvement

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## ABSTRACT

Molecular markers usually do not have any biological effect. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted from one generation to the next. Marker assisted selection (MAS) is a novel technique that can complement traditional breeding methods for rapid genetic gains. Genetic gain through selective breeding is the objective of a breeder to achieve long term improvement in animal and plant genomes; however the pace of improvement is inversely proportional to the Generation Interval. Genetic improvement in livestock, particularly those with long generation intervals, requires decades for tangible results. Successful MAS breeding programmes require gene mapping, marker genotyping, quantitative trait loci (QTL) detection, genetic evaluation and finally MAS. Genomic selection is a form of marker assisted selection. Using markers covering the whole genome could mean potentially that all the genetic variance is explained; and the markers are assumed to be in linkage disequilibrium with the QTL so that the number of effects per QTL to be estimated is small. MAS drastically reduces generation interval and increases selection accuracy. Therefore, a breeding strategy based upon markers making the best use of the two approaches can facilitate rapid genetic gain though selection of markers related to economic traits such as milk and meat production.

**Keywords :** Molecular markers, disequilibrium, meat production.

## INTRODUCTION

Marker assisted selection or marker aided selection (MAS) is an indirect selection process where a trait of interest is selected based on a marker (morphological, biochemical or DNA/RNA variation) linked to a trait of interest (e.g.

productivity, disease resistance, a biotic stress tolerance, and quality), rather than on the trait itself. This process is used in plant and animal breeding.For example, using MAS to select individuals with disease resistance involves identifying a marker allele that is linked with disease resistance rather than the level of disease resistance. The assumption is that the marker associates at high frequency with the gene or quantitative trait locus (QTL) of interest, due to genetic linkage (close proximity, on the chromosome, of the marker locus and the disease resistance-determining locus). MAS can be useful to select for traits that are difficult or expensive to measure, exhibit low heritability and/or are expressed late in development. At certain points in the breeding process the specimens are examined to ensure that they express the desired trait.

## Molecular markers

Molecular markers usually do not have any biological effect. They are identifiable DNA sequences, found at specific locations of the genome, and transmitted from one generation to the next. Their identification relies on a DNA assay, in contrast to morphological markers that are based on visible traits, and biochemical markers based on proteins produced by genes. Different kinds of molecular markers exist. They may differ in a variety of ways – such as the amount of genetic variation at each marker. The information provided to the breeder by the markers varies depending on the type of marker system used.

## Successful MAS breeding programmes require advances in five areas

1. **Gene mapping:** Identification and mapping of genes and genetic polymorphisms.
2. **Marker genotyping:** Genotyping of large numbers of individuals for large numbers of markers at a reasonable cost for QTL detection and routine application for MAS.
3. **QTL detection:** Detection and estimation of associations of identified genes and genetic markers with economic traits.
4. **Genetic evaluation:** Integration of phenotypic and genotypic data in statistical methods to estimate breeding values of individuals in a breeding population.
5. **MAS:** Development of breeding strategies and programmes for the use of molecular genetic information in selection and mating programmes.

## Steps involved in MAS

**1.** Validation of molecular markers: Extract the DNA from test individuals and find out whether there is one-to-one relationship with marker and the trait.
**2.** Extract the DNA of breeding population at the early stage and apply MAS. Select the individuals on the basis of presence of desired molecular markers for the concerned trait.

## From markers to MAS

The molecular marker systems described above allow high-density DNA marker maps (*i.e.* with many markers of known location, interspersed at relatively short intervals throughout the genome) to be constructed for a range of economically important farm animal species, thus providing the framework needed for eventual application of MAS. The next step is that putative genes affecting traits of interest can be detected by testing for associations between marker variants and any trait of interest. These traits might be genetically simple – for example, many disease resistance traits in plants are controlled by one or a few genes (Ruane and Colleau, 1996; Rao, Lakshminarasu and Jena, 2002). Alternatively, they could be genetically complex quantitative traits, involving many genes (*i.e.* so-called quantitative trait loci (QTLs)) and environmental effects. Yue *et al*. (2005) using 280 molecular markers(comprising 134 RFLPs, 131 AFLPs and 15 microsatellites) detected a number ofputative QTLs for drought resistance in rice.

For example, consider a hypothetical situation where a molecular marker M (with two alleles M1 and M2), that we can identify using a DNA assay, is known to be located on a chromosome close to a gene of interest Q (with a variant Q1 that increases yield and a variant Q2 that decreases yield), that is, as yet, unknown. If an individual has the alleles M1 and Q1 on one chromosome and M2 and Q2 on the other any of its progeny receiving the M1 allele will have a high probability of also carrying the favorable Q1 allele, and would be preferred for selection. With conventional selection, relying on phenotypic values, it is not possible to use this kind of information. The success of MAS is influenced by the relationship between the markers and the genesof interest. Dekkers (2004) distinguished three kinds of relationship:

1. The molecular marker is located within the gene of interest (*i.e.* within the gene Q, using the example above). In this situation, we can refer to gene-assisted selection (GAS). This is the most favourable situation. On the other hand, it is most difficult to find these markers.
2. The marker is in linkage disequilibrium (LD) with Q throughout the whole population. LD is the tendency of certain combinations of alleles (*e.g.* M1 and Q1) to be inherited together. Population-wide LD can be found when markers and genes of interest are physically very close to each other and/or when lines or breeds have been crossed in recent generations. Selection using these markers can be called LD-MAS.
3. The marker is in linkage equilibrium (LE) with Q throughout the whole population. Selection using these markers can be called LE-MAS. This is the most difficult situation for applying MAS.

MAS can be applied to support existing conventional breeding programmes. These programmes use strategies such as: recurrent selection (*i.e.* within-breed or within-line selection, important in livestock); development ofCrossbreds or hybrids (by crossing several improved lines or breeds) and introgression (where a target gene is introduced from a low-productive line or breed (donor) into a productive line (recipient) that lacks the target gene (a strategy especially important in plants).

## Limitations of MAS

- Cost
- Requirement of technical skill
- Automated techniques for maximum benefit
- DNA markers are not affected by environment but traits may be affected by the environment and show G x E interactions. Therefore, while developing markers, phenotyping should be carried out in multiple environments, and implications of G x E should be understood and markers should be used judiciously.
- DNA marker has to be validated for each the breeding population. Assumptions regarding the validity of markers may be disastrous.

## Current application of MAS in livestock

The first reported map in livestock was for the chicken in 1992, which was quickly followed by publication of maps for cattle, pigs and sheep. Microsatellite markers have been of major importance. Markers have been identified for almost all farm animal species, including against milk production in dairy cattle (Ansari-Mahyari *etal*., 2008; Lipkin *et al.,* 2008), buffalo (Sarika *et al.,* 2013), growth and carcass traits of beef cattle (Carr *et al*., 2006), chicken (Lipkin *et al*., 2002; Lahav *et al*., 2006), and goat (Shen *et al*., 2004). There are reports of methodology for MAS (Hayes *et al*., 2007, Kwame AD and Lawrence BS, 2012), genomic selection strategies (Thomasen *et al.,* 2013; Buch LH *etat.,* 2012a), use of molecular technologies for the advancement of animal breeding (Spelman *et al.,* 2013*)*, the efficiency of MAS (Lande and Thompson, 1990) and genomic selection (Florian *et al.,* 2013; Roos *et al.,* 2011), QTLs and epistatic effects (Liu *et al*., 2003), types of selection model (Luo *et al.,* 1997), genome-wide screening for markers (Meuwissen *et al.,* 2007), MAS in dairy breeding (Meuwissen and Van Arendonk, 1992), relationship between MAS and linkage analysis (Ollivier, 1998), relationship between MAS and inbreeding (Pedersen *et al*., 2009) and selection for sex limited

characters (Ruane and Colleau, 1996). ISAG–FAO recommended some microsatellite markers for cattle, buffalo, sheep, goat, horse, donkey, camelid, pig, chicken. Holstein Association, USA developed genomic testing technologies and offer a wide array of tests. An AnimalQTL database (Animal QTLdb) strives to collect all publicly available trait mapping data, *e.g.* QTL (phenotype/expression, eQTL), candidate gene and association data (GWAS). Copy number variations (CNV) mapped to livestock animal genomes was constructed recently, to facilitate locating and comparing discoveries within and between species.

## CONCLUSION

Marker-assisted selection is used in the livestock breeding industry, primarily through gene-assisted selectionand linkage disequilibrium markers-assisted selection. Use of linkage equilibrium markers-assisted selection has been limited and is hampered by implementation issues. Opportunities for the application of marker-assisted selection exist, in particular for gene-assisted selection and linkage disequilibrium markers-assisted selection and, to a lesser degree, for linkage equilibrium markers-assisted selection because of greater implementation requirements. Regardless of the strategy, successful application of marker-assisted selection requires a comprehensiveintegrated approach with continued emphasis onphenotypic recording programs to enable quantitative trait loci detection, estimation and confirmation of effects, and use of estimates in selection.

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REVIEW ARTICLE

# Conservation of Animal Genetic Resources, Strategies and Future Prospects

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## ABSTRACT

Conservation is an action undertaken to ensure that the diversity of farm animal genetic material is being maintained for contribution to food production, agricultural production and productivity through planning, strategies and policies for future purposes. Effective conservation of genetic resources is possible only if the breeds are identified and documented adequately, and there is a full participation towards conservation efforts of communities keeping the animals. In developing countries, few breeds of the five major species are covered by conservation programmes, and programmes are of variable quality; the focus is typically on *invivo* conservation (FAO, 2007). The establishment of gene banks for local breeds and backups for commercial breeds is needed. The role and contribution of Farm Animal Genetic Resources (FAnGR) have often been overlooked, as they had to compete against high input and output breeds. However, indigenous FAnGR carry genes that enable them to tolerate harsh environments, cope with thorny vegetation in drought-prone areas, walk long distances and repel attacks by diseases and pests. In general, indigenous breeds provide the necessary genetic diversity needed by modern agriculture as a means to ensure stability and are vital building blocks for future livestock breeding programmes. As such, conserving them is important, not only for the communities who keep the animals but also for the future of modern agriculture. However, these animal resources are constantly being eroded and are nearing extinction

**Keywords :** Animal genetic resources, conservation, livestock population prioritization, Genetic diversity

## INTRODUCTION

AnGR comprises all animal species, breeds and strains that have economic, scientific and cultural value to mankind in terms of food and agricultural production for the present and the posterity (Gibson *et al.,* 2005; Toro *et al*., 2009). Conservation refers to all human activities including strategies, plans, policies and actions undertaken to ensure that the diversity of animal genetic resources being maintained to contribute to food and agricultural production and productivity, or to maintain other values of these resources (ecological, cultural) now and in the future. The effort to improve food security in developing countries lies in wise use of genetic diversity (Philipsson *et al*., 2011). Livestock populations developed in different ecological or geographical areas will become genetically distinct as a result of genetic drift and differential selection pressures, provided they have also been reproductively isolated from other populations developed under different conditions. The predominant species include cattle, sheep, goats, pigs, chickens, horses and buffalo (Hoffmann, 2010).The great concerns are the inflated loss of indigenous breeds impacting the livelihood options for the poor owing to utilization and management of these genetic resources (Tisdell, 2003).To secure against disasters, it is necessary to characterize animal genetic resources and subsequently to build inventories, including information on the spatial distribution of breeds and valuable breeding stocks. This may include precautionary cryo-conservation of genetic material, or other measures to ensure genetic recovery following a disaster.

### Reasons and Threats of conservation of farm animal genetic resources

To meet present socio-economic demand. (FAnGR are a source of income for poor rural communities, losing them will be detrimental to their livelihoods). Insurance against future changes in production circumstances. Cultural and historical reasons. (Cultural and historical values of most communities are reflected by the type of breeds they keep, therefore, conserving them is necessary to maintain their identity), Opportunities to meet future demands, Regenerating population after disease outbreaks, Rescuing rare or endangered species or breeds, Providing a source of genetic material for research purposes, Supplying germplasm for the development of new breeds, Maintaining indigenous livestock gene pool diversity. Threats includes the indiscriminate cross-breeding practices, expansion of intensive agriculture, change in the economy, establishment of protective areas, lack of market demand, dis appearance of traditional livelihoods and loss of indigenous knowledge institutions.

### Conservation methods

Major strategies should normally be followed in conservation of farm animal genetic resources. The first involves conservation of living population, i.e. in situ conservation as well as ex situ in vivo. The in situ conservation means conservation of animal genetic re-sources in their adaptive environment (original and natural condition).In-situ conservation this is the preservation of animal genetic diversity in the original production environment. This can be done in two ways: viz. on-farm or community-based conservation. Community-based conservation combines the sustainable use of a breed with the empowerment of rural people who keep it.

In Ex-situ conservation, preservation of genetic material outside its original production context. This is done in two ways: cryopreservation (dip-freezing) of genetic material, e.g. semen, oocytes, embryo and DNA, or as live populations where animals are kept in zoos and experimental or show farms. Ex situ conservation means conservation of animal genetic re-sources away from its original production systems where they were developed or are now normally found and bred (FAO, 2013; Kasso and Balkrishnan, 2013). This implies keeping animals (often a very limited number) outside their natural habitat. If reconstruction of a population with frozen semen is required, it might be very helpful to use the few purebred ex situ in vivo conserved females as founders. In practice, ex situ in vivo conservation program suffers from disadvantages of variation of herd management on the farms from management of the herd in the field. Unlike herds under farmers' management, animals in the station may be spared

migration, drought, and diseases and subjected to a different pattern of evolutionary processes. This means natural selection is usually no longer effective in its role of ensuring the adaptation of the population (Kasso and Balkrishnan, 2013). Gene bank should have separate long term storage facility. Room for long term cryopreservation should be separate from laboratory facility. It will be better to maintain two separate storage facilities desirably in different geographical locations (FAO, 2012).

In situ and ex situ conservation methods are complementary. Combining the two approaches can provide a powerful conservation strategy. The most common form of ex situ conservation is in vitro cryoconservation of gametes or embryos in a gene bank. Cryoconservation can be supported by ex situ in vivo conservation. The latter approach implies the conservation of a limited number of live animals in a small breeding herd or a zoo. Cryo-conservation can serve as a contingency plan when a breed population needs to be restored or when a breed has become extinct, as well as for breed improvement. This process benefits companies and researchers by making genetic materials available (FAO, 2012).

## Prioritization of breed for conservation

Criteria for selecting breeds for conservation in view of a number of breeds that are considered endangered and the financial implication that comes with the programme to conserve Animal Genetic Diversity, it is imperative that strict selection pertaining to AnGR to be conserved be made. Selection criteria should include degree of endangeredness, adaptation traits, traits of economic importance, unique traits.

- A paradigm within FAnGR for the past 15 years concerns the use of genetic data, alongside other information in prioritization of livestock populations and breeds for conservation (Weitzman, 1992; Simianer et al., 2003; Boettcher et al., 2010; Ginja et al., 2013).
- While prioritization may be less of a priority in the world's richest regions, it is not expected to be the case in developing countries, where extinction may take a number of forms, including genetic erosion (Berthouly-Salazar et al., 2012; FAO, 2015a,b)
- When a number of breeds are assigned to risk classes, then there is need to prioritize the breeds for conservation.
- Any breed under high risk status should be given higher weightage or priority for conservation.
- Conserving the most genetically diversed breed will be the most efficient way to conserve the diversity of a species.
- Breeds with unique traits should also be given priority for conservation.
- Prioritization of breeds with molecular genetic information (Weitzman 1992).

## Animal Genetic Resources Strategies

## Implementation at national level

- Establish or strengthen national institutions, including national focal points, for planning and implementing animal genetic resources measures, for livestock sector development.
- Establish or strengthen national educational and research facilities.
- Raise national awareness of the roles and values of animal genetic resources.
- Review and develop national policies and legal frameworks for animal genetic resources.

### Implementation at international level

- Establish or strengthen international information sharing, research and education.
- Strengthen international cooperation to build capacities in developing countries and countries with economies in transition.
- Raise regional and international awareness of the roles and values of animal genetic resources.
- Review and develop international policies and regulatory frameworks relevant to animal genetic resources. (FAO, 2007).

### Prospects of animal genetic resources

### Genomic Diversity Conservation

The genome data and technologies only now allow the development of breed management programs able to achieve this aim.(Herrero-Medrano *et al.*2014),using genome resequencing and SNP arrays discovered almost 100 non-synonymous polymorphic nucleotides nearly fixed in commercial pig breeds but with an alternative allele in non-commercial populations, affecting 65 genes in total.The chicken breeds examined functional variation in copy number variants (CNV) at over 200 genes overlapping 1000 quantitative trait loci, including some putatively involved in traits such as skin color and skeletal characteristics (Han *et al.,* 2014).

### Old DNA Studies

Established as a major route into a deeper understanding of livestock evolution and diversity (Larson *et al.,* 2010), old DNA studies have been hampered by a number of constraintsthese also, alternative sources of material such as parchment are, however, providing promising outcomes (Teasdale *et al.,* 2015).

### Studies based on Genome-wide Diversity

It is well known that ascertainment bias of SNP arrays can strongly underestimate the diversity of the (usually autochthonous and less commercial) breeds not used to design the arrays (Porto Neto and Barendse, 2010).The combination of parameters will be required to adequately summarize genome diversity (e.g., heterozygosity and effective population size and inbreeding), as no single all-encompassing statistic to summarize all of a population's genomic diversity and history exists, despite of how tempting it may be to define such statistic (e.g., for policy makers). Effective population size (Ne) estimates can be obtained with as little as a single genome using methods such as the Pairwise Sequential Markovian Coalescent, although these analyses can prove inconclusive if genome coverage is insufficient or if admixture pertains (Li and Durbin, 2011; Orozco-terWengel and Bruford, 2014; Schiffels and Durbin, 2014; Frantz *et al*., 2015).

### Availability of Data

Animal genetic resources data play important role when many genotyping projects on commercial livestock breeds are funded by industry, rendering all except summary data unavailable in many cases, in principle raw data from publicly funded projects should be made publicly available.When data are open, it first makes the information more credible, makes data re-usable, and also enables reproducibility an important scientific principle (Ertz *et* al., 2014).The ownership and hosting of such a resource would be logistically and financially challenging, and could provide an opportunity for the agri-industry to contribute toward conservation of the genetic resources it has utilized in the past and may need again in the future.

## CONCLUSION

It is important to document the status of the animal genetic resources with respect to demographic data, geographical distribution, physical conformations of the animals, performance characteristics, and socio-economic aspects of breed utilization by the stack holders and the utility of the breed.The priorities for breeds to be conserved should be determined. Certain breeds which are threatened or endangered due to neglect, natural disaster and other factors such as poor productivity, low marketability etc. It is highly essential to prepare a watch list of breeds which are under the categories of endangered and threatened breeds as well as genetically eroded breeds. The use of appropriate tools like field recording, cross breeding, different methods like progeny testing etc. and biotechnical methods like AI, ET, and molecular genetics could be exploited for conservation and improvement of domestic animals. The participation-farmer, NGO's and research and development organization is the only way for better conservation of animal biodiversity.Research priorities must include understanding of the functional genetics and genomics of adaptation traits. The various methods of conservation along with the modern technologies will go a long way in conserving the genetic resources along with their improvement for future use. Sustainable utilization of the AnGR will ensure continued propagation of breeds along with their preservation.

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**Table 1.Conservation goals**

| | | | |
|---|---|---|---|
| **Flexibility of country's AGR to meet changes** | Insurance against changes in production conditions | Safeguarding against diseases, disasters, etc. | Opportunities for genomic research |
| **Genetic Factors** | Allowing continued breed evolution/genetic adaption | Increasing knowledge of phenotypic characteristics of breed | Minimizing exposed to genetic drafts |
| **Sustainable utilization of total areas** | Opportunities for development in rural areas | Maintenance of agro-ecosystem diversity | Conservation of rural culture diversity |

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RESEARCH ARTICLE

# Effect of Vermicompost and Mycorrhizal Consortia on Growth of Jatropha Seedlings

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## ABSTRACT

Composting emerges as the most widely applicable process for handling diverse wastes in recycling. Organic wastes are composted to mitigate the environmental consequence of direct land application; composting also helps to meet the demand of organic manure for intensive farming. A very wide variety of organic residues from sources of plant, animal and industrial wastes can be composted to evolve a stable eco-friendly product of utility.The environmental friendly 'vermicomposting technology' can very well be adopted for converting these wastes into wealth. 'Vermicomposting' is a process of composting, featuring the addition of certain species of earthworms to enhance the process of waste conversion and to produce a better end product.Nursery experiments were carried out to find the effect of vermicompost on Jatropha seedlings. Seeds of one month old seedlings of Jatropha were planted in the polybags with various treatments involving different types of litters.The impact of the composts on growth and development of jatropha seedlings were studied.The growth parameters were recorded 15, 30, 45, 60 and 75 days after planting. Coffee pulp vermicompost application registered the highest collar diameter for jatropa seedlings among all other treatments, followed by pungam vermicompost.

**Keywords :** Vermicomposting, organic manure, pungam vermicompost, *jatropha.*

## INTRODUCTION

Composting emerges as the most widely applicable process for handling diverse wastes in the entire area of waste recycling. Organic wastes are composted in an appropriate manner depending on their physico-chemical nature to

mitigate the environmental consequence of direct land application; composting also helps to meet the demand of organic manure for intensive farming. A very wide variety of organic residues from sources of plant, animal and industrial wastes can be composted to evolve a stable eco-friendly product of utility. Lot of waste materials such as tree litter, coffee and tea wastes, vegetable wastes etc. are available in and around Nilgiri hills of Tamil Nadu. The degradable organic matter from these wastes when dumped in open undergoes either aerobic or anaerobic degradation. These unengineered dumpsites permit fine organic matter to become mixed with percolating water to form leachate. The potential of the leachate to pollute adjoining water and soil is high. The environmental friendly 'vermicomposting technology' can very well be adopted for converting these wastes into wealth. 'Vermicomposting' is a kindred process to composting, featuring the addition of certain species of earthworms to enhance the process of waste conversion and to produce a better end product. Vermicomposting differs from conventional composting in several ways. Chiefly, vermicomposting is a mesophilic process and the process is considered faster than normal composting. Since the material passes through the earthworm gut undergoes a significant but not yet fully understood transformation; hence, the resulting earthworm castings are abundant in microbial activity, plant growth regulator and fortified with pest repellery attributes as well.

Considerable work has been carried out on vermicomposting of various organic materials and it has been established that epigeic forms of earthworms can hasten the composting process to a significant extent, with production of a better quality of composts as compare with those prepared through traditional methods. The viability of using earthworms as a treatment or management technique for numerous organic wastes has been investigated by number of workers (Hand, 1988; Logsdan, 1994; Singh and Sharma 2002). Similarly numerous industrial wastes have been vermicomposted and turned into nutrient rich manure (Sundaravadivel *et al*., 1995). But there is a dearth of information regarding the vermicomposting of wastes viz., tree litters, coffee, tea processing wastes, pungam and simaruba shell, vegetable mandy wastes, etc. In order to "Evaluate the efficacy of vermiconpost from different wastes" a study has been formulated with the following objective.

"To evaluate the efficacy of vermicompost and mycorrhizal consortia on growth and development of Jatropha seedlings under nursery conditions".

## MATERIALS AND METHODS

### Effect of vermicompost and mycorrhizal consortia on growth of Jatropha seedlings

Nursery experiments were carried out to find out the effect of vermicompost on Jatropha. The polybag experiment had 12 treatments replicated thrice in a randomized block design. Thirty plants were maintained for each replication. The bags were filled with nursery mixture of sand: red soil: FYM combination. The farmyard manure was replaced by the same quantum of compost, each containing 10 g compost. The mycorrhizal consortia consisting of *Glomus mosseae, Gigaspora margarita* and *Acaulospora laevis* was used @ of 5g. to each polybag. Seeds of Jatropha were planted in the polybags.

### Treatments

$T_1$ – $VC_1$ (Teak litter vermicompost) + mycorrhizal consortia
$T_2$ – $VC_2$ (Albizia litter vermi compost) + mycorrhizal consortia
$T_3$ – $VC_3$ (Simaruba shell vermicompost) + mycorrhizal consortia
$T_4$ – $VC_4$ (Pungam shell vermicompost) + mycorrhizal consortia
$T_5$ – $VC_5$ (Coffee pulp vermicompost) + mycorrhizal consortia
$T_6$ – Control + mycorrhizal consortia

$T_7$ – $VC_1$ (Teak litter vermicompost)
$T_8$ – $VC_2$ (Albizia litter vermicompot)
$T_9$ – $VC_3$ (Simaruba shell vermicompost)
$T_{10}$ – $VC_4$ (Pungam shell vermicompost)
$T_{11}$ – $VC_5$ (Coffee pulp vermicompost)
$T_{12}$ - Control
Design: RBD

The growth parameters were recorded 15, 30, 45, 60 and 75 days after planting.

### Biometrical observation

Growth parameters like leaf number, shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, total dry matter production, seed germination and collar diameter were recorded for Jatropha**.**

### Examination of mycorrhizal infection in roots

The extent of VAM infection was estimated by following the method of Philips and Hayman (1970).Plant roots were collected and washed carefully to remove adhering soil particles.The roots were cut into approximately two centimeter segments. The Potassium hydroxide (10%) was used to digest the roots by autoclaving at 10 lbs for 15 min. After this, the solution was decanted and neutralized with one per cent hydrochloric acid. The root pieces were then washed with tap water and stained with 0.008 per cent tryphan blue in lactic acid: glycerol: distilled water (1:2:2 v/v) for 24 hours. The excess stain was removed by treating the root pieces with lactophenol. The mycorrhizal infection in the root pieces was observed using a binocular microscope (10 x).

The per cent mycorrhizal colonization was then calculated.

$$\text{Percent infection} = \frac{\text{Number of positive segments}}{\text{Total number of roots segments observed}} \times 100$$

### Statistical analysis

The data obtained from various experiments were analysed statistically using the Factorial Randomized Block Design, as described by Gomez and Gomez (1984).

## RESULTS AND DISCUSSION

### Nursery Experiment- Biometrical observation of jatropha seedlings

Coffee pulp vermicompost application recorded the maximum shoot fresh weight of 54.3 g which was significantly greater than the remaining treatments. This treatment was followed by pungam shell vermicompost (52.1 g). The same trend was noticed during various stages of growth under investigations (Table1) .Applications of various vermicompost significantly influenced the shoot dry weight of Jatropha seedlings. Among the treatments, coffee pulp registered maximum shoot dry weight (8.92 g), which was significantly higher than other treatments. Plants receiving coffee pulp vermicompost recorded the highest value of 8.92 g at 75 DAP (Table1,2,3 &4) .Shoot length of the Jatropha seedlings were greatly influenced by the application of various composts. Considering the treatments, coffee

pulp vermicompost application produced maximum shoot length of 30.63cm. The statistical analysis indicated that there was a significant interaction between treatments and periods of growth .It was observed that the coffee pulp vermicompost application recorded the maximum root length (3.057 cm), the least root length was recorded by control. With regards to interaction between treatment and periods, there was a significant variation among the treatments.

As far as root fresh weight is considered, there was no difference due to various treatments till sixty days after sowing. Then the variation was among treatments. The maximum root fresh weight of 2.88 g was registered in coffee pulp vermicompost, which was significantly superior to all other treatments. This was followed by teak litter vermicompost (2.66 g). The control recorded the least value of 1.37 g.All the treatments were significantly superior to control. The maximum root dry weight was recorded in coffee pulp vermicompost (1.21 g) .This was followed by Teak vermicompost + mycorrhizal consortia (0.87 g) and control recorded the minimum of 0.6 g.

Application of various vermicomposts significantly increased the number of leaves. Seeds treated with the coffee pulp vermicompost recorded highest leaves, which was significantly superior to the other treatments. With regarding percentage of seed germination, coffee pulp vermicompost recorded the highest seed germination, which was superior (79.0%) to others, followed by mycorrhizal consortia treatment (77.0%).With respect to dry matter production of the Jatropha seedlings, a significant variation due to treatments during different periods of observation was noticed. Coffee pulp vermicompost registered maximum dry matter production of 10.1 g and it was superior to rest of the treatments.

Coffee pulp vermicompost application registered the highest collar diameter (4.56 cm) among all other treatments, followed by pungam vermicompost (4.39 cm). There was significant difference among all other treatments. Control recorded least value (2.86 cm).In order to study the efficacy of vermicompost from various substrates, experiments were carried out and the results of the experiments are discussed hereunder .Coffee pulp vermicompost application recorded the highest total dry matter production in Jatropha and was superior to rest of the treatments .

### Impact of vermicompost and mycorrhizal consortia on mycorrhizal infection in jatropha seedlings

A comparison of the mean value of all treatments of Jatropha seedlings were registered significant values for VAM infection. $T_7$ - Teak vermicompost had registered higher value of 99.0 per cent among all other treatments, followed by pungam vermicompost. $T_1$ - teak vermicompost + mycorrhizal consortia, $T_9$ - simaruba shell vermicompost + mycorrhizal consortia and $T_{12}$ - control were statistically on par with each other. Also, $T_5$ - coffee vermicompost + mycorrhizal consortia and $T_{11}$ - coffee vermicompost were statistically on par with each other (Table 5).

This could be due to the richness of applied vermicompost with nutrients and indole acetic acid which is evidenced from the nutritional analyses of composts. There are reports that certain metabolites produced by earthworms could be responsible for plant growth (Gavrilov, 1962 and Nielson, 1965). Wilson and Carlile (1989) reported better growth of tomatoes, lettuce and pepper in vermicomposted duck wastes than in unprocessed wastes. Domniguez *et al.* (1997) also reported that vermicastings are rich in humus which contains essential plant nutrients, micronutrients, vitamins, beneficial microorganisms, antibiotics, enzymes etc., that are available for long term nutritional needs for plant growth. Karmegam *et al.* (1999) reported that germination efficiency of green gram was greater in vermicompost medium. Hidalgo *et al.* (1999) reported that 95 percentage germination was observed in cucumber when the seeds were sown with soil mixture and vermicompost.

Coffee pulp vermicompost recorded maximum value for all growth indices, like collar diameter, shoot length, root length, shoot dry weight, shoot fresh weight, percentage of seed germination, number of leaves, root fresh weight and root dry weight. Earthworm casting contain humic substances that could influence plant growth via physiological effects (Muscolo *et al.*, 1999). Sivasubramanian (1999) has reported that the introduction of the

earthworm *Eudrilus eugeniae* alone and with cowdung and mulch effectively increase the vegetative growth of marigold and chrysanthemum through plant height, number of leaves, number of laterals produced and leaf area. Sivasubramanian and Ganesh Kumar (2000) have reported that spraying vermiwash had a very positive effect on the plant height of vanilla. Harti *et al.* (2001) reported that the worm extract contained hydroxy-indole carboxylic acid. The findings of current study are in confirmation with the findings of Gopi (2002) who reported that application of vermicompost (1:1:1) as soil mixtures significantly increased the growth and biomass productivity of forest seedlings such as *Tectona grandis, Casuarina equisetifolia, Simarouba glauca, Pongamia pinnata* and *Delonix regia.*

## CONCLUSION

Vermicompost obtained by composting teak litter and coffee pulp along with mycorrhizal consortia has significantly increased the growth and biomass of Jatropha seedlings.

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**Table 1. Effect of application of vermicompost and mycorrhizal consortia on shoot fresh and dry weight (g) of Jatropha seedlings**

| Treatments | Shoot fresh weight (g) | | | | | Shoot dry weight (g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DAS | | | | | DAS | | | | |
| | 15 | 30 | 45 | 60 | 75 | 15 | 30 | 45 | 60 | 75 |
| $T_1$ | 8.43$^{c}$ | 12.2$^{c}$ | 18.6$^{de}$ | 25.4$^{f}$ | 34.5$^{i}$ | 1.60$^{ab}$ | 1.83$^{abc}$ | 3.23$^{a}$ | 3.42$^{a-d}$ | 5.06$^{bcd}$ |
| $T_2$ | 7.02$^{e}$ | 10.2$^{e}$ | 16.6$^{i}$ | 26.1$^{e}$ | 35.0$^{h}$ | 1.21$^{b}$ | 1.57$^{bc}$ | 2.26$^{bc}$ | 3.88$^{ab}$ | 5.26$^{bc}$ |
| $T_3$ | 6.04$^{fg}$ | 9.52$^{f}$ | 17.1$^{h}$ | 27.1$^{d}$ | 34.2$^{i}$ | 1.33$^{ab}$ | 1.74$^{abc}$ | 2.46$^{abc}$ | 3.03$^{b-e}$ | 4.94$^{bcd}$ |
| $T_4$ | 6.37$^{f}$ | 12.2$^{c}$ | 18.9$^{d}$ | 30.1$^{a}$ | 36.4$^{g}$ | 1.67$^{ab}$ | 2.37$^{ab}$ | 2.70$^{ab}$ | 3.56$^{abc}$ | 5.13$^{bc}$ |
| $T_5$ | 5.65$^{gh}$ | 10.6$^{e}$ | 18.3$^{ef}$ | 26.1$^{e}$ | 37.1$^{f}$ | 1.52$^{ab}$ | 1.83$^{abc}$ | 2.59$^{ab}$ | 3.13$^{b-e}$ | 4.54$^{cde}$ |
| $T_6$ | 8.30$^{c}$ | 11.4$^{d}$ | 19.4$^{c}$ | 29.4$^{b}$ | 39.0$^{e}$ | 1.23$^{ab}$ | 1.44$^{c}$ | 2.64$^{bc}$ | 3.33$^{a-d}$ | 4.47$^{cde}$ |
| $T_7$ | 10.4$^{b}$ | 14.14$^{b}$ | 20.2$^{b}$ | 26.1$^{e}$ | 44.1$^{d}$ | 1.92$^{ab}$ | 1.85$^{abc}$ | 2.20$^{bc}$ | 2.81$^{cde}$ | 4.28$^{de}$ |
| $T_8$ | 5.45$^{h}$ | 10.63$^{e}$ | 18.1$^{f}$ | 24.4$^{h}$ | 39.4$^{e}$ | 1.50$^{ab}$ | 1.82$^{abc}$ | 2.08$^{bc}$ | 2.59$^{de}$ | 5.28$^{bc}$ |
| $T_9$ | 8.31$^{c}$ | 12.2$^{c}$ | 17.6$^{g}$ | 25.1$^{fg}$ | 48.2$^{c}$ | 1.79$^{ab}$ | 2.06$^{abc}$ | 2.46$^{abc}$ | 3.33$^{a-d}$ | 8.76$^{a}$ |
| $T_{10}$ | 7.62$^{d}$ | 11.4$^{d}$ | 17.4$^{gh}$ | 24.7$^{gh}$ | 52.1$^{b}$ | 2.13$^{a}$ | 2.44$^{ab}$ | 2.71$^{ab}$ | 3.70$^{ab}$ | 5.42$^{b}$ |
| $T_{11}$ | 12.6$^{a}$ | 15.47$^{a}$ | 21.4$^{a}$ | 28.1$^{c}$ | 54.3$^{a}$ | 1.69$^{ab}$ | 2.49$^{a}$ | 3.28$^{a}$ | 4.00$^{a}$ | 8.92$^{a}$ |
| $T_{12}$ | 5.45$^{h}$ | 9.29$^{f}$ | 16.2$^{i}$ | 24.3$^{h}$ | 28.9$^{j}$ | 1.00$^{c}$ | 1.27$^{c}$ | 2.00$^{c}$ | 2.47$^{e}$ | 4.06$^{e}$ |

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

**Table 2. Effect of application of vermicompost and mycorrhizal consortia on shoot and root length (cm) of Jatropha seedlings**

| Treatments | Shoot length (cm) | | | | | Root length (cm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DAS | | | | | DAS | | | | |
| | 15 | 30 | 45 | 60 | 75 | 15 | 30 | 45 | 60 | 75 |
| $T_1$ | 5.86$^{a}$ | 8.40$^{ab}$ | 9.86$^{de}$ | 12.73$^{c}$ | 17.60$^{g}$ | 3.13$^{a}$ | 4.03$^{b}$ | 7.43$^{b}$ | 8.00$^{bc}$ | 9.66$^{de}$ |
| $T_2$ | 5.13$^{ab}$ | 8.36$^{ab}$ | 9.60$^{de}$ | 14.50$^{b}$ | 16.43$^{g}$ | 2.96$^{a}$ | 5.46$^{b}$ | 7.60$^{b}$ | 9.23$^{b}$ | 11.06$^{d}$ |
| $T_3$ | 5.30$^{ab}$ | 8.56$^{ab}$ | 9.66$^{de}$ | 17.60$^{a}$ | 19.50$^{f}$ | 3.36$^{a}$ | 5.30$^{b}$ | 6.50$^{bc}$ | 7.70$^{bc}$ | 9.40$^{de}$ |
| $T_4$ | 4.33$^{ab}$ | 9.26$^{a}$ | 10.93$^{cd}$ | 17.40$^{a}$ | 19.90$^{ef}$ | 3.53$^{a}$ | 4.83$^{b}$ | 6.80$^{bc}$ | 8.40$^{bc}$ | 10.63$^{d}$ |
| $T_5$ | 4.93$^{ab}$ | 8.60$^{ab}$ | 10.96$^{cd}$ | 18.30$^{a}$ | 20.33$^{def}$ | 3.33$^{a}$ | 5.30$^{b}$ | 6.76$^{bc}$ | 8.76$^{bc}$ | 10.26$^{de}$ |
| $T_6$ | 4.60$^{ab}$ | 9.66$^{a}$ | 12.4$^{bc}$ | 18.63$^{a}$ | 21.86$^{d}$ | 3.23$^{a}$ | 5.13$^{b}$ | 6.66$^{bc}$ | 8.56$^{bc}$ | 9.50$^{de}$ |
| $T_7$ | 4.60$^{ab}$ | 8.70$^{ab}$ | 13.66$^{ab}$ | 18.16$^{a}$ | 20.26$^{ef}$ | 2.96$^{a}$ | 5.13$^{b}$ | 6.43$^{bc}$ | 8.70$^{bc}$ | 10.96$^{d}$ |
| $T_8$ | 4.80$^{ab}$ | 8.83$^{ab}$ | 14.46$^{a}$ | 18.20$^{a}$ | 21.20$^{de}$ | 3.00$^{a}$ | 5.93$^{b}$ | 6.86$^{bc}$ | 8.83$^{bc}$ | 15.46$^{c}$ |
| $T_9$ | 4.90$^{ab}$ | 9.33$^{a}$ | 13.46$^{ab}$ | 17.56$^{a}$ | 23.43$^{c}$ | 2.66$^{a}$ | 3.96$^{b}$ | 5.46$^{bc}$ | 8.30$^{bc}$ | 16.43$^{c}$ |
| $T_{10}$ | 4.13$^{b}$ | 9.26$^{a}$ | 12.43$^{bc}$ | 18.43$^{a}$ | 28.93$^{b}$ | 2.80$^{a}$ | 4.33$^{b}$ | 6.43$^{bc}$ | 8.53$^{bc}$ | 24.70$^{b}$ |
| $T_{11}$ | 4.36$^{ab}$ | 9.33$^{a}$ | 14.13$^{a}$ | 18.43$^{a}$ | 30.63$^{a}$ | 3.63$^{a}$ | 8.13$^{a}$ | 15.13$^{a}$ | 17.40$^{a}$ | 30.57$^{a}$ |
| $T_{12}$ | 4.00$^{b}$ | 7.30$^{b}$ | 9.26$^{e}$ | 11.90$^{c}$ | 13.51$^{h}$ | 2.53$^{a}$ | 4.00$^{b}$ | 4.63$^{c}$ | 6.66$^{c}$ | 8.23$^{e}$ |

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

**Table 3.Effect of application of vermicompost and mycorrhizal consortia on root fresh and dry weight (g) of Jatropha seedlings**

| Treatments | Root fresh weight (g) | | | | | Root dry weight (g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | DAS | | | | | DAS | | | | |
| | 15 | 30 | 45 | 60 | 75 | 15 | 30 | 45 | 60 | 75 |
| $T_1$ | 0.46$^{a}$ | 0.65$^{a}$ | 0.81$^{a}$ | 1.03$^{bc}$ | 1.94$^{ab}$ | 0.12$^{cd}$ | 0.32$^{b}$ | 0.40$^{b}$ | 0.59$^{ab}$ | 0.87$^{b}$ |
| $T_2$ | 0.39$^{a}$ | 0.55$^{a}$ | 0.71$^{a}$ | 1.01$^{bc}$ | 1.98$^{ab}$ | 0.14$^{bcd}$ | 0.15$^{ef}$ | 0.28$^{cd}$ | 0.54$^{b}$ | 0.79$^{cd}$ |
| $T_3$ | 0.35$^{a}$ | 0.54$^{a}$ | 0.77$^{a}$ | 1.24$^{abc}$ | 2.11$^{ab}$ | 0.12$^{cd}$ | 0.21$^{c-f}$ | 0.30$^{cd}$ | 0.58$^{b}$ | 0.78$^{cd}$ |
| $T_4$ | 0.42$^{a}$ | 0.52$^{a}$ | 0.66$^{a}$ | 1.22$^{abc}$ | 2.21$^{a}$ | 0.19$^{bc}$ | 0.24$^{cd}$ | 0.29$^{cd}$ | 0.53$^{b}$ | 0.85$^{bc}$ |
| $T_5$ | 0.40$^{a}$ | 0.56$^{a}$ | 0.78$^{a}$ | 1.32$^{ab}$ | 2.34$^{a}$ | 0.19$^{bc}$ | 0.29$^{bc}$ | 0.35$^{bc}$ | 0.51$^{b}$ | 0.83$^{bc}$ |
| $T_6$ | 0.48$^{a}$ | 0.67$^{a}$ | 0.70$^{a}$ | 1.26$^{abc}$ | 2.18$^{a}$ | 0.11$^{cd}$ | 0.13$^{f}$ | 0.29$^{cd}$ | 0.42$^{cd}$ | 0.86$^{bc}$ |
| $T_7$ | 0.39$^{a}$ | 0.55$^{a}$ | 0.70$^{a}$ | 1.23$^{abc}$ | 2.66$^{a}$ | 0.22$^{b}$ | 0.25$^{bcd}$ | 0.31$^{cd}$ | 0.53$^{c}$ | 0.80$^{bc}$ |
| $T_8$ | 0.61$^{a}$ | 0.61$^{a}$ | 0.71$^{a}$ | 1.27$^{ab}$ | 2.05$^{ab}$ | 0.18$^{bcd}$ | 0.19$^{def}$ | 0.33$^{bcd}$ | 0.36$^{cd}$ | 0.63$^{e}$ |
| $T_9$ | 0.60$^{a}$ | 0.73$^{a}$ | 0.83$^{a}$ | 0.90$^{bc}$ | 1.94$^{ab}$ | 0.19$^{bc}$ | 0.22$^{cde}$ | 0.34$^{bcd}$ | 0.39$^{cd}$ | 0.71$^{d}$ |
| $T_{10}$ | 0.63$^{a}$ | 0.72$^{a}$ | 0.83$^{a}$ | 0.85$^{bc}$ | 2.44$^{a}$ | 0.19$^{bcd}$ | 0.21$^{def}$ | 0.31$^{cd}$ | 0.37$^{cd}$ | 0.78$^{cd}$ |
| $T_{11}$ | 0.46$^{a}$ | 0.58$^{a}$ | 0.83$^{a}$ | 0.62$^{a}$ | 2.88$^{a}$ | 0.31$^{a}$ | 0.43$^{a}$ | 0.59$^{a}$ | 0.67$^{a}$ | 1.21$^{a}$ |
| $T_{12}$ | 0.22$^{a}$ | 0.47$^{a}$ | 0.55$^{a}$ | 0.75$^{c}$ | 1.37$^{c}$ | 0.10$^{d}$ | 0.13$^{f}$ | 0.26$^{d}$ | 0.34$^{d}$ | 0.60$^{e}$ |

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

**Table 4. Effect of application of vermicompost and mycorrhizal consortia on number of leaves and percentage of seed germination of Jatropha seedlings**

| Treatments | No. of leaves | | | | | Percentage of seed germination |
|---|---|---|---|---|---|---|
| | DAS | | | | | DAS |
| | 15 | 30 | 45 | 60 | 75 | 15 |
| $T_1$ | 2.0$^{a}$ | 3.7$^{a}$ | 6.3$^{cd}$ | 9.3$^{b-e}$ | 12.00$^{de}$ | 51.00$^{i}$ |
| $T_2$ | 2.0$^{a}$ | 4.0$^{a}$ | 5.7$^{d}$ | 8.0$^{de}$ | 10.70$^{de}$ | 57.00$^{h}$ |
| $T_3$ | 2.0$^{a}$ | 3.7$^{a}$ | 6.7$^{cd}$ | 8.0$^{de}$ | 11.70$^{de}$ | 42.00$^{j}$ |
| $T_4$ | 2.0$^{a}$ | 4.0$^{a}$ | 9.0$^{abc}$ | 8.0$^{de}$ | 12.30$^{de}$ | 69.00$^{e}$ |
| $T_5$ | 2.0$^{a}$ | 4.0$^{a}$ | 8.7$^{bc}$ | 10.0$^{bcd}$ | 13.00$^{d}$ | 74.00$^{c}$ |
| $T_6$ | 2.0$^{a}$ | 4.0$^{a}$ | 10.0$^{ab}$ | 11.0$^{abc}$ | 11.70$^{de}$ | 77.00$^{b}$ |
| $T_7$ | 2.0$^{a}$ | 3.0$^{a}$ | 11.0$^{ab}$ | 11.7$^{ab}$ | 11.70$^{de}$ | 61.00$^{g}$ |
| $T_8$ | 2.0$^{a}$ | 4.0$^{a}$ | 10.0$^{ab}$ | 10.3$^{a-d}$ | 24.00$^{b}$ | 65.00$^{f}$ |
| $T_9$ | 2.0$^{a}$ | 4.0$^{a}$ | 9.0$^{abc}$ | 9.7$^{b-e}$ | 29.00$^{a}$ | 70.00$^{e}$ |
| $T_{10}$ | 2.0$^{a}$ | 4.0$^{a}$ | 8.7$^{bc}$ | 8.7$^{cde}$ | 19.00$^{c}$ | 72.00$^{d}$ |
| $T_{11}$ | 2.0$^{a}$ | 4.0$^{a}$ | 11.7$^{a}$ | 13.0$^{a}$ | 29.30$^{a}$ | 79.00$^{a}$ |
| $T_{12}$ | 2.0$^{a}$ | 3.3$^{a}$ | 5.0$^{d}$ | 7.0$^{e}$ | 10.00$^{e}$ | 40.00$^{k}$ |

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

**Table 5. Examination of mycorrhizal infection in roots**

| Jatropha seedlings | Mean value |
|---|---|
| $T_1$ | 50.3[c] |
| $T_5$ | 11.0[e] |
| $T_6$ | 31.0[d] |
| $T_7$ | 99.0[a] |
| $T_9$ | 51.0[c] |
| $T_{10}$ | 71.33[b] |
| $T_{11}$ | 11.0[e] |
| $T_{12}$ | 51.66[c] |

In a column, means followed by a common letter are not significantly different at 5% level by DMRT

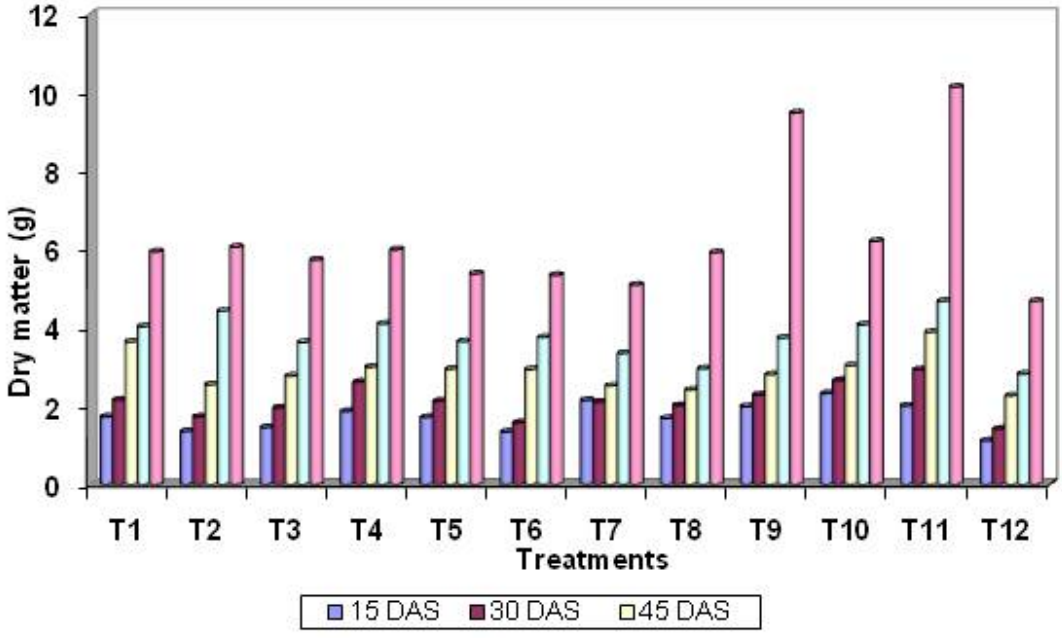


**Fig. 1. Effect of vermicompost and mycorrhizal consortia on dry matter production (g) of Jatropha seedlings**

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REVIEW ARTICLE

# *Emblica officinalis* Linn. (Indian Gooseberry) - The Role of Ayurvedic Therapeutic Herb in Cancer

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## ABSTRACT

*Emblica officinalis* is used therapeutically in Indian system of medicine. Owing to its several therapeutic effects on various organs, it has been found to be as a remedial for the diseases such as, asthma, bronchitis, digestive ailments such as indigestion, hyperacidity and ulcers and anaemia, jaundice, diabetes, hemorrhage conditions, eye diseases, allergic skin problems, and gynaecological problems. Many ailments are treated through the fruit which is used either alone or in combination with other fruit. There is well known information emanating from both in vitro and in vivo studies indicating fruit extract of the Emblica commonly referred to as Indian Gooseberries, has potent anticancer properties. It possesses antipyretic, analgesic, antitussive, adaptogenic, gastroprotective, antiatherosclerotic, antiatherogenic, antianemic, antihypercholesterolemic, wound healing, cardioprotective, antidiarrheal, hepatoprotective, nephroprotective, and neuroprotective properties as confirmed in numerous preclinical studies. Besides that experimental studies have accounted that *E. officinalis* of its phytochemicals also reveal anticarcinogenic properties. The review summarizes the results correlated to these properties and also highlights the aspects that future research establishing its activity and utility as a cancer preventive and therapeutic drug in human beings.

**Keywords :** *Emblica officinalis*, preventive measurement, phtytochemicals, therapeutic applications.

## INTRODUCTION

Cancer is a worldwide epidemic with approximately fourteen million cases was diagnosed each year, leading to an annual death toll of about eight million [1]. It can be characterized by the failure of regulation of tissue growth which results in the abandoned multiplication of the normal cells to form tumors which in furthermore invades into nearby parts of the body[2].There is two important factor which can be cause the cancer such as carcinogenic factors and also hereditary. It is considered as a curable disease since the majority of the cancer was caused by the environmental factors [3]. To encourage a disease free healthy life Nature has gifted mankind medicinal plants. Abundant medicinal plants are available in a group of herbal preparations for concerning Indian traditional health. *Phyllanthus emblica* Linn., commonly known as Indian gooseberry (*Amla)*, belongs to the family of Euphorbiaceae, is a chief herbal drug employed in Unani (Graceo-arab) and ayurvedic systems. It is used evenly as a medicine and as a tonic to build up lost energy and vigor. *E. officinalis* is tremendously nutritious and chief dietary source of vitamin C, amino acids, and minerals. Whole parts of the plant are used for medicinal purposes, predominantly the fruit, which has been utilized in Ayurveda as a powerful *rasayana* and in customary medicine for the management of diarrhea, jaundice and inflammation.Moreover, plant parts show antidiabetic, antiulcerogenic, hypolipidemic, hepatoprotective, antibacterial, antioxidant, gastroprotective, and chemopreventive [4].

### Chemical Constituents

Amla is one of the most extensively studied plants(fig.1). Many reports suggested that it contains tannins, alkaloids and phenols [5]. The active ingredient that has significant pharmacological action in amla is chosen by Indian scientist as "Phyllemblin". The fruit of Emblica is rich in quercetin, phyllaemblic compounds, flavonoids, gallic acid, tannins, pectin, and vitamin C and contains various polyphenolic compounds. A broad variety of phytochemical components includes alkaloids, terpenoids, flavonoids, and tannins have been exposed to posses useful biological [6,7]. Ellagic acid and lupeol is in root and bark contains leucodelphinidin in amla fruit. The seeds yield a fixed oil of 16% which is brownish-yellow in color. It also have fatty acids such as linolenic (8.8%), linoleic (44.0%), oleic (28.4%), stearic (2.15%), palmitic (3.0%) and myristic (1.0%) [8].Identified chemical compounds where isolated from Emblica many such as gallic acid, ellagic acid, quercetin, 1-O-galloyl-beta-D-glucose, chebulinic acid, chebulagic acid, 3,6-di-O-galloyl-Dglucose, corilagin, isostrictiniin 1, 3 Ethylgallic acid (3 ethoxy 4,5 dihydroxy benzoic acid) and 6-di-O -galloyl beta D glucose [9].

### Nutritional Properties

Amla is a well-known fruit which has more nutritional qualities. The fruit of amla is rich in minerals, polyphenols and also contain vitamin C between the range of 200-900 mg per 100 g [10,11]. Nutritional components of Emblica are reported in Table 1.

### Antioxidant Properties

Nature has gifted us with defensive antioxidant mechanisms-superoxide dismutase, catalase, GSH peroxidases, reductase, glutathione (GSH), vitamin E (tocopherols and tocotrienols), vitamin C, etc., along with several dietary components. Higher consumption of components/nutrients with antioxidant capabilities has been associated with lower frequency of abundant human morbidities or mortalities are per many epidemiological studies. A different probable application of antioxidant or free radical manipulations in prevention/control of the disease has been revealed by ongoing research. Natural products from different dietary aspects such as Indian spices and medicinal plants are known to have antioxidant activity[12].In fruits and vegetables especially vitamins and polyphenols are considered to be responsible for the antioxidant activity, with polyphenols being the most active [13,14].The natural antioxidants are expected to be an alternative one instead of using synthetic antioxidants, because of their potential

health benefits [15].Antioxidant activities in many spices, fruits, vegetables, medicinal plants and microalgae were evaluated, and the results were found that some of them might be rich sources of natural antioxidants [16].

### Applications of *Emblica officinalis* in Cancer

Natural products of plant origin currently comprise a considerable quantity of commercially available antineoplastic drugs[17]. Polyphenols act on many targets in pathways and mechanisms related to tumor cell proliferation and death, carcinogenesis, inflammation, metastatic spread, angiogenesis, drug and radiation resistance.

Ngamkitidechakul *et al*., examined the anticancer effects of aqueous extract of *E. officinalis* in four different ways are against cancer cell lines, *in vitro* apoptosis, mouse skin tumourigenesis and *in vitro* invasiveness. The *E. officinalis* extract at 50-100 µg/mL significantly inhibited the growth of cancer cell in six human cancer cell lines such as A549-lung, HepG2-liver, HeLa-cervical, SK-OV3-ovarian, MDA-MB-231-breast and SW620-colorectal. Nevertheless, the extract was not toxic against MRC 5 (normal lung fibroblast). These results recommend *E. officinalis* revealed that anticancer activity against selected cancer cells, thus warrants further study as a possible chemo preventive and ant invasive agent [18].

Chemoprevention with phytochemicals especially in fruits and vegetables is presently considered as one of the most important approaches to control cancer.Emblica is valued for its unique tannins and flavanoids, which show very powerful antioxidant qualities.The inhibition of tumor incidences by fruit extract of this amla has determined by two-step process on skin carcinogenesis in Swiss albino mice. Chemopreventive potential of Emblica fruit extract on 7,12-dimethylbenz(a)anthracene where induced skin tumorigenesis in Swiss albino mice have initiated [19].Only very rare studies have speculated that *P.emblica* had chemopreventive effects on liver cancer. It was evaluated in vivo wistar rats which treated along with carcinogen Diethylnitrosamine at 200mg/kg b.wt.i.p to induce liver cancer. The result of the study revealed that methanolic fruit extract which was a pretreatment demonstrated significant pathological manifestation at both the doses of 100 and 200 mg/kg b.w. *Emblica officinalis* has the great potential to be useful in ameliorating the carcinogen-induced response in rat [20].

## CONCLUSION

Research in medicinal plants has increased a renewed focus recently. The most important reason is that various system of medicine even though it is effective along with a lot of side effects that often lead to associated complications. The fruit of amla is used to be an ingredient for the preparation of many Ayurvedic medicines and tonics as it removes extreme salivation, nausea, vomiting, giddiness, spermatorrhoea, maintain the body temperature and menstrual disorders. Vitamin C content of amla increases in the sun dried amla for example if 100 gm. of fresh amla gives out 600 mg of Vitamin C, then when it is sun-dried, its content increases to 1500 to 1600 mg. Plant-based system of medicine being natural does not cause this serious problems. Further studies must be carrying out to clarify the molecular mechanism of interaction in various plant based drugs with a human body in the different type of diseases.

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**Fig.1.Fruit of *Emblica officinalis (Amala)***

**Tabel.1.Nutritional components of *Emblica officinalis***

| S.No. | Nutritional parameters | Percentage % |
|---|---|---|
| 1. | Moisture | 81.2 % |
| 2. | **Macronutrients** | |
| | Protein | 0.5 |
| | Fat | 0.1 |
| | Mineral matter | 0.7 |
| | Fibre | 3.4 |
| | Carbohydrate | 14.1 |
| 3. | **Micronutrients** | |
| | Calcium | 50mg/100 |
| | Phosphorous | 20mg/100 |
| | Iron | 1.2mg/100 |
| | Vitamin C | 600mg/100 |
| | Nicotinic acid | 0.2mg/100 |

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RESEARCH ARTICLE

# Phytochemical Screening and Nutritional Composition of *Solanum anguivi* L. Fruit

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## ABSTRACT

*Solanum anguivi* (L.) is a wild or semi-domesticated vegetable locally consumed in India. The present study aimed to study the medicinal and nutritional characteristics of *Solanum anguivi* L. fruit. The phytochemical constituents were investigated qualitatively and results revealed the presence of alkaloids, flavonoids, tannins, saponins, phenols, steroids and glycosides which has more medicinal values. The fruit comprises 83% of moisture and the proximate analysis on dry basis showed that the fruit was rich in protein (11.26±0.1%), crude fiber (16.36±0.05%) and ash (16.23±0.2%). *Solanum anguivi* L. fruit had also revealed substantial amount of minerals like calcium (321±1.7mg), sodium (350.1±2.007mg), potassium (423.33±2.3mg), phosphorus (223.1±0.2mg), zinc (5.56±0.2mg), iron (18.46±0.02mg) and copper (18.45±0.02mg).Hence, *Solanum anguivi* L. could be used as an herbal alternative medicine to synthetic therapy for the treatment of anemia and other oxidative stress.

**Keywords :** *Solanum anguivi* L., fruit extract, phytochemicals, proximate composition, minerals

## INTRODUCTION

Plants are an important resource of conventional medicines used against different ailments. Rural people who have century's old traditional knowledge transferred from generation to generation still rely on plant resources for variety of purposes such as food, fodder and medicines [1]. Phytochemicals are group of plant inherent bioactive substances

that are responsible for protection of such plants from environmental stress, microbial attack, insects and other external aggression. These phytochemicals are localized to fruit, seed, stem epidermis, flower and other peripheral surfaces of plants9. Fruits comprise a variety of phytochemical such as tannins, saponins, flavonoids, terpenes, phenolic compounds etc [2].Various researchers have shown the disease protective capability of these phytochemical. Besides that it helps in prevention and elucidation of several micronutrient deficiencies, most especially in less developed countries. There are wide array of research investigations highlighting the chemical, nutritional and health beneficial properties of fruits and vegetables [3,4,5,6].

*Solanaceae* is a plant family comprising about 2300 species, nearly one-half of which belong to the genus *Solanum* [7]. The fruit is used in folklore medicine in the treatment of high blood pressure[8]; roots are useful in treatment of cough, ulcers, asthma, nervous disorder and fever[ 9]. The berries of *S. anguivi* L. can be consumed fresh, semi-ripe, ripe, dried or ground into flour. *S. anguivi* L. berries are especially characterized by their bitterness due to the presence of various phenolic compounds conferring them antioxidant properties[10]. Proximate and nutritional composition of five different *Solanum* species commonly found in Nigeria were investigated by some researchers[11, 12]. Despite the great nutritional value and medicinal importance of *S. anguivi* L. fruits, there is little or paucity of information on the nutritional and phytochemical investigation of *S.anguivi* L. fruits in India. Hence the present study was aimed to evaluate proximate, mineral and phytochemical composition of the fruits of *S. anguivi* L.

## MATERIALS AND METHODS

### Chemicals and Instruments

The analytical grade chemicals such as sulphuric acid, Mayer's reagent, sodium hydroxide, ferric chloride, chloroform, hydrochloric acid, petroleum ether, nitric hydrochloric acid, deionized water and sodium borohydride was obtained from Sigma-Aldrich chemicals, USA.

### Plant Materials

Fresh green fruits of *Solanum anguivi* L. were purchased from Salem local market. The fruits were identified and authenticated by the Institute of Herbal Science, Plant Anatomy Research Centre, Chennai (PARC/2016/3297, PARC/2016/3296). The fruits were thoroughly washed with distilled water and used for analysis.

### Preparation of Extracts

The fresh fruit was crushed and immersed in water, methanol and chloroform solution in a 100ml flat bottom flask and was macerated for one week respectively to separate the medicinally active portions. The collected extract was filtered and used for the qualitative phytochemical analysis [13, 14].

### Phytochemcial Screening

***Test for Alkaloids***:To few ml of filtrate, a drop or 2ml of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate indicated the test as positive.

***Test for Flavonoids***: The filtrate of 1ml was mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on addition of few drops of diluted hydrochloric acid which indicated the presence of flavonoids.

***Test for Phenolic Compounds*:** To 1ml of the filtrate, few ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

***Test for Tannin:*** The filtrate of 1ml was mixed with 2ml of 2% solution of $FeCl_3$. Blue green or black coloration indicated the presence tannin.

***Test for Saponin*:**1ml of filtrate was diluted with 20ml of distilled water and shaken well in a graduated cylinder for 15 mins. The formation of foam to a length of 1cm indicated the presence of saponin.

***Test for Steriods:*** The filtrate of 1ml was mixed with 2ml of chloroform and concentrated sulfuric acid was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids.

***Test for Glycosides*:** To a few ml of filtrate, 1ml of water and then diluted NaOH solution were added. Formation of yellow color indicates the presence of glycosides.

***Test for Anthocyanin:*** One ml of filtrate, 2ml of 2N HCL and 2ml of ammonia were mixed. Initial appearance of pink-red color turning into blue-violet indicates the presence of anthocyanin.

***Test for Coumarin*:** To 1ml of filtrate, 3ml of 10% NaOH was added. The formation of yellow color indicates the presence of coumarin.

### Nutritional Composition Analysis

Five gram of the fresh sample of *S. anguivi* L. was quantified for its moisture, ash, protein, fat and minerals according to AOAC methods of determination [15 ]. Moisture content was determined by drying 2g of the sample to a constant weight in a crucible placed inside oven at temperature of 105°C. Ash content was determined by igniting one gram sample placed in a muffle furnace maintained at 550°C for 5 hr. The protein content was calculated by multiplying the total organic nitrogen by 6.25 16. Fat was obtained by extracting 5g of the sample in a Soxhlet apparatus using petroleum ether with boiling range 60-80° C. Fiber and Carbohydrate content was determined according to Oyeyemi et al (2015) and Onwuka (2005) calculation equation respectively. Each analysis was carried out in triplicate. The minerals such as Copper, Calcium, Potassium, Sodium, Iron, Zinc, Phosphorus and Manganese were determined by atomic absorption spectrophotometer (AA-6300 Thermo Fisher) using air-acetylene flame.

## RESULTS AND DISCUSSION

### Phytochemical Screening

The aqueous, methanol and chloroform extracts of *S. anguivi* L. was screened for different bioactive compounds such as alkaloids, flavonoids, tannins, saponins, phenols, steroids, glycosides, anthocyanin and coumarin (Table 1). The strong presence of alkaloids (white or creamy precipitate), flavonoids (yellow coloration), tannin (dark green coloration) and phenol (white precipitate) were evidenced by the high intense color and formation of precipitate in *S. anguivi* L. The saponin was noted by stable foam formation in all the extracts. The steroids (red color produced in the upper layer and yellow color in lower layer) and glycosides (mild brown ring) were present in all studied extract.

According to the study of Oyeyemi et al (2015), all phytochemical compounds were present except glycoside in *S. anguivi* L. The anthocyanin and coumarin were not observed in aqueous extract of *S. anguivi* L, but was present in methanol and chloroform extract. Similar result was obtained by Manjulika et al (2014) in *S. indicum* which belongs to the variety of egg plant. Gnana et al (2013) found that phenol, tannin and steroid in aqueous extract; alkaloid and

tannin in chloroform extract were not shown in *Solanum torvum* fruit. Whereas saponin was found in all the extracts of Solanum family reported by Gnana et al (2013); Oyeyemi et al (2015). Saponin found to possess a wide range of medicinal property which may help in protection against various diseases such as hypercholesterolemia, hyperglycemia and obesity (Mohanta *et al.,* 2007). The difference in the observation of phytochemical compound could be due to varietal difference and geographical distribution of Solanum family.According to the presence of phytochemical compound, *S. anguivi* L. could be medicinal fruit and be included in the diet to increase the immune status as suggested by Dan et al (2014).

**Proximate Composition of *S. anguivi* L. Fruit**

The moisture content of fresh *S. anguivi* L. fruit (Table 2) was similar to the level reported by Adeyeye et al (2006) (91.9%) in *S. anguivi* L. fruit; Denis et al (2010)(87%) in *Solanum indica* fruit. The obtained result on ash content on dry basis was higher than the content reported by Adedeye et al (2006) (8.7%); Oyeyemi et al (2005) (8.89%); Dan et al (2014) (6.9%) in *S. anguivi* L. fruit.

The proximate composition on dry basis such as carbohydrate, protein and fat content respectively (29.10±0.4%; 11.26±0.1%; 2.2±0.1%) of *S. anguivi* L. (Table 2) revealed higher level than reported by Adeyeye et al (2006) (23.3%; 5.4%; 7.6%) in *S. anguivi* L. fruit; Dan et al (2014) (2.4%; 13.4%; 1.4%) except protein in *S. anguivi* L. fruit. Oyeyemi et al (2015) (28.98%; 36.3%; 5.68%) reported similar carbohydrate content but greater protein and fat content in *S. anguivi* L. fruit. Although the obtained result on proximate composition was found to be lower than the report of Ali (2012) (40.67±0.68%; 23.47±0.27%; 5.26±0.50%) in *Solanum indica* fruit, low fat content observed in *S. anguivi* L. (2.2±0.1) could favour for the prevention of constipation and colon cancer[20]. The high fiber content of *S. anguivi* L. fruit (16.36±0.05%) clearly indicates that the fruit could be ranked as fiber rich vegetable which could be employed in the treatment of diseases such as obesity, diabetes, cancer and abdominal disorder.The result on fiber content of *S. anguivi* L. fruit was comparable with the result of Dan et al (2014) (11.8±0.7%) in *S. anguivi* L.; Adeyeye et al (2006) (15.5%) in *S. anguivi* L.

The mineral content of *S. anguivi* L. revealed that the fruit is rich in minerals by revealing good percentage of copper (18.45 mg %), calcium (321 mg %), potassium (423.33 mg %), sodium (350.1 mg %), iron (18.46mg %), zinc (5.56mg %), phosphorus (223.1mg %) and manganese (0.58 mg %). The obtained result was higher than the content reported by Dan et al (2014); Adeyeye et al (2006) and Oyeyemi et al (2015).The sodium content of fresh *S. anguivi* L. fruit was higher than the level reported by Dan et al 2014 (0.03 mg %); Oyeyemi et al (2015) (7.5 mg %) but similar result was obtained by Adeyeye et al (2006) (268.4mg %). The obtained content of potassium was lower than the report of FAO (1967) (2000mg) and Dan et al (2014) (2059.7mg) and higher than the content reported by Oyeyemi et al (2015) (2.03mg %). The manganese content of *S. anguivi* L. was higher than the result of Dan et al (2014) (0.2mg %) but lower than the value reported by Oyeyemi et al (2015) (27.65±0.19mg %).

Agrahar-Murugkar and Subbulakshmi (2005) found mineral content in different wild edible fruits and revealed that calcium was rich in *Solanum indicum*; phosphorus and magnesium was rich in *Solanum gilo*; iron and manganese was rich in *Prunus nepalensis* and *Viburnum corylifolia* respectively; *Solanum xanthocarpum* contain higher amount of sodium and copper. *Vangeria spinosa* was higher in zinc. *Gomphogy necissiformis* was rich in potassium.

According to the report of the study of Adepoju (2009), sodium content of *S. anguivi* L. fruit was similar to the sodium content of *Sponiasmombim* fruit (465.0 ± 21.21 mg%); potassium, calcium and phosphorus content were similar to the level respectively in *Mordiiwhytii* fruit (potassium - 410.0 ± 12.20 mg%, calcium - 300.0 ± 12.20mg% and phosphorus - 170.0 ± 7.50mg%); *S. anguivi* L. fruit revealed higher zinc content and lower manganese content than the zinc (2.2 ± 0.12mg%) and manganese (6.2 ± 0.15mg%) content of *Mordiiwhytii* fruit.While comparing the result reported by Valvi and Rathod (2011) on mineral composition of wild edible fruits, *S. anguivi* L. fruit was rich in copper and zinc than *Flacourtia indica* fruit (7.6±0.06mg%) and *Cordiadichotoma* fruit (3.85±0.03 mg%) respectively.

## CONCLUSION

The phytochemistry of *S. anguivi* L. fruit suggested the presence of all screened phytochemicals which predicted the good medicinal characteristics of the fruit. *S. anguivi* L. fruit was also considered as a highly nutritious fruit in terms of protein, ash, crude fiber, copper, calcium, potassium, sodium, iron, zinc and phosphorus. Further study is needed to identify and study different class of compounds of *S. anguivi* L. fruit and its biological activity.

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**Table 1: Qualitative phytochemical screening of *S. anguivi* L. fruit**

| S. No. | Parameters | Fruit Extract | | |
|---|---|---|---|---|
| | | ***Water*** | ***Methanol*** | ***Chloroform*** |
| 1. | Alkaloids | ++ | ++ | ++ |
| 2. | Flavonoids | ++ | ++ | ++ |
| 3. | Tannin and Phenols | ++ | ++ | ++ |
| 4. | Saponin | ++ | ++ | ++ |
| 5. | Steroid | ++ | ++ | ++ |
| 6. | Glycosides | ++ | ++ | ++ |
| 7. | Anthocyanin | - | + | + |
| 8. | Coumarin | - | + | + |

"+" indicates the presence of phytochemical and "–"indicates the absence of phytochemical

**Table 2: Proximate Composition of *S. anguivi* L. fruit**

| S. No. | Constituents | Percentage composition |
|---|---|---|
| 1. | Moisture (%) | 83.03±0.1 |
| 2. | Ash (%) | 16.23±0.2 |
| 3. | Fat (%) | 2.2±0.1 |
| 4. | Protein (%) | 11.26±0.1 |
| 5. | Crude fibre (%) | 16.36±0.05 |
| 6. | Carbohydrate (%) | 29.10±0.4 |
| 7. | Copper (mg) | 18.45±0.02 |
| 8. | Calcium (mg) | 321±1.7 |
| 9. | Potassium(mg) | 423.33±2.3 |
| 10. | Sodium(mg) | 350.1±2.007 |
| 11. | Iron(mg) | 18.46±0.02 |
| 12. | Zinc(mg) | 5.56±0.2 |
| 13. | Phosphorus (mg) | 223.1±0.2 |
| 14. | Manganese (mg) | 0.58±0.2 |