



## Evaluation of Selenium and Iron Levels in Shatt Al-Arab Sediment and the Iraqi Marine Environment

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### ABSTRACT

Sixteen Samples of sediment were collected from Shatt Al-Arab river and the Iraqi marine environment in southern of Iraq. The samples were distributed one station on Euphrates river before its confluence with Tigris river, seven stations along Shatt Al-Arab river and eight stations selected from the Iraqi Marine environment. All samples were collected from surface sediment at different waters Column in low tide time. Selenium was measured by Spectrophotometric method through using 4-Methyl-o- phenylene diamine as complex agent in acidic medium (pH= 1.5). The Iron was measured by Spectrophotometric method also by using complex formation with Potassium thiocynat. The results of the total selenium measurement and total iron showed the values at extent (1.928-13.818  $\mu\text{g/g}$ ) , (2298.418 -4238.702  $\mu\text{g/g}$ ) respectively in Shatt Al-Arab sediment , while total Selenium and total iron in the marine sediment was recorded at range (1.044-11.449  $\mu\text{g/g}$ ) ,(1822.789-3996.228  $\mu\text{g/g}$ ) respectively . Standard deviation for all the stations (n=3) of Selenium and Iron was calculated and showed at extent (0.00160- 0.03032) , ( 0.25225- 4.69526) respectively .

**Keywords** : Spectrophotometric method , Total Selenium , Total Iron.

### INTRODUCTION

Selenium exists in trace amounts in most crustal materials of earth , the concentration of total selenium in most soil lies within the range of 0.01 - 2  $\mu\text{g Se /g}$  [Mayland et al.,1989 , EPA, 1976] , but it's concentration rarely exceeds





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500µg/g[UNEP, 1988]. The selenium is found in nature in several states of oxidation and some of its chemical forms are volatile and has the four states from oxidation which are elemental selenium (0), selenide (II), selenite (IV) and selenate (VI) [Bao et al., 2012, Abdulnabi et al., 2015]. The chemical forms of Selenium present in soils and sediments are closely related to the oxidation-reduction potential and depend on pH in sediment and soil and the role of microorganisms[Gonzalez et al.,2006, Oldfield, 1972]. Selenide (II) can be aerobically oxidised to selenium (0) and selenite (IV) at the pH of sea water can be oxidised to selenate (VI), but in biological systems selenite can also be reduced by thiol groups to selenide to form a selenotrisulphide complex. Selenium-protein binding can occur, as in the synthesis of seleno-amino acids from inorganic selenium and their subsequent incorporation into peptide chains. This process is biologically mediated, involving carbon-selenium bonding[EPA, 1976]. Selenium in soil and sediment binds with Fe<sub>2</sub>O<sub>3</sub> in clay minerals and organic material and the binding strength increases as the pH decreases [Oldfield, 1972]. The most of sources selenium in soil was divided to natural source such as volcanic eruptions, movement of wind, rain water and weathering of rocks and industrial sources such as stations of generation of electric power and combustion of coal and oil [Schneider et al.,2015, Staicu et al.,2015, Bauer, 1997], these parameters released volatile selenium which is subsequently deposited over the surface of the earth[UNEP, 1988, Eisler, 1985] and thus it's transformed to an aquatic system via several operations and reaches to sediment by deposition operation through biochemical cycles [EPA, 1976, Porcella et al., 1991]. Sedimentary rocks usually contain concentrations of selenium much higher than the earth's. The selenium content sometimes reaches 0.5 mg/g in limonite rocks, 2.6 mg/g in vanadium-uranium rocks[Deepa and Lingappa, 2014, Bem, 1981] while the concentration of selenium in sandstones are shows at range 0.05-1.12 µg/g, 0.0-30 µg/g for carbonate rocks[UNEP, 1988] and contains at extent 1-100 µg/g in phosphorites rocks [Mayland et al.,1989] and the ratio between the selenium to sulfur in igneous rocks are 1:6000 [UNEP, 1988]. Iron metal is the fourth most abundant between the elements and second most abundant metal in the Earth's crust after aluminium. It has the two states from oxidation in natural, ferrous ion (Fe<sup>+2</sup>) and ferric ion (Fe<sup>+3</sup>). It is one of the seven metals known in antiquity along with gold, silver, copper, mercury, tin and lead. It has both properties lithophile and chalcophile. Iron exist in several common minerals such as pyrite FeS<sub>2</sub>, magnetite Fe<sub>3</sub>O<sub>4</sub>, haematite Fe<sub>2</sub>O<sub>3</sub> and siderite FeCO<sub>3</sub>. It is also found in many rock-forming minerals such as mica, garnet, amphibole, pyroxene and olivine. The abundance of iron in sedimentary rocks is determined by various factors such as provenance and pH conditions[EPA, 1994, James, 1966]. Iron rich sedimentary rocks contain 15% or more from iron. However, most sedimentary rocks contain iron in varying degrees such as sedimentary ironstone, where the dominant iron minerals are siderite, ankerite or oxides of the goethite-limonite type, may contain >30% Fe<sub>2</sub>O<sub>3</sub>. Typical levels of iron in sedimentary rocks are given as described: limestone 0.33%; sandstone 0.98%, shale 4.7%, and banded iron formation 28% [James, 1966, EPA, 2003]. This study focuses on two important elements in sediment which are selenium and iron because they are essential nutrients that enter in the food chain of animals and humans[Abdulnabi et al., 2015]. In addition to that selenium is related with iron in differential sediments it depends on pH value. Therefore by knowing the concentrations and distribution of metals in sediments the sources contaminants in aquatic system can be known. Furthermore, many studies worldwide used the sediment of rivers, estuaries and seas as indicators for pollution by trace metals[Al-Khuzai, 2015, Benzer et al.,2013].

## Experimental Part

### Site Selection

Sixteen stations of sediment samples were Selected from sediment rivers and marine sediment in southern of Iraq. The stations were distributed as one station(S<sub>1</sub>) on Euphrates river before its confluence with Tigris river and Shatt Al-Arab river formation. Seven stations (S<sub>2</sub> - S<sub>8</sub>) were selected along Shatt Al-Arab river from north of Basra city to south of it. These areas are important as they involve many activities such as population, agriculture, industry and commerce [Abdulnabi et al., 2015]. Eight stations (S<sub>9</sub> - S<sub>16</sub>) were selected from the Iraqi marine environment, they are distributed in three sites(S<sub>9</sub> - S<sub>11</sub>) in the iraqi marine region from southern Fao city towards Khor Abdullah and five stations (S<sub>12</sub> - S<sub>16</sub>) from nearby region of Um Qasr port towards Khor alZubair port in the nearby region from



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confluence with Basra canal . This area is important in marine navigation for large number and different loads of Ships pass through it to Arabian gulf. All samples were collected in winter season, December in 2014 and spring season, March in 2015 , in different regions from surface sediment in low tide and kept in plastic bags [Al-Saad et al. ,2007] undercooling and then transferred to laboratory for carry out different operations . Fig.(1) are shown below describing the selected sites .

**MATERIAL AND CHEMICALS**

Nitric acid (65%), Sulfuric acid(97%) and Hydrochloric acid (37%) were obtained from Scharlau .Selenium dioxide (Purity99.8%) and KSCN were supplied by Sigma-Aldrich . Perchloric acid(70%) and Hydrofluoric acid (40%) were supplied by Himedia . Haxan was purchased from J.T.Baker , Ferric Sulfate was obtained from BDH , 4-MOPDA was supplied by Merck and EDTANa<sub>2</sub> was obtained from G.C.C . Deionized water was used for the preparation of all solutions.

**Instrumentation**

Different complex agents were used for the determination of selenium and Iron through complex formation in optimal conditions ,selenium was measured in acidic medium at 332nm through using MOPDA,while the Iron was measured through using the potassium thiocyanate at 462 nm , all spectral measurements through using UV-Vis spectrophotometer double- beam from type (Shimadzu 1800 PC , Japan) with 1.0 cm quartz cell . Magnetic stirrer was used to mix all samples . Thermometer was used for adjust solution temperature. Grab sampler were used for collecting all samples from surface sediment.

**Procedure****Digestion of sample**

All the samples of sediment were collected from various regions of the river sites and marine sites in Basra city . These samples were preserved in cooling , after that , all samples left to dry at room temperature and then grinded and sieved through a 63 µm screen to obtain homogeneous particles [Al-Saad et al. ,2007] . Digestion operation carried out for all samples with various acids through weight less then 1g from sieved samples in beaker teflon and then 9ml of concentrated nitric acid was added with 1ml from hydrochloric acid (1N) and allow for the solution stand overnight at room temperature , after that all samples were digested as in the method described in methods of soil analysis [Sparks et al. ,1996] .

**Determined of Selenium in the samples**

Total selenium was measured through complex formation between 4-Methyl-o-phenylene diamine as complex agent with selenite (Se<sup>+4</sup>) through taking 25 ml from digestion solution and then adding an amount of disodium ethylene diamine tetra acetic acid and Potassium thiocyanate , they are used as for eliminating interferences for different ions such as (Fe<sup>+3</sup> , Cd<sup>+2</sup> , Cu<sup>+2</sup>) with measurement of Selenium . After that, the same procedure previously described was carried out for the analysis Selenium (IV) [Abdulnabi et al., 2015] . All the results for determining total selenium are shown in table 1 and figure 2 .

**Determined of Iron in the samples**

Spectrophotometric method for the determination of Iron as ferric ion in solution in acidic medium was used through complex formation , reddish brown color was obtained with potassium thiocyanate [Jeffery et al. ,1989 , Janardhan et al., 2014] . This procedure is rapid and easy and include preparation of standard solution of Iron (1000 ppm) , from this solution serial dilutions were made to obtain different concentration levels of (1 ,5 ,10 ,20 ,30 ,40 ,50 ,60 , 70 ,80 ,90



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,100 ppm) from ferric Ion , after that , 5ml from standard solution and adding 0.1ml potassium thiocyanate (0.05M) for this solution then the Iron was measured directly at 462nm and made standard curve according for Beer law . This step was repeated on samples through 5ml from digestion solution for sample and 0.1ml of potassium thiocyanate was added and measured at 462nm [Janardhan et al., 2014] . Furthermore , the blank solution was used in order to obtain high accuracy of results . All the results for the determination of total Iron are shown in table 1 and figure 1.

**RESULTS AND DISCUSSION**

Iron for all samples were measured by spectrophotometric method at 462nm[Janardhan et al., 2014] . The results are shown in figure (1) and table (1) for Iron measurement. Station (S<sub>3</sub>) showed the highest value when compared with all the sites measurements while the highest value of marine sediment was recorded at station (S<sub>14</sub>). The data of Iron measurement were compared in all the stations of Shatt Al-Arab river , the data recorded the highest value in station (S<sub>3</sub>) and the lowest value in station (S<sub>6</sub>) because the Hartha region (S<sub>3</sub>) has not undergone dredging operations and also it contains the generating station for electrical energy , while the regions of stations (S<sub>6</sub>) and (S<sub>7</sub>) have undergone continuous dredging works because they are regions of commercial port and furthermore the Seba site (S<sub>7</sub>) is an important region between Iraq and Iran as a navigation line. Also noted the high value was recorded in station (S<sub>5</sub>) because this region contains different immersed iron bodies and has undergone drainage water and waste water release without treatment , while the highest value in the Qurna Station (S<sub>1</sub>) was recorded compared with some other stations on Shatt Al-Arab river because the Iron concentration in sediment depends on the type and nature of sediment whether it's sand sediment or clay sediment or silt sediment [EPA , 2003] and also depends on different activities as the anthropogenic, agriculture and industry [Al-Khuzai ,2015, Al-Saad et al. ,2007] . In order to compared the results of Iron concentration between the marine sediment stations under study , the high value of total Iron concentration was recorded in station (S<sub>14</sub>) while the lowest value was recorded in station (S<sub>13</sub>) . Also a gradual increase in the value of total Iron concentration was noted from station (S<sub>9</sub>) towards (S<sub>11</sub>) and then it decreased in stations (S<sub>12</sub>) and (S<sub>13</sub>) . Furthermore the concentration value of Iron was noted increasing from station (S<sub>15</sub>) towards (S<sub>16</sub>) because station (S<sub>15</sub>) has undergone dredging operations because of its location as commercial port and an important oil port . Station (S<sub>16</sub>) is the confluence region between the basra canal and Khor al-Zubair port , the Shatt al-Basra canal is a drainage channel, where to the wastewater and industrial waste are released into canal without treatment and this explains the increase of iron concentration at this station in comparison with (S<sub>15</sub>) site . The results of Iron analyses in this study has recorded the lowest values when compared with the recorded data in previous studies[Mahmood ,2008 , Hassan ,2007] for some of the stations studied here , while it is consistent with the data recorded in other studies [Alshmary ,2013].The levels of Iron is exist the most abundant in earth's crust and exists more in sediment , especially in clay sediment [EPA ,2003]. On the other hand , the iron levels in sediment depend on multi operations occurring in aquatic system such as adsorption and ion exchange on outer surfaces for some particles of clay and organic material , they also depend on deposition of some elements in sediment through operations of weathering rocks , most of the heavy metals existed within the crystal lattice of the sediment particles [Mahmood ,2008] . Furthermore the increase of the concentration levels of Iron in the sediment, might be attributed to anthropogenic sources such as domestic wastes , industrial wastes and petroleum refinery operations . All these activities were released to the aquatic system without treatments [Al-Khuzai , 2015 , Al-Saad et al. ,2007] .

Total selenium for all the samples was measured by spectrophotometric method at 332nm by using complex agent in acidic medium . The results are shown in table1 and figure2. This data records the highest value in station (S<sub>2</sub>) when compared with the results of all the rest sites. Results of selenium was compared for all sites along Shatt Al-Arab river from Qurna (S<sub>1</sub>) towards Fao station (S<sub>8</sub>) ,the measurement showed high value in station (S<sub>1</sub>) when compared with all the other stations except for (S<sub>2</sub>) site because the qurna station undergoes different anthropogenic activities such as agriculture , domestic wastes and river navigation . This activities increase the selenium concentration in the aquatic system [Abdulnabi et al., 2015, Staicu et al., 2015] . After that selenium was converted to Selenium metal through biochemical pathways and deposited in sediment, these stages are called biogeochemical cycle for Selenium





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[Porcella et al., 1991] . Similarly the concentration value of selenium increased from station (S<sub>1</sub>) to station (S<sub>2</sub>). After that the concentration of selenium decreased from station (S<sub>2</sub>) towards station (S<sub>6</sub>) because , in station (S<sub>2</sub>) this region has not undergone dredging works but was subjected to various pollutants such as wastewater release and industrial waste to river without treatment [Abdulnabi et al., 2015] . Additionally , the region was affected by agricultural activities and burning natural gas processes which usually accompany extraction operations of oil and gas . All these processes cause the ecosystem pollution of selenium [Schneider et al., 2015, Staicu et al., 2015] . Moreover the concentration of selenium was increased in station (S<sub>7</sub>) and (S<sub>8</sub>) when compared with sites (S<sub>4</sub>-S<sub>6</sub>) . The results of the selenium concentration were compared for all the marine stations(S<sub>9</sub>-S<sub>16</sub>) . The data has shown gradual increase of selenium concentration from station (S<sub>8</sub>) towards (S<sub>11</sub>) site and recording the highest value at station (S<sub>11</sub>) in marine stations that might be attributed to marine navigation [Abdulnabi et al., 2015] or its depend on the type and nature components of the sediment . After then the concentration of selenium was decreased from station (S<sub>11</sub>) towards (S<sub>14</sub>) and the station (S<sub>13</sub>) recorded lowest value in the concentration of selenium in marine stations . The frequent Increase in the concentration of selenium was noted in station (S<sub>15</sub>) because this area undergoes the loading and unloading operations of commercial loads and oil loads inside the Khor Al-Zubair port , while station (S<sub>16</sub>) recorded low concentration of selenium when compared with station (S<sub>15</sub>) and high value compared from station (S<sub>12</sub>) to station (S<sub>14</sub>) . The most important sources of selenium pollution of aquatic system are weathering of rocks, combustion of coal and oil , movement of wind, irrigation and drainage operations, wastewater and industrial waste. All these processes cause the ecosystem pollution of selenium [Abdulnabi et al., 2015, Schneider et al., 2015, Staicu et al., 2015] .

## REFERENCES

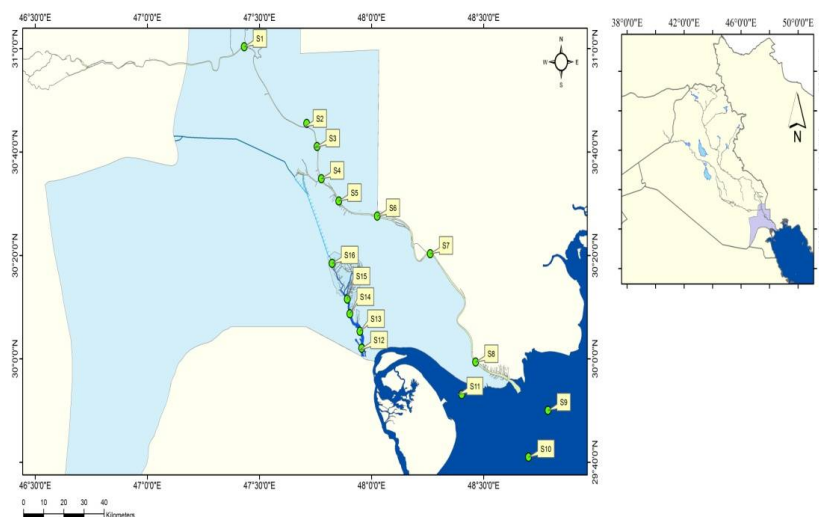
1. Mayland,H.F.,James,L.F.Panter,K.E. and Sonderegger,J.L. , Selenium in seleniferous Environments . Soil Science Society of America and American Society of Agronomy , SSSA Special Publication no. 23. , pp.15-50 , 1989 .
2. EPA (Environmental Protection Agency) . Selenium .Office of research and development . Health effects research laboratory . EPA-600/1-76-014 , 1976 .
3. UNEP (United Nations Environment Programme) . Arsenic, mercury and selenium in the marine environment . UNEP Regional Seas Reports and Studies No. 92 , 1988 .
4. Bao,P. , Huang,H. , Hu,Z.-Y. , Haggblom,M.M. and Zhu,Y.-G., Impact of temperature, CO<sub>2</sub> fixation and nitrate reduction on selenium reduction, by a paddy soil clostridium strain. Journal of Applied Microbiology . Vol.114 , pp.703-712, 2012 .
5. Abdulnabi,Z.A. , Hassan,W.F. , Al-Khuzai,D.K.K., Saleh,S.M. and Hashim,M.G. , Evaluation of selenium levels for the water surfaces in southern of Iraq . Journal of Chemical and Pharmaceutical Research. Vol. 7, No.10, pp.495-501, 2015.
6. Gonzalez, R.C.-, Simunek, J. , Sauve,S. and Adriano, D. , Mechanisms and pathways of trace element mobility in soils . Advances in Agronomy.Elsevier .Vol. 91, pp.111-177 , 2006.
7. Oldfield,J. E. , Selenium Deficiency in Soils and Its Effect on Animal Health . Geological Society of America Bulletin . Vol. 83 , pp. 173-180 , 1972 .
8. Schneider, L. ,Maher, W.A. ,Potts, J.Taylor, A.M.,Batley, G.E. , Krikowa, F. ,Chariton, A.A. and Gruber, B., Modeling food web structure and Selenium biomagnification in lake Macquarie ,new south wales ,Australia , usingstable carbon andnitrogen isotopes Environmental Toxicology and Chemistry. Vol.34 , No.3 ,pp.608-617,2015.
9. Staicu,L.C. , Hullebusch,E.D.V. , Oturan,M.A. , Ackerson,C.J. and Lens,P.N.L. , Removal of colloidal biogenic Selenium from wastewater .Chemosphere.Vol. 125 , pp. 130-138 , 2015.
10. Bauer, F,Selenium and Soils in the Western United States.Electronic Green Journal .Vol.1, No. 7 , Article 3 , 1997
11. Eisler, R. , Selenium hazards to fish, wildlife and invertebrates: a synoptic review . U.S. Fish and Wildlife Service Biological Report 85 (1.5). 1985.
12. Porcella,D. B. , Bowie,G.L. , Sanders,J.G. and Cutter,G.A., Assesing Se Cycling and toxicity in aquatic ecosystems .Water, Air and Soil Pollution.Vol.57-58, No.1, pp. 3-11, 1991.





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13. Deepa,K. and Lingappa,Y. , Spectrophotometric determination of selenium in industrial and environmental samples using vanillin-2-aminocotinic acid (VANA) . Der Pharma Chemica . Vol.6 , No.1, pp.52-60 , 2014 .
14. Bem,E. M. , Determination of Selenium in the Environment and in Biological Material . Environmental Health Perspectives .Vol. 37, pp. 183-200, 1981.
15. EPA (Environmental Protection Agency) . Technical resource document , extraction and beneficiation of ores and minerals , Volume 3, Iron . Office of Solid Waste Special Waste Branch . EPA 530-R-94-030 , 1994 .
16. James,H.L. , Data of Geochemistry . Chemistry of the Iron-rich Sedimentary Rocks , Chapter W. , 6th ed , United States Government Printing Office, Washington , 1966 .
17. EPA (Environmental Protection Agency) . Ecological Soil Screening Level for Iron . Office of Solid Waste and Emergency Response , November 2003 .
18. Al-Khuzai,D.K.K. , Assessment of sediment quality collected from shatt al-arab river, Basra, southern Iraq . Journal of international academic research for multidisciplinary . Vol.3, No. 6 , 2015 .
19. Benzer,S. , Arslan,H. , Uzel,N. , Gül,A. and Yılmaz,M. , Concentrations of metals in water, sediment and tissues of Cyprinus carpio L., 1758 from Mogan Lake (Turkey) . Iranian Journal of Fisheries Sciences . Vol.12, No.1, pp. 45-55 , 2013 .
20. Al-Saad,H.T., Al-Imarah,F.J. M., Saeed,M.A. R. , Distribution and levels of trace metals in the red sea coasts and comparison with their levels in the Arabian Gulf . MARINA MESOPOTAMICA . Vol.22 , No.1, pp.1-16 , 2007 .
21. Sparks,D.L. , Page,A. I. , Helmke,D.A. , Loeppert,R.H. , Soltanpour,P.N., Tabatabai,M.A. , Johnston,C.T. and Sumner, M. E. , Method of soil analysis Part. 3. Chemical methods. Madison Wisconsin, U.S.A. 1996 .
22. Jeffery,G.H. , Bassett,J. , Mendham,J. and Denney,R.C. , Vogels , Text Book of Quantitative Chemical analysis . 5th ed , John wiley & Sons , Inc., New York , 1989 .
23. Janardhan,P. , Dhanalakshmi,R. and Ruby,J.J. , Study of invitro anti-oxidant activity of ipomea pes-caprae . Intercontinental Journal of Pharmaceutical Investigation and Research , Vol.1 , No.1 , pp. 25-31 , 2014 .
24. Mahmood,A. A. , Concentrations of pollutants in water , sediments and aquatic plants in some wetlands in south of Iraq. Ph.D.Thesis . University of Basrah . Iraq . 2008.
25. Hassan,W. F. , Geochemical and hydrochemical Study at Shatt Al-Arab Canal Sediments and Overlying Water . Ph.D.Thesis . University of Basrah . Iraq . 2007.
26. Alshmary,A.Y. H. , Estimation of some heavy metals in clams, sediments and water from Shatt Al-Arab and treatment rocks porcellanite .M.Sc.Thesis .University of Basrah. Iraq.2013.



**Fig 1 : Locations the sample selected in Southern of Iraq**





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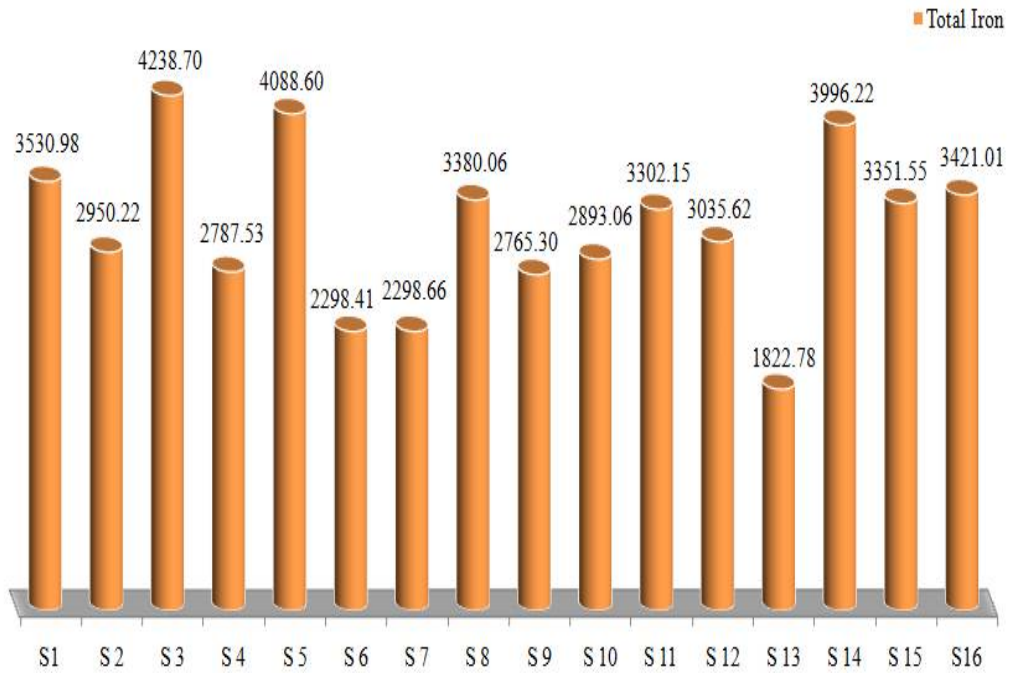


Fig. 2: Concentration of total Iron µg/g

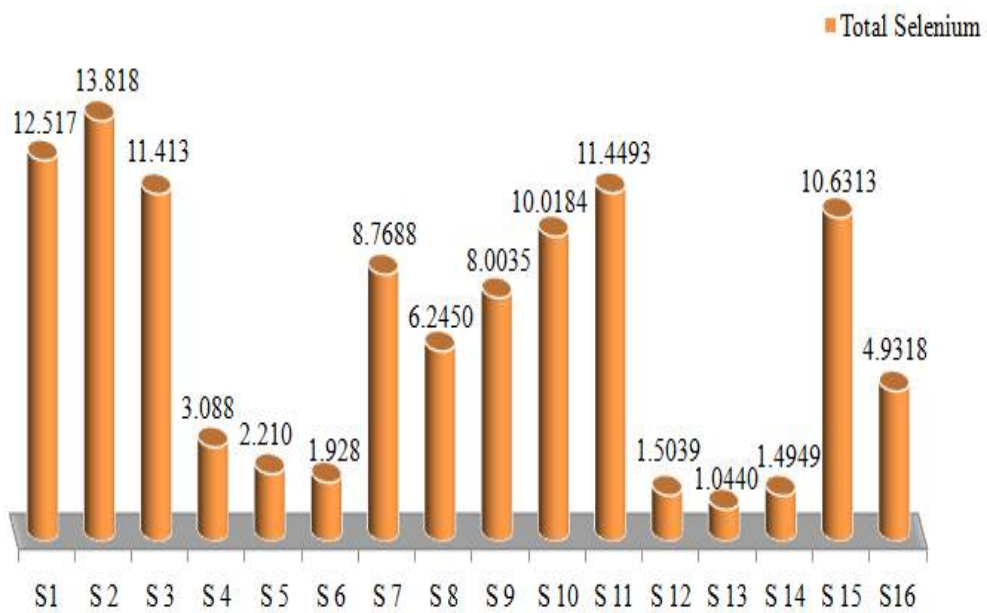


Fig. 3 : Concentration of total Selenium µg/g





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**Table 1: Concentration of total Selenium and total Iron of stations selected from surface sediment samples**

No. of samples	Mean(n=3) of Conc. of Selenium $\mu\text{g/g}$	Standard Deviation (SD)	Mean(n=3) of Conc. of Iron $\mu\text{g/g}$	Standard Deviation (SD)
S <sub>1</sub>	12.5171	0.01769	3530.9826	1.80851
S <sub>2</sub>	13.8181	0.01508	2950.2284	0.25225
S <sub>3</sub>	11.4135	0.03032	4238.7022	0.92949
S <sub>4</sub>	3.08873	0.01763	2787.5308	4.36139
S <sub>5</sub>	2.21071	0.01565	4088.6053	2.75607
S <sub>6</sub>	1.92871	0.00368	2298.4189	0.68903
S <sub>7</sub>	8.76880	0.01031	2298.6669	1.27416
S <sub>8</sub>	6.24506	0.00771	3380.0613	2.34808
S <sub>9</sub>	8.00352	0.01580	2765.3078	1.98205
S <sub>10</sub>	10.0184	0.01669	2893.0633	2.07168
S <sub>11</sub>	11.4493	0.02742	3302.1541	2.51323
S <sub>12</sub>	1.50397	0.00675	3035.6283	4.69526
S <sub>13</sub>	1.04407	0.00645	1822.7891	3.23124
S <sub>14</sub>	1.49494	0.01923	3996.2288	2.73351
S <sub>15</sub>	10.6313	0.00160	3351.5558	4.22377
S <sub>16</sub>	4.93188	0.00697	3421.0106	4.18162







## RESEARCH ARTICLE

## Phytochemical Investigation of Vacuum Dried and Freeze Dried Jamun (*Syzygium cumini*) Pulp Powder

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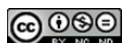
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### ABSTRACT

Phytochemical analysis was done for both vacuum and freeze dried jamun pulp samples. The samples were extracted with crude methanol and ethanol. This study was mainly contributed to know the presence of flavonoids, alkaloids, amino acids, glycosides, steroids, triterpenoid, reducing sugar and tannins and the absence of saponin, anthroquinones as the chemical class present in the extracts. As a result of this study we found that all the biologically active phytochemicals were present in both vacuum and freeze dried jamun pulp powder when subjected to both methanol and ethanol extract. Further this study will be helpful for the production of jamun pulp products and quantifying the active components in the products.

Keywords: Vacuum dryer, Freeze dryer, Phytochemicals

### INTRODUCTION

The underutilized fruit *Syzygium cumini* (Family Myrtaceae) is also known as *Syzygium jambolanum* and *Eugenia cumini*. The common names Jambul, Black Plum, Java Plum, Indian Blackberry, Jamblang, Jamun etc. mostly these three are grown in subcontinents of (1). In India these large trees were cultivated as an edible fruit. *Syzygium cumini* (L.) is belonging to the family Myrtaceae. It was also reported in many studies that jamun pulp contains vitamin C, gallic acid, tannins, anthocyanins, includes cyanidin, petunidin, malvidinglucoside and other components. The ripe fruit juice was used for the preparation of vinegar that is considered to be a stomachic, carminative and diuretic.



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Jamun food products like jam jellies and squashes were prepared. The fruits are astringent. A wine is prepared from the ripe fruits (2). Preserving these nutritional enriched jamun pulp with drying was studied in this study. The very old method for preservation was drying and it is a difficult food processing operation mainly because undesirable changes in quality. The advent of modern medicine, herbal medicine suffered a setback, but during last two or three decades advances in phytochemistry and in identification of plant compounds effective against certain diseases have renewed the interest in herbal medicines (3). Different drying methods like vacuum and freeze drying are used for drying of fruits, berries and vegetables. In vacuum temperate is maintained under vacuum pressure and in freeze drying the drying was carried out under very low temperature. However, these method leads to retain taste colour and nutritional value of the product, decrease in the density and water absorbance capacity and migration of the solutes from the internal part of the drying material to the surface, due to the long drying period and high temperature.

**MATERIAL AND METHODS****Plant Materials**

For this study the jamun fruits directly obtained from producers in the region of Pollachi. The matured fruits were selected and washed in normal tap water. Using hair dryer the excess moisture present in the outside of the jamun was removed. Pre weighed 100g of the Jamun fruit was packed in each PP zip lock bag and kept in deep freezer at -30°C for further use.

**Drying condition**

The stored Jamun fruits were taken from the deep freezer and kept in room temperature to reach its normal state. Jamun pulp was extracted manually by separating the pulp from the seed. Approximately 500g of pulp was taken for drying experiment. Vacuum shelf tray with 2'x16'x1.25', heat load- 27Kw) with temperature varied from 40 °C, 50 °C and 60 °C and freeze drier -20°C, -30°C and -40 °C is used for drying of Jamun pulp. The drying process was performed in duplicate for each drying temperature.

**Preparation of sample extracts****Solvent extraction**

Soxhlet extraction method was used in this study. Powdered samples of approximately 20 g was packed in thimble and extracted with 300 ml of different solvents separately. Methanol and ethanol Solvents were used. The process of extraction continues for 24 hours. After the extraction process the extract was taken in a beaker and kept in hot water bath and heated to 30-40°C till all the solvent get evaporated. Dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis (4).

**Preliminary Phytochemical screening:**

One gram of the methanol and ethanol extract of *Syzygium cumini* pulp was dissolved in 100ml of its solvents to obtain a stock of concentration 1% (v/v). The extract thus obtained were subjected to phytochemical screening following the methodology of (5,6).





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**Screening procedure**

**Test for Alkaloids**

Five ml of the extract was added to 2ml of HCL. To this acidic medium, 1 ml of Dragendroff's reagent was added. An orange or red precipitate produced immediately indicated the presence of alkaloids.

**Test for amino acids**

One ml of the extract was treated with few drops of Ninhydrin reagents. Appearance of purple colour showed the presence of amino acid.

**Test for Reducing sugar Test**

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

**Test for Flavonoids**

Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turned colourless indicated the presence of flavonoids.

**Test of Saponins**

The extract was diluted with 20ml of distilled water and it is agitated in a graduated cylinder for 15 min. the formation of 1cm layer of foam showed the presence of saponins.

**Test for Steroids**

One ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tubes. The upper layer turned red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids

**Test for Tannins**

Crude extract was mixed with 2ml of 2% solution of FeCl<sub>3</sub>. A blue-green or black coloration indicated the presence of phenols and tannins.

**Test for Triterpenoids**

Ten mg of the extract was dissolved in 1ml chloroform; 1ml of acetic anhydride was added following the addition of 2 ml of con, sulphuric acid. Formation of reddish violet colour indicated the presence of triterpenoids.

**Test for Glycosides.**

The extract was hydrolyzed with HCl solution neutralized with NaOH solution. A few drops of Fehling's solution A and B were added. Red precipitate in indicated the presence of glycosides.



**Reginold Jebitta et al.****Test for Anthraquinones**

Five ml of extract solution was hydrolyzed with diluted conc sulphuric acid, extracted with benzene. 1ml of dilute ammonia was added to it, rose pink colour suggested the positive response for anthraquinones.

**RESULTS AND DISCUSSION**

In this study the photochemical screening were performed with ethanol and methanol extract of Jamun pulp powder dried under six different temperature. The results obtained in the present investigation are presented in Table No.1 and Table No.2. The jamun pulp powder dried in all the temperature gave good results. Syzygium cumini pulp was rich in flavonoids, alkaloids amino acids, glycosides, steroids, triterpenoids, reducing sugar and tannins and the absence of saponin and anthraquinones. From this study we conclude that most of the phytochemicals were present in both methanol and ethanol extract of pulp powder. Solubility of each constituent in an herb is very specific to different solvents used in the extraction process. The Ethanolic extract of both plant's leaves contain phytosterols, tannins and phenolic compounds, free amino acids and flavonoids. The preliminary phytochemical analysis of the extract revealed the presence of tannin, flavonoid and phytosterols. These compounds have been reported to inhibit bacterial growth. (7) Since there is no degradation in phytochemical as the temperature increases and decreases, it is found that cross flow dryer with temperature and it is suitable to dry Jamun pulp with all phytonutrients. These dried pulp powder can be used further for any food product which will give a positive impact on anti diabetic and analgesic activities.

**CONCLUSION**

From the present study we found that phytochemical which is present in raw Jamun pulp were also found in Jamun pulp powder dried under vacuum and freeze dried samples which was extracted with ethanol and methanol. Due to the presence of these above mention phytochemicals in jamun fruit these dried powder will also have diabetic and analgesic activities properties. This study can be move further for the production of Jamun pulp products and quantifying the active components in the products. It can also work in isolation and purifying of active compounds which is highly used in pharmaceutical studies.

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**REFERENCES**

1. P. Warriar, V. Nambiar and C. Ramankutty, (1996), *Indian Medical Plants*, Orient Longman Ltd: Hyderabad, India, 5:225- 228.
2. *Wealth of India Raw material*, New Delhi: Csir, Vol IX 1976, 100-104.
3. H. Syed Jahangir and R. Nazeerullah (2011), Screening of Antimicrobial Activity of Selected Medicinal Plants *Cassia surattensis* Burm.f and *Rhinacanthus nasutus* (L) Kure, *Indian Journal Of Natural Sciences International*, 2(8):588-589.
4. RNS Yadav and Munin Agarwala (2011), "Phytochemical analysis of some medicinal plants, *Journal of Phytology*" 3 (12):10- 14.
5. Kumar.A., Ilavarasan. R., Jayachandran .T (2009), "Phytochemicals Investigation on a Tropical plant, *Syzygium Cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India", *Pakistan Journal Of Nutrition*, 8: 83-85.





**Reginold Jebitta et al.**

6. S. Shyamala Gowri1 and K. Vasantha, (2010) "Phytochemical Screening and Antibacterial Activity of Syzygium cumini (L.) (Myrtaceae) Leaves Extracts", *International Journal of PharmTech Research*, (2), 156-157.
7. Clark, W. S. (1981). Antimicrobial Activities of phenolic constituents of *Mangolia gradiflora*, L. *J. Pharm. Sci.* 10: 951.

**Table1: Preliminary Phytochemical Investigation of Jamun Pulp Powder Dried under Vacuum**

SI.No.	Phytochemicals	Methanol			Ethanol		
		50°C	60 °C	70 °C	50°C	60 °C	70 °C
1	Alkaloids	+++	+	+	+++	+	+
2	Amino Acids	+++	++	+	+++	++	+
3	Reducing sugar	+++	++	+	+++	++	+
4	Flavonoids	+++	++	+	+++	++	+
5	Saponins	-	-	-	-	-	-
6	Steroids	++	+	+	+	+	+
7	Tannins	+++	++	+	+++	++	+
8	Triterpenoids	+++	++	+	+++	++	+
9	Glycosides.	+++	+	+	+++	+	+
10	Anthraquiones	+	+	+	+	+	+

**Table 2: Preliminary phytochemical investigation of Jamun pulp powder dried under Freeze Dried powder**

SI.No	Phytochemicals	Methanol			Ethanol		
		-20°C	-30°C	-40 °C	-20°C	-30 °C	-40 °C
1	Alkaloids	+++	+++	+++	+++	+++	+++
2	Amino Acids	+++	+++	+++	+++	+++	+++
3	Reducing sugar	+++	+++	+++	+++	+++	+++
4	Flavonoids	+++	+++	+++	+++	+++	+++
5	Saponins	-	-	-	-	-	-
6	Steroids	++	++	++	++	++	++
7	Tannins	+++	+++	+++	++	++	++
8	Triterpenoids	++	++	++	++	++	++
9	Glycosides.	+++	+++	+++	+++	+++	+++
10	Anthraquiones	++	++	++	++	++	++

+ - present less

++ -present moderate

+++ -present high -Not present





## Anti-Biofilm Properties of *Melissa officinalis* Essential Oil

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### ABSTRACT

In the paper, we report the activity of *Melissa officinalis* L. essential oil against bacterial biofilm. This study tested the effects of essential oil of *Melissa officinalis* L. on six bacterial clinical isolates biofilm-forming bacteria. The minimum inhibitory concentration (MIC) and biofilm inhibitory concentration (BIC) assays were performed in microtitre plates using a twofold dilution series. The results showed that the essential oil of *Melissa officinalis* produced inhibitory effects against all isolates. The MIC values were in the range of 0.25-1 mg/mL while BIC values were between 0.5-8 mg/mL. In addition *Melissa officinalis* essential oil was able to inhibit initial adherence in the most tolerant isolate (*Pseudomonas aeruginosa*) at sub-inhibitory concentrations. Conclusions: *Melissa officinalis* essential oil represents a potent source of antimicrobial agents to prevent and treat biofilms.

**Keywords:** MIC, BIC, biofilm, *Melissa officinalis*, resistance, essential oil.

### INTRODUCTION

Biofilm is a group of microorganisms sticking together on a surface and embedded in a complex protective matrix [1]. The matrix, secreted by the cells within the biofilm, is a mixture of extracellular nucleic acids, proteins, lipids and polysaccharides [2]. It is the most important structure of a biofilm that is essential for the existence of the biofilm. Bacteria in a biofilm show extreme tolerance of antimicrobial agents and phagocytes. It is thought that the EPS in



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biofilms protect cells by preventing or reducing access of the antimicrobial agents [3]. There are six forces that drive the formation of biofilm: default mode (mechanism), community, favourable habitat, defence, availability of nutrition and environmental conditions [4]. The gene expression in biofilm cells differs from planktonic cells. In *Pseudomonas aeruginosa* biofilm more than three hundred new proteins were found that were not detectable in planktonic cells [5]. Biofilms are involved in chronic persistent infections that affect millions of people each year that may cause death. Chronic persistent infections including pneumonia in cystic fibrosis patients, chronic wounds, chronic otitis media and implant associated infections [4]. The hallmark of biofilms is their resistance to antimicrobial agents. The microbial resistance to antimicrobial agents specifically in biofilm increases the need for new drugs. Essential oils could be an alternative to antibiotics. Essential oils are volatile compounds synthesized by aromatic plants. They are used in food preservation, antimicrobial agents, analgesic, sedative, and anti-inflammatory [6]. Clinical and in vitro studies showed that some essential oils were more effective than antibiotics against antibiotic-resistant strains such as MRSA [7]. *Melissa officinalis* L. (Lamiaceae) also known as Lemon balm has been used as a medicinal plant in the Mediterranean region and in Europe [8]. It is used for the treatment of gastrointestinal disorders, nervousness, rheumatism and headaches [9]. The essential oil of *Melissa officinalis* has antibacterial and antifungal and antioxidant properties [8, 9]. The aim of the present study was to determine the effects of essential oil of wild *Melissa officinalis* grown in Jordan on the growth of biofilm-forming bacterial clinical isolates.

**MATERIALS AND METHODS****Essential oil of *Melissa officinalis***

Fresh amount of the *Melissa officinalis* was collected from mountains of Qumeim, Irbid, north Jordan, before the flowering period. The plant materials were taxonomically identified and authenticated by the Botanical Survey of Yarmouk University. The composition of the essential oil from *Melissa officinalis* was determined using gas chromatography-mass spectrometry (GC-MS) [10]. Forty five components accounting for 99.01% of the oil were identified. The major compounds (more than 1%) were Ethyl nerolate 24.56%, Geranyl formate 15.78%, Methyl geranate 8.18%, Limonene 7.92%,  $\delta$ -Cadinene 7.66%,  $\alpha$ -Cadinene 4.75%, 1,8-Cineole 4.69%, Selina-3,11-dien-6- $\alpha$ -ol 3.93%, 9-epi-E-Caryophyllene 3.10%, E-beta-Ocimene 2.48%, 1-para-Menthene 1.96%, Geranial 1.54%, Thymoquinone 1.38%, Sequilavandulol 1.37%, and Sabinene 1.25% [10].

**Cultures and media**

The effect of *Melissa officinalis* essential oil on bacterial biofilm formation was examined using six bacterial clinical isolates including: three strains of Gram positive bacteria: Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus epidermidis*, and *Bacillus subtilis*, and three strains of Gram negative bacteria: *Escherichia coli*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa*. These clinical isolates were obtained from human patients. Cultures were stored on tryptone soya agar (TSA) (Oxoid, Hampshire, UK) at 2-4°C and subcultured every 2 months or whenever required. Isolates were purified on specific nutrient agar plates and characterized by standard microbiological and biochemical methods like Gram stain, catalase test, coagulase test and an API system (bioMerieux, France).

**Biofilm formation and broth microdilution assays**

Biofilm formation was quantified in microtitre plates using the method described by Rachid et al [11]. Bacteria were grown overnight to mid-log phase by inoculating in 10 ml tryptone soya broth (TSB) and incubating at 37°C until the OD at 600 nm (OD<sub>600</sub>) reached approximately 0.6. Strains were then diluted in fresh TSB supplemented with 0.5% glucose to give cell density of approximately 10<sup>6</sup> cfu/ml. For each test strain, 200  $\mu$ l of inoculum was added to 72 wells of a 96-well plate. A quantity of 200  $\mu$ l TSB was added to the remaining 24 wells and the plate incubated for 24 h at 37°C. The optical density at 600 nm (OD<sub>600</sub>) was measured to serve as an indicator of bacterial growth, the plate contents emptied out and washed three times with phosphate-buffered saline (PBS) (Sigma Aldrich). The plates were



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air-dried and the cells that remained adhered to microwells stained with 0.4% crystal violet (Sigma Aldrich). Optical density at 490 nm (OD<sub>490</sub>) nm was determined using microplatereader (**Synergy™ HTX Multi-Mode Microplate Reader USA**) to quantify the amount of crystal violet-stained biofilm. Each strain was assayed in triplicate.

**MIC assay**

MIC was determined using 96 well microtitre plates as described by Rachid et al [11]. Serial two fold dilutions of *Melissa officinalis* essential oil in TSB were carried out in microtitre plates; 100 µl of bacterial cells with density of approximately 10<sup>6</sup> cfu/ml were added to the wells, mixed and then incubated at 37°C for 24 h aerobically. The MIC was read as the minimum antibiotic concentration needed to inhibit visible growth of the strain. The MIC for each antibiotic/strain was carried out in triplicate in three independent experiments. The positive control used for MRSA, *Staphylococcus epidermidis*, and *Bacillus subtilis* was vancomycin, for *E. coli* and *Enterobacter aerogenes* was chloramphenicol for *P. aeruginosa* was ceftazidime. The absorbance was measured at 600 nm to serve as an indicator of bacterial growth.

**Biofilm inhibitory concentration (BIC) assay**

BIC was determined using 96 well microtitre plates as described by Rachid et al [11]. Serial two fold dilutions of *Melissa officinalis* essential oil in TSB were carried out in microtitre plates; 100µl of the diluted bacterial cells were added to the wells, mixed and then incubated at 37°C for 24 h aerobically. The wells were washed three times with PBS. The plates were dried using air, and the remaining surface-adsorbed cells of the individual well were stained with 0.1% (w/v) crystal violet. The wells were then washed three times with PBS and allowed to air-dry for 60 min. The crystal violet-stained biofilm was solubilized using 95% (v/v) ethanol and the absorbance read at 490 nm. A well, with no cells and sterile TSB was used as blank (negative control), and a well with cells and TSB but without *Melissa officinalis* essential oil was used as a control. The positive control used for MRSA, MSSA, and *S. epidermidis* was vancomycin, for *E. coli* and *K. pneumonia* was chloramphenicol for *P. aeruginosa* was ceftazidime and finally for *P. mirabilis* was ampicillin. BIC was determined as the minimum concentration that caused 30% decrease in optical density. Assays were performed three times on different days for each individual strains and the same result was obtained on each occasion.

**Adherence of bacterial cells to polystyrene**

Initial adherence of bacterial cells to polystyrene was determined using a previously reported method [12]. Briefly, bacteria were grown overnight in 10 ml TSB at 37°C and then diluted 1 : 100 in fresh TSB containing *Melissa officinalis* essential oil at the required concentration. A quantity of 5 ml of the bacterial suspensions was then poured into Petri dishes and incubated for 30 min at 37°C. The plates were washed five times using 5 ml PBS, air dried and stained for 1 min with 0.4% crystal violet. The number of adhered cells was determined microscopically (CETI 60243T UK) by counting the number of bacteria in 20 fields of view. The essential oil concentrations were 1/10 of MIC, 1/2 MIC, and the MIC concentration. Adherence was calculated as the total number of cells adhered per square centimetre examined. Each *Melissa officinalis* essential oil concentration was assayed in triplicate and the adherence of *Melissa officinalis* essential oil treated cells compared with untreated controls. Assays were performed three times on different days and the same result was obtained on each occasion

**RESULTS****MIC and BIC**

*Melissa officinalis* essential oil produced inhibitory effects against all isolates but with considerable variation in susceptibility. Table 1 shows the MIC and BIC values of *Phlomis brachydon* essential oil (mg/mL). The MIC values





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were in the range of 0.25-1 mg/mL while the BIC values were in the range of 0.5-8 mg/mL. The most susceptible in planktonic growth was *E. aerogenes* with an MIC value of 0.25 mg/mL while the most susceptible in biofilm growth was MRSA with BIC of 0.25 mg/mL. The most resistant isolate both in planktonic and biofilm growth were *P. aeruginosa* with MIC value of 1 mg/mL and BIC of 8 mg/mL. As expected BIC values were higher than MIC values for all isolates. The BIC for *P. aeruginosa* was eight folds higher than MIC values while for *E. Coli* the BIC value was four folds higher than the MIC value. *Melissa officinalis* essential oil was more active on Gram positive bacteria than Gram negative bacteria. The most unexpected results were for the MRSA. The MIC and BIC value of 0.5 mg/mL were the lowest in BIC and very close to lowest in MIC.

**Inhibition of *P. aeruginosa* adherence to polystyrene by *Phlomis brachydon* at sub-MIC levels**

*P. aeruginosa* was the most resistant isolate in planktonic and biofilm growth to *Melissa officinalis* essential oil. This is why it was chosen to test the effect of sub-inhibitory concentrations (sub-MICplank) on its adherence to polystyrene. Figure 2 shows the effect of *Melissa officinalis* on initial adhesion of *P. Aeruginosa* using 1/10 of the MIC, 1/2 of the MIC, the MIC and without EO. The results show that adding sub-inhibitory concentrations (sub-MICplank) of *Melissa officinalis* to polystyrene Petri dishes containing a suspension culture of the *P. aeruginosa* strain reduced the number of individual cells adhering to the polystyrene surface after 30 minutes incubation period (Fig. 2).

**DISCUSSION**

Bacterial biofilms show high resistance to antibiotics. Therefore there is a need for antibiotics alternatives that are able to overcome biofilm resistance mechanisms. Essential oils could fulfil the need. *Melissa officinalis* is a medicinal plant used for its antiseptic, antimicrobial, antioxidative, carminative, digestive, and calmative activities [13]. Essential oils are strong antimicrobial agents with broad spectrum activity with antimicrobial, fungicidal and insecticidal activities [14]. The results showed that *Melissa officinalis* essential oil was more active on Gram positive bacteria than on Gram negative bacteria. The mechanisms of action include the degradation of the cell wall and damage of the cytoplasmic membrane. Generally, Gram-negative bacteria are more resistant to essential oil than Gram-positive bacteria due to difference in the structures of the cell walls [15]. The cell wall of Gram-positive bacteria is mostly made up of peptidoglycan. Essential oils are able to penetrate the cell wall of Gram-positive bacteria and destroy bacteria cell [16, 17]. In Gram-negative bacteria the peptidoglycan layer is covered by a hydrophilic outer membrane. Thus the outer membrane is impermeable to hydrophobic substances like essential oil [18, 19]. The presence of this outer membrane in the cell wall of Gram negative bacteria makes them more resistant to essential oil than Gram-positive bacteria.

The most resistant biofilm growth was *P. aeruginosa* with BIC value of 8 mg/mL. *P. aeruginosa* is the most frequent cause of chronic pulmonary infection in people with cystic fibrosis [20]. In cystic fibrosis the infection with *P. aeruginosa* starts with the organism in planktonic form which is then followed by chronic infection by the biofilm. *P. aeruginosa* planktonic infection can be eradicated successfully using antibiotics but once a biofilm has formed, eradication is very difficult. Antimicrobial resistance of *P. aeruginosa* biofilms is complex and not well understood [21, 22]. The resistance mechanisms include the matrix that limits the penetrations of antimicrobial agents, low metabolic activity of cells grown within biofilm, the presence of persisters within biofilm cells populations and *P. aeruginosa* biofilm cells are anaerobic since many agents are inactive or less active under anaerobic conditions [22, 23, 24]. The essential oil of *Melissa officinalis* was able to eradicate *P. aeruginosa* biofilm with higher efficiency than some reported antibiotics in use, making them interesting candidates for the treatment of biofilms. It was able to penetrate and kill biofilm. Essential oils are mixtures of many components so that many different mechanisms of action are responsible that enable them to overcome microbial resistance [7]. *Melissa officinalis* essential oil was able to inhibit *P. aeruginosa* adherence to polystyrene at subinhibitory level. Essential oils and their components have activity against a variety of targets, particularly the membrane and cytoplasm [18]. It is believed that the sub-inhibitory level of *Melissa officinalis*





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essential oil that did not cause killing of the microorganism might have caused damage and changes to *P. aeruginosa* cell membrane and prevented it from adhering to the polystyrene surface.

## REFERENCES

1. Abdel-Aziz SM, Aeron A. Bacterial Biofilm: Dispersal and Inhibition Strategies. SAJ Biotechnol 2014;1(1):105.
2. Flemming HC, Wingender J. The biofilm matrix. Nature Reviews Microbiology 2010;8:623-633.
3. Sutherland IW. Biofilm exopolysaccharides: a strong and sticky Framework. Microbiology 2001;147: 3–9.
4. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS Suppl 2013;136:1-51.
5. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. *Pseudomonas aeruginosa* displays multiple phenotypes during development as a biofilm. J Bacteriol 2002;184:1140-54.
6. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils - A review. Food and Chemical Toxicology 46;2008:446–475.
7. Yap PSX, Yiap BC, Ping HC, Lim SHE. Essential oils, a new horizon in combating bacterial antibiotic resistance. The Open Microbiology Journal 2014;8:6-14.
8. Mencherini T, Picerno P, Russo P, Meloni M, Aquino R. Composition of the fresh leaves and stems of *Melissa officinalis* and evaluation of skin irritation in a reconstituted human epidermis model. J Nat Prod 2009;72:1512-1515.
9. Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of *Melissa officinalis* L. (Lamiaceae) essential oil. J. Agric. Food Chem 2004;52:2485-2489.
10. Al-Sarayreh S, Rawashdeh A, Al-Tarawneh I, Al-Qudah M. Chemical Composition and In Vitro Antimicrobial Activity of Essential Oil of *Melissa officinalis* L. From Jordan. Indian Journal Of Natural Sciences 2015;6(31):8823-8829
11. Rachid S, Ohlsen K, Witte W, Hacker J, Ziebuhr W. Effect of subinhibitory antibiotic concentrations on polysaccharide intercellular adhesion expression in biofilm-forming *Staphylococcus epidermidis*. Antimicrob Agents Chemother 2000;44(12):3357-3363.
12. Heilmann C, Gerke C, Premington FP, Gotz F. Characterization of Tn917 insertion mutant of *Staphylococcus epidermidis* affected in biofilm formation. Infect Immun 1996;64:277-282.
13. Rehman S-U, Latief R, Bhat KA, Khuroo MA, Shawl AS, Chandra S. Comparative analysis of the aroma chemicals of *Melissa officinalis* using hydrodistillation and HS-SPME techniques. Arabian Journal of Chemistry 2013, <http://dx.doi.org/10.1016/j.arabjc.2013.09.015>
14. Hamdan DI, Mohamed ME, Abdulla RH, Mohamed SM, El-Shazly AM. Anti-inflammatory, insecticidal and antimicrobial activities and chemical composition of the essential oils of different plant organs from navel orange (*Citrus sinensis* (L.) Osbeck var. Malesy) grown in Egypt. Journal of Medicinal Plants Research 2013;7(18):1204-1215
15. Trombetta D, Castelli F, Sarpietro MG, Venuti V, Cristani M, Daniele C, Saija A, Mazzanti G, Bisignano G. Mechanisms of antibacterial action of three monoterpenes. Antimicrob Agents Chemother 2005;49:2474-2478.
16. Kavanaugh NL, Ribbeck K. Selected Antimicrobial Essential Oils Eradicate *Pseudomonas* spp. and *Staphylococcus aureus* Biofilms. J Appl Environ Microbiol 2012;78:4057-4061.
17. Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 2003;67:593-656.
18. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of essential oils on pathogenic bacteria. Pharmaceuticals 2013;6:1451-1474.
19. Gao Y, Van Belkum MJ, Stiles ME. The outer membrane of Gram negative bacteria inhibits antibacterial activity of brochocin-C. J Appl Environ Microbiol 1999;65:4329-4333.
20. Fitzsimmons S. The Cystic Fibrosis Foundation Patient Registry Report 1996. Pediatric Pulmonology 1996;21:267-75.
21. Drenkard E. 2003. Antimicrobial resistance of *Pseudomonas aeruginosa* biofilms. Microbes and Infection 2003;5(13):1213-1219



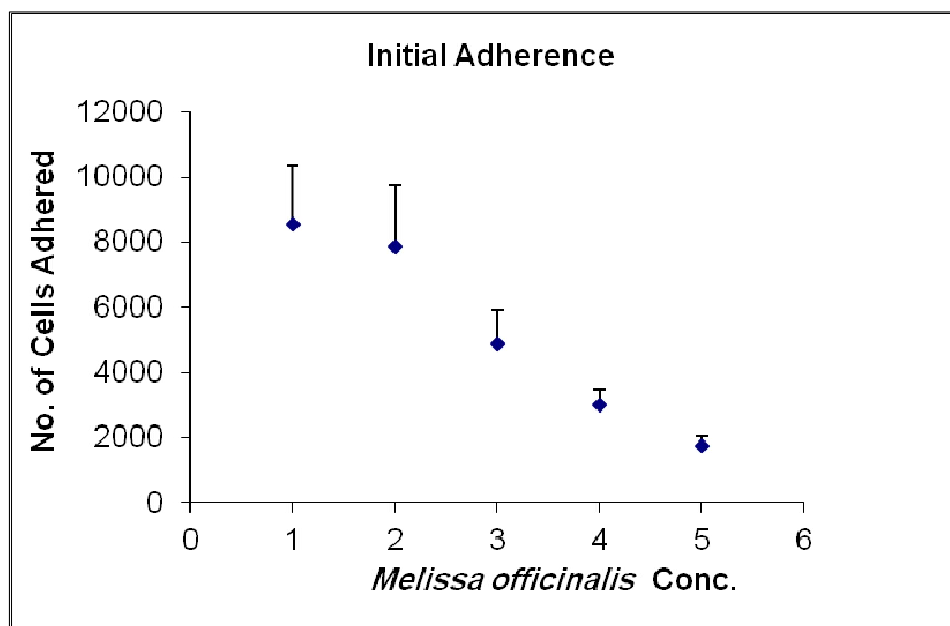


**Sameeh A Al- Sarayreh et al.**

22. Hoiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. Int. J. Antimicrob. Agents 2010;35:322-332.
23. Schobert M, Tielen P. Contribution of oxygen-limiting conditions to persistent infection in *Pseudomonas aeruginosa*. Future Microbiol 2010;5:603-621.
24. Lewis K. Multidrug tolerance of biofilms and persister cells. Curr. Top. Microbiol. Immunol 2008;322:107-131.

**Table 1. MIC and BIC of *Melissa officinalis* (mg/mL) for the bacterial isolates used for the study.**

Isolate Number	Isolate name	MIC <sub>plank</sub>	BIC <sub>biofilm</sub>
1	MRSA	0.5	0.5
2	<i>P. aeruginosa</i>	1	8
3	<i>E. aerogenes</i>	0.25	1
4	<i>E. coli</i>	0.5	2
5	<i>B. subtilise</i>	0.5	1
6	<i>S. epidermidis</i>	0.5	1



**Figure 1. Effect of *Melissa officinalis* on initial adhesion of *P. aeruginosa*; 1: without EO, 2: 1/10×MIC 3: 1/2×MIC, 4: MIC.**





## Standardization of Scale to Measure Participation of Extension Workers

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### ABSTRACT

An attempt was made to develop and standardize the measuring the participation of EWs in ATMA based on Likert's method of summated rating. Total 21 dimensions of participation in activities of BTT were identified. These participation dimensions were mailed to a panel of judges and 17 participation dimensions were selected which had relevancy weightage of more than 0.75. The items were given to a panel of 50 judges to indicate relevancy to each item for inclusion in the scale to measure the participation of extension worker in ATMA. A list of 57 items having more than 0.75 relevancy weightage, was administered to 32 extension workers from non- sampled area. Response for the items was obtained on five point continuum. The participation scores of each respondent was obtained by summing up the scores over all items. On the basis of the total score, the respondents were arranged in a descending order. Then 25 percent EWs with highest total scores and 25 percent Ews with the lowest scores were selected. The split-half and test-retest techniques for testing reliability were used. The criteria of content and construct validity were applied for testing the validity of participation of extension workers scale. The final format of participation scale with 57 items representing 17 participation dimensions can be administered to the EWs with a five point response continuum. The participation score was calculated by adding up the scores obtained by respondents on all the statements and was considered as individual's score.

**Key words:** Participation, Extension Workers, ATMA



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## INTRODUCTION

The state government of Maharashtra has taken number of initiatives in agricultural development as well as in extension to benefit the farmers through reorganization of state agriculture extension from time to time. The government of Maharashtra has introduced the innovation in technology transfer the agricultural technology management agency (ATMA) on experimental basis in four districts since 1998- 99. The farm information and advisory center (FIAC) at the block is under control the of ATMA organization. It works through block technology team consisting of extension workers of development departments and the farm advisory committee (FAC) having farmers representatives on it. It is responsible for programme planning and implementation. The efficiency and effectiveness of the ATMA programmes mainly depends upon the participation of block technology team. The participation of extension workers were middle level functionaries therefore must have been affected. No comprehensive scale was available to measure the participation of Extension Workers in ATMA. IN order to augment this attempt was made to develop and standardize a scale for measuring the participation of EWs in ATMA. According to Webster's Dictionary, participation means to take a part or to have shared in common or to have a share of profit or benefit in common with others. In the context of present study participation was operationally defined as to taking part in prioritization and diagnosis of farmers' needs and problems, setting extension priorities, planning and designing solutions, approving block action plans, actual implementation, evaluation of extension programmes within the block and providing feedback.

### Procedure for Standardization of scale

In order to develop and standardize the measuring instrument the method of summated rating (Likert 1932) was followed. The detail procedure adopted for this purpose is described here under.

### Collection of participation dimension

Studies on participation of agricultural extension functionaries were reviewed. Further studies on the participation of personnel working in the domains of industry and education were traced through internet and from journals, books, etc and reviewed. After reviewing the literature, job chart of extension workers and discussion with the experts in the field of extension education, psychology, management and social sciences, 21 dimensions of participation in activities of BTT were identified.

### Deciding relevancy of participation dimensions

It was quite possible that all the 21 participation dimensions identified may not be equally relevant in measuring participation of extension workers in the activities of BTT working in ATMA. Hence, these dimensions were subjected to scrutiny by an expert panel of judges to determine the relevancy and their subsequent screening. These participation dimensions were mailed to a panel of 100 judges in the field to extension education, psychology, management, administration and social sciences. These judges were requested to indicate appropriateness (relevancy) of the dimensions for inclusion in the scale to measure participation of extension workers in working of ATMA. The responses of judges were secured on three point continuum namely, most relevant, somewhat relevant and not relevant and scored as 3, 2 and 1, respectively. In 62 judges could respond in stipulated time. These judgements were used for working out the relevancy weightage (RW) of the each participation dimension by using the procedure given by Patil *et al.* (1996). Considering the relevancy weightages, the participation dimensions were screened for their relevancy. Accordingly, dimensions having relevancy weightage of more than 0.75 were considered. Using these criteria, 17 participation dimensions having more than 0.75 relevancy weightage were selected.



**Kinkhedkar et al.****Construction of items for the scale**

An important aspect in the development of a scale is the constitution of the item pool. The item pool was developed by thoroughly reviewing related literature, job chart and discussions with the experts in extension education and extension workers. An item pool of participation items was related to 17 participation dimensions generated initially. The identified items formulas were carefully edited by Edwards (1957). The identified participation dimension along with number of items initially formulated under each are given below in Table.1

**Deciding relevancy of items of the scale**

These items were subjected to scrutiny by an expert panel of judges to determine the relevancy and their subsequent screening. For this purpose, the items were given to a panel of 50 judges in the field of Extension Education, Psychology, Management, Social Science and State Department of Agriculture. The judges were asked to indicate relevancy to each item for inclusion in the scale to measure the participation of extension worker in ATMA. The responses were obtained on three point continuum viz. relevant, somewhat relevant and not relevant with scores of 3, 2, and 1 respectively. In all, 35 judges could respond stipulated time. On the basis of the judges response the relevancy weightages were worked out for the items by adopting procedure given by Patil et.al (1996). Applying the criteria of more than 0.75 relevancy weightage 57 items were selected and others were rejected.

**Item analysis**

Further, it was considered essential to delineate the item that discriminates between persons having different participation. A list of 57 items having more than 0.75 relevancy weightage, was administered to 32 extension workers from non- sampled area. The response from them were elicited on five point continuum namely always, sometime, rarely, very rarely and never and were recorded as 5, 4, 3, 2 and 1, respectively. The participation scores of each Extension Workers were obtained by summing up the scores over all items. Considering the total score earned by each Extension Workers they were arranged in a descending order. Then 25 percent Ews with highest total scores and 25 percent Ews with the lowest scores were selected. These two groups provided the criterion groups as 'high' and 'low' groups to evaluate the individual items. The critical ratio ( $t$ ) for each item was worked out by the formula given by Edwards (1957). The ' $t$ ' value is a measure of the extent to which a given items differentiate between the high group from the low group.

**Selection of items for inclusion in final scale.**

The value of critical ratio ( $t$ ) for all the 57 items were computed and arranged in descending orders. The value of critical ratio 2.14 was observed to be significantly differentiating between 'high and 'low' group. The items having greater than 2.14 ' $t$ ' value were then selected for inclusion in the final format of the scale. By this procedure 57 items were retained and included in final format of participation scale (Table. 2)

**Testing reliability of the scale**

The split-half and test-retest techniques for testing reliability were used.

**Split-half method**

The format of the scale containing 57 participation items was administered to 25 extension workers of non-sample area. The responses were rated on five point continuum as always, sometime, rarely, very rarely and never with a score of 5, 4, 3, 2 and 1. For working out split-half reliability the scores earned by all the EWs on add and even items





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were added together separately and were correlated. The reliability coefficient calculated for the participation scale by following this procedure was 0.8108 and found significant at 0.01 level of probability.

**Test-retest method**

The format of scale having 57 items was administered twice to 25 EWs with an interval of fifteen days. The responses were secured on a five point continuum as always, sometime, rarely, very rarely and never with a score of 5,4,3,2, and 1 respectively. The total scores of all 25 EWs for each item were calculated separately.

The scores were then correlated. The value of 'r' was 0.8613 and was reported to be significant. It has indicated the participation of extension workers scale constructed was reliable.

**Testing validity of the scale**

The criteria of content and construct validity were applied for testing the validity of participation of extension workers scale.

**i) Content validity**

The contents of the scale were derived from the job chart of EWs review of literature, consultation with experts and securing judge's opinion on appropriateness of items included in the scale. It was assumed that the scores obtained by administering the scale measured the participation of extension workers and nothing else.

**ii) Construct validity**

The construct validity was tested by using 't' test. The items were grouped into two groups high and low group and the difference in means was tested by 't' test . The items with significant't' value among high and low groups were than selected in inclusion of scale. Thus the construct validity of participation scale developed for measurement of participation of extension workers reasonably established.

**Norms for use of scale**

The final format of participation scale with 57 items representing 17 participation dimensions can be administered to the EWs with a five point response continuum namely, Strongly agree, Agree, Undecided, Disagree and Strongly disagree. With the scores of 5, 4, 3, 2, and 1, respectively. The participation score was calculated by adding up the scores obtained by extension worker on all the statements and was considered as individual's score. The participation score on this scale range from a minimum 57 to a maximum of 285. The categorization of the EWs was done on the of four quartiles considering the obtainable index ranges was made as Low (Up to 25 index), Medium ( 26 to 50 index ), High( 51 to 75 index), and Very high ( Above 75 index).

**CONCLUSION**

The scale was found to be reliable and valid .Therefore; it can correctly measure the participation of Extension Workers in activities of Blocks Technology Team in Agricultural Technology Management Agency to maximum precision possible and can yield consistent results when used on different occasions involving the same / different respondents. This scale could also be used to measure participation of EWs in the other organization even beyond study area with necessary modifications in the wordings of the scale items.





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**REFERENCES**

1. Edwards, A.L.1957. Techniques of attitude scale construction, app.let on century,Inc.New York.
2. Likert, R.1932. A technique for the measurement of attitudes. Archives Psychology, No.140:44-55.
3. Patil, S. L., Sundara Swamy and V.G.Patil 1996. Development of scale to measure perception of Farmers about usefulness of NEAP, Maharashtra J.Exten Education.XV:125-131

**Table.1. Details about participation items developed & finally Retained in Participation**

Sr.No.	Dimensions	Total number of items identified	Number of items retained after relevancy test	Number of items retained after item analysis
01	Diagnosis of needs and problems	11	03	03
02	Prioritization of needs of problems	09	04	04
03	Planning and designing technological solutions	12	05	05
04	Implementation of programme	07	03	03
05	Monitoring and evaluation of programme	11	03	03
06	Feedback related with programme	05	03	03
07	Providing consultation	07	03	03
08	Functional participation	05	03	03
09	Interactive participation	07	04	04
10	Self mobilization	05	03	03
11	Consensus building	05	03	03
12	Participation in decision making	06	03	03
13	Regularity in attending meeting	08	04	04
14	Sharing of responsibility	05	03	03
15	Conflict resolution	07	04	04
16	Partnership building	07	03	03
17	Collaborative working	06	03	03
	<b>Total</b>	<b>123</b>	<b>57</b>	<b>57</b>

**Table.2. Final format of Participation scale constructed and Standardized for Measurement of Participation of Representative Extension workers in the activities of Block Technology Team. Participation response Continuum: Strongly agree (5), Agree (4), undecided (3), Disagree(4) and Strongly disagree (1)**

Sr. No.	Items
<b>I.</b>	<b>Diagnosis of needs and problems</b>
1.	Identify problems based on interests of the farmers
2.	Identification of farmers felt needs







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3.	Diagnose farmers problems and workout solutions
<b>II.</b>	<b>Prioritization of needs of problems</b>
4.	Determine priorities of problems
5.	Place priority on felt needs of farmers
6.	Determine priorities for extension activities
7.	Bringing the priorities of opportunities to farmers
<b>III.</b>	<b>Planning and designing technological solutions</b>
8.	Formulate plan of work to overcome the problems
9.	Plan and design developmental programme
10.	Designing the technological solutions for identified problems
11.	Deciding the calendar of activities of the developmental programme
12.	Involve farmers in programme planning
<b>IV.</b>	<b>Implementation of programme</b>
13.	Encourage the farmers for actualization of programme
14.	Shoulder responsibility for programme implementation
15.	Provide technical guidance for implementation of programme
<b>V.</b>	<b>Monitoring and evaluation of programme</b>
16.	Watch the progress of programme activities
17.	Provide constructive suggestions of modification during implementation of programmes
18.	Evaluate programme on feedback information
<b>VI.</b>	<b>Feedback related with programme</b>
19.	Encourage feedback from extension machinery
20.	Welcome feedback from farmers
21.	Make use of feedback information to strengthen research and extension activities
<b>VII.</b>	<b>Providing consultation</b>
22.	Workout solutions for problems posed by farmers
23.	Provide solutions to problems within time period
24.	Provide consultation to enhance the performance of developmental programme
<b>VIII.</b>	<b>Functional participation</b>
25.	Participate in every meeting to discuss the pre-determined programme
26.	Offer constructive modification in pre-determined programme objectives in changed situation
27.	Take part in farmer group discussion
<b>IX.</b>	<b>Interactive participation</b>
28.	Encourage tackling of farmers problems by different departments
29.	Extend full co-operation in implementing need based programmes
30.	Ensure participation of representatives of development departments during programme planning
31.	Ensure participation of representative of development departments during programme implementation
<b>X.</b>	<b>Self mobilization</b>





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32.	Provide active initiative during implementation of programme
33.	Willing to provide needed help during programme development
34.	Willing to provide needed services during programme implementation
<b>XI.</b>	<b>Consensus building</b>
35.	Promote agreement on opinions of majority of members
36.	Reach general agreement on decision related to conflict issues.
37.	Build consensus of all stakeholders on programme objectives
<b>XII.</b>	<b>Participation in decision making</b>
38.	Promote identification of alternative courses action
39.	Promote selection of appropriate course of action among available alternatives
40.	Arrive at collective decision on programme related activities
<b>XIII.</b>	<b>Regularity in attending meeting</b>
41.	Arriving for meeting on the fixed scheduled time
42.	Leave the meeting only after all the issues are discussed
43.	Keep regularity in attending meeting
44.	Making available for urgently called meeting
<b>XIV.</b>	<b>Sharing of responsibility</b>
45.	Share responsibility for generating group goals
46.	Take responsibility for technology dissemination
47.	Shoulder responsibility in programme implementation
<b>XV.</b>	<b>Conflict resolution</b>
48.	Try to arrive at consensus decisions
49.	Follow group norms to enforce discipline during meetings
50.	Allow members to feel feel to express their opinion during conflict resolution
51.	Try to overcome conflict situation through democratic means
<b>XVI.</b>	<b>Partnership building</b>
52.	Promote co-ordination between various development department
53.	Play a role of an active partner in programme implementation
54.	Ensure co-ordination to avoid wastage of scare resources
<b>XVII.</b>	<b>Collaborative working</b>
55.	Work jointly with different department for technological solutions
56.	Promote co-ordination between the members
57.	Co-ordinate work to achieve group goals





## Standardization of Scale to Measure Participation of Farmers

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### ABSTRACT

An attempt was made to develop standardize a scale for measuring the participation of representative farmers in ATMA based on Likert method of summated rating. Total 21 dimensions of participation in activities of FAC were identified. These participation dimensions were mailed to a panel and 17 participation dimensions were selected which had relevancy weightage more than 0.75. The items were given to 50 judges indicate appropriateness (relevancy) of each item for inclusion in the scale to measure the participation of representative farmers in ATMA. A list of 53 items having more than 0.75 relevancy weightage was administered to 32 farmers from non- sampled area. Responses for the items were obtained on five point continuum. The participation scores of each farmer were obtained by summing up the scores over all items. On the basis of the total score, the respondents were arranged in a descending order. Twenty five per cent of the respondents with highest total scores and Twenty five per cent with lowest scores were selected. The split-half and test-retest techniques for testing reliability were used. The criteria of content and construct validity were applied for testing the validity of participation of farmers scale. The final format of participation scale had 53 items representing 17 participation dimensions can be administered to the farmers with a five point continuum. The participation score was calculated by adding up the scores obtained by respondents on all the statements and was considered as individual's score.

**Key words:** Participation, Farmers, ATMA





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## **INTRODUCTION**

The government of Maharashtra has introduced the innovation in technology transfer the Agricultural Technology Management Agency (ATMA) on experimental basis in four districts since 1998-99. The Farm Information & Advisory Center (FIAC) at the block is under control the of ATMA organization. It works through block technology team consisting of extension workers of development department and the Farm Advisory Committee (FAC) having farmers representative on it. It is responsible for programme planning & implementation .The efficiency & effectiveness of the ATMA programme mainly depends upon the participation of the farmers' Advisory Committee members in the programme planning & implementation. In view of the crucial nature of the role that farmers are expected to play in the new strategy of agricultural production therefore must have been affected .No comprehensive scale was available to measure the participation of farmers' in ATMA. In order to augment this attempt was made to develop & standardize a scale for measuring the participation of farmers in ATMA.According to Webster's Dictionary, participation means to take a part or to have share in common or to have a share of profit or benefit in common with others. In the context of present study participation was operationally defined as the to the degree of participation of representative farmers in the programmes and activities of Farmers Advisory Committee.In operational terms the present study refers to the extent of sharing or taking part in meetings, asking questions, making suggestions, proposing extension programmes, giving related information required for discussion, organising the extension programmes and giving feedback about the programme.

### **Procedure for standardization of scale**

In order to develop and standardize the measuring instrument the methods of summated rating by Likert (1932) was followed. The detail procedure adopted for this purpose is described here under.

### **Collection of participation dimensions**

Studies on participation's were reviewed. Further studies on the participations of farmers working in the domains of various schemes, programmes and various schemes, programs, and various rural development programs were traced through internet and from journals, books etc. and reviewed. After reviewing the literature, and discussion with expert in the field of extension education, psychology, management and social Sciences, 21 dimensions / components of participation in activities of FAC were identified.

### **Deciding relevancy of participation dimensions**

It was quite possible that all the 21 participation dimensions identified may not be equally relevant in measuring participation of representative farmers in the activities of FAC working in ATMA. Hence these dimensions were subjected to scrutiny by an expert panel of judges to determine the relevancy and their subsequent screening. The operational definitions of dimensions were also included for their reference. These participation dimensions were mailed to a panel of 100 judges in the field of extension education, psychology, management, administration and social sciences. These judges were requested to indicate appropriateness (relevancy) of dimensions for inclusion in the scale to measure participation of representative farmers working in ATMA. The responses of judges were secured on three point continuum namely, most relevant, somewhat relevant and not relevant and scored as 3,2 and 1, respectively. In all 62 judges could respond in stipulated time . These judgment were used for working out the relevancy weightage (RW) of the each participation dimension by using the procedure given by Patil et.al (1996). Considering the relevancy weightages, the participation dimensions were screened for their relevancy. Accordingly, dimensions having weightage of more than 0.75 were considered. Using these criteria, 17 participation dimensions having more than 0.75 relevancy weightage were selected.



**Kinkhedkar et al.****Construction of items for the scale**

An important aspect in the development of a scale is the constitution of the item pool. The item pool was developed by thoroughly reviewing related literature and discussions with the experts in extension education, psychology, management, extension personnel of State Department of Agriculture. An item pool of participation items was related to 17 participation dimensions generated initially. The identified items were carefully edited by Edward (1957). The identified participation dimension along with number of items initially formulated under each are given in Table. 1

**Deciding relevancy of items of the scale**

These items were subjected to scrutiny by an expert panel of judges to determine the relevancy and their subsequent screening. For this purpose, the items were given to a panel of judges in the field of extension education, psychology, management, social science and state Department of Agriculture. The judges were requested to indicate appropriateness (relevancy) of each item for inclusion in the scale to measure the participation of representative farmers in ATMA. The responses were obtained on three point continuum viz. relevant, somewhat relevant and not relevant with scores of 3,2 and 1 respectively. In all, 35 judges could respond in stipulated time. On the basis of the judges responses, the relevancy weightages were worked out for the items by adopting the procedure given by Patil et.al (1996 ).Applying the criteria of more than 0.75 relevancy weightage 53 items were selected and others were rejected.

**Item analysis**

Further, it was considered essential to delineate the item that discriminates between persons having different participation. A list of 53 items more than 0.75 relevancy weightage was administered to 32 farmers from non-sampled area. The responses from them were elicited on a five point continuum namely always, sometime, rarely, very rarely and never carrying the score of 5,4,3,2, and 1, respectively. The participation scores of each farmer were obtained by summing up the scores over all items. Considering the total score earned by each farmer was arranged in a descending order. Then 25 per cent of the farmers with highest total scores and 25 per cent of the farmers with lowest scores were selected. These two groups provided the criterion group as high and low groups to evaluate the individual items .The critical ratio (+) for each item was worked out by the formula given by Edward's (1957). The 't' value of is a measure of the extent to which a given item differentiate between the high group from the low group.

**Selection of items for inclusion in final scale.**

The values of critical ratio (t) for all the 53 items were computed and arranged in descending order. The value of critical ratio 2.14 was observed to be significantly differentiating between 'high' and 'low' group. The items having greater than 2.14 't' value were then selected for inclusion in the final format of the scale. By this procedure 53 items were retained and included in final format of 'Participation scale' (Table no 2).

**Testing reliability of the scale**

The split-half and test-retest techniques for testing reliability were used.

**a) Split-half method**

The format of the scale containing 53 participation items was administered to 25 farmers of non-sampled area. The responses were rated on a five point continuum as always, sometime, rarely, very rarely and never having a scores of 5, 4, 3, 2 and 1. For working out split-half reliability the scores earned by all the farmers on odd and even items





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were added together separately and were correlated. The reliability coefficient calculated for the participation scale by following this procedure was 0.8326 and found significant at 0.01 level of probability.

**b) Test- retests method**

The format of scale having 53 items was administered twice to 25 farmers with an interval of 15 days. The responses were secured on five point continuum as always, sometime, rarely, very rarely and never with a score of 5, 4, 3, 2 and 1, respectively. In this way, two administrations of the same test yielded two independent sets of scores. The total score of all 25 farmers for each item were calculated separately. The score were then correlated. The value of 'r' was 0.8643 and was reported to be significant. It has indicated the participation of farmers scale constructed was reliable.

**Testing validity of the scale**

The criteria of content and construct validity were applied for testing the validity of participation of farmers scale.

**a) Content validity**

The content of the scale were derived from the review of literature, consultation with experts and securing judge's opinion on appropriateness of items included in the scale. It was assumed that the scores obtained by administering the scale measured the participation of farmers and nothing else.

**b) Construct validity**

The construct validity was tested by using 't' test. The items were grouped into two groups high and low groups and the difference in means was tested by 't' test. The items with significant 't' value among high and low groups were than selected in inclusion of scale Thus the construct validity of participation scale developed for measurement of participation of farmers.

**Norms for use of scale**

The final format of participation scale had 53 items representing 17 participation dimensions can be administered to the farmers with a five point response continuum namely Strongly agree, Agree, undecided, Disagree and Strongly disagree with the scores of 5,4,3,2 and 1, respectively. The participation score was calculated by adding up the scores obtained by farmers on all the statements and was considered as individual's score. The participation score on this scale range from a minimum 53 to a maximum of 265. The categorization of the respondents was done on the basis of four quartiles considering the obtainable index range was made as low (Up to 25 index), Medium (26 to 50 index), High (51 to 75 index) and Very high (Above 75 index).

**CONCLUSION**

The scale was found to be reliable and valid. Therefore, it can correctly measure the participation of representative farmers in activities of Farmers Advisory Committee in Agricultural Technology Management Agency to maximum precision possible and can yield consistent results when used on different occasions involving the same / different respondents .This scale could also be used to measure participation of farmers in the other organization even beyond the study area with necessary modification in the wordings of the scale items.





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## REFERENCES

1. Edwards, A.L.1957. Techniques of attitude scale construction, app.let on century,Inc.New York.
2. Likert, R.1932. A technique for the measurement of attitudes. Archives Psychology, No.140:44-55.
3. Patil, S. L., Sundara Swamy and V.G.Patil 1996. Development of scale to measure perception of Farmers about usefulness of NEAP, Maharashtra J.Exten Education.XV:125-131

**Table.1 Details about Participation items developed & finally retained in Participation**

Sr.No	Dimensions	Total number of items identified	Number of items retained after relevancy test	Number of items retained after item analysis
01	Diagnosis of needs and problems	07	03	03
02	Prioritization of needs and problems	05	03	03
03	Planning and designing technological solutions	06	04	04
04	Implementation of programme	09	04	04
05	Feedback related with programme	07	03	03
06	Providing consultation	04	03	03
07	Functional participation	04	03	03
08	Interactive participation	08	03	03
09	Self mobilization	05	03	03
10	Consensus building	05	03	03
11	Participation in decision making	05	03	03
12	Regularity in attending meeting	08	03	03
13	Sharing of responsibility	05	03	03
14	Conflict resolution	07	03	03
15	Agreement on risk sharing	04	03	03
16	Partnership building	04	03	03
17	Collaborative working	04	03	03
	Total	97	53	53





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**Table.2:Final format of Participation scale constructed and standardized for Measurement of Participation of Representative farmers in the Activities of Farmers Advisory Committee. Participation response Continuum: Strongly agree (5), Agree (4), undecided (3), Disagree (4) and Strongly disagree(1)**

Sr.No.	Items
<b>I.</b>	<b>Diagnosis of needs and problems</b>
1.	Ensure correct identification of needs of farmers
2.	Ensure correct identification of problems of farmers
3.	Identify production constraints faced by farmers
<b>II.</b>	<b>Prioritization of needs and problems</b>
4.	Determine priorities of needs
5.	Determine priorities of problems
6.	Help extension workers to determine priorities of tasks
<b>III.</b>	<b>Planning and designing technological solutions</b>
7.	Help in formulating plan of work to overcome the problems
8.	Help in formulating plan of work to overcome constraints confronting the farmers
9.	Properly designing the technological solutions to tackle identified problems
10.	Play an active role in preparation of action plan
<b>IV.</b>	<b>Implementation of programme</b>
11.	Mobilize farmers to take part in implementation of programme
12.	Encourage the farmers to take active part in implementation of programme
13.	Implement plan in realistic manner
14.	Share responsibility in implementation of programme
<b>V.</b>	<b>Feedback related with programme</b>
15.	Provide regular feedback about the programme
16.	Provide feedback regarding research
17.	Provide feedback about extension activities
<b>VI.</b>	<b>Providing consultation</b>
18.	Provide information about the village situation needed by extension workers
19.	Try to solve the problem posed by extension workers
20.	Provide needed information to extension workers to solve the problems
<b>VII.</b>	<b>Functional participation</b>
21.	Participate in every meeting to discuss the pre-determined programme objective
22.	Develop awareness about pre-determine programme objectives
23.	Offer constructive modification in pre-determined programme objectives in changed situation
<b>VIII.</b>	<b>Interactive participation</b>
24.	Extend full co-operation to extension agency in implementing need based programme
25.	Encourage formation of new work groups







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26.	Strengthen functioning of existing work groups
<b>IX.</b>	<b>Self mobilization</b>
27.	Willing for implementation of programme
28.	Provide active initiative during implementation of programme
29.	Willing to provide needed help during programme development
<b>X.</b>	<b>Consensus building</b>
30.	Reach to general agreement on opinion of all members
31.	Build consensus of farmer representatives on programme objectives
32.	Promote understanding on different perspectives on developmental activities
<b>XI.</b>	<b>Participation in decision making</b>
33.	Promote decision after through discussion in meeting
34.	Involve in selecting appropriate course of action among available alternatives
35.	Promote collective decision on various issues related with programme
<b>XII.</b>	<b>Regularity in attending meeting</b>
36.	Arriving for meeting on the fixed scheduled time
37.	Leave the meeting only after all issues are discussed
38.	Keep regularity in attending meeting
<b>XIII.</b>	<b>Sharing of responsibility</b>
39.	Accept any responsibility entrusted by members
40.	Voluntarily come forward to accept the responsibility in implementing the programme
41.	Share responsibility for generating group goals
<b>XIV.</b>	<b>Conflict resolution</b>
42.	Try to arrive at consensus discussions
43.	Follow group norms to enforce discipline during meetings
44.	Try to overcome conflict situation through democratic means
<b>XV.</b>	<b>Agreement on risk sharing</b>
45.	Make decision together and share the risk
46.	Knows the consequences of agreement at local level
47.	Predict the unanticipated consequences of programmes
<b>XVI.</b>	<b>Partnership building</b>
48.	Ensure proper co-ordination between representatives of different farmers group
49.	Facilitate exchange of experiences with extension workers
50.	Ensure co-ordination of different farmers groups to avoid wastage of scarce resources
<b>XVII.</b>	<b>Collaborative working</b>
51.	Ensure participation of all farmers groups during implementation of programme
52.	Establish collaborative relationship to solve joint problems
53.	Ensure co-operative efforts to achieve common goal





RESEARCH ARTICLE

## Character Association Analysis in Back Cross Derived Populations Involving Foliar Disease Susceptible and Resistant Parents in Groundnut( *Arachis hypogaea* L.)

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### ABSTRACT

The present study was made to understand the association for kernel yield and yield contributing traits in BC<sub>2</sub>F<sub>1</sub> population of three crosses involving foliar disease susceptible (ICGV 00350, ICGV 03128 and VRI 2) and resistant parents (GPBD4) in groundnut. The trait kernel yield per plant had positive and significant association with number of pods per plant, hundred pod weight, shell weight per plant, shelling percentage and pod yield per plant in all three crosses. Whereas late leaf spot and rust disease scores exhibit negative correlation with kernel yield per plant in one cross and no association in other two crosses. Thus, the late leaf spot and rust may affect the improvement of kernel yield per plant depending upon the parents involved.

**Key words:** Groundnut, Correlation, foliar disease resistance and kernel yield

### INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the principal economic oilseed crops of the world. Groundnut is grown on nearly 23.95 million ha worldwide with the total production of 36.45 million tons and an average yield of 1520 kg/ha in 2009 (FAOSTAT, 2011). Since the economic yield is contributed by the pods formed under the ground, the yield



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potential of groundnut is known only when the crop is harvested. It is almost difficult to predict pod yield based on aerial morphological characters (Weiss, 2000). The low productivity of the crop in India and several African countries is ascribed to many biotic and abiotic stresses in the cultivation of the crop. Among the biotic stresses, two fungal diseases namely late leaf spot (LLS) caused by *Phaeoisariopsis personata* and rust caused by *Puccinia arachidis* are widespread and economically most important. These diseases often occur together and cause yield loss up to 50–70% in the crop (Subrahmanyam *et al.* 1984). Besides adversely affecting the yield and quality of pod, it also affects the yield and quality of haulm. Searle (1965) suggested that the average merit of a character in a population could be changed by means of selection programme based on the basis of phenotype of the main trait concerned. However, such an improvement would be more reliable if indirect selection based on another correlated trait. Understanding the relationships among yield and yield components is of paramount importance for making the best use of these relationships in selection. The correlation coefficient may be confounded with indirect effect due to common association inherent in trait interrelationships. In view of above, the present study was undertaken to assess correlation among important agronomic traits on kernel yield per plant and to formulate an efficient selection strategy in foliar disease resistance breeding programme.

**MATERIALS AND METHODS**

The present experimental material comprised of three crosses viz., ICGV 00350× GPBD 4 (cross 1), ICGV 03128× GPBD 4 (cross 2) and VRI 2 × GPBD 4 (cross 3). The parent ICGV00350, ICGV 03128 and VRI 2 were susceptible to late leaf spot (LLS) and rust diseases but having high pod yield and oil content. To incorporate resistance to these diseases, resistant donor GPBD 4 was used in crossing programme and the recurrent parents were again backcrossed with F<sub>1</sub>'s and BC<sub>1</sub> F<sub>1</sub>'s. The BC<sub>2</sub> F<sub>1</sub> populations of three crosses were used to investigate the relationship among yield and yield component characters. The crop was raised during *kharif* 2014, at the Oilseeds Farm, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore (Tamil Nadu), India. Recommended agronomic practices were followed under irrigated condition. Observations were recorded in each cross for 10 characters viz., number of pods per plant, hundred pod weight (g), hundred kernel weight (g), shell weight (g), shelling percentage, sound mature kernel (SMK) (%), late leaf spot (LLS) score, rust score, pod yield per plant (g) and kernel yield per plant (g). Shell weight per plant was calculated by the difference between pod yield per plant and kernel yield per plant and expressed in grams. To screen the lines for sources of resistance to late leaf spot and rust, one (Resistant) to nine (susceptible) point disease scale suggested by Subrahmanyam *et al.* (1995) was employed. Simple correlation coefficient analysis for yield and yield components were computed utilizing the formula proposed by Al-Jibouri *et al.* (1985).

**RESULTS AND DISCUSSION****Correlation Analysis**

The correlation coefficients provide a reliable measure of association among the characters and help to differentiate vital associates useful in breeding from those of non-vital ones (Falconer, 1981). The result pertaining to this has been presented in Table 1 to 3.

**Correlation between kernel yield per plant and other yield contributing traits**

The kernel yield per plant showed positive and significant association with number of pods per plant, hundred pod weight, hundred kernel weight, shell weight per plant, shelling percentage and pod yield per plant in the crosses ICGV00350× GPBD 4 and ICGV 03128 × GPBD 4. Whereas the cross VRI 2 × GPBD 4 exhibits positive and significant correlation with traits viz., number of pods per plant, hundred kernel weight, shell weight per plant, shelling percentage and pod yield per plant towards kernel yield per plant. The cross ICGV00350× GPBD 4 had negative





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significant correlation with both the disease scores in lieu with kernel yield per plant. Whereas other two crosses *viz.*, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4 exhibits no association with foliar disease scores of LLS and rust. These results are affirmed with findings of Prabhu *et al.* (2015) for number of pods per plant, Mothilal (2003) for hundred pod weight, Shoba *et al.* (2012) for hundred kernel weight (Anitha, 2013) for shell weight (Prabhu *et al.*, 2014) for pod yield per plant (Kwaga, 2014) for plant height (Nandini and Savithramma, 2012) for sound mature kernel per cent. Hence for improving kernel yield per plant in groundnut, it is suggested that selection has to be exercised number of pods per plant, hundred kernel weight, shell weight per plant, shelling percentage and pod yield per plant for all the three crosses were studied., while hundred pod weight may also be use as a selection indices for the crosses ICGV00350× GPBD 4 and ICGV 03128 × GPBD 4 alone to improving kernel yield. Careful attention to be made for selecting high kernel yield genotypes based on disease scores in the cross ICGV 00350× GPBD 4 which had negative significant correlation with LLS and rust scores.

#### Correlation between pod yield per plant and other yield contributing traits

The trait pod yield per plant in back cross progenies of groundnut recorded positive and significant correlation with number of pods per plant, hundred pod weight, hundred kernel weight, shell weight per plant and shelling percentage in the crosses of ICGV00350× GPBD 4 and ICGV 03128 × GPBD 4. The trait cross pod yield per plant exhibits significant and positive association with traits *viz.*, number of pods per plant, hundred pod weight shell weight per plant and shelling percentage in the cross VRI 2 × GPBD 4 . Whereas LLS and rust score recorded negative and significant correlation with the trait pod yield per plant in the cross ICGV00350× GPBD 4. While the cross ICGV 03128× GPBD 4 and VRI 2 × GPBD 4 having no association with LLS and Rust on either side towards pod yield per plant. Similar results were reported by Rao *et al.* (2014) for number of pods per plant (Priyadarshini, 2012) for hundred kernel weight (Anita, 2013) for shell weight, Prabhu *et al.* (2015) for hundred pod weight and sound mature kernel per cent. Hence, these positive and significant traits in each cross may be considered and taken into account as selection indices for improving pod yield per plant in foliar disease resistance groundnut breeding program.

#### Correlation between Number of pods per plant and other yield contributing traits

Traits *viz.*, hundred pod weight, hundred kernel weight, shell weight per plant and shelling percentage registered positive and significant correlation with the trait number of pods per plant in the cross ICGV00350× GPBD 4. The cross ICGV 03128×GPBD 4 recorded significant correlation towards number of pods per plant with the traits like hundred pod weight, shell weight per plant and shelling percentage. A significant positive association was noticed towards this trait in the backcross population of the cross VRI 2 × GPBD 4 with the traits shell weight per plant and shelling percentage. While the trait LLS and rust score having negative significant correlation with number of pods per plant in the cross ICGV 00350 × GPBD 4. Whereas there is no observation of association between foliar disease scores and number of pods per plant in the back cross derived population of ICGV 03128× GPBD 4 and VRI 2 × GPBD 4. These findings were also reported by Prabhu *et al.* (2015) and Anitha (2013) for hundred pod weight, hundred kernel weight, shell weight and shelling percentage. This suggests that selection for higher number of pods per plant results in reduced LLS disease score in selected progenies of ICGV 00350 × GPBD 4, in other hand the foliar disease scores having no scope as a selection indices for improving number of pods per plant in rest of two crosses.

#### Correlation between hundred pod weight with other yield contributing traits

The trait hundred pod weight reveals significant and positive and significant correlation with hundred kernel weight and shell weight per plant in the cross ICGV 00350 × GPBD 4. while the cross ICGV 03128 × GPBD 4 registered significant and positive correlation with hundred kernel weight , shell weight per plant , sound mature kernel percent (SMK) and LLS score in this back cross population. The cross VRI 2 × GPBD 4 revealed significant and positive correlation with traits *viz.*, hundred kernel weight , shell weight per plant , LLS score and rust score in



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association of the trait hundred pod weight. Similar results were reported by Priyadharsini (2012), Pavithradevi (2013), Anitha (2013) and Prabhu *et al.* (2015).

**Correlation between hundred kernel weight with other yield contributing traits**

The trait hundred kernel weight exhibit significant and positive correlation with shell weight per plant and shelling percentage, whereas it had negative significant correlation with LLS and rust disease scores in the cross derivatives of ICGV 00350 × GPBD 4. The traits viz., shell weight per plant, shelling percentage and LLS score having significant positive correlation with hundred kernel weight trait in the backcross population of ICGV 03128 × GPBD 4. The cross VRI 2 × GPBD 4 recorded significant positive correlation with LLS disease score towards hundred kernel weight. Similar results were reported by Priyadharsini (2012), Pavithradevi (2013), Anitha (2013) and Prabhu *et al.* (2015).

**Correlation between shell weight per plant, shelling percentage and sound mature kernel percent (SMK) with other yield contributing traits**

The cross ICGV 00350 × GPBD 4 revealed negative significant correlation with foliar disease scores viz., LLS and rust towards shell weight per plant and shelling percentage. Whereas the cross ICGV 03128 × GPBD 4 registered significant positive correlation with LLS scores towards shelling percentage. There is no significant association between foliar disease scores towards shell weight per plant and shelling percentage in the cross VRI 2 × GPBD 4 was also observed. The cross ICGV 03128 × GPBD 4 recorded significant positive correlation with LLS scores towards sound mature kernel percent (SMK).

**Correlation between LLS disease score and rust disease score**

All the said three crosses shows the positive and significant correlation between late leaf spot score and rust score in this population denotes that co-occurrence of these two diseases in groundnut in most of the cases. These results are in accordance with the findings of Prabhu *et al.* (2015). The crosses with no association between disease score and yield traits, improvement on yield component trait are possible irrespective of disease incidence. However, in case of crosses with significant association between yield traits and disease score, caution is necessary as selection on yield trait may also have an effect on disease reaction. The negative association can be effectively exploited for simultaneous improvement on yield and less disease score. Stratified selection programme may be followed to improve both yield and disease score in the presence of association between yield and disease scores.

It was apparent from the present investigation of correlation that the traits viz., number of pods per plant, hundred pod weight, hundred kernel weight, shelling per cent and pod yield per plant are desirable selection indices for increasing kernel yield per plant in all the three crosses viz., ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4, for the improvement of the kernel yield per plant in cross VRI 2 × GPBD 4 the same selection indices in cross 1 and cross 2 may be chosen except the trait hundred pod weight. Hence these traits may be considered as the important yield attributing characters and due emphasis should be placed while breeding for high kernel yield in foliar disease resistance groundnut breeding program.

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## REFERENCES

1. Al-jibouri, H. A., P. A. Miller and H. P. Robinson. (1985). Genotypic and environmental variances and covariances in upland cotton cross of interspecific origin. *Agronomy Journal*, 50: 633-636.
2. Anitha, B. K. (2013). Identification of quantitative trait loci for oil yield and marker assisted backcross for high oleic acid in groundnut (*Arachis hypogaea* L.). *Ph.D. (Ag.) Thesis* submitted to the Tamil Nadu Agricultural University, Coimbatore.
3. Falconer, D. S. 1981. *Introduction to Quantitative Genetics*, Longman Inc.Ltd., New York.
4. Kwaga, Y. M. (2014). Correlation coefficients between kernel yield of groundnut (*Arachis hypogaea* L.) under infestation of *Alectravogelii* (Benth) in the Northern Guinea Savanna ecology of Nigeria. *American Journal of Research and Communication*, 2(2): 82-90.
5. Mothilal, A. (2003). Genetics of vegetative and reproductive traits in groundnut (*Arachis hypogaea* L.). *Ph.D. (Ag.) Thesis* submitted to Tamil Nadu Agricultural University, Coimbatore.
6. Nandini, C. and D. L. Savithamma (2012). Character association and path analysis in F8 recombinant inbred line population of the cross NRCG 12568 × NRCG 12326 in groundnut (*Arachis hypogaea* L.). *Asian Journal of Biological Sciences*, 7(1) : 55-58.
7. Pavithradevi, S. (2013). Identification of Quantitative Trait Loci (QTLs) for yield and yield component traits under drought stress in Spanish bunch groundnut (*Arachis hypogaea* L.). *Ph.D. (Ag.) Thesis* submitted to Tamil Nadu Agricultural University, Coimbatore.
8. Prabhu, R., N. Manivannan, A. Mothilal and S. M. Ibrahim (2015). Correlation Coefficient analysis for yield and yield attributes in groundnut (*Arachis hypogaea* L.). *Plant Archives*, 15(2):685-689.
9. Prabhu, R., N. Manivannan, A. Mothilal and S. M. Ibrahim (2014). Magnitude and direction of association for yield and yield attributes in groundnut (*Arachis hypogaea* L.). *Electronic Journal of Plant Breeding*, 5(4) : 824-827
10. Priyadarshini, M. (2012). Molecular marker analysis for yield and yield component traits under non stress and drought stress conditions in groundnut (*Arachis hypogaea* L.). *Ph.D. (Ag.) Thesis* submitted to Tamil Nadu Agricultural University, Coimbatore.
11. Rao, T. V., V. Venkanna, D. Bhadraru and D. Bharathi (2014). Studies on variability, character association and path analysis on groundnut (*Arachis hypogaea* L.). *International Journal of Pure and Applied Bioscience*, 2(2): 194-197
12. Searle, S.R. 1965. The value of indirect selection. I. Mass selection. *Biometrics*, 21: 682-707.
13. Shoba, D., N. Manivannan, P. Vindhiyavarman and S. N. Nigam (2012). SSR markers associated for late leaf spot disease resistance by bulked segregant analysis in groundnut (*Arachis hypogaea* L.). *Euphytica*, 188(2) : 265-272
14. Subrahmanyam, P., McDonald, D., Waliyar, F., Reddy, L.J., Nigam, S.N., Gibbons, R.W., Rao, V.R., Singh, A.K., Pande, S., Reddy, P.M. and Subba, R.P.V. 1995. Screening methods and sources of resistance to rust and late leaf spot of groundnut. International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, Information. Bulletin No. 47: 24
15. Subrahmanyam P, Williams JH, McDonald D, Gibbons RW (1984). The influence of foliar diseases and their control by selective fungicides on a range of groundnut (*Arachis hypogaea* L.) genotypes. *Ann Appl Biol*, 104:467-476.





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**Table. 1. Simple correlation coefficients between pod yield and yield component traits in BC<sub>2</sub>F<sub>1</sub> population of the cross ICGV 00350× GPBD 4**

Character	Number of pods per plant	Hundred pod weight (g)	Hundred kernel weight (g)	Shell weight per plant (g)	Shelling percentage	SMK (%)	LLS score	Rust score	Pod yield per plant (g)
Hundred pod weight (g)	0.294*								
Hundred kernel weight (g)	0.288**	0.776**							
Shell weight per plant (g)	0.771**	0.741**	0.554**						
Shelling percentage (%)	0.502**	-0.131	0.267*	0.110					
SMK (%)	-0.112	0.161	0.073	0.029	0.063				
LLS score	-0.513**	-0.164	-0.272*	-0.379**	-0.351*	0.122			
Rust score	-0.459**	-0.212	-0.341*	-0.364**	-0.307*	-0.088	0.906**		
Pod yield per plant (g)	0.910**	0.601**	0.570**	0.914**	0.437**	0.001	-0.484**	-0.472**	
Kernel yield per plant (g)	0.922**	0.491**	0.541**	0.812**	0.573**	-0.014	-0.505**	-0.496**	0.979**

\*, \*\* Significant at 5 % and 1 % level of probability, respectively

**Table. 2. Simple correlation coefficients between pod yield and yield component traits in BC<sub>2</sub>F<sub>1</sub> population of the cross ICGV 03128× GPBD 4**

Character	Number of pods per plant	Hundred pod weight (g)	Hundred kernel weight (g)	Shell weight per plant (g)	Shelling percentage (%)	SMK (%)	LLS score	Rust score	Pod yield per plant (g)
Hundred pod weight (g)	0.278**								
Hundred kernel weight (g)	0.176	0.709**							
Shell weight per plant (g)	0.767**	0.471**	0.203*						
Shelling percentage (%)	0.563**	0.102	0.217*	0.172					
SMK (%)	0.088	0.342**	0.011	0.011	0.102				
LLS score	0.002	0.230*	0.260**	-0.045	0.155*	0.215*			
Rust score	0.084	0.044	0.039	0.057	0.059	0.052	0.197*		
Pod yield per plant (g)	0.902**	0.531**	0.339**	0.893**	0.504**	0.171	0.057	0.082	
Kernel yield per plant (g)	0.901**	0.522**	0.378**	0.780**	0.621**	0.210	0.100	0.088	0.978**

\*, \*\* Significant at 5 % and 1 % level of probability, respectively





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**Table. 3. Simple correlation coefficients between pod yield and yield component traits in BC<sub>2</sub>F<sub>1</sub> population of the cross VRI 2 × GPBD 4**

Character	Number of pods per plant	Hundred pod weight (g)	Hundred kernel weight (g)	Shell weight per plant (g)	Shelling percentage (%)	SMK (%)	LLS score	Rust score	Pod yield per plant (g)
Hundred pod weight (g)	-0.080								
Hundred kernel weight (g)	-0.113	0.628**							
Shell weight per plant (g)	0.676**	0.436**	0.081						
Shelling percentage (%)	0.564**	-0.246	0.132	0.065					
SMK (%)	0.003	0.236	-0.014	0.016	0.016				
LLS score	-0.325	0.345**	0.278*	0.089	-0.225	-0.066			
Rust score	-0.079	0.265*	0.218	0.136	0.034	-0.037	0.634**		
Pod yield per plant (g)	0.847**	0.310*	0.220	0.855**	0.518**	0.155	-0.017	0.135	
Kernel yield per plant (g)	0.843**	0.215	0.265*	0.694**	0.688**	0.149	-0.067	0.120	0.967**

\*, \*\* Significant at 5 % and 1 % level of probability, respectively







RESEARCH ARTICLE

## Combining Ability and Line x Tester Analysis on Heat Tolerance in Cotton (*Gossypium hirsutum* L.)

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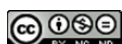
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### ABSTRACT

In recent years cotton breeders have started to interest more and more attention for improving high temperature tolerant cotton varieties due to increased global warming. For this purpose 54 cross combinations (6x9) were created in 2010 and 54 F<sub>1</sub> hybrid and their 15 parents, totally 69 genotypes were tested at the experimental area of GAP International Agricultural Research and Training Center in 2011. In the populations seed cotton yield, ginning percentage, fiber yield, photosynthetic yield, chlorophyll content and photosynthetically active radiation expressed non-additive gene action (dominant or epistatic) and GCA variance was higher than SCA variance only for fluorescence reflecting the role of additive type of gene action. It was determined that SJ-U86, AGC 375, Fiber Max 819, AGC 208 and STV 453 for seed cotton yield; Fiber Max 819 and Fiber Max 832 for ginning percentage; SJ-U86, DP 90, AGC 375, Fiber Max 819 and STV 453 for fiber yield; Fiber Max 832 and STV 453 for photosynthetic yield; AGC 375, SJ-U 86 and DP 396 for chlorophyll content; Fiber Max 819 and STV 474 for fluorescence; AGC 208 and DP 499 for photosynthetically active radiation (PAR) were the best parent cotton varieties and also having the best (GCA) general combining abilities. In the study specific combining ability (SCA) of hybrids were investigated and some promising cross combinations were selected and transferred to the next generation.

**Key words:** Cotton, heat tolerance, photosynthesis, chlorophyll content, fluorescence



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## INTRODUCTION

With climate change and global warming, high temperature stress has become one of major constrain factor influence on cotton production and productivity (Bibi et al., 2008). Global warming is the rise in the average temperature of Earth's atmosphere and oceans and its projected continuation (Oosterhuis, 2013). By the end of the twenty first century, global climate change is projected to cause increased temperatures of up to 4.0 °C (IPCC, 2007). Some prediction studies of climate change under various emission scenarios shown that cotton yield will decrease 29% under medium or high emission gases by 2097 (Lee and Six., 2010). Cotton is one of the most important industrial crops in many countries of the world including Turkey. In the cotton production areas of Turkey, especially in the Southeastern Anatolia Region extreme heat stress usually occur in mid- July and mid-August, during the peak time of flowering and boll loading, resulting in lower lint yield and fiber quality. An optimum temperature range of 20 to 30°C has been reported for cotton (Reddy et al, 1991); adverse temperatures can affect all stages of development, the crop seems to be particularly sensitive to adverse temperatures during reproductive development (Oosterhuis, 2002; Burke and Chen, 2015). Heat stress also effect boll number, boll size, number of seeds per boll (Oosterhuis, 2013). Early-season dry matter accumulation, rates of developmental events, root growth and development, leaf and stem growth patterns and fruit retention were found related to different temperature and CO<sub>2</sub> conditions (Reddy and Hodges., 2007). Limitations to normal growth and development in cotton under heat stress result from numerous adverse effects on the physiology of cotton. High temperature influences photosynthesis, electron transport rate (ETR), chlorophyll content, enzyme activity, pollen viability and photorespiration (Rodriguez-Garay and Barrow, 1988; Kakani et al., 2005; Liu et al., 2006; Snider et al, 2009; Cottee et al., 2010; Song et al., 2015). Photosynthesis in cotton is highly sensitive to temperatures above 35°C (Bibi et al., 2008; Oosterhuis et al., 2011), optimum temperature for photosynthesis being 28 °C (Burke et al., 1988). Taiz and Zeiger, 2002 revealed that both photosynthesis and respiration are inhibited at high temperatures, but as temperature increases, photosynthetic rates drop before respiration rates. Cotton has an optimal thermal kinetic window of 23.5 to 32°C in which metabolic activity is most efficient (Burke et al., 1988). High temperatures (>35°C) throughout the growing season are commonplace among the cotton production areas and exceed the thermal kinetic window for which metabolic activity is most efficient in cotton plants (Asha and LalAhamed, 2013). The concept of stress is intimately associated with that of stress tolerance, which is the plant's fitness to cope with an unfavorable environment. In the literature the term stress resistance is often used interchangeably with stress tolerance, although the latter term is preferred (Taiz and Zeiger, 2002). The effects of high temperature on germination, seedling growth, vegetative growth and crop development have been well documented in the literature (Hodges et al., 1993; Reddy et al., 1996; Oosterhuis and Snider, 2011). Cotton developmental events occur much more rapidly as maximum temperatures increase (Reddy et al., 1996). One of the most important and economic ways to overcome negative effects of heat stress is to identify and/or develop heat-tolerant cultivars (Singh et al., 2007). The development of heat-tolerant varieties is feasible and will help mitigate the effects of climate change (Azhar et al., 2009). The primary objective of this study was to develop heat tolerant cotton lines and varieties and determine general combining abilities and specific combining abilities in early generations for developing heat tolerant cotton cultivars.

## MATERIALS AND METHODS

The genetic material was developed by crossing six female/line, including DP 396, DP 90, DP 499, Stoneville 453, Stoneville 468 and Stoneville 474, with nine cotton varieties (male/tester) including SJ-U86, AGC 85, AGC 208, AGC 375, Fiber Max 819, Fiber Max 832, Fiber Max 958, Acala 1517-95 and Acala 1517-99 in a line x tester mating design. Six cotton genotypes were selected as female parents based on their agronomic and technological performance and nine cotton varieties were selected as male parents for heat tolerance. Fifteen cotton varieties were hand crossed using the line x tester mating design at the GAP International Agricultural Research and Training Center's experimental area in 2010. Fifteen cotton varieties and their 54 F<sub>1</sub> cross combinations were grown in the randomized complete block design with three replications at the same experimental area in 2011. Each genotype was planted as 6





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m long row, the distance between and within the rows spacing were 0.70 m and 0.15 m, respectively. Sowing was made with hand on 31 May 2011; all plots received 140 kg ha<sup>-1</sup> N and 60 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>. Half of the nitrogen and all phosphorus were applied at sowing time and the remaining N was given as ammonium nitrate (33%) at the square stage before the first irrigation. Recommended cultural practices such as insect and weed control methods were employed. During cotton growing period daily temperature data were recorded by data logger (Figure 1). The plots were harvested twice by hand for yield determination on 24 October 2011 and second on 24 November 2011 and the seed cotton yield was calculated based on the hand-harvest date. After ginning lint yields were calculated by multiplying seed cotton yield by lint percentage. In the study seed cotton yield, fiber yield, ginning percentage, chlorophyll content, photosynthetic active radiation (PAR), fluorescence and photosynthetic yield were investigated. Physiological measurements were determined during peak flowering stage, at this stage measurements were taken during mid-day (11:00 h and 13:00 h) on the fifth fully expanded leaf below the terminal with one reading per leaf of the plant according to Johnson and Sounders (2003). Photosynthetic yield (μmol CO<sub>2</sub> m<sup>-2</sup>s<sup>-1</sup>), photosynthetically active radiation (PAR) and fluorescence were measured using the EARS-PPM Plant Photosynthesis System. All of parameters were analyzed with the TarPopGen computer program which developed by Ozcan and Açıkgöz (1999) and differences were scrutinized for significance using LSD<sub>0.05</sub> test. The GCA variance effects of the parents and the SCA variance effects of the hybrids were estimated by the using of the line x tester analysis method described by Kempthorne (1957) and adopted by Sing and Chaudhary (1985). Regarding statistical analysis, the combined analysis of parents and crosses was done as suggested by Arunachalam (1974) and for combining ability analysis, the following model was used:

$$y_{ijk} = \mu + f_i + m_j + (mf)_{ij} + bk + e_{ijk}$$

where,  $y_{ijk}$  = value of the observation recorded on the (i x j) th cross in the k th replication;  $\mu$  is the general effect;  $f_i$  is the effect of the i th line;  $m_j$  is the effect of the j th tester;  $(mf)_{ij}$  is the specific combining ability (sca) effect of the (ixj) th cross;  $b_k$  is the k th block effect and  $e_{ijk}$  is the environmental effect associated with the ij k th observation which is assumed to be normally and independently distributed with a mean of zero and variance ( $\sigma^2$ ).

## RESULTS

The results for the analysis of variance revealed that genetic differences among the genotypes were highly significant ( $P \leq 0.01$ ) for seed cotton yield, ginning percentage, fiber yield, photosynthetic yield, chlorophyll content, fluorescence and photosynthetic active radiation (Table 1). This findings indicating the present of a considerable genetic variability between genotypes, hence subsequent analysis for combining ability was performed. The total genetic variability was partitioned to general combining ability and specific combining ability. Line x tester analysis showed that parents were statistically significant for seed cotton yield, ginning percentage and fiber yield and parents against hybrids were also significant for all the investigated traits except photosynthetic yield and photosynthetically active radiation (PAR). Hybrids were also significant at ( $P \leq 0.01$ ) probability level for all investigated traits. General Combining Ability (GCA) was highly significant for testers in terms of all investigated traits except PAR; revealing important role of additive type of gene effects in these traits, on the other hand there were non-significant differences for general combining ability (GCA) for lines. Specific Combining Ability (SCA) was highly significant for hybrids (line x testers) for all investigated traits revealing non-additive gene effects as dominance or epistatic.

From the Table 1, the variance due to GCA was lower than SCA for seed cotton yield, ginning percentage, fiber yield, photosynthetic yield, chlorophyll content and photosynthetically active radiation expressed non-additive gene action (dominance or epistatic) and GCA variance was higher than SCA variance only for fluorescence reflecting the role of additive type of gene action. The values of general combining ability of parents (lines and testers) are given in Table 2. From the Table 2, among the 15 parents, significant and positive general combining ability (GCA) effects for seed cotton yield were estimated for SJ-U86, AGC 375, Fiber Max 819, AGC 208 and STV 453; whereas negative GCA





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effects were estimated for Acala 1517-95, Acala 1517-99 and DP 90, respectively. Positive GCA effects for ginning percentage were predicted for Fiber Max 832 and Fiber Max 819, while negative GCA effects were predicted for Acala 1517-95, Fiber Max 958 and DP 90. Five of fifteen parents showed positive and significant GCA effects for fiber yield and positive GCA effects were estimated for SJ-U86, DP 90, AGC 375, Fiber Max 819 and STV 453; whereas negative GCA effects were estimated for Acala 1517-95 and Acala 1517-99. Only two parents (Fiber Max 832 and STV 453) showed significant and positive GCA effect for photosynthetic yield. Negative GCA effects for photosynthetic yield were observed for Acala 1517-95, DP 90 and Fiber Max 958. Parental lines AGC 375, SJU-86 and DP 396 were estimated to be best general combiners for chlorophyll content and Fiber Max 958 and Acala 1517-95 were estimated negative GCA effect for chlorophyll content. Positive GCA effects for fluorescence were estimated for two genotypes (Fiber Max 819 and STV 474) and negative GCA effects were observed for only one genotype (Acala 1517-95). Positive GCA effects for photosynthetically active radiation (PAR) were predicted for AGC 208 and DP 499, while negative GCA effects were observed for only one parent (Acala 1517-95). Parental varieties showed significantly differences in GCA effects, for example SJ-U 86 and AGC 375 showed positive effects for seed cotton yield, fiber yield and chlorophyll content, AGC 208 for seed cotton yield and photosynthetically active radiation, STV 453 for seed cotton yield, fiber yield and photosynthetic yield, STV 474 for fluorescence, DP 396 for chlorophyll content, DP 90 for fiber yield and DP 499 for photosynthetically active radiation (PAR).

Specific combining ability effects of 54 hybrids for seed cotton yield, ginning percentage, fiber yield and other physiological measurements are presented in Table 3. Based on the results of SCA effects for hybrids, DP 90 x SJ-U86 (2x7), DP 499 x AGC 85 (3x8), Stoneville 453 x Fiber Max 958 (4x13), Stoneville 453 x Acala 1517-99 (4x15), Stoneville 468 x Fiber Max 958 (5x13) and Stoneville 474 x Fiber Max 832 (6x12) for seed cotton yield; DP 90 x Fiber Max 832 (2x12), DP 499 x Fiber Max 958 (3x13), Stoneville 453 x Acala 1517-95 (4x14), Stoneville 474 x Acala 1517-99 (6x15) for ginning percentage and DP 90 x SJ-U86 (2x7), DP 499 x AGC 85 (3x8), DP 499 x FM 958 (3x13), Stoneville 453 x Fiber Max 958 (4x13), Stoneville 453 x Acala 1517-99 (4x15), Stoneville 468 x Fiber Max 958 (5x13) and Stoneville 474 x Fiber Max 832 (6x12) for fiber yield were found to be the best specific combinations. Significant and positive SCA effects for photosynthetic yield were observed in only one cross combination DP 396 x Acala 1517-99 (1x15) as this hybrid combination was found to be the best combination for photosynthesis, however negative SCA effects were observed for four cross combinations for photosynthesis. Significant and positive specific combining ability effects for chlorophyll content were observed in three crosses of the fifty-four cross combinations, DP 396 x Acala 1517-99 (1x15), DP 499 x FM 958 (3x13) and Stoneville 453 x Acala 1517-95 (4x14) cross combinations exhibited the greatest SCA effect and found to be the best desirable for this trait. Positive and significant SCA effects were observed for only two hybrids in terms of fluorescence and photosynthetically active radiation, Stoneville 468 x FM 958 (5x13) and STV 474 x Acala 1517-99 (6x15) hybrid combinations were observed to be best for fluorescence and DP 499 x AGC 85 (3x8) and Stoneville 468 x Acala 1517-99 (5x15) for PAR. The promising hybrid combinations and their SCA effects for all investigated characteristics can be seen in Table 4. The proportional contributions of lines, testers and their interactions (line x testers) to the total variance for investigated traits were presented in Table 5. It was shown that in the Table 5, maximum contributions to total variance of all characters were made by line x tester interactions and testers (male parents), on the other hand lines (female parents) had small contributions to all traits.

## DISCUSSION

In this study line x tester analysis method were used to determine the best hybrid combinations in early generations in terms of seed cotton yield, ginning percentage, fiber yield, photosynthetic yield, chlorophyll content, fluorescence and photosynthetically active radiation. Variance component analysis indicated that all traits except fluorescence exhibited non-additive gene action effects (dominance or epistatic). Sudhanshu (1997) observed non-additive gene action for seed cotton yield and yield related characters. Zhang et al., 1994, indicated that seed cotton yield was controlled by additive major genes. Panchal et al (1995) observed additive gene effects for lint percentage and non-additive gene effects for seed cotton yields, following line x tester analysis of *Gossypium barbadense* varieties. Walarmathi and Jehangir., 1998 and Rajan et al., 1999 have reported additive genetic effect for seed cotton yield. Some



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other researchers reported the involvement of both additive and non-additive variances for these traits (Kumerasan et al., 1999; Liu and Han., 1998). According to the results of this study generally, GCA variance was lower than SCA variance; therefore selection in advanced generations may be more appropriate for these traits under non-additive genetic effects. Among the cotton varieties used as parents, five parents (SJ-U86, AGC 375, Fiber Max 819, AGC 208 and Stv 453) showed significant positive GCA effects for seed cotton yield, two parental lines (Fiber Max 832 and Fiber Max 819) showed significant and positive GCA effects for ginning percentage and five parents (SJ-U86, DP 90, AGC 375, Fiber Max 819 and Stv 453) showed positive GCA effects for fiber yield and these cotton cultivars were predicted to be best general combiner for these traits. On the other hand, for photosynthetic yield only two parents (Fiber Max 832 and Stv 453) showed significant and positive GCA effects. Azhar et al., 2005 studied to improve heat tolerance they revealed that importance of non-additive gene effects controlling heat. However, some researcher reported that genetic variability included both additive and non-additive components, but proportion of additive genetic variability was high for heat tolerance index (Rahman, 2006). ESTs genes could play a significant role in heat tolerance (Demirel et al., 2014). Rana et al., 2011 suggested that use of chlorophyll content for choosing the best single parents for stress breeding and they reported a decreased chlorophyll ratio in crop plants under drastic factor conditions shows increased resistance to heat and vice versa. AGC 375, SJ-U86 and DP 396 were observed to be best combiner for chlorophyll content, and only two parents exhibited positive GCA effects for fluorescence (Fiber Max 819 and Stv, 474).

For photosynthetically active radiation (PAR) only two parents (AGC 208 and DP 499) exhibited positive and significant GCA effects. Earlier studies revealed that two major genes controlling net-photosynthesis rate with close additive and dominance effects and net photosynthesis rate controlled by polygenes (Wang et al., 2013). In the present study, only one hybrid combination (Deltapine 396 x Acala 1517-99) exhibited positive SCA effect for photosynthetic yield. Pettigrew et al. (1993) suggested that photosynthetic rate could be a selection criterion for plant breeders. Although Fiber Max 832 and Stv 453 cotton varieties were best general combiner for photosynthetic rate, any hybrids were not obtained from cross combinations. For this trait only one reading was performed from one leave because of time consuming. This situation may be related to the one reading, the age of leave, plant growing stage or the duration of measurement. Snider et al., 2014 indicated that even under optimum temperature conditions and water availability, heat tolerance could be influenced by plant developmental stage. Similar results were reported by Zu et al., 2008, they reported that photosynthesis is affected not only by genotype, but also by environmental factors such as light intensity and quality, moisture and temperature. Mathur et al., 2014 revealed that under high temperature conditions, plants exhibit short-term avoidance or acclimation mechanisms such as changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions.

**CONCLUSION**

The objective of this study was to assess the effect of high temperature stress on cotton yield and physiological parameters and also determine the best hybrid combinations for heat tolerance. For this aiming 54 hybrid combinations were screened for yield and physiological parameters, and six hybrid combinations were found to be best for seed cotton yield, four hybrid combinations were found to be best for ginning percentage and seven hybrid combinations were found to be best for fiber yield and only one hybrid combination exhibited positive SCA effect for photosynthetic yield. The results of this study indicated that presence of non-additive gene action suggested that selection in advanced generations may be more appropriate.

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## REFERENCES

1. Arunachalam, V. 1974. The fallacy behind the use of modified lint tester design. Indian Journal of Genet. and Plant Breed. 34:280- 287.
2. Asha, R., Lal Ahmed, M., 2013. Screening of Cotton Genotypes for Heat Tolerance. International J. of Bio-resource and Stress Manag. 4: 599-604.
3. Azhar, M. T., Khan, A. A., Khan, I. A., 2005. Combining Ability Analysis of Heat Tolerance in *Gossypium hirsutum* L. Czech J. Genet. Plant Breed. 41: 23-28.
4. Azhar, F. M., Ali, Z., Akhtar, M. M., Khan, A. A., Trethowan, R., 2009. Genetic variability of heat tolerance, and its effect on yield and fibre quality traits in upland cotton (*Gossypium hirsutum* L.). *Plant Breeding*, 128, 4: 356-362.
5. Bibi, A. C., Oosterhuis, D. M., Gonias, E. D., 2008. Photosynthesis, quantum yield of photosystem II and membrane leakage as affected by high temperatures in cotton genotypes. The Jour. of Cotton Sci. 12: 150-159.
6. Burke, J. J., Mahan, J. R., Hatfield, J. L., 1988. Crop-specific thermal kinetic windows in relation to wheat and cotton biomass production. Agron. Journal 80: 553-556.
7. Burke, J. J., Chen, J., 2015. Enhancement of Reproductive Heat Tolerance in Plants. PLoS One 10: e0122933. doi: [10.1371/journal.pone.0122933](https://doi.org/10.1371/journal.pone.0122933)
8. Demirel, U., Gür, A., Can, N., 2014. Identification of heat responsive genes in cotton. *Biologia Plantarum*. 58: 515-523.
9. Cottee, N. S., Tan, D. K. Y., Bange, M. P., Cothren, J. T., Campbell, L. C., 2010. Multi-level determination of heat tolerance in cotton (*Gossypium hirsutum* L.) under field conditions. *Crop Sci*. 50: 2553-2564.
10. Hodges, H. F., Reddy, K. R., McKinion, J. M., Reddy, V. R., 1993. Temperature effects on cotton. Mississippi Agr. & Forestry Exp. Sta., Mississippi State University. 1-15.
11. IPCC, 2007. Intergovernmental Panel on Climate Change. Impacts, Adaptation and Vulnerability. [http://www.meteotrentino.it/clima/pdf/rapporti\\_meteo/IPCC\\_Impacts\\_Adaptation\\_and\\_Vulnerability.pdf](http://www.meteotrentino.it/clima/pdf/rapporti_meteo/IPCC_Impacts_Adaptation_and_Vulnerability.pdf).
12. Johnson, J. R., Saunders, J. R., 2003. Evaluation of Chlorophyll Meter for Nitrogen Management in Cotton. <http://msucare.com/nmrec/reports/2002/>
13. Kakani, V. G., Reddy, K. R., Koti, S., Wallace, T. P., Prasad, V. V. V., Reddy, V. R., Zhao, D., 2005. Differences in in vitro pollen germination and pollen tube growth of cotton cultivars in response to high temperature. *Annals of Botany*. 96: 59-67.
14. Kempthorne, O., 1957. An Introduction to Genetic Statistics. John Wiley and Sons. Inc., New York. U.S.A.
15. Kumerasan, D., Senthilkumar, P., Ganesan, J., 1999. Combining ability studies for quantitative traits in cotton (*Gossypium hirsutum* L.). *Crop Res. Hisar*. 18: 430-432.
16. Lee, J., Six, J., 2010. Effect of climate change on field crop production and greenhouse gas emissions in California's Central Valley. 19<sup>th</sup> World Congress of Soil Science, Soil Solutions for a Changing World. 1-6 August, Brisbane, Australia, 52-55.
17. Liu, Y. X., Han, X. M., 1998. Research on the combining ability and inheritance of 12 economic characters in upland cotton. *China Cottons*. 25: 9-11.
18. Liu, Z., Yuan, Y. L., Liu, S. Q., Yu, X. N., Rao, L. Q., 2006. Screening for high temperature tolerant cotton cultivars by testing in vitro pollen germination, pollen tube growth and boll retention. *J. Integrat Plant Biol*. 48: 706-714.
19. Mathur, S., Agrawal, D., Jajoo, A., 2014. Photosynthesis: Response to high temperature stress. *J. of Photochemistry and Photobiology*. 137: 116-126.
20. Oosterhuis, D. M., 2002. Day or night high temperature: A major cause of yield variability. *Cotton Grower* 46: 8-9.
21. Oosterhuis, D. M., Pretorius, M. M., Loka, D. A., FitzSimons, T. R., 2011. High Temperature Tolerance in Cotton. AAES Research Series 602: 53-56. <http://arkansasagnews.uark.edu/602-9.pdf>
22. Oosterhuis, M. D., Snider, J. L., 2011. High Temperature Stress on Floral Development and Yield of Cotton. [http://www.cotton.org/foundation/upload/Stress-Physiology-in-Cotton\\_Chapter\\_1.pdf](http://www.cotton.org/foundation/upload/Stress-Physiology-in-Cotton_Chapter_1.pdf)
23. Oosterhuis, D. M., 2013. Global Warming and Cotton Productivity. ICAC 72<sup>nd</sup> Plenary Meeting, September 29-October 4, p 1-13. Cartagena, Colombia.





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24. Ozcan, K., Açıkgöz, N., 1999. A Statistical Program for Population Genetics. 3. The Symposium of Computer Applications for Agric. Cukurova Univ. Adana-Turkey.
25. Rajan, G. A. S., Kandasamy, G., Vanniarajan, C., 1999. Combining ability studies for quality characters in American cotton. *Crop Res Hisar*. 18: 273-277.
26. Panchal, S. S., Patel, J. A., Saiyad, M. R., Patel, S. B., Patel, S. A., 1995. Line x tester analysis of combining ability in Egyptian cotton (*Gossypium barbadense*). *Gujarat Agric. Univ. Res J.* 20: 58-63.
27. Pettigrew, W. T., Heitholt, J. J., Vaughn, K. C., 1993. Gas exchange and comparative anatomy among cotton leaf-type isolines. *Crop Sci.* 33: 1295-1299.
28. Rahman, H., 2006. Environmental interaction, additive and non-additive genetic variability is involved in the expression of tissue and whole-plant tolerance in upland cotton (*Gossypium hirsutum* L.). *Genetics and Molecular Biology*. 29: 525-532.
29. Rana, R. M., Khan, S. H., Ali, Z., Khan, A. I., Khan, I. A., 2011. Elucidation of thermotolerance diversity in cotton (*Gossypium hirsutum* L.) using physio-molecular approaches. *Genetics and Molecular Res.* 10: 1156-1167.
30. Reddy, V. R., Baker, D. N., Hodges, H. F., 1991. Temperature effect on cotton canopy growth, photosynthesis and respiration. *Agron. J.* 83: 699-704.
31. Reddy, V. R., Hodges, H. F., McCarty, W. H., McKnight, J. M., 1996. Weather and cotton growth: Present and Future. *Mississippi Agr. & Forestry Exp. Sta., Mississippi State Univ. Starkeville, Miss.*
32. Reddy, R. K., Hodges, H. F., 2007. Exploring the Limitations for Cotton Growth and Yield. *J. of New Seeds*. 8: 1-22.
33. Rodriguez-Garay, B., Barrow, J. R., 1988. Pollen selection for heat tolerance in cotton. *Crop Sci.* 28: 857-859.
34. Singh, R. K., Chaudhary, B. D., 1985. Line x Tester Analysis. *Biometrical Methods in Quantitative Genetic Analysis*. Kalyani Publishers New Delhi, India.
35. Singh, R. P., Prasad, P. V. V., Sunita, K., Giri, S. N., Reddy, K. R., 2007. Influence of high temperature and breeding for heat tolerance in cotton: a review. *Advances in Agron.* 93: 313-385.
36. Snider, J. L., Oosterhuis, D. M., Skulman, B. W., Kawakami, E. M., 2009. Heat stress-induced limitation to reproductive success in *Gossypium hirsutum*. *Physiologia Plant.* 137: 125-138.
37. Snider, J. L., Chastain, D. R., Collins, G. D., 2014. Field-grown Cotton Exhibits Seasonal Variation in Photosynthetic Heat Tolerance without Exposure to Heat-stress or Water-deficit Conditions. *J. of Agron. and Crop Sci.* DOI: 10.1111/jac.12113.
38. Song, G., Wang, M., Zeng, B., Zhang, J., Jiang, C., Hu, Q., Geng, G., Tang, C., 2015. Anther response to high-temperature stress during development and pollen thermotolerance heterosis as revealed by pollen tube growth and in vitro pollen vigor analysis in upland cotton. *Planta*. 241: 1271-85.
39. Sudhanshu, J., 1997. Studies on combining ability in intra and interspecific crosses of cotton. *Crop Res. Hisar*. 14: 91-95.
40. Taiz, L., Zeiger, E., 2002. *Plant Physiology*. 3rd ed. USA.
41. Walarmathi, M., Jehangir, K. S., 1998. Line x tester analysis for combining ability in *Gossypium hirsutum* cotton. *Madras-Agric. J.* 85: 103-105.
42. Wang, Y., Shuang, Y., Irfan, M., Zhang, C., Sun, Q., Wu, S., Lin, F., 2013. Genetic analysis of carbon metabolism-related traits in maize using mixed major and polygenic models. *Australian J. of Crop Sci.* 7: 1205-1211.
43. Zhang, J. F., Deng, Z., Sun, J. Z., Liu, J. L., 1994. Heterosis and combining ability in interspecific crosses between *Gossypium hirsutum* and *Gossypium barbadense*. *J. Huazhong Agric. Univ.* 13: 9-14.
44. Zhu, W., Liu, K., Wang, X. D., 2008. Heterosis in yield, fiber quality, and photosynthesis of okra leaf-oriented hybrid cotton (*Gossypium hirsutum* L.). *Euphytica*. 164: 283-291.





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**Table 1. Analysis of variance for genotypes and combining ability for investigated traits in line x tester analysis method.**

Source of Variation	DF	Seed Cotton Yield	Ginning Percentage	Fiber Yield	Photosynthetic Yield	Chlorophyll Content	Fluorescence	Photosynthetic Active Radiation (PAR)
Replication	2	248422.01**	90.14**	34389.21**	879.59*	153.00*	6293918.93**	2114.10
Genotypes	68	28446.13**	30.18**	3999.77**	522.94**	115.96**	2200695.61**	4379.86**
Parents	14	10564.57*	16.38**	1456.71*	119.32	59.05	571324.54	2658.07
Parents vs Hybrids	1	120378.95**	446.70**	14595.57**	659.04	902.28**	5198426.45*	616.13
Hybrids	53	31434.99**	25.97**	4471.60**	626.99**	116.15**	2574534.55**	4905.69**
GCA (Lines)	5	23476.37	26.45	4118.26	733.80	109.80	3087314.07	4876.11
GCA (Testers)	8	90155.65**	63.43**	12611.66**	1290.14*	220.37**	565861.23*	7495.10
SCA (Line x Tester)	40	20685.68**	18.42**	2887.75**	481.01**	96.10**	1893671.78*	4391.50**
Error	136	5146.15	7.08	724.41	238.31	46.97	1275286.68	2348.02
$\sigma^2$ (GCA)		3167.42	2.20	454.75	43.02	6.82	206772.17	62.86
$\sigma^2$ (SCA)		5179.84	3.78	721.11	80.90	16.38	206128.37	681.16
$\sigma^2$ GCA/ $\sigma^2$ SCA		0.61	0.58	0.63	0.53	0.42	1.00	0.09

\* and \*\*, Significantly different from zero at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.  $\sigma^2$ GCA: Variance of general combining ability,  $\sigma^2$ SCA: variance of specific combining ability, DF: Degrees of freedom, SE: Standard error.

**Table 2. Predicted general combining ability effects (GCA) of parents for investigated traits.**

Parents	SCY	GP	FY	PY	CC	FLR	PAR
<b>Line (Female)</b>							
1. DP 396	-0.93	-0.43	0.11	-2.89	3.26*	-170.40	-9.04
2. DP 90	-47.57**	-1.78**	22.08**	-8.22**	-0.04	-230.70	-6.84
3. DP 499	5.33	0.34	4.89	0.00	-2.15	-395.26	19.79*
4. STV 453	37.99**	0.21	12.24*	7.15*	1.31	196.81	13.27
5. STV 468	19.88	0.78	9.11	2.69	-0.68	61.53	-3.15
6. STV 474	-14.70	0.88	-4.26	1.28	-1.69	538.68*	-14.03
SE $\pm$	13.80	0.51	5.18	2.97	1.31	217.33	9.32
<b>Tester (Male)</b>							
7. SJ-U86	97.02**	0.53	36.16**	1.04	3.31*	98.05	4.38
8. AGC 85	8.07	1.07	3.21	-1.30	1.30	318.11	8.63
9. AGC 208	40.09*	-0.41	11.55	3.59	2.84	242.00	23.63*
10. AGC 375	42.13*	1.22	17.17**	4.43	3.43*	116.33	-5.23
11. Fiber Max 819	41.93*	1.43*	16.19*	4.54	-0.58	601.88*	4.83
12. Fiber Max 832	-1.49	1.91**	0.33	8.51*	0.95	517.25	14.30
13. Fiber Max 958	-22.37	-1.82**	-7.56	-7.91*	-4.75**	-358.77	-11.51
14. Acala 1517-95	-146.60**	-4.02**	-55.45**	-18.69*	-6.44**	-1212.11**	-47.17**
15. Acala 1517-99	-58.80**	0.09	-21.59**	5.79	-0.05	-322.75	8.13
SE $\pm$	16.90	0.62	6.34	3.63	1.61	266.17	11.42

\* and \*\*, Significantly different from zero at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively. SE: Standard error, SCY: Seed Cotton Yield, GP: Ginning Percentage, FY: Fiber Yield, PY: Photosynthetic Yield, FLR: Fluorescence, PAR: Photosynthetic Active Radiation







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Table 3. Predicted specific combining ability (SCA) of 54 hybrids for investigated traits

Cross Combination	SCY	GP	FY	PY	CC	FLR	PAR
DP 396 x SJ-U86	-16.07	1.13	-3.82	-8.67	3.40	581.90	-19.40
DP 396 x AGC 85	3.64	1.57	4.91	16.67	-4.08	-101.81	-27.99
DP 396 x AGC 208	5.55	1.28	4.37	1.78	1.07	-476.70	6.52
DP 396 x AGC 375	49.15	0.15	18.78	-1.06	5.05	274.29	6.21
DP 396 x FM 819	70.77	-0.42	26.44	-22.83*	2.99	634.74	-9.18
DP 396 x FM 832	34.43	-1.21	9.79	1.53	-1.97	775.38	18.68
DP 396 x FM 958	-136.38**	-2.24	-53.14**	-12.72	-8.13*	-835.92	-29.85
DP 396 x ACALA 1517-95	-13.15	-1.38	-5.92	-1.28	-7.10	18.07	5.49
DP 396 x ACALA 1517-99	2.06	1.12	-1.41	26.58**	8.77*	-869.95	49.52
DP 90 x SJ-U86	171.45**	-0.55	59.93**	9.67	3.73	493.20	42.06
DP 90 x AGC 85	-56.66	2.71	-19.78	-6.33	1.61	200.48	-37.86
DP 90 x AGC 208	56.16	-0.62	16.75	5.11	4.84	-24.40	32.15
DP 90 x AGC 375	-53.42	-2.53	-30.81*	11.61	4.51	377.92	23.34
DP 90 x FM 819	50.75	0.42	20.43	8.17	-0.43	-240.96	15.95
DP 90 x FM 832	-51.07	3.61*	-15.92	-4.81	3.56	702.34	4.48
DP 90 x FM 958	-91.40*	-1.22	-30.95	-6.72	-4.82	-774.96	-32.05
DP 90 x ACALA 1517-95	30.16	1.31	17.27	4.39	-3.47	78.03	3.28
DP 90 x ACALA 1517-99	-55.97	-3.13*	-16.93	-21.08*	-9.52*	-811.65	-51.36
DP 499 x SJ-U86	-72.72	0.42	-28.18	8.11	1.04	-163.90	22.10
DP 499 x AGC 85	85.28*	-0.12	32.31*	6.78	-0.30	274.03	70.52*
DP 499 x AGC 208	21.51	1.60	12.17	2.56	0.32	488.81	-16.82
DP 499 x AGC 375	-49.58	2.26	-15.75	-4.94	-1.30	-335.18	-17.62
DP 499 x FM 819	-8.90	-0.57	-4.94	0.94	-2.95	-637.07	-32.68
DP 499 x FM 832	72.22	-1.10	25.48	5.31	3.44	132.56	41.52
DP 499 x FM 958	80.79	3.92*	33.17*	14.06	8.85*	644.25	33.65
DP 499 x ACALA 1517-95	-19.74	-0.82	-10.03	-4.17	-1.02	242.92	-22.68
DP 499 x ACALA 1517-99	-108.87*	-5.59**	-44.23**	-28.64**	-8.07*	-646.43	-77.99**
STV 453 x SJ-U86	-34.36	-1.40	-14.44	-4.70	0.34	-456.98	-18.05
STV 453 x AGC 85	-32.31	-1.48	-13.15	-15.70	-5.27	-164.70	-15.97
STV 453 x AGC 208	-92.43*	-0.75	-34.69*	2.74	-5.88	-177.92	10.70
STV 453 x AGC 375	33.63	-0.13	17.00	4.91	1.13	-190.25	-26.11
STV 453 x FM 819	-82.44	-0.52	-31.28*	-3.54	-1.95	211.85	31.51
STV 453 x FM 832	-100.92*	-0.47	-36.53*	-6.84	-10.35*	-1164.50	21.37
STV 453 x FM 958	147.34**	1.33	54.34**	3.57	3.15	684.51	15.17
STV 453 x ACALA 1517-95	36.05	4.49**	14.17	14.02	15.25**	566.18	2.51
STV 453 x ACALA 1517-99	125.43**	-1.06	44.58**	5.55	3.58	691.82	-21.13
STV 468 x SJ-U86	-69.82	0.97	-22.41	-1.91	-4.01	-587.70	-39.29
STV 468 x AGC 85	-11.42	-1.43	-6.96	12.09	1.19	-1420.09*	-2.21
STV 468 x AGC 208	-20.82	-1.07	-10.18	-18.13*	-0.24	-8.98	-37.21





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STV 468 x AGC 375	33.49	-0.24	14.09	-14.30	-7.70	43.01	-35.68
STV 468 x FM 819	79.72	-1.58	29.45	5.26	3.27	641.13	29.27
STV 468 x FM 832	-83.81*	1.86	-28.16	2.79	3.31	47.60	-51.04
STV 468 x FM 958	122.59**	1.77	45.01**	17.70	4.12	1826.13**	37.60
STV 468 x ACALA 1517-95	-33.96	-1.59	-14.60	-7.19	-2.15	-213.87	0.60
STV 468 x ACALA 1517-99	-15.98	1.31	-6.25	3.68	2.22	-327.23	97.96**
STV 474 x SJ-U86	21.51	-0.56	8.93	-2.50	-4.51	133.48	12.58
STV 474 x AGC 85	11.46	-1.25	2.66	-13.50	6.86	1212.09	13.50
STV 474 x AGC 208	30.03	-0.43	11.58	5.94	-0.10	199.20	4.66
STV 474 x AGC 375	-13.28	0.49	-3.31	3.78	-1.69	-169.80	49.86
STV 474 x FM 819	-109.90**	2.67	-40.10*	12.00	-0.92	-609.68	-34.86
STV 474 x FM 832	129.15**	-2.69	45.33**	2.03	2.01	-493.38	-35.00
STV 474 x FM 958	-122.94**	-3.55*	-48.43**	-15.89	-3.17	-1544.01*	-24.53
STV 474 x ACALA 1417-95	0.63	-2.02	-0.88	-5.78	-1.49	-691.35	10.80
STV 474 x ACALA 1517-99	53.33	7.35**	24.24	13.92	3.02	1963.45**	3.00
SE ±	41.41	1.53	15.33	8.91	3.95	651.99	27.97

SE: Standard error, \* and \*\*, Significantly different from zero at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively. SCY: Seed Cotton Yield, GP: Ginning Percentage, FY: Fiber Yield, PY: Photosynthetic Yield, FLR: Fluorescence, PAR: Photosynthetic Active Radiation

**Table 4. Promising hybrid combinations for investigated traits and their specific combining abilities.**

Traits	Cross Code	Cross Combinations	Specific Combining Ability
Seed Cotton Yield	2x7	DP 90 x SJU-86	171.45**
	3x8	DP 499 x AGC 85	85.28*
	4x13	Stoneville 453 x Fiber Max 958	147.34**
	4x15	Stoneville 453 x Acala 1517-99	125.43**
	5x13	Stoneville 468 x Fiber Max 958	122.59*
	6x12	Stoneville 474 x Fiber Max 832	129.15**
Ginning Percentage	2x12	DP 90 x Fiber Max 832	3.61*
	3x13	DP 499 x Fiber Max 958	3.92*
	4x14	Stoneville 453 x Acala 1517-95	4.49**
	6x15	Stoneville 474 x Acala 1517-99	7.35**
Fiber Yield	2x7	DP 90 x SJ-U86	59.93**
	3x8	DP 499 x AGC-85	32.31*
	3x13	DP 499 x FM 958	33.17*
	4x13	Stoneville 453 x Fiber Max 958	54.34**
	4x15	Stoneville 453 x Acala 1517-99	44.58**
	5x13	Stoneville 468 x Fiber Max 958	45.01**
	6x12	Stoneville 474 x Fiber Max 832	45.33**
Photosynthetic Yield	1x15	DP 396 x Acala 1517-99	26.58**
Chlorophyll Content	1x15	DP 396 x Acala 1517-99	8.77*





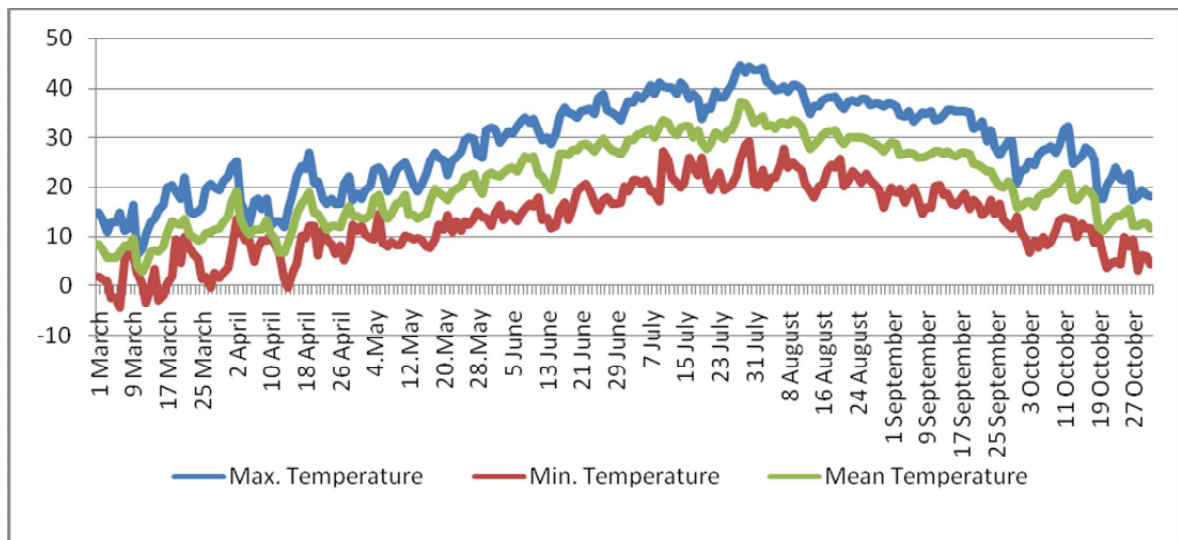
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	3x13	DP 499 x Fiber Max 958	8.85*
	4x14	Stoneville 453 x Acala 1517-95	15.25**
Fluorescence	5x13	Stoneville 468 x Fiber Max 958	1826.13**
	6x15	Stoneville 474 x Acala 1517-99	1963.45**
Photosynthetically Active Radiation (PAR)	3x8	DP 499 x AGC 85	70.52*
	5x15	Stoneville 468 x Acala 1517-99	97.96**

\* and \*\*, Significantly different from zero at  $P \leq 0.05$  and  $P \leq 0.01$ , respectively.

**Table 5. Proportional contributions of lines, testers and their interactions to total variance for investigated traits.**

Source of variation	SCY	GP	FY	PY	CC	FL	PAR
Contribution of Lines	7.04	9.60	8.68	11.04	8.91	11.31	9.37
Contribution of Testers	43.29	36.86	42.57	31.05	28.63	33.17	23.06
Contribution of Lines x Testers	49.66	53.52	48.74	57.90	62.44	55.51	67.56



**Figure 1. Daily average, maximum and minimum temperature (°C) during x periment.**





## Rainfall is an Initiating Factor and Food Availability is a Conserving Factor of Reproductive Season in the Indian Flying Fox, *Pteropus giganteus*

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### ABSTRACT

Bats are slow breeding, mostly with one or two offspring a year hence every reproductive season is very important for their successful reproduction. The reproduction of *P. giganteus* has been understood as mostly influenced by either rainfall or ambient temperature or food availability while studying them directly under natural conditions. We studied the influence of ambient temperature, food availability and rainfall on the reproductive timing in *P. giganteus*. Mating in *P. giganteus* starts from July; mean time the monthly rainfall starts at low level and attains the peak in November, simultaneously most number of mating was observed. Fruit availability was low at the time of reproductive season; however, after parturition, more number of young ones was observed with respect to the peak of fruits availability. Our result suggests that among the exogenous factors rainfall and food availability play predominant role in influencing the reproduction of *P. giganteus*.

**Keywords:** bat, reproduction, climatic factors, fruits, survival, young one



**Maruthupandian Jeyabalan et al.****INTRODUCTION**

Reproduction and fertility in tropical mammals are known to be influenced by environmental factors such as photoperiod, temperature, rainfall, food availability and other seasonal parameters like humidity and cyclone seasons (O'Brien, 1996). Bats are reported to have long gestation period and are unable to react quickly to short-term environmental fluctuations, but may cue into predictable seasonal changes and optimize their time of reproduction (Heideman, 1995). It would be advantageous to give birth during the peak availability of food (Clutton-Brock *et al.*, 1982) but in bats, where earlier birth affects are reported as affecting juvenile survival and fitness in greater horseshoe bats (Ransome, 1989). Bats reproduction, particularly lactation is energetically costly (Loudon and Racey, 1987) it appears that food availability is an important factor in determining the ability of females to allocate energy to reproduction (Thompson, 1992). However, wherever food supplies vary noticeably, animals may only be able to meet the costs of reproduction during periods of maximum food availability and the success of a species may reflect its ability to produce offspring at a propitious time (Clarke, 1981). The information on the timing of reproduction in *P. giganteus* and the role of exogenous factors involved in the breeding season is still scanty, hence this study was made on factors that are most likely influence the breeding season in *P. giganteus*.

**MATERIALS AND METHODS**

The study was conducted from July to July (2010–2011; 13 months) near the Nallachchampatti village (77°48'59.83"E; 10°3'10.79"N), located approximately 32 km west of MKU campus. A colony of about 420 individuals of *P. giganteus* occupied a single *Ficus religiosa* tree (Family: Moraceae) situated adjacent to private agricultural land. A total of 57 acts of copulation occurred during the rest phase of 07:00–09:30 h between July and January. During this study we visited the colony monthly twice and phenology of plants and fruits source availability around the area was observed regularly. These observations were made using binoculars (Balileo 20 × 50 Gross feld) from 02:30 h (day 1) to 23:30 h (day 2) that yielded a total of 1170 hour observations. In addition, their copulatory behaviours were videographed (Sony DCR-SR47) and photographed (Nikon D3000). In order to study the effect of environmental factors such as rainfall, temperature and humidity on reproduction of *P. giganteus*, they were recorded using digital Hygro-thermometer and rainfall data was obtained from India Meteorological Department (Hydromet Division) Madurai, Tamil Nadu. A significant correlation was observed between the food availability before and after breeding season with that of the presence of young ones of *P. giganteus* in the colony.

**RESULTS**

During the breeding season, we have observed availability of various food sources around the study site. The breeding season has started from July, before breeding season (Table 1) *Achras sapota* (Sapotaceae), *Carica papaya* (Caricaceae), *Psidium guajava* (Myrtaceae) and *Ficus benghalensis* (Moraceae) were in their fruiting stage and bats were provided with surplus fruits and have extracted the juices from leaves as well (Fig. 1). The reproductive peak (Fig. 2) was observed during October to December 2010 and during that peak *P. giganteus* was observed to prefer leaves of *F. religiosa* till February 2011 (Table 1 & Fig. 1). *Ceiba pentandra* (Bombacaceae) was also available with its nectar resources at the end of mating season (January and February). After the breeding season these bats fed fruits from *Mangifera indica* (Anacardiaceae), *F. religiosa*, *A. sapota*, *P. guajava* and *C. papaya* as they were available tremendously (Table 1 & Fig. 6). Mating started initially (Fig. 2) from July (n = 5), and has peaked from September (n = 8) and continued through October (n = 10), November (n = 11) and December (n = 9), subsequently peak has declined by January (n = 3) and again mating resumed from July (n = 5). We correlate the monthly rainfall with the number of mating occasions. In fact, more number of mating incidents were observed on November (n = 11), mean time the monthly rainfall (mm) level was also high. Similarly with decrease in monthly rainfall (mm) level number of mating incidents was also low (Fig. 3). In the observations scheduled from July to July (Fig 4.) temperature and humidity was interlinked with mating. It shows that during the peak of mating season the temperature level



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( $68.5 \pm 2.2\%$ ;  $n = 26$ ) was low and humidity level ( $28.7 \pm 0.7^\circ\text{C}$ ;  $n = 26$ ) was high when compared to other months. Fig. 5 (a, b, c, d) shows the relationship between the environmental factors namely rainfall, temperature and humidity with mating occasions of *P. giganteus*. It is evident that more mating incidents incurred at the time of more rainfall, high humidity and low temperature.

**DISCUSSION**

Environmental factors are also known to influence reproduction in mammals including food availability, variety of social cues, the day/night cycle, temperature, humidity and rainfall (Bronson, 1985). In our study also these environmental factors play some crucial roles in determining the breeding season of *P. giganteus*. In the environments, rainfall seems to be the most important factor affecting bat reproductive cycles, by acting as a direct trigger of reproductive activity or by its indirect effect on the availability of food sources (Heideman, 1995, 2000); (Mello et al., 2004). In this study we also observed that *P. giganteus* mating starting from the month of July ( $n = 6$ ) mean time the monthly rainfall (mm) was at low level and attains the peak in November, and by the same time number of mating were also observed in peak ( $n = 11$ ), which highlights the coincidences of rainfall peak in November. This result clearly confirms that rainfall is one of the important environmental factors that trigger the reproduction of *P. giganteus*. Ambient temperature is well known to influence reproductive pattern of bats in the temperate zone (Crichton and Krutzsch, 2000) it may also be more important in the tropics (Mello et al., 2004). Most of mating incidences were observed in the morning time between 07:00 and 09:30 h from July to July schedule, and the mating peak was observed between 07:00 and 08:30 h. These results clearly indicate that early morning time was the preferred time of mating, may be due to the favourable temperature prevailing within, which is one of the factors that determine the mating stimulation. Ambient temperature in tropics may be a stronger selection pressure than food availability in determining reproductive timing of bats (Mello et al., 2009). We correlated the number of mating occasions with rainfall, temperature and humidity and it shows that mating was at peak when high level of rainfall prevailed, with high humidity and low level of temperature. This result clearly indicates that environmental factors play an important role in the breeding season of *P. giganteus*. Favorable conditions with enhanced food availability has the positive effect on the reproduction as it is energetically costly, where in mammals lactation period is energy demanding period upon which each lactating females could demand energy by 66–133 percent more (Migula, 1967); (Randolph et al., 1977). We observed that *P. giganteus* give birth to young ones during March to July, at the same time fruits availability has attained the peak with maximum availability to young ones. This result clearly indicates that parturition in *P. giganteus* and fruit availability coincides exactly. Fruits are generally rich in carbohydrates although they are typically low in fats and proteins (Corlett, 1996); (Herrera 1987); (Martinez et al., 1993); (Mattson, 1980) leaves of several plant species are rich in protein (Kunz and Diaz 1995); (Telek and Martin, 1983) which is supposed to provide an important source of nitrogen for plant-visiting bats. We have earlier reported that *P. giganteus* mostly preferred *F. religiosa* for roosting; hence, during breeding season bats in the colony chewed the leaves of *F. religiosa* occasionally and extracted the juices from the leaves. Calcium is supposed to be necessary for reproduction in animals (Currey 1984, 1990); (Robbins, 1993) (Sadlier, 1969) for maintaining normal cellular neuromuscular, enhance the metabolic activities and skeletal functions. We assume this as an energy supplementing posture due to the need of nutrients for mating or lactation as many researchers have previously reported that birth and lactation were energetically costly. Hence we suggest that the *P. giganteus* prefer nutritious food followed by parturition for the development of young ones and their by increases its survival rate. Furthermore, our key findings clearly confirm that food source plays role as conserving factor for successful breeding in *P. giganteus*.

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## REFERENCES

1. Bronson F.H. Mammalian reproduction: An ecological perspective. *Biology of Reproduction*1985; 32: 1–26.
2. Clarke J.R. Physiological problems of seasonal breeding *in* eutherian mammals. Ed. C.A. Finn. Clarendon press: Oxford. *Oxford Reviews of Reproductive Biology*1981; 3: 244–312.
3. Clutton-Brock T.H, Guinness F.E and Albon S.D. Red deer: Behaviour and Ecology of Two Sexes1982; University of Chicago Press, Chicago.
4. Corlett R.T. Characteristics of vertebrate-dispersed fruits in Hong Kong. *Journal of Tropical Ecology*1996; 12: 819–833.
5. Crichton E.G, Krutzsch P.H Reproductive biology of bats 2000. Academic Press. London.
6. Currey J.D. Physical characteristics affecting the tensile failure properties of compact bone 1990; *Journal of Biomechanics* 23: 837–844.
7. Currey J.D. *The Mechanical Adaptations of Bones*. Princeton University Press, Princeton, New Jersey1984.
8. Heideman P.D. Synchrony and seasonality of reproduction in tropical bats. *Symp Zool Soc Lond*1995; 67: 151–165.
9. Heideman P.D. Environmental regulation of reproduction, *In: Reproductive Biology of Bats*, (Crichton, E.G and Krutzsch, P.H eds). New York. 2000; 469–499 pp.
10. Herrera C.M. Vertebrate-dispersed plants of the Iberian Peninsula, as study of fruit characteristics. *Ecological Monographs*1987; 57: 305–331.
11. Kunz T.H, Diaz C.A. Folivory in fruit-eating bats. With new evidence from *Artibeus jamaicensis* (Chiroptera: Phyllostomidae). *Biotropica* 1995; 27: 106–120.
12. Loudon A.S.I, Racey P.A. Reproductive energetics in mammals. *Symp. Zool. Soc. Lond* 1987; 57, 371 pp
13. Martinez Del Rio C, RESTREPO C. Ecological and behavioral consequences of digestion in frugivorous animals. *Vegetatio*1993; 107: 205–216.
14. Mattson W.J. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics*1980; 11: 119–161.
15. Mello, M.A.R, Kalko E.K.V and Silva W.R. Ambient temperature is more important than food availability in explaining reproductive timing of the bat *Sturnira lillium* (Mammalia: Chiroptera) in a montane Atlantic Forest. *Canadian Journal Zoology* 2009; 87: 239–245.
16. Mello, M.A.R, Schittini G.M, Selig P and Bergallo H.G. A test of the effects of climate and fruiting of Piper species (Piperaceae) on reproductive patterns of the bat *Carollia perspicillata* (Phyllostomidae). *Acta Chiropterologica* 2004; 6: 309–318.
17. Migula P. Bioenergetics of pregnancy and lactation in the European common vole. *Acta Theriologica*1967; 14: 167–179.
18. O'brien G.M. Seasonal reproduction in flying foxes, reviewed in the context of other tropical mammals. *Reproduction Fertility Development* 1993; 5: 499–521.
19. Randolph P.A, Randolph J.C, Mattingly K and Foster M.M. Energy costs of reproduction in the cotton rat, *Sigmodon hispidus*. *Ecology*1977; 58: 31–45.
20. Ransome R.D. Population changes of greater horseshoe bats studied near Bristol over the last twenty-six years. *Biological Journal of the Linnean Society*1989; 38: 71–82.
21. Robbins C.T. *Wildlife Feeding and Nutrition*. Academic Press, New York1993.
22. Sadler, R.M.F.S. *The Ecology of Reproduction in Wild and Domestic Animals*. Methuen, London1969.
23. Telek L, Martin F.W. Tropical plants for leaf protein concentrates, *In: Leaf Protein Concentrates*, L. Telek and H.D. Graham (eds.). AVA Publishing, Inc, Westport, Connecticut, 1983; 81–116 pp.
24. Thompson, S.D. Gestation and lactation in small mammals: basal metabolic rate and the limits of energy use, *In: Mammalian Energetics: Interdisciplinary Views of Metabolism and Reproduction*, (T.F. Tomasi and T.H. Horton, eds), Cornell University Press, New York, 1992; 213–259 pp.





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**Table 1. Mating occasions and fruits availability during the study period**

Study period	Time of mating (h)	No. of mating (n)	Food sources (Plant species)	Phenology of Plant species
July (2010)	7.30, 7.45, 8.10, 7.35, 7.50, 8.10	6	<i>Psidium guajava</i> (Myrtaceae), <i>Achras sapota</i> (Sapotaceae), <i>Ficus benghalensis</i> (Moraceae)	Fruit
August	7.15, 7.40, 8.20, 7.20, 7.45	5	<i>Carica papaya</i> (Caricaceae), <i>Achras sapota</i> (Sapotaceae)	Fruit
September	7.35, 7.45, 8.30, 8.40, 9.00, 7.30, 7.45, 7.55	8	<i>Carica papaya</i> (Caricaceae), <i>Ficus benghalensis</i> (Moraceae)	Fruit
October	7.25, 7.35, 7.50, 8.10, 8.35, 7.40, 7.55, 8.10, 8.20, 8.35	10	<i>Achras sapota</i> (Sapotaceae), <i>Ficus religiosa</i> (Moraceae)	Fruit & Leaves
November	7.55, 8.15, 8.25, 8.40, 8.50, 7.55, 8.05, 8.15, 8.30, 8.50, 9.00	11	<i>Ficus religiosa</i> (Moraceae)	Leaves
December	7.15, 7.40, 8.10, 8.40, 8.50, 8.40, 7.40, 8.10, 8.28	9	<i>Ficus religiosa</i> (Moraceae), <i>Achras sapota</i> (Sapotaceae)	Fruit & Leaves
January (2011)	8.20, 7.10, 8.04	3	<i>Ficus religiosa</i> (Moraceae), <i>Ceiba pentandra</i> (Bombacaceae), <i>Achras sapota</i> (Sapotaceae)	Fruit & Leaves
July	7.42, 7.25, 8.10, 7.34, 7.51	5	<i>Psidium guajava</i> (Myrtaceae), <i>Achras sapota</i> (Sapotaceae), <i>Ficus benghalensis</i> (Moraceae), <i>Carica papaya</i> (Caricaceae)	Fruit



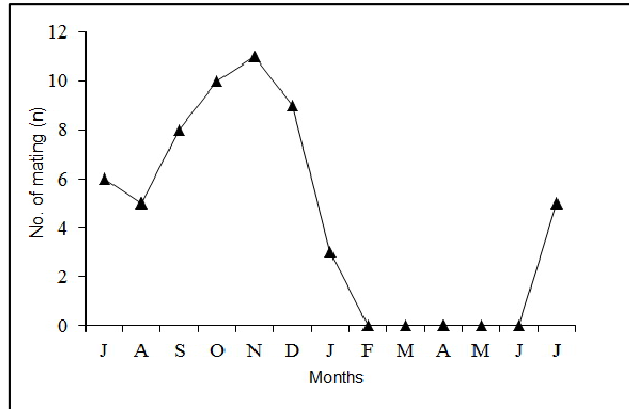
**Fig.1. Chewed leaves of *Ficus religiosa* by *P. giganteus* during breeding season**



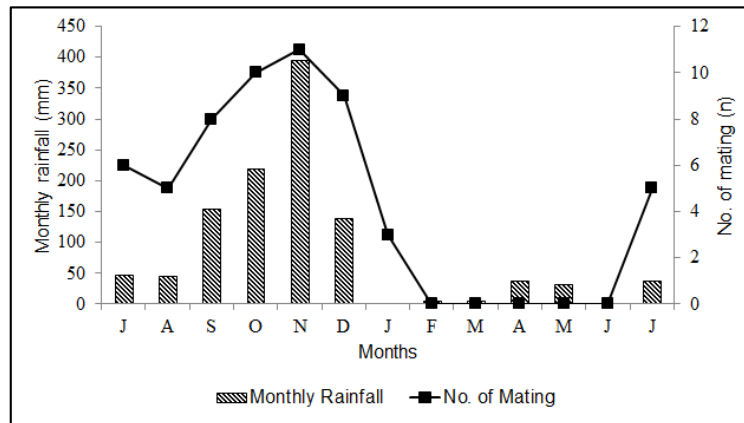




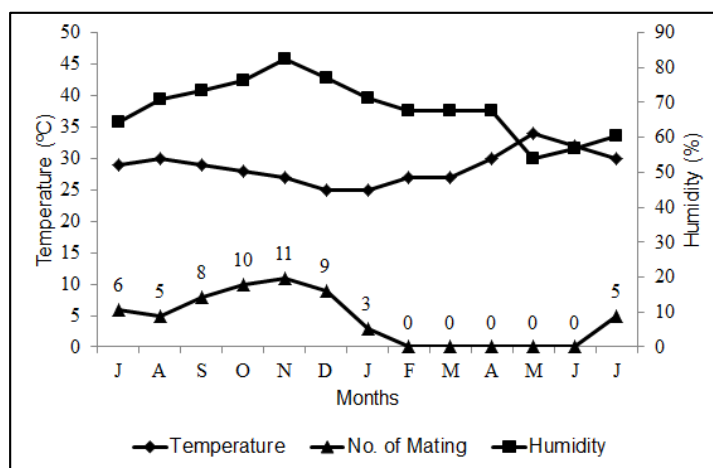
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**Fig. 2. No. of mating occasions during the study period**



**Fig. 3. Relationship between mating occasions and monthly rainfall**



**Fig. 4. Relationship between mating occasions and temperature and humidity**





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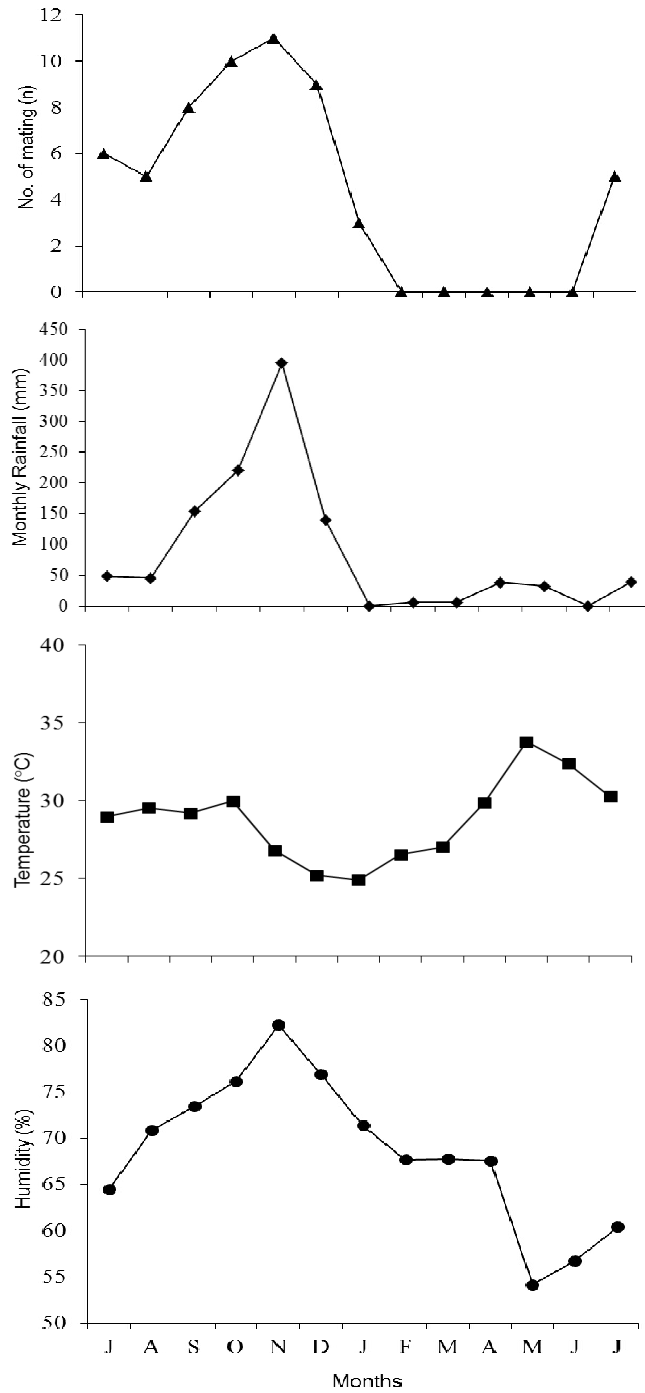
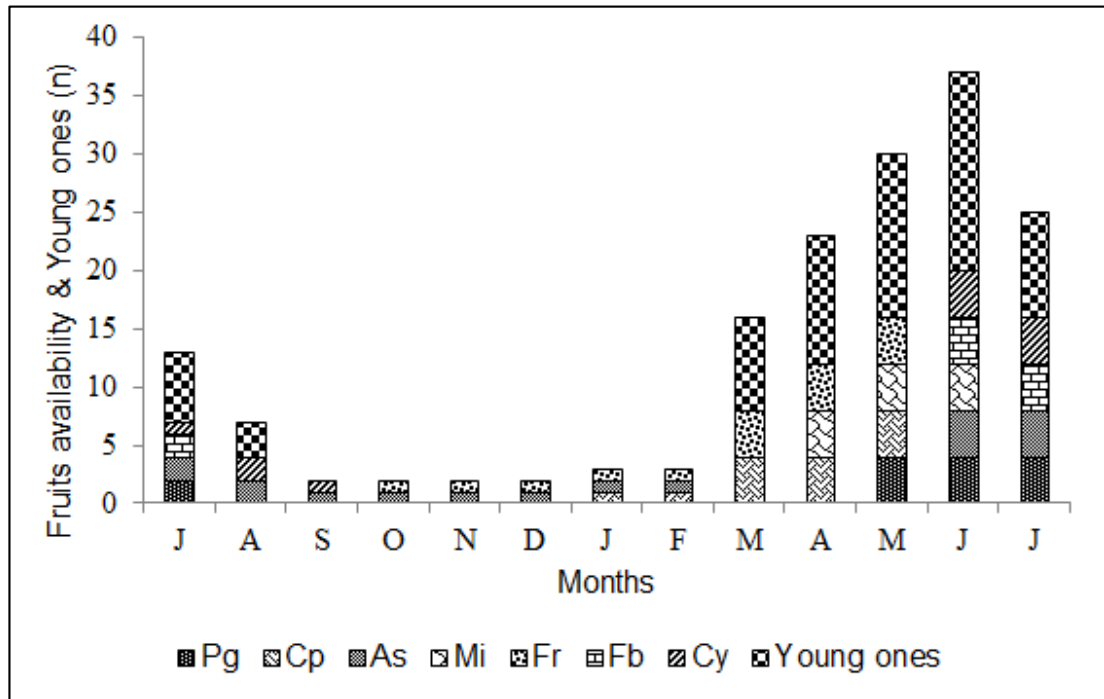


Fig. 5. Relationship between mating occasions, monthly rainfall, temperature and humidity





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Pg-*Psidium guajava*, Cp-*Ceiba pentandra*, As-*Achras sapota*, Mi-*Mangifera indica*,  
Fr-*Ficus religiosa*, Fb-*Ficus benghalensis*, Cy-*Carica papaya*

**Fig. 6. Relationship between fruits availability & young ones after breeding season**





## RESEARCH ARTICLE

## Studies on Genetic Variability for Important Traits in Back Cross Population Involving Foliar Disease Resistant and Susceptible Genotypes in Groundnut (*Arachis hypogaea* L.)

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### ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop in the tropical and subtropical countries of the world. There are several biotic and abiotic stresses that adversely affect groundnut production. Among them, late leaf spot (LLS) and rust are the major foliar diseases that not only reduces pod yield but also severely affect the fodder and seed quality. A marker assisted backcrossing program was carried out using three disease susceptible recurrent parents (ICGV 0035, ICGV 03128 and VRI 2) along with donor parent (GPBD 4). Three crosses of BC<sub>2</sub>F<sub>1</sub> population (ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4) along with their parental lines were evaluated for variability, heritability and genetic advance during *khariif*, 2014. Observations on ten quantitative characters were recorded. The PCV and GCV values for most of the traits were high in range for the crosses ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4. Whereas the cross VRI 2 × GPBD 4 recorded high PCV and GCV for most of the traits except hundred pod weight, shelling percentage, sound mature kernel percent and Late Leaf Spot score which recorded moderate PCV and GCV. All the three crosses exhibit high/moderate heritability accompanied with high/moderate genetic advance for the all traits studied in this population. This indicates the influence of additive gene action and scope for improvement of these traits through simple selection.

**Keywords:** Groundnut, genetic advance, heritability, kernel yield and variability.



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## INTRODUCTION

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an important oilseed crop in tropical and subtropical regions of the world. It is grown in six continents but mainly in Asia, Africa and America in over 100 countries with a world production of 37.10 m tons from an area of 23.11 m ha (FAO 2007). Late leaf spot (LLS) caused by *Phaeoisariopsis personata* (Berk. & Curt.) and rust (caused by *Puccinia arachidis* Speg.) are the most important leaf diseases in groundnut (*Arachis hypogaea* L.) causing yield losses in excess of 50% in semi-arid tropic regions (Subrahmanyam *et al.*, 1984; Subrahmanyam *et al.*, 1985a; Waliyar, 1991). Although the diseases can be controlled by fungicides, adoption of resistant cultivars by semi-arid tropic farmers is the best option to minimize losses at farm level and maintain good product quality (Dwivedi *et al.*, 1993). Identification of resistant sources and knowledge of components and mechanism of resistance are the pre-requisite for the success of disease resistance breeding programs. The occurrence of natural variability in the crop is negligible due to limited natural crossing. In such cases, creations of new variability through hybridization followed by selection will be the best option for the improvement of crop plants. With this framework, hybridization were attempted to develop three BC<sub>2</sub>F<sub>1</sub> cross derivatives by marker assisted backcrossing approach to estimate variability parameters for yield and yield attributes. The GCV along with heritability estimates provide reliable estimates of the amount of genetic advance to be expected through phenotypic selection (Burton, 1952). Estimate of genetic advance is more useful as a selection tool when considered jointly with heritability estimates (Johnson, 1955). With this background, the present study was made to assess the selection potential for kernel yield and component characters with foliar fungal disease resistance in groundnut.

## MATERIAL AND METHODS

The present experimental material comprised of three crosses *viz.*, ICGV 00350 × GPBD 4 (cross 1), ICGV 03128 × GPBD 4 (cross 2) and VRI 2 × GPBD 4 (cross 3). The recurrent parents *viz.*, ICGV00350, ICGV 03128 and VRI 2 were susceptible to late leaf spot (LLS) and rust diseases but having high pod yield and oil content. To incorporate resistance to these diseases, resistant donor GPBD 4 was used in crossing programme and the recurrent parents were again backcrossed with F<sub>1</sub>'s and BC<sub>1</sub> F<sub>1</sub>'s. The BC<sub>2</sub> F<sub>1</sub> populations of three crosses were used to investigate the genetic variability among yield and yield component characters. The crop was raised during *kharif* 2014, at the Oilseeds Farm, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore. Recommended agronomic practices were followed under irrigated condition. Observations were recorded in each cross for 10 characters *viz.*, number of pods per plant, 100-pod weight (g), 100-kernel weight (g), shell weight (g), shelling percentage, sound mature kernel (SMK) (%), late leaf spot (LLS) disease score, rust disease score, pod yield per plant (g) and kernel yield per plant (g). Shell weight per plant was calculated by the difference between pod yield per plant and kernel yield per plant and it's expressed in grams.

### Statistical analysis

Standard statistical procedures were adopted for calculating the mean, range and various genetic parameters like phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h<sup>2</sup>) in broad sense and genetic advance as per cent of mean (GAM). The range of coefficient of variation (CV) was categorized as per Sivasubramanian and Madhavamenon (1973): < 10% - low coefficient of variation; 10-20% - Medium coefficient of variation; > 20% - high coefficient of variation. As suggested by Robinson *et al.* (1949), the heritability range was classified as: < 30% - low heritability; 30%-60% - moderate heritability; > 60% - high heritability. Similarly, the range of genetic advance as per cent of mean (GAM) was grouped as: < 10% - low GAM; 10%-20% - medium GAM; > 20% - high GAM (Johnson *et al.* 1955). To screen the lines for sources of resistance to late leaf spot and rust, one (resistance) to nine (susceptible) point disease scale suggested by Subrahmanyam *et al.* (1995) was employed.



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## RESULTS AND DISCUSSION

A survey of genetic variability with the help of suitable parameters such as genotypic coefficient of variation, heritability estimates, genetic advance are absolutely necessary to start an efficient breeding program (Atta *et al.*, 2008). The results on the mean, range, variability, heritability and genetic advance as per cent of mean for 10 characters in three backcross populations viz., ICGV 00350 × GPBD, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4 of groundnut are presented in Table 1 to 3, respectively. Studies on genetic parameters such as PCV, GCV, heritability and GAM provide basic fact regarding the genetic properties of the population, based on which breeding methods are formulated for further improvement of the crop. Current study revealed the presence of wide range of phenotypic and genotypic coefficient of variation for all the characters studied. The estimates of GCV and PCV indicated that the values of PCV were always higher than GCV suggesting the influence of environmental factors. Less difference was observed between PCV and GCV in certain cases indicated greater role of genetic components and less influence by environment. Similar kind of results were obtained by Ladoleet *et al.* (2009), Shinde *et al.* (2010), Sunil (2014), Sunil *et al.* (2015) and Prabhu *et al.* (2015).

### Number of pods per plant

All the three crosses (ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4) recorded high PCV, GCV, heritability and genetic advance as per cent of mean for this trait. High PCV and GCV values for number of pods per plant were reported by Shinde *et al.* (2010), Priyadharsini (2012), Anitha (2013), Makinde and Ariyo (2013) and Prabhu *et al.* (2015), whereas high heritability with high GAM values for number of pods per plant was reported by Zaman *et al.* (2011), Priyadharsini (2012), Anitha (2013), Padmaja *et al.* (2013 a) and Prabhu *et al.* (2015).

### 100 pod weight (g)

The cross ICGV 00350 × GPBD 4 recorded high PCV along with moderate GCV for this trait coupled with both high heritability and GAM. Whereas the backcross ICGV 03128 × GPBD 4 exhibit high PCV, GCV, heritability and GAM for this trait. The cross VRI 2 × GPBD 4 recorded moderate PCV and GCV along with moderate heritability with moderate genetic advance. Ali *et al.* (2000), Ladole *et al.* (2009) and Prabhu *et al.* (2015) also expressed the same for the trait 100-pod weight.

### 100-kernel weight (g)

The values of PCV and GCV were moderate for 100-kernel weight in the cross ICGV 00350 × GPBD 4. whereas, high PCV and moderate GCV were exhibited in other two crosses for the same trait. These results are in accordance with Makinde and Ariyo (2013), Padmaja *et al.* (2013 a), Padmaja *et al.* (2013 b) and Thirumala *et al.* (2014) for high PCV and GCV values. Zaman *et al.* (2011), Anitha (2013) and Ashutosh and Prashant (2014) also reported medium GCV for this trait. All the three crosses recorded higher heritability and GAM for this trait. This is accordance with findings of Zaman *et al.* (2011), Padmaja *et al.* (2013 a), Padmaja *et al.* (2013 b), Ashutosh and Prashant (2014), Thirumala *et al.* (2014) and Prabhu *et al.* (2015).

### Shell weight per plant (g)

This trait revealed high PCV and GCV values by all the three cross derivatives. The crosses viz., ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4 exhibits higher heritability coupled with higher GAM for shell weight per plant. Anitha (2013) reported high PCV, GCV, heritability and GAM values for this trait. Prabhu *et al.* (2015) reported that both higher heritability and moderate heritability accompanied with higher GAM for this trait.



**Gopikrishnan et al.****Shelling percentage (%)**

Shelling percentage in crosses of ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 exhibited high magnitudes of PCV and GCV indicating the limited wide scope of selection for this trait. Whereas the cross VRI 2 × GPBD 4 revealed moderate PCV and GCV values for this trait. All the three backcross derivatives exhibits high heritability coupled with high GAM for shelling percentage. Similar results were also obtained by Zaman *et al.* (2011), Anitha (2013) and John *et al.* (2013) for higher heritability for this trait.

**Sound mature kernel (%)**

This trait recorded low PCV and GCV values by the cross ICGV 00350 × GPBD 4. whereas, moderate PCV and GCV were exhibited in other cross derivatives of ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4. The cross ICGV 00350 × GPBD 4 showed high heritability coupled with moderate GAM for this trait. High heritability coupled with high GAM was noticed in crosses of ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4 for this trait. High heritability and high GAM for sound mature kernel per cent were earlier reported by Hiremath *et al.* (2011) and Prabhu *et al.* (2015).

**Late leaf spot score**

Both the cross derivatives showed higher PCV and GCV values for late leaf spot score. Moderate PCV and GCV values recorded in the cross derivatives of VRI 2 × GPBD 4. High PCV, GCV values were noticed earlier by Khedikar *et al.* (2009), Venkataravana and Injeti (2008), Narasimhulu *et al.* (2013), Padmaja *et al.* (2013 a), Ashish *et al.* (2014) and medium GCV values by Vishnuvardhan *et al.* (2012) and Padmaja *et al.* (2013 b). High heritability coupled with high GAM for the trait late leaf spot were recorded in both the backcross combinations. Whereas the cross VRI 2 × GPBD 4 recorded high heritability accompanied with moderate GAM for this trait. Venkataravana and Injeti (2008), Khedikar *et al.* (2009), Vishnuvardhan *et al.* (2012), Narasimhulu *et al.* (2013), Padmaja *et al.* (2013 a), Padmaja *et al.* (2013 b), Ashish *et al.* (2014) and Prabhu *et al.* (2015) reported the high heritability with high GAM for late leaf spot score.

**Rust score**

High PCV and GCV for rust were recorded by all the three cross derivatives viz., ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4. High PCV and GCV values were earlier reported by Venkataravana and Injeti (2008), Narasimhulu *et al.* (2013), Ashish *et al.* (2014), Shridevi *et al.* (2014) for the trait rust disease score. The same crosses registered high heritability and high GAM values for this trait. High GAM results are in accordance with John *et al.* (2008), Venkataravana and Injeti (2008), Vishnuvardhan *et al.* (2012), Narasimhulu *et al.* (2013), Ashish *et al.* (2014) and Shridevi *et al.* (2014) and Prabhu *et al.* (2015) for rust score.

**Pod yield per plant (g)**

The backcross derivatives of all three crosses exhibit higher PCV and GCV values. The crosses ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 recorded high heritability and high GAM for pod yield per plant. Whereas the cross VRI 2 × GPBD 4 recorded moderate heritability with high GAM. High PCV, GCV, heritability and GAM values for pod yield per plant were earlier observed by Narasimhulu *et al.* (2012), Priyadharsini (2012), Anitha (2013), John *et al.* (2013), Narasimhulu *et al.* (2013), Mukesh *et al.* (2014) Thirumala *et al.* (2014) and Prabhu *et al.* (2015).

**Kernel yield per plant (g)**

High PCV and GCV values coupled with high heritability and GAM were exhibited by the entire three cross derivatives viz., ICGV 00350 × GPBD 4, ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4 for kernel yield per plant trait.





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These findings were similar to the findings of Narasimhulu *et al.* (2012), Priyadharsini (2012), Anitha (2013), John *et al.* (2013), Narasimhulu *et al.* (2013), Mukesh *et al.* (2014), Thirumala *et al.* (2014) and Prabhu *et al.* (2015) for the trait kernel yield per plant.

## CONCLUSION

Based on the foregoing discussion, it can be concluded that traits *viz.*, number of pods per plant, 100-pod weight, 100-kernel weight, shell weight, shelling percentage, sound mature kernel, late leaf spot (LLS) score, rust score, pod yield per plant and kernel yield per plant in the crosses ICGV00350 × GPBD 4 ICGV 03128 × GPBD 4 and VRI 2 × GPBD 4 and exhibit high/medium coefficient of variation accompanied with high/moderate heritability and high/moderate genetic advance as per cent of mean which implies the existence of additive gene effect in all the three backcross derivatives. The presence of wide spectrum of genetic variation for almost all the characters in these crosses along with additive gene action will help in the development of promising genotypes with high kernel yield and resistance to foliar diseases.

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## REFERENCES

1. Ali, N., Malik, S.N., Bashir, K. and Mirza, M.Y. 2000. Genetic variability, heritability and correlation studies in groundnut. *Sarhad Journal of Agriculture*, 16: 533-536.
2. Anitha, B.K. 2013. Identification of quantitative trait loci for oil yield and marker assisted backcross for high oleic acid in groundnut (*Arachis hypogaea*L.). *Ph.D. (Ag.) Thesis*. Submitted to the Tamil Nadu Agricultural University, Coimbatore, India.
3. Ashish, J., Nadaf, H.L. and Gangadhara, K. 2014. Genetic analysis of rust and late leaf spot in advanced generation recombinant inbred lines of groundnut (*Arachis hypogaea*L.). *International Journal of Genetic Engineering and Biotechnology*, 5(2): 109-114.
4. Ashutosh, K.S. and Prashant, K.R. 2014. Evaluation of groundnut genotypes for yield and quality traits. *Annals of Plant and Soil Research* 16(1): 41-44.
5. Atta, B. M., M. A. Haq and T. M. Shah. 2008. Variation and inter relationships of quantitative traits in chickpea (*Cicer arietinum* L.). *Pak. J. Bot.*, 40(2): 637 – 647.
6. Burton, G. W. 1952. Quantitative inheritance in grasses. *Proc. 6th. Intl. Grassland Congr.*, 1: 277 - 283.
7. Dwivedi, S.L., S.N. Nigam, P. Subrahmanyam, R. Jambunathan, G.V.S. Nagabhushanam, P.M Reddy, K. Raghunath & D. Mc- Donald, 1993. Effect of foliar diseases control by Chlorothalonil on pod yield and quality characteristics of confectionery groundnuts (*Arachis hypogaea*L.). *J Sci Food Agric.* 63: 265–271.
8. Hiremath, C.P., Nadaf, H.L. and Keerthi, C.M. 2011. Induced genetic variability and correlation studies for yield and its component traits in groundnut (*Arachis hypogaea*L.). *Electronic Journal of Plant Breeding*, 2(1): 135-142.
9. John, K., Vasanthi, R.P., Sireesha, K. and Giridharakrishna, T. 2013. Genetic variability studies in different advanced breeding genotypes of Spanish bunch groundnut (*Arachis hypogaea*L.). *International Journal of Applied Biology and Pharmaceutical Technology*, 4(2): 185-187.
10. Johnson, H. W, H. E. Robinson and R. E. Comstock. 1955. Estimates of genetic and environmental variability in soybean. *Agron. J.*, 47: 314 - 318.
11. Johnson, H.W., Robinson, H.F. and Comstock, R.E. 1955. Estimates of genetic and environmental variability in soybean. *Agronomy Journal*. 47: 314-318.







**Gopikrishnan et al.**

12. Khedikar, Y.P. 2008.Molecular tagging and mapping of resistance to late leaf spot and rust in groundnut (*ArachishypogaeaL.*).*Ph.D. (Ag.) Thesis*. Submitted to University of Agricultural Science, Dharwad, India
13. Ladole, M.Y., Wakode, M.M. and Deshmuk, S.N. 2009. Geneticvariability and character association studies for yield and yield contributing traits in groundnut (*Arachishypogaea L.*).*Journal of Oilseeds Research*,26: 123-125.
14. Ladole, M.Y., Wakode, M.M. and Deshmuk, S.N. 2009. Genetic variability and character association studies for yield and yield contributing traits in groundnut (*Arachishypogaea L.*).*Journal of Oilseeds Research*,26: 123-125.
15. Makinde, S.C.O. and Ariyo, O.J. 2013. Genetic divergence, character correlations and heritability study in 22 accessions of groundnut (*ArachishypogaeaL.*). *Journal ofPlant Studies* 2(1): 143-145.
16. Mukesh, K.M., Rai, P.K., Kumar, A., Singh, B.A. and Chaurasia, A.K. 2014. Study on genetic variability and seed quality of groundnut (*ArachishypogaeaL.*) genotypes.
17. Narasimhulu, R., Kenchanagoudar, P.V. and Gowda, M.V.C. 2012.Study of genetic variability and correlations in selected groundnut genotypes.*International Journal ofApplied Biology and Pharmaceutical Technology*,3(1): 355-358.
18. Narasimhulu, R., Kenchanagoudar, P.V., Gowda, M.V.C. and Sekhar, L. 2013. Genetic variability and correlation studies for selection of multiple diseasesresistance lines in two crosses of peanut.*Bioinfole*,10(1B): 183-186.
19. Padmaja, D., Brahmeswara, R.M.V., Eswari, K.B. and Madhusudhan, R.S. 2013 b. Genetic variability, heritability for late leaf spot tolerance and productivity traits in recombinant inbred line population of groundnut (*ArachishypogaeaL.*). *Journal of Agriculture and Veterinary Sciences*, 5(1): 36-41.
20. Padmaja, D., Eswari, K.B., Brahmeswara, R.M.V. and Madhusudhan, R.S. 2013 a. Genetic variability parameters for yield components and late leaf spot tolerance in BC<sub>1</sub>F<sub>2</sub> population of groundnut (*ArachishypogaeaL.*). *InternationalJournal of Innovative Research and Development*,2(8): 348-354.
21. Prabhu, R., Manivannan, N., Mothilal, A. and Ibrahim, S.M. 2015. Estimates of genetic variability parameters for yield and yield attributes in groundnut (*ArachishypogaeaL.*). *International Journal of Agriculture, Environment and Biotechnology*. 8(3): 551-559.
22. Priyadharshini, M. 2012. Molecular marker analysis for yield and yield component traits under non stress and drought stress conditions in groundnut (*ArachishypogaeaL.*). *Ph.D. (Ag.)Thesis*.Submitted to Tamil Nadu Agricultural University, Coimbatore, India.
23. Shinde, P.P., Khanpara, M.D., Vachhani, J.H., Jivani, L.L., Kachhadia, V.H. 2010.Genetic variability in *Virginia* bunch groundnut.*Plant Archives*.10(2): 703-706.
24. Shridevi, A.J., Nadaf, H.L., Gowda, M.V.C., Bhat, R.S., Patil, R.K., Motagi, B. Kenchanagowda, P., Mukri, G., Archana, B., Ganagshe y, P., Gangadhar, K. and Jaggal, L. 2014. Marker detection and genetic analysis for rust resistanceof recombinant and backcross inbred lines in groundnut (*ArachishypogaeaL.*). *Indian Journal of Genetics*74(2): 213- 221.
25. Sivasubramanian, S. and P. Madhavamenon. 1973. Genotypic and phenotypic variability in rice. *Madras Agric. J.*, 60: 1093 - 1096.
26. Subrahmanyam P, Williams JH, Mcdonald D, Gibbons RW (1984). The inXuence of foliar diseases and their control by selective fungicide on a range of groundnut (*ArachishypogaeaL.*) genotypes.*Ann Appl Biol.*,104:467–476.
27. Subrahmanyam, P., L.J. Reddy, R.W. Gibbons & D. McDonald, 1985a. Peanut rust: A major threat to peanut production in the semi-arid tropics. *Plant Dis.*, 69: 813–819.
28. Subrahmanyam, P., McDonald, D., Waliyar, F., Reddy, L.J., Nigam, S.N., Gibbons, R.W., Rao, V.R., Singh, A.K., Pande, S., Reddy, P.M. and Subba, R.P.V. 1995.Screening methods and sources of resistance to rust and late leaf spot of groundnut.International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India, Information. Bulletin No. 47: 24
29. Sunil, K. 2014. Genetic variability, heritability, genetic advance and correlation coefficient for vegetative and floral characters of gerbera (*Gerbera jamesonii*).*International Journal of Agriculture, Environment and Biotechnology* 7(3): 527-533.
30. Sunil, P., Akhilesh, T., Satish, K. and Tarun, J. 2015.Genetic variability, heritability and genetic advance in tomato (*Solanum lycopersiconMill.*).*International Journal ofAgriculture, Environment and Biotechnology*8(2): 245-251.





**Gopikrishnan et al.**

31. Thirumala, R.V., Venkanna, V., Bhadru, D. and Bharathi, D. 2014. Studies on variability, character association and path analysis on groundnut (*Arachis hypogaea*L.). *International Journal of Pure and Applied Bioscience*, 2(2): 194-197.
32. Venkataravana, P. and Injeti, S.K. 2008. Assessment of groundnut germplasm and advanced breeding lines and isolation of elite foliar disease resistant genotypes for southern Karnataka. *Journal of Soils and Crops*, 18(2): 282-286.
33. Vishnuvardhan, K., Vasanthi, R.P., Reddy, K.H.P. and Reddy, B.V.B. 2012. Genetic variability studies for yield attributes and resistance to foliar diseases in groundnut (*Arachis hypogaea*L.). *International Journal of Applied Biology and Pharmaceutical Technology*, 3(1): 390-394.
34. Waliyar, F., 1991. Evaluation of Yield Losses due to Groundnut Leaf Diseases in West Africa. pp. 32–33. Summary Proceedings of the second ICRISAT regional groundnut meeting for West Africa, 11–14 September 1990. ICRISAT Sahelian Centre, Niamey, Niger. ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) Patancheru, Andhra Pradesh India.
35. Zaman, M.A., Khatun, M.T., Ullah, M.Z., Moniruzzaman, M. and Alam, K.H. 2011. Genetic variability and path analysis of groundnut (*Arachis hypogaea*L.). *The Agriculturists* 9(1&2): 29-36.

**Table 1. Estimates of genetic variability parameters in BC<sub>2</sub>F<sub>1</sub> generation for the cross in groundnut ICGV 00350 × GPBD 4**

S.No.	Character	Mean	SE	Minimum	Maximum	PCV (%)	GCV (%)	Heritability (BS) (%)	GAM (%)
1.	Number of pods per plant	12.45	1.10	3.00	25.00	49.41	39.37	63.49	68.08
2.	100-pod weight (g)	71.06	2.72	17.50	94.80	21.32	18.81	77.82	36.00
3.	100-kernel weight (g)	25.58	0.86	16.00	36.73	18.85	15.27	65.67	26.85
4.	Shell weight per plant (g)	4.37	0.21	1.37	6.28	26.57	20.59	60.04	34.61
5.	Shelling percentage (%)	56.08	2.18	15.18	100	33.05	32.38	95.99	68.85
6.	SMK (%)	91.13	1.60	68.75	100	9.81	7.86	64.15	13.66
7.	LLS score	5.16	0.23	2.00	7.00	25.06	23.06	84.67	46.05
8.	Rust score	3.58	0.21	1.00	5.00	35.89	33.47	86.89	67.74
9.	Pod yield per plant (g)	8.64	0.72	1.62	16.12	46.72	39.14	70.19	71.15
10.	Kernel yield per plant (g)	5.26	0.56	0.24	11.96	59.89	50.35	70.69	91.87

**Table 2. Estimates of genetic variability parameters in BC<sub>2</sub>F<sub>1</sub> generation for the cross in groundnut ICGV 03128 × GPBD 4**

S.No.	Character	Mean	SE	Minimum	Maximum	PCV (%)	GCV (%)	Heritability (BS) (%)	GAM (%)
1.	Number of pods per plant	13.49	0.812	2.00	45.00	59.63	48.28	65.55	81.69
2.	100-pod weight (g)	87.88	2.26	49.4	165.2	25.55	24.35	90.85	48.52
3.	100-kernel weight (g)	32.39	0.96	15.28	67.66	29.44	28.53	93.88	57.77
4.	Shell weight per plant (g)	4.10	0.24	0.55	17.21	60.24	54.81	82.78	104.22





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5.	Shelling percentage (%)	57.72	1.77	11.21	93.03	30.40	28.85	90.04	57.21
6.	SMK (%)	89.09	1.08	37.50	100	12.05	10.80	80.45	20.25
7.	LLS score	2.71	0.07	1.00	5.00	27.49	23.01	70.06	40.25
8.	Rust score	1.29	0.04	1.00	3.00	37.90	27.40	52.27	41.40
9.	Pod yield per plant (g)	11.30	0.75	1.58	38.55	66.34	58.39	77.49	107.43
10.	Kernel yield per plant (g)	7.20	0.54	0.24	28.13	75.21	65.20	75.16	118.40

**Table 3. Estimates of genetic variability parameters in BC<sub>2</sub>F<sub>1</sub> generation for the cross in groundnut VRI 2 × GPBD 4**

S.No.	Character	Mean	SE	Minimum	Maximum	PCV (%)	GCV (%)	Heritability (BS) (%)	GAM (%)
1.	Number of pods per plant	11.90	0.86	3.00	22.00	48.23	38.31	63.09	66.03
2.	100-pod weight (g)	100.67	2.50	82.60	132.5	14.53	11.13	58.69	18.51
3.	100-kernel weight (g)	35.29	1.20	18.41	52.80	21.12	18.96	73.47	35.27
4.	Shell weight per plant (g)	4.68	0.33	1.44	10.40	42.89	34.60	65.05	60.55
5.	Shelling percentage (%)	55.49	1.90	33.61	100	18.16	17.11	88.68	34.96
6.	SMK (%)	87.72	2.07	50.00	100	15.33	12.79	69.56	23.14
7.	LLS score	5.80	0.11	4.00	7.00	12.74	10.00	61.49	17.01
8.	Rust score	2.33	0.12	1.00	4.00	30.91	27.50	79.13	53.09
9.	Pod yield per plant (g)	11.86	0.92	2.21	23.52	50.43	38.64	58.68	64.23
10.	Kernel yield per plant (g)	7.17	0.66	0.425	15.67	59.93	49.21	68.53	88.40





RESEARCH ARTICLE

## Constraints in Implementation of Livestock Development for Livelihood Support Programme in Wayanad District of Kerala State

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### ABSTRACT

Constraints in implementation Livestock Development for Livelihood Support (LDLS) programme was studied among the selected implementing officers working in the Animal Husbandry Department, Kerala. The constraints were studied using the Delphi method. The study revealed that the constraints perceived most severe was political interference in the selection of eligible beneficiary followed by inadequate quantity of concentrate feed supplied, cumbersomeness of regular animal health check-up, unavailability of the required number of pregnant heifers for distribution, unavailability of poultry medicine in hospitals and incapability of beneficiaries to win the confidence of bank personnel to avail loan which in turn resulted in more time consumption. The solutions most suggested to tide over these constraints were ranking the eligible farmers on the basis of production performance of their livestock and selecting accordingly, increasing the quantity of subsidized feed, beneficiaries regularly communicating with the veterinarians regarding the health status and productivity of their animals through the co-operative society personnel, purchasing heifers from SLBP beneficiaries, making available poultry medicines in every veterinary hospital and permitting willing beneficiaries to meet the non-subsidy expenses from own resources.

**Keywords:** Constraints, Programme Implementation, LDLS Programme.



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## INTRODUCTION

Success of any development programmes depends on many internal and external factors of the programme. Among them, the constraints encountered during the implementation phase of programme are more important as it affect the further progress and successfully achieving the desired set objectives. Programme implementation comprises of providing publicity and information of scheme to the targeted community, accepting application, selection of eligible beneficiaries, assisting them in getting financial and material resources, maintaining proper records, providing technical assistance, continuous monitoring and evaluation. Difficulties of implementing officer in any above mentioned responsibilities will hamper the success of programme ultimately. In this context a study was conducted to understand the constraints encountered while implementation of the Livestock Development for Livelihood Support (LDLS) programme. LDLS was implemented during 2011-2012 by the Department of Animal Husbandry, Government of Kerala to uplift the socio-economic conditions of the livestock farmers. Under the programme one pregnant heifer, two adult female goats and ten layer chicks were distributed. Pregnancy ration of 50 Kg concentrate cattle feed per month for a three month period was also distributed to each beneficiary.

## MATERIALS AND METHODS

The Delphi technique was employed to study the constraints in implementation of LDLS programme. Hsu and Sanford (2007) point out that "The Delphi technique is a widely used and accepted method for gathering data from respondents within their domain of expertise. The technique is designed as a group communication process which aims to achieve a convergence of opinion on a specific real world issue. The Delphi technique is well suited as a method for consensus-building by using a series of questionnaires delivered using multiple iterations to collect data from a panel of selected subjects.

### Application of Delphi method

Delphi procedure was followed to reach a consensus among the implementing officers of LDLS regarding the constraints experienced by them. For the purpose of the present study the following procedure was adopted

**Step 1:** Collecting the constraints from a panel of 15 implementing officers.

**Step 2:** Pooling the constraint items and presenting the same to the same panel of implementing officers and getting the consensus on what are the severe constraints and the not severe constraints. For this purpose a questionnaire was prepared incorporating the pooled constraint items those constraints judged to be severe by more than 50 per cent of the implementing officers were subsequently chosen.

**Step 3:** At this last stage solutions to the chosen constraint items were sought from the implementing officers.

## RESULTS AND DISCUSSION

Data in Table 1 showed that in case of the domain 'beneficiary selection' the severe constraints felt by implementing officers were political interference in the selection of eligible beneficiary and difficulty in selection due to more number of eligible farmers. This finding is in agreement with that of Kumar and Khan (2003) and Kumar *et al.* (2011) who observed the political and public representative interference in Krishi Vigyan Kendras and Agricultural Technology Management Agency respectively. Natchimuthu and Ramkumar (2004) also reported that beneficiaries had realized about the undue benefits taken by officials, politicians, and a segment of farmers who were in close proximity to officials and politicians. In case of the domain 'time', the severe constraints felt were much time needed for Procuring loan from bank and hindrance to proper implementation of programme due to other routine official duties. Kumar and Khan (2003) and Jeengeret *al.* (2010) also found that non-availability of loans in time and the

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complicated procedure to avail the loan were some of the major constrains faced by Krishi Vigyan Kendra officials and District Poverty Initiative Project implementing officers respectively. Ahmad (2011) in his study suggested that there is a need for community based credit providing agencies to tide over the problem of availing loan from banks.

Under the domain 'finance' the severe constraints were unpredictable cost of animals in the market, fund allotted to purchase the animals was insufficient, fund allotted to various items of programme was insufficient and fund allotted for veterinary health care was insufficient, With respect to the domain 'feed and fodder' the severe constraint felt by implementing officers was complaints regarding inadequate quantity of concentrate feed. Beneficiaries unable to win the confidence of bank officials by producing the required essential documents and non-repayment of loan by beneficiaries in time were the severe constraints perceived by implementing officers under domain 'beneficiary'. Planning Commission (2000) found that there was willful default of loan repayment even by those possessing repayment capacities among Integrated Rural Development Programme beneficiaries, and it also suggested that recovery procedure need to be streamlined and simplified. As far as the domain 'post implementation works' concerned, the severe constraints felt were difficulty in settling the accounts of the scheme, amidst the other routine responsibilities and animal health checkup every month is cumbersome. In case of the 'heifer' domain, the constraints felt by implementing officers as severe were unavailability of the required number of pregnant heifers and difficulty in judging the production capacity of the procured heifers. Under the domain 'poultry' the most felt severe constraint was non availability of poultry medicine in the hospital.

#### Solutions suggested for constraints

Data in Table.2 indicated the solutions suggested by implementing officers to overcome the constraints in implementing the LDLS programme. Political interference in the selection of eligible farmers was one of the constraints mentioned in the selection of beneficiaries. The solutions suggested by the implementing officers, included ranking the eligible farmer candidates based on the production performance of their livestock and selecting the beneficiary as per his rank (26.66%) and ensuring more transparency while selecting beneficiaries taking up equal responsibility by all the selection committee members (13.33%). Regarding the constraint, difficulty in selection due to more number of eligible farmers, the solutions suggested were, a farmer shall not be a beneficiary of more than one scheme for a period of ten years (26.66%) and selecting beneficiaries based on their aptitude (13.33%). With respect to the constraint, much time is required to procure loan from bank, the solutions suggested were if beneficiaries are able to bear the non-subsidy amount from their own sources, availing the same must be made universally non-mandatory (33.33%) and there shall be a change in the credit policy including interest amount (20.00%). As for the constraint, hindrance to proper implementation of programme due to other routine official duties the solutions suggested were bifurcating the extension administration work and clinical work, so that these are entrusted with separate personnel (33.33%) and appointing a special officer for a group of panchayats to implement programme (13.33%).

The solutions suggested to overcome the constraint, fund allotted to purchase the animals was insufficient were allotting extra fund for purchasing animals (26.66%), purchasing heifers from the organized / government farms, so that middlemen could be kept away (13.33%) and purchasing heifers from within the state itself to save the transportation charges (6.66%). Regarding the constraint, fund allotted to various items was insufficient; the solutions suggested were increasing the fund allotment for other items of programme (40.00%) and including only the most relevant associated item viz., concentrate feed and fodder, so that budget outlay can be effectively utilized (6.66%). Solutions suggested to the constraint fund allotted for veterinary health care was insufficient, were that there must be enhancement in the fund allotment, and distributing medical kit to the beneficiaries which contains mineral mixture, vitamins, dewormer etc. As for the constraint, unpredictable cost of animals in the market the solutions suggested were Bringing in to force an official animal pricing committee which values the animal based on breed and production performance since, brokers and cattle merchants exploit the situation by unjustifiably demanding high cost after knowing that the cattle is for distribution under government subsidy programme (26.66%) and supplying animals from the government farm (26.66%). Solution suggested for the constraint, inadequate



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quantity of concentrate feed was to increase the quantity of feed supply (53.33%). Regarding the constraint, beneficiaries were unable to win the confidence of bank officials by producing the required essential documents, solutions suggested were for those beneficiaries who were unable to procure loan in time may be permitted to meet the non-subsidy expenses from own resources (40.00%) and beneficiaries who were unable to submit required documents may be replaced with those in the waiting list (26.66%). The solution suggested for the constraint, non-repayment of loan by beneficiaries in time was deducting the amount directly from milk co-operatives where the milk is poured (53.33%). As for the constraint, difficulty in settling the accounts of the scheme amidst the routine official duties the solution suggested was appointing separate staff to look after account related clerical work of the programme (40.00%). Regarding the constraint, animal health check up every month is cumbersome the solutions suggested were beneficiaries should communicate regularly to the veterinarians about health status and productivity of animals through the co-operative society personnel (20.00%), having veterinary staff under mobile veterinary unit for routine health visit (13.33%) and reducing the number of visits in difficult terrains like in Wayanad district (13.33%). As for the constraint, Unavailability of the required number of pregnant heifers the solutions suggested were heifers with SLBP scheme beneficiaries may be purchased (40.00%), calf feed subsidy programme should be enlarged to cover more number of calf (13.33%) and purchasing heifers from government / organized farms (13.33%). With respect to the constraint difficulty in judging the production capacity of the procured heifers, the solution suggested were purchasing lactating or first parity cattle so that production capacity can be judged (40.00%) and approaching the trusted sources (13.33%). As for as constraint unavailability of poultry medicine in hospital, the solution suggested was that poultry medicines must be made available in every veterinary hospital (53.33%). Solutions suggested by implementing officers to overcome the constraints in executing LDLS programme

**REFERENCES**

1. Ahmad, S. *Community empowerment through livestock development and credit project. Aid-for-trade case story: Pakistan*. Organization for economic cooperation and development 2011; 5p.
2. Hsu, C.C. and Sanford, B.A. The Delphi technique: making sense of consensus. Practical assessment, research & evaluation. [on line] 2007; 12(10). Available: <http://pareonline.net/getvn.asp?v=12&n=10>. [24 Sep. 2013].
3. Jeenger, H.K., Snkhala, G. and Kadian, K.S. Constraints perceived by the beneficiaries of the district poverty initiative project in Rajasthan. *Indian J. Dairy sci* 2010; **63**: 324-331.
4. Kumar, M. and Khan, I.M. Constraints faced by the trainers of the KVKs run by different agencies in Rajasthan. *Indian J. Social Res.* 2003; **47**: 45-50.
5. Kumar, A., Eshwarappa, G. and Manjunatha, B.N. Constraints faced by stakeholders in implementation of agricultural technology management agency programme. *Karnataka J. Agric. Sci.* 2011; **24**: 255-257.
6. Natchimuthu, K. and Ramkumar. Constraints in the utilization of dairy development programmes in Pondicherry. *Indian J. of Dairy Sci.* 2004; **57**: 198-202.
7. PC [Planning Commission]. *Impact assessment study of socio-economic development programmes – A case study of Himachal Pradesh*. Asia Pacific Socio-Economic Research Institute, New Delhi, sponsored by Government of India 2000; 85 p.





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**Table 1. Implementing officers' constraints in executing LDLS programme**

Domain	Sl. No	Constraints	Severe constraints (in percent)	Rank
Beneficiary selection	1	Political interference in the selection of eligible beneficiary	53.33	III
	2	Difficulty in selection due to more number of eligible farmers	53.33	III
Time	3	Much time needed for Procuring loan from bank	66.66	I
	4	Hindrance to proper implementation of programme due to other routine official duties	53.33	III
Finance	5	Fund allotted to purchase the animals was insufficient	53.33	III
	6	Fund allotted to various items of programme was insufficient	53.33	III
	7	Fund allotted for veterinary health care was insufficient	53.33	III
	8	Unpredictable cost of animals in the market	60.00	II
Feed and fodder	9	Inadequate quantity of concentrate feed	53.33	III
Beneficiary	10	Beneficiaries unable to win the confidence of bank officials by producing the required essential documents	66.66	I
	11	Non-repayment of loan by beneficiaries in time	53.33	III
Post implementation works	12	Difficulty in settling the accounts of the scheme, amidst the other routine responsibilities	53.33	III
	13	Animal health checkup every month is cumbersome	53.33	III
Heifer	14	Unavailability of the required number of pregnant heifers	66.66	I
	15	Difficulty in judging the production capacity of the procured heifers	53.33	III
Poultry	16	Unavailability of poultry medicine in hospital	53.33	III

**Table 2. Solutions suggested by implementing officers to overcome the constraints in executing LDLS programme**

Constraints	Solution measures suggested by implementing officers for constraints	Opinion (in per cent)
Political interference in the selection of eligible beneficiary	Ranking the eligible farmers based on performance of their livestock and selecting them according to rank	26.66
	Ensuring more transparency while selecting beneficiaries taking up equal responsibility by all the selection committee members	13.33
Difficulty in selection due to more number	A farmer shall not be a beneficiary of more than one scheme for a period of 10 years	26.66







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of eligible farmers	Giving priority to weaker sections of livestock farming community	13.33
	Selecting beneficiaries based on their aptitude	13.33
Much time needed for Procuring loan from bank	If beneficiaries are able to bear the non-subsidy amount from their own sources, availing the same must be made universally non-mandatory	33.33
	There shall be a change in the credit policy including interest amount.	20.00
Hindrane to proper implementation of programme due to other routine official duties	Bifurcating the extension administration work and clinical work	33.33
	Appointing a special officer for a group of panchayats to implement animal husbandry development programmes	13.33
Fund allotted to purchase the animals was insufficient	Allotting extra fund for purchasing animals	26.66
	Purchasing heifers from the organized / government farms, so that middlemen could be kept away	13.33
	Purchasing heifers from within the state itself saves the transportation charges	6.66
Fund allotted to various items of programme was insufficient	Increase the fund allotment for other items of programme	40.00
	Including only the most relevant associated items so that budget outlay can be effectively utilized	6.66
Fund allotted for veterinary health care was insufficient	Enhancement in the fund allotment	40.00
	Distribution of medical kit to the beneficiaries which contains mineral mixtures, vitamins, dewormer etc.,	13.33
Unpredictable cost of animals in the market	Supplying animals from the government farm	26.66
	Bringing in to force an official animal pricing committee which values the animal based on breed and production performance since, brokers and cattle merchants exploit the situation by unjustifiably demanding high cost after knowing that the cattle is for distribution under government subsidy programme	26.66
Inadequate quantity of concentrate feed	Increase the quantity of subsidized feed supply	53.33
Beneficiaries unable to win the confidence of bank officials by producing the required essential documents	For those beneficiaries who are unable to procure loan in time may be permitted to meet the non-subsidy expenses from own resources	40.00
	Beneficiaries who are unable to submit required documents may be replaced with those in the waiting list	26.66
Non-repayment of	Deducting the amount directly from milk co-operatives where	53.33





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loan by beneficiaries in time	the milk is poured	
Difficulty in settling the accounts of the scheme, amidst the other routine responsibilities	Appointing separate staff to look after account related clerical works of the programme	40.00
Animal health check-up every month is cumbersome	Beneficiaries should communicate regularly to the veterinarians about health status and productivity of animals through the co-operative society personnel.	20.00
	Having veterinary staff under mobile veterinary unit for routine health visit	13.33
	Reducing the number of visits in difficult terrains like in Wayanad district.	13.33
Unavailability of the required number of pregnant heifers	Heifers from SLBP scheme beneficiaries may be purchased	40.00
	Calf feed subsidy programme should be enlarged to cover more number of calves.	13.33
	Purchasing heifers from government / organized farms	13.33
Difficulty in judging the production capacity of the procured heifers	Lactating or first parity cattle could be purchased to have better idea on its production potential	40.00
	Approaching the trusted sources	13.33
Unavailability of poultry medicine in hospital	Poultry medicines must be made available in every veterinary hospitals	53.33





RESEARCH ARTICLE

## Income and Employment Generation of Livestock Development for Livelihood Support (LDLS) Programme among Beneficiaries Household in Wayanad District of Kerala State

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### ABSTRACT

Income and employment generation due to implementation of the LDLS project was assessed among beneficiary families of the project. The results showed that majority of beneficiary household (42.66%) derived a medium level of gross annual income followed by a low level of income (33.33%) and a high level of income (24.00%) in the studied area. The employment generation among the beneficiary household revealed that it provided 6.12 hours, 1.16 and 0.35 hours per day to dairy farming, goat and poultry farming respectively. The level of employment generated was medium among majority of beneficiaries household (45.33%), which was followed by low level of employment (29.33%) and high level of employment (25.33%) respectively.

**Key words:** Income generation, Employment generation, LDLS programme

### INTRODUCTION

Livestock development programmes are meant not only for the development of livestock resources but also to support the livelihood of the poor livestock farmers and other stakeholders. Livestock development programmes play significant role in income and employment generation of the livestock keepers and in the overall economic growth of a developing country. Many state and centrally sponsored livestock development programmes are being





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implemented in different states. Among such programmes, Livestock Development for Livelihood support programme (LDLS) was implemented during 2011-12 by the Department of Animal Husbandry, Government of Kerala to uplift the socio economic conditions of the livestock farmers. LDLS was implemented in entire Kerala state with special preference to Wayanad district. The total outlay of the programme was Rs. 20 crore, and the programme cost per individual beneficiary was Rs. 50,000/-. Economic assistance of 50 per cent (Rs. 25,000/-) in the form of subsidy was provided from the animal husbandry department. An amount of Rs. 19,000/- was sanctioned in the form of bank loan and the remaining amount of Rs. 6,000/- was borne by beneficiaries from their own sources. Under the programme one pregnant heifer, two adult female goats and ten layer chicks were distributed. Pregnancy ration of 50 Kg concentrate cattle feed per month for a three month period was also distributed to each beneficiary. The programme was intended to augment milk and egg production to ensure livelihood support and food security. Goats were distributed to beneficiaries for source of subsidiary income and chicks to augment income from backyard poultry farming.

## MATERIALS AND METHODS

The study was conducted in the Wayanad district of Kerala among 150 beneficiaries of LDLS programme. The research design adopted was one group after only design. Respondents of the study were selected by applying stratified multistage random sampling technique. There are a total of twenty five grama panchayats in Wayanad district of which five panchayats each were randomly selected from all three taluks (Vythiri, Sulthan Batheri and Mananthavady). From each grama panchayat an equal number of 10 beneficiaries were selected randomly.

### Gross family income

In the present study the gross family income meant the total amount of money generated per annum from all the sectors of animal husbandry viz., milk production, goat production, egg production, chicken production, dung production, selling of kids & calf, selling of productive and culled animals and income derived from chicks, as said by the respondents at the time of interview. On the basis of income levels obtained by the beneficiaries they were classified into three categories as low, medium and high income generation of families using Dalenius and Hodges Cumulative Square root Frequency method

### Family employment

In the present study the family employment meant the number of hours family members were employed in dairy farming, goat farming and backyard poultry farming, as said by respondents at the time of interview. Also the number of family members involved in various livestock activities was measured. On the basis of employment generated the beneficiaries were classified into three categories as low, medium and high employment generation using Dalenius and Hodges Cumulative Square root Frequency method

## RESULTS AND DISCUSSIONS

The results of present study are as follows Data in Table 1 showed that a medium level of gross annual income was derived in 42.66 per cent of household followed by a low level of income in 33.33 per cent households and a high level of income in 24.00 per cent of household.

### Employment generated to the beneficiary families

Data in table 2. indicated that the average working hours from the animal husbandry activities. The average working hours in dairy farming was 6.12 hours per day, where as in goat and poultry farming was 1.16 and 0.35 hours





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respectively. Data in Table 3 showed that a medium level of employment was generated in 45.33 per cent of household followed by a low level of employment in 29.33 per cent of households and a high level of employment in 25.33 per cent of household. It is worthwhile that as for as income and employment generation for beneficiary families in the animal husbandry sector; the direction of income and employment generated was towards low to medium level. This is not at all a favourable trend in view of efforts taken by the LDLS scheme. The direction should have been at least towards medium to high level. Alam (1997), Birch and Shuria (2001), Deepika *et al.* (2002), Chauhan and Kundu (2005), Mavi *et al.* (2006) and FAO (2010), studied the projects Smallholder Livestock Development Project, Wajir Pastoral Development Project, Aseel poultry projects, Intensive Cattle Development Programme, self-employment project and Integrated Dairy Development Projects respectively reported that the income of the beneficiary households increased under these projects.

The role of employment in poverty-reduction programmes in developing countries has received considerable attention worldwide, in development strategies and policies (Omoret *et al.*, 2004). With regard to employment generation among beneficiary families, Chauhan and Kundu (2005) mentioned that average daily labor utilization per household in all the dairy operations was found to be 5.62 man hours for the beneficiary household which was significantly higher than 4.90 man hours for the non-beneficiary households. Singh *et al.* (2011) studied the impact of women dairy cooperative societies on the beneficiaries in Haryana state and found that the farm families of member group were spending more time on dairy farming activities than non-member group. The direction of income and employment generated among LDLS beneficiaries was towards low to medium level only, which is not a favourable situation in view of the efforts taken under the LDLS scheme.

### REFERENCES

1. Alam, J. Impact of smallholder livestock development project in some selected areas of rural Bangladesh. *Livestock Rural Dev.* [on line] 1997;9(3). Available: <http://www.lrrd.org/lrrd9/3/bang932.htm>. [12 Jan. 2014].
2. Birch, I. and Shria, H.A.O. *Perspectives on pastoral development.* (1<sup>st</sup> Ed.). Oxam G B publications 2001;45p.
3. Deepika, G., Women's Gotti., Anthra. And Yakshi. Community efforts of in-situ conservation of aseel poultry breed. In: *Adivasi areas of east Godavari district in Local livestock breeds for sustainable rural development: Towards community based approach for animal genetic resource conservation. Proceedings of a workshop; 1<sup>st</sup> to 4<sup>th</sup> November, 2002, Udaipur and Sadri, Rajasthan, India.* Lokhit Pashu-Palak Sansthan and League for Pastoral Peoples. pp.91-103.
4. Chauhan, A.K. and Kundu, T.R. Impact of new dairy farming technology on employment and income of rural households in Haryana. *Indian J. Dairy Sci* 2005;58: 293-297.
5. Mavi, K.S., Chauhan, J.P.S. and Das, B.C. Constraints in adoption of improved dairy farming practices by dairy farmers. *Indian J. Ext. syst* 2006;1:63-67.
6. FAO [Food and Agricultural Organization]. Status of Prospects for Small holder Milk Production – A Global Perspective. (1<sup>st</sup> Ed.) by T. Hemme and J. Otte. Rome 2010;179p.
7. Omoret, A., Mullindo, J.C., Islam, S.M.F., Nurah, G., Khan, M.I., Staal, S.J. and Dugdill, B.T. *Employment generation through small-scale dairy marketing and processing.* Food and Agricultural organizations of United Nations, Rome 2004;47 p.
8. Singh, U., Vijay, A.N.A. and Malik, B.S. Economic impact of women dairy cooperative societies on its beneficiaries: A study in Haryana state. *Indian J. of Dairy sci* 2011;64: 428-434.





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**Table 1. Distribution of beneficiaries based on gross annual income from animal husbandry sector.**

**n = 150**

Sl. No.	Annual income (in Rupees)	Frequency	Percentage
1	Low (< 145000)	50	33.33
2	Medium (145001 to ≤ 215000)	64	42.66
3	High (> 215001)	36	24.00
	Total	150	100

**Table 2. Employment generated to the beneficiary family in the animal husbandry sector**

Sl. No.	Animal husbandry activity	Average time spent (in hours) per day
1	Dairy farming	6.12
2	Goat farming	1.16
3	Poultry farming	0.35

**Table 3. Distribution of beneficiaries based on employment generated to the beneficiary family in the animal husbandry sector**

**n = 150**

Sl. No.	Employment generated (In hours)	Frequency	Percentage
1	Low (≤ 6)	44	29.33
2	medium (6.01 to ≤ 8.10)	68	45.33
3	High (> 8.11)	38	25.33
	Total	150	100





## Antibiogram Profile of *Listeria monocytogenes* Isolated from Bovine Mastitic Milk

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### ABSTRACT

*Listeria monocytogenes* is a pathogenic bacterium that can cause Listeriosis in humans and various animal species. The present study was conducted to ascertain the antibiotic sensitivity of *Listeria monocytogenes* isolated from bovine mastitic milk. A total of 100 mastitic milk (clinical and subclinical) samples were collected from dairy farms of three different panchayaths namely Thariyode, Pozhuthana and Kalpetta municipality of Wayanad district, Kerala based on California Mastitis Test and changes in udder. Out of 100 mastitic milk samples, *L. monocytogenes* was isolated from six (six per cent) mastitic milk.

**Key words:** *Listeria monocytogenes*, bovine mastitic milk, antibiogram profile.

### INTRODUCTION

"Listeriosis is one of the emerging food borne infection" (Abay et al., 2012). *Listeria monocytogenes* is a multi-systemic invasive pathogen, capable of colonising multiple host tissues, causing a range of clinical conditions in animals and human beings. This causes considerable morbidity and mortality in humans and livestock. The case fatality rate for this foodborne pathogen ranges from 15.0 to 30.0 per cent with the highest hospitalization rates (90.5 per cent) amongst known food-borne pathogens (CDC, 2000). It is a significant food borne pathogen due to its ability to survive in a wide range of environmental conditions including refrigeration temperature. Although 13 different serotypes of *Listeria monocytogenes* are known, more than 95% of isolates are from food and cases of listeriosis belong to 1/2a, 1/2b and 4b serotypes (Wagner and Allerberger, 2003).



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*Listeria spp.* Are recognised as a causative organism for mastitis in cattle (Yadav et al., 2010). Even though listerial intramammary infections (IMI) are rare, contaminated milk and milk products have been involved in several outbreaks of listeriosis (Fleming et al., 1985). Even though *Listeria monocytogenes* is common in the faeces of cattle and in the farm environment, only a few cases of listerial mastitis have been reported in the literature (Sharp, 1987). The internalin gene *inIA* is necessary for the internalization of *Listeria spp.* into intestinal epithelial cells; the presence of *inIC* and *inIJ* in *L. monocytogenes* has been used to differentiate virulent and avirulent strains (Liu et al., 2007). The objectives of the present study were to isolate *Listeria spp.* from bovine mastitis cases and to analyse the antibiogram profile of the isolates.

## MATERIALS AND METHODS

### Collection of mastitic milk samples

The study was undertaken for a period of 10 months from June 2014 to March 2015. On the basis of CMT and consistency of milk and udder changes a total of 100 mastitic milk which included 56 sub-clinical and 44 clinical mastitic milk samples were collected from three different regions namely, from Kapletta 34, from Thariyode 33 and from Pozhuthana 33. The milk samples were transported to laboratory in insulated containers and processed in lab within 6 hours of collection.

### Isolation of *L. monocytogenes*

A standard protocol described by Thomas et al. (1991) was followed with slight modifications for the isolation of *L. monocytogenes*. A total of 10g sample (millilitres in case of liquid samples) were transferred into a sterile stomacher bags containing 0.5 per cent sterilized peptone water and homogenized. In this method, a two-step enrichment procedure using UVM I and II broth was followed. In primary enrichment, UVM I was used. Acriflavin (6 mg) and Nalidixic acid (10 mg) are the selective agents used in the UVM I media. One ml of the homogenized suspension was transferred to 9ml of UVM I and incubated at 37°C for 24 h. The secondary selective enrichment was carried out in UVM II broth, which contained the selective agents at higher concentration (Acriflavin 12.5 mg and Nalidixic acid 10 mg). From the primary enriched UVM I broth culture, 0.1 ml was transferred to 10 ml of UVM II broth and incubated at 37°C for 24 h. A loopful of the inoculum from the enrichment broth was streaked onto duplicate plates of PALCAM agar medium. The plates were incubated at 37°C for 24 h. At the end of incubation, colonies showing characteristic appearance (grey-green with black centre colonies surround with black halo) on PALCAM agar medium were selected and transferred onto nutrient agar slants and incubated at 37°C overnight. The isolates were stored at refrigeration temperature for characterization. The isolates were subjected to staining, biochemical and molecular test (PCR).

### Molecular confirmation of *Listeria monocytogenes*

The PCR confirmation *L. monocytogenes* isolates as per the protocol described by McClain and Lee (1998).

### PCR Technique

The primer pair consisting of primer *hlyA1* 5'- GCAGTTGCAAGCGCTTGGAGTGAA-3' and *hlyA2* 5'- GCAACGTATCCTCCAGAGTGATCG-3' was used for the amplification of a 432 bp region of the *hlyA* gene. PCR was performed in a 25µl reaction mixture with a PCR buffer containing 200 µm concentration of each deoxynucleoside triphosphate (dNTP), 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 1 unit of Taq polymerase (Promega), 0.25 µM concentration of each primer and 2.5 µl of DNA template. DNA amplification was carried out for 34 cycles in 25 µl of reaction mixture as follows: denaturation at 94°C for 50 seconds, annealing at 60°C for 50 seconds, extension at 72°C for 90 seconds, with final extension at 72°C for 5 minutes. The amplified DNA was analyzed by agarose gel





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electrophoresis on a 1.5 % agarose (GeNei™, Mumbai) gel prepared in 1X Trisacetate EDTA buffer (40mM Tris, 20mM acetic acid and 1mM EDTA) stained with ethidium bromide (0.5µg/ml). A 100 bpDNA ladder(Bangalore Genei, India) was used as a reference marker. Tris-acetate EDTA (0.5<sup>x</sup>) was used as the running buffer and the gel was viewed using UV transillumination at a wavelength of 254 nm.

**Antibiotic Susceptibility Test**

The antibiotic susceptibility profile of the isolates of *L. monocytogenes* was studied as per the guidelines provided by Clinical Laboratory Standards Institute (2010). The bacterial isolates obtained in the present study were subjected to disc diffusion assay as per the method described by Bauer *et al.* (1966). Selection of antibiotics was based on the treatment information obtained from Veterinary and Medical Clinicians. The antibiotic discs used in the study were procured from HiMedia Laboratories Ltd., Mumbai. *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 83452 were used as a quality control strains for *L. monocytogenes* isolates.

A loopful of pure culture for each test isolate recovered was transferred into a tube containing five ml of nutrient broth medium. The broth culture was incubated in shaker water bath at 37°C to obtain the turbidity of the 0.5 McFarland standard tube. After incubation, the test isolate culture was spun at 5000 rpm for five min, to obtain a pellet which was later dissolved using one ml of sterile normal saline solution resulting in an approximately 1 to 2 × 10<sup>8</sup> cfu/ml count for each test isolate. This culture was evenly spread MH agar containing five per cent sheep blood for *L. monocytogenes* isolates, using a sterile cotton swab. Antibiotic discs were placed on inoculated agar surface at about two to three cm apart. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and incubated at 37°C overnight. The zones of inhibition diameter were measured for each antibiotic, initially for quality control strain and also for all test strains. The obtained data was compared with interpretative chart furnished by the manufacturer to grade the test isolates as sensitive, intermediate and resistant for respective antibiotics.

All the isolates were tested against 12 different antibiotics viz. Amoxycylav (30µg), Ampicillin (10µg), Ceftriaxone (30µg), Erythromycin (30µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Co-Trimoxazole (25µg), Gentamicin (10µg), Methicillin (5µg), Tetracycline (30µg), Vancomycin (30 µg) and streptomycin (10µg).

**RESULTS**

Out of 100 mastitic milk samples, six (6 per cent) samples were positive for *L. monocytogenes*. In Kalpetta three (8.82 per cent) samples were positive for *L. monocytogenes*. *L. monocytogenes* was confirmed in two (6.06 per cent) samples of Thariyode. In Pozhuthana, one (3.03 per cent) sample was positive for *L. monocytogenes* (Table 29).

**Antibiotic susceptibility of *L. monocytogenes* isolates**

The *L. monocytogenes* isolates were found highly sensitive to ceftriaxone (83.33 per cent), ciprofloxacin (83.33 per cent) and least sensitive to erythromycin (33.33 per cent), ampicillin (33.33 per cent) and co-trimoxazole (16.66 per cent) and completely resistant to methicillin and amoxycylav.

**Discussion and Conclusion**

In the present study, *L. monocytogenes* was isolated from six per cent mastitic milk samples from all the three regions. Yadav et al. (2010) isolated *L. monocytogenes* from 3.52 per cent of the mastitic milk samples collected from various regions of Gujarat. The present findings were in agreement with observations made by Vilar et al. (2007) who isolated this organism from 6.1 per cent of the raw milk samples in Spain. In conclusion, recovery of *L. monocytogenes* from bovine mastitic milk samples underscores the zoonotic potential of listeriosis. Thus, studies regarding





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epidemiological and zoonotic potential of *L. monocytogenes* are essentially required with special emphasis being given for improved diagnosis, prevention and control measures.

The isolates were found highly sensitive to ceftriaxone (83.33 per cent), ciprofloxacin (83.33 per cent) and least sensitive to erythromycin (33.33 per cent), ampicillin (33.33 per cent) and co-trimoxazole (16.66 per cent) and completely resistant to methicillin and amoxycylav. These findings were in accordance Dehkordi et al. (2013) who reported higher sensitivity of *L. monocytogenes* isolates from raw milk samples to ciprofloxacin and lower sensitivity to penicillin in Iran. As the isolates were found highly resistant to the antibiotics there is a need for judicious use of antibiotics.

## REFERENCES

1. Abay, S., Aydin, F. and Sumerkan, A.B. 2012. Molecular typing of *Listeria* spp. isolated from different source. *Ankara Univ. Vet. Fak. Derg.* 59: 183-190.
2. Centers for Disease Control and Prevention. Annual Report-2000. CDC/USDA/FDA Food borne Disease Active Surveillance Network. CDC's Emerging Infections Program. Revised 27 June 2003.
3. Wagner, M., Allerberger, F., 2003. Characterization of *Listeria monocytogenes* recovered from 41 cases of sporadic listeriosis in Austria by serotyping and pulsed-field gel electrophoresis. *FEMS Immunol. and Med. Microbiol.* 35: 227–234.
4. Yadav, M.M., Roy, A., Bhandari, B., Joshi, C., 2010. Pheno-genotypic characterization of *Listeria monocytogenes* from bovine clinical mastitis. *Buffalo Bulletin.* 29: 29
5. Fleming, W.R., Gannon, M. and Sherris, M.W. *FEMS Immunol. and Med. Microbiol.* 89: 389–399.
6. Sharp, M.W. 1987. Bovine mastitis and *Listeria monocytogenes*. *Vet Rec.* 121: 512-513.
7. Liu, D., Lawrence, M.L., Austin, F.W., Ainsworth, A.J., 2007. A multiplex PCR for species- and virulence-specific determination of *Listeria monocytogenes*. *J. Microbiol. Methods.* 71: 133–140.
8. Thomas, E.J., King, R.K., Burchak, J. and Gannon, V.P. 1991. Sensitive and specific detection of *L. monocytogenes* in milk and ground beef with the polymerase chain reaction. *Appl. Environ. Microbiol.* 57: 92576-2580.
9. McClain, D. and Lee, W.H. 1988. Development of USDA-FSIS for isolation of *L. monocytogenes* from raw meat and poultry. *J. Ass. Off. Analyt. Chem.* 71: 660-663.
10. Clinical and Laboratory Standards Institute. 2010. Performance standards for antimicrobial susceptibility testing: Twentieth informational supplement M100-S20. CLSI, Wayne, PA, USA.
11. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.* 45: 493–496.
12. Vilar, M.J., Yus, E., Sanjuan, M.C., Dieguez, F.J. and Rodriguez, J.L. 2007. Prevalence and risk factors for *Listeria* spp. on dairy farms. *J. Dairy Sci.* 90(11): 5083-5088
13. Dehkordi, F.S., Barati, S., Momtaz, H., Ahari, S.N.H. and Dehkordi, S.N. 2013. Comparison of shedding, and antibiotic resistance properties of *L. monocytogenes* isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo and camel species in Iran. *Jundishapur J. Microbiol.* 6(3): 285-294.

**Table.1. Collection of clinical and subclinical mastitic milk samples**

Regions	Subclinical mastitic milk samples	Clinical mastitic milk samples
Kalpetta	15	19
Thariyode	17	16
Pozhuthana	24	9
Total	56	44





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**Table 2. Details of primers used in this study**

Gene	Target organism	Primer sequence	Amplification on size	Reference
hlyA	<i>L. monocytogenes</i>	F 5'- GCAGTTGCAAGCGCTTGGAGTG AA-3'	432bp	Furreretal. (1991)
		R 5'- GCAACGTATCCTCCAGAGTGAT CG-3'		
prfA	<i>L. monocytogenes</i>	F 5'- 5'CTGTTGGAGCTCTTCTGGTGA AGCAATCG-3'	1031bp	Paziak- Domanska etal. (1999)
		R 5'- AGCAACCTCGGTACCATATACT AACTC-3'		
plcA	<i>L. monocytogenes</i>	F 5'- CTGCTTGAGCGTTCATGTCTCAT CCCCC-3'	1484bp	Notermans et al. (1991a)
		R 5'- CATGGGTTTCACTCTCCTTCTAC -3'		

**Table.3. Occurrence of *L. monocytogenes* in bovine mastitic milk**

Sl. No.	Areas of collection	No of samples collected	Samples positive for <i>L. monocytogenes</i>	
			By culture method No (Per cent)	PCR method
1	Kalpetta	34	3 (8.82)	3 (8.82)
2	Thariyode	33	3 (9.09)	2 (6.06)
3	Pozhuthana	33	1 (3.03)	1(3.03)
Total		100	7 (7.00)	6 (6.00)





RESEARCH ARTICLE

## Comparison of Histology of Cardiac Muscle using Different Infiltrating Media

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### ABSTRACT

Plastinated heart samples used by different infiltrating media such as Von-Gunther's biodur infiltration, Jelly wax, teacup and thermacol resin and Pongamia oil were studied for the histological details such as cross striations and intercalated disk. It was found that the specimens using Plastic teacup & thermacol resin was considered to be best for the histological study of cardiac muscle fiber.

**Key words:** Histology ,cardiac muscle, infiltrating medias, comparsion

### INTRODUCTION

Plastination is a method of preservation of biological organs using resin or other infiltrating medias in a dry form. However, the histological study of such Plastinated specimens have not been compared using different infiltrating media to identify the best infiltrating media for more detail histology and staining affinity, the present study has been undertaken. This will help the histologist to choose the correct infiltrating media for other tissues and different staining methods.

### MATERIALS AND METHODS

Cardiac tissue infiltrated with Biodur [Fig.V] was procured from Netherlands. Similarly, heart samples from local abattoir were collected and were fixed in 10% Neutral buffer formalin and processed by routine method [Singh and sulochana, 1996], but instead of routine paraffin infiltration, different infiltration media like with jelly wax[Fig. VI],



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Plastic tea cup & thermacol resin[Fig.VIII] and Vegetable Oil (pongamia oil)[Fig.VII] were used. By dissolving jelly wax, plastic teacup & thermacol in organic solvent respective resin was prepared, pongamia oil is readily available in the market. Before blocking tissues with paraffin, cardiac tissues were subjected to deplastination in organic solvent. All the blocks were cut at 6 $\mu$  and stained with H& E (Culling, 1974).

**RESULTS AND DISCUSSION**

The infiltrated heart samples were processed and stained with Haematoxylin and eosin, it was found that the biodur infiltrated heart muscle (Fig. 1) did not show clear striations and also the sections were not uniform. Because the biodur has infiltrated intracellularly and even after deplastination, it was not compatible for tissue sectioning and staining. The use of biodur infiltrating media was costly as compared to other infiltrating media which is not affordable in developing countries. In jelly wax infiltration the sections were uniform but did not show the striation and intercalated disc (Fig. 2). The cytoplasmic and nuclear stain of the fibers were more clear in the oil infiltrated sample (Fig. 3) which were similar to that of jellywax. (Manjunath, 2014) has studied the light microscopic architecture in spleen, liver, kidney of pig by using plastic teacup and thermacol resin infiltration media and showed excellent tissue morphology and concluded that this method can be considered for histological and histopathological study. (Griodan et al 1994; Ripani-1996) employed silicon resin as infiltrating media and deplastinated with sodium methoxide and observed that Plastinated specimens could be considered viable for structural details. (Ravi and Bhat, 2011) also used silicon embedded tissue sections after proper deplastination and suggested that this method can be used for both light and electronic microscopic study, However, the procedure was a prolonged method. (Ramya, 2015) also studied different infiltrating media including double infiltration technique and concluded that the double infiltration method using 15% jellywax as a first infiltrating media with 3 changes with 1 hour interval at room temperature and second infiltrating media as paraffin wax at 58°C with 3 changes of 1 hour interval. she mentioned that double infiltration method, with this combination showed better results and cellular details in heart muscle as compared to paraffin and paraffin with cerasin infiltration method. Hence, in the present study the plastic teacup and thermacol infiltrated cardiac muscle fibers showed uniform sectioning and staining with distinct striations and intercalated disc with uniform distribution of cytoplasm and nuclear stain (Fig. 4). This method is short and economical compared to other methods, Hence, this method can be recommended for the histological study of different organs.

**CONCLUSION**

Among the four infiltrating media studied, the plastic tea cup and thermacol resin is economical and best infiltrating media for studying histology of cardiac muscle fibers. Such, infiltrating media can be used for plastination of organs collected from endangered species / near extinct species after their death and which can be further used for studying histoarchitecture of the collected organs.

**REFERENCES**

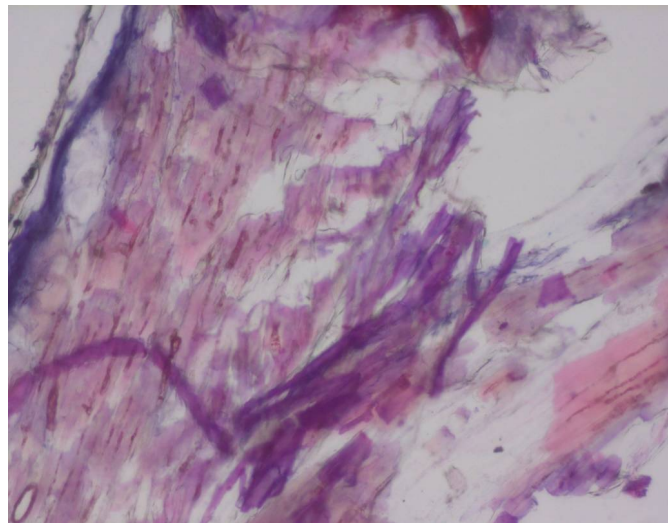
1. Singh UB, Sulochana S. Handbook of Histological and Histochemical Techniques. 2<sup>nd</sup> ed. Premier Publishing House: Hyderabad; 1996.
2. Culling CFA. Hand Book of Histological and Histochemical Techniques. 3<sup>rd</sup> ed. Butterworths: London; 1974.
3. Manjunath K, Prasad RV, Jamuna KV, Placid E. D'souza, Suguna rao and Ramkrishna, V. Comparison of Histological Architecture of Paraffin Embedded and Indigenously Plastinated Tissues. Indian Journal of Veterinary Anatomy 2014; 26 (2):132-133.
4. Grondin G, Grondin GG, Talbot BG. A study of criteria permitting the use of plastinated specimens for light and electron microscopy. Biotechnique and Histochemistry 1994;69:219-234.



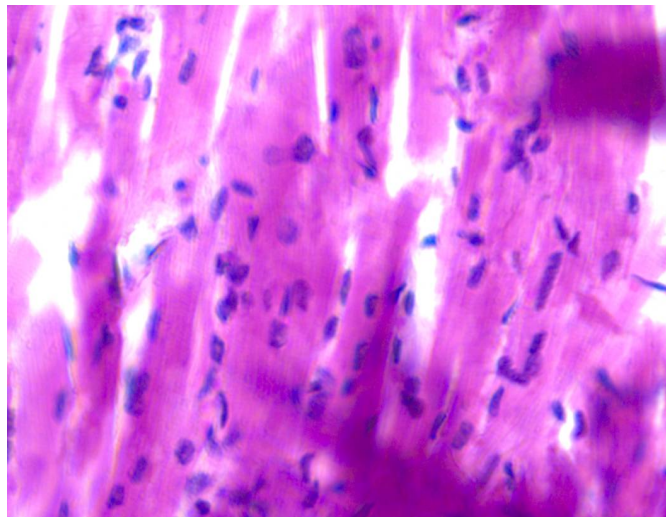


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5. Ripani M, Boccia L, Cervone P, Macciucca DV. Light microscopy of plastinated tissue. Can Plastinated organs be considered viable for structural observation. Journal of the International Society for Plastination 1996;11: 28-30.
6. Ravi SB, Bhat VM. Plastination: A novel, innovative teaching adjunct in oral pathology. Journal of Oral and Maxillofacial Pathology 2011;15:133-137.
7. Ramya. Comparative Histomorphology of liver, spinal cord,cardiac muscle, kidney and lymph node of pig using different embedding media.[dissrtation] India:KVAFSU; 2015.



**Fig. 1: Biodur infiltrated cardiac tissue did not show clear striations and also the sections were not uniform -100X**



**Fig. 2 Jelly Wax infiltrated cardiac tissue sections were uniform but did not show the striation and intercalated disc -400X**





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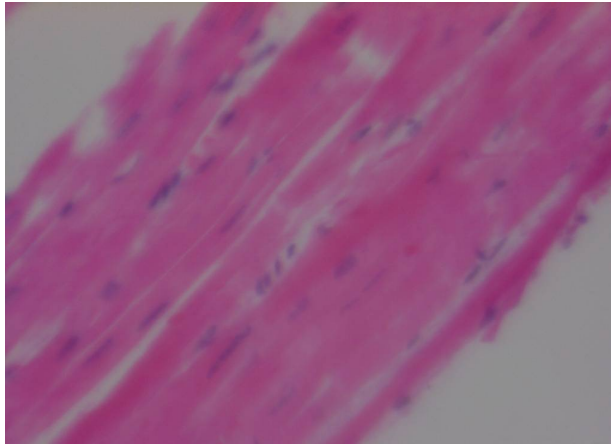


Fig. 3 Vegetable oil (Pongamia oil) infiltrated cardiac tissue .The cytoplasmic and nuclear stain of the fibers were more clear in the oil infiltrated sample - 400x

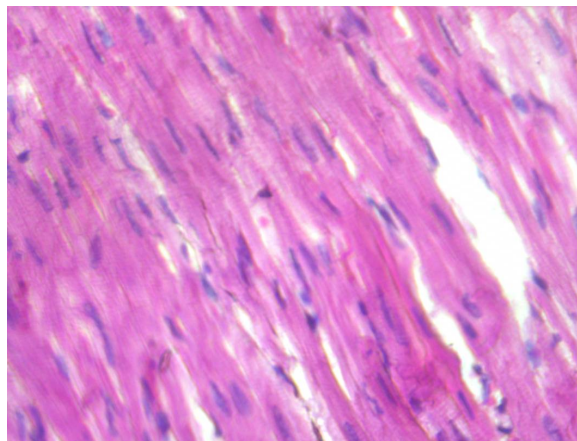


Fig. 4 Plastic Teacup and Thermacol resin infiltrated cardiac tissue showing uniform sectioning and staining with distinct striations and intercalated disc with uniform distribution of cytoplasm and nuclear stain - 400x

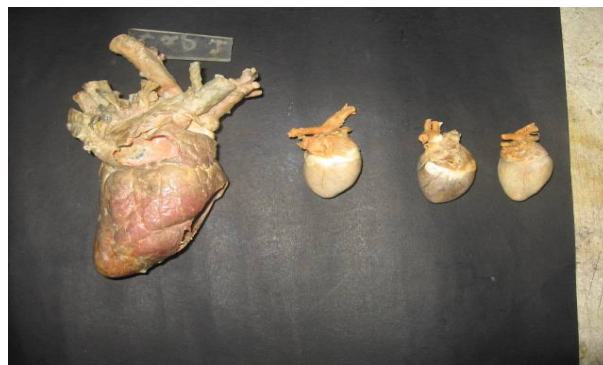


Fig. 5 Plastinated heart using biodur infiltrating media





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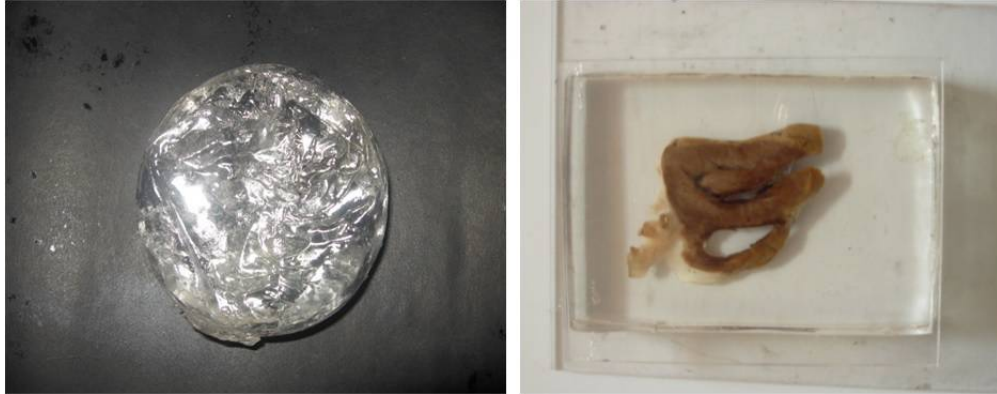


Fig. 6. Jelly wax infiltrated heart



Fig. 7. Vegetable Oil (pongamia oil) as an Infiltrating Media for cardiac tissue







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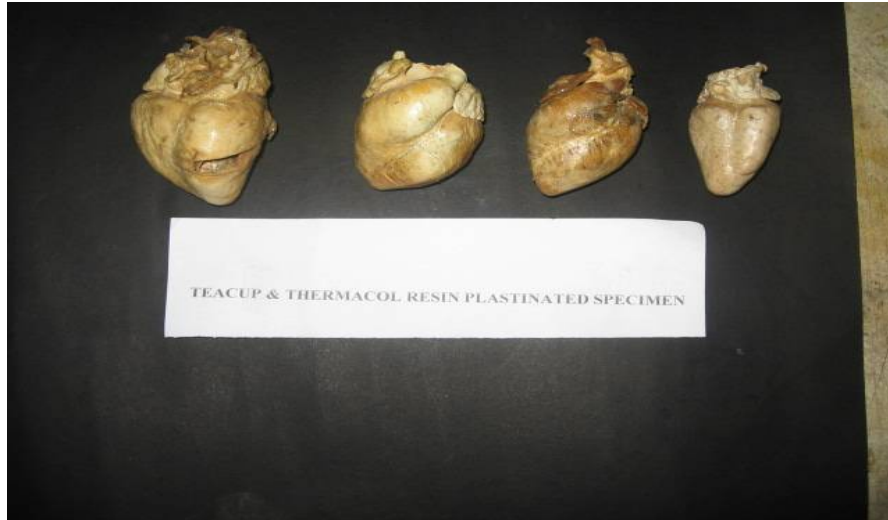


Fig.8.Tea-cup & Thermocol resin infiltrated Heart specimens





RESEARCH ARTICLE

## Allelic Polymorphism in the Second Exon of *CAHI-DRB3* in Resistance to Gastrointestinal Nematodes of Kumaon Hill Goats

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### ABSTRACT

A number of studies show that, the QTLs (Quantitative Traits Loci) for resistance to gastrointestinal nematodes in ruminants are located on chromosomes. The MHC has been consistently associated with nematode resistance and the polymorphism in MHC class II genes is playing an important role in resistance to gastrointestinal nematodes. The polymorphisms of MHC genes strongly influence the outcome of infection and lead to genetic resistance to infections. The widest polymorphism among the MHC genes is found in locus DRB and DQA. The most frequently investigated fragment of the DRB and DQA gene covers exon 2, which codes the binding site for a foreign protein. The present study was to know the polymorphism in the *cahi-DRB3* gene in resistance to Kumaon hill goats. A total of 60 animals were used for analysing resistance of Kumaon hill goats. The blood samples were collected and genomic DNA was isolated. The *cahi-DRB3* gene was amplified from the genomic DNA, purified and sequenced for the polymorphism. The *cahi-DRB3* sequences revealed a single band of 319bp including 267bp exon 2 encoding 89 amino acid. The sequence obtained was analysed and found the existence of ten *cahi-DRB3* alleles in Kumaon hill goats. The occurrence of alleles varied with different proportion among the population.

**Key words:** DRB3, polymorphism, MHC, Gastrointestinal nematodes



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## INTRODUCTION

Grazing animals are permanently exposed to infection with larvae of gastrointestinal nematodes. Parasitic infection is one of the main problems causing considerable losses in ruminants causing decrease in productivity (Perry and Randolph, 1999), mortality (Sykes, 1994), and high economic losses (Iqbal *et al.*, 1993) in small and marginal farming communities. Among the diseases that constrain the survival and productivity of sheep and goats, gastrointestinal nematodes (GINs) infection ranks highest on a global index, with *Haemonchus contortus* being of overwhelming importance (Perry *et al.*, 2002). Along with *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Oesophagostomum columbianum* are common in small ruminants in India (Annual report GIP, 2010). The economic losses in ruminants due to parasites in the United States are estimated at more than \$ 3 billion per year (Smith, 2002). The annual losses due to GINs in sheep and goats in Australia are estimated 400 million Australian dollars (AUD) (Sackett *et al.*, 2006). The mortality due to sheep helminths in Australia accounts for 41 million AUD (McLeod, 1995). The projected losses due to strongyle infection in goats in Madhya Pradesh were Rs. 657 million, while it was Rs.73 million in Meghalaya. The mean loss per animal in sheep and goat was Rs.336 and 580, respectively (Annual report on GIP, 2011). The control of the nematode infestations in ruminants relies mainly on the proper organization of grazing and (or) use of anthelmintic agents. One of the novel approaches is the characterization and utilization of host genetic variation for resistance or resilience to endoparasites. It is carried out by selection and breeding of animals genetically resistant to nematodes (Baker *et al.*, 1999; Bishop and Stear, 1999). The selection of animals, which are genetically resistant to infection by gastrointestinal nematode parasite, is an attractive approach because resistance traits are readily disseminated through the sale of resistant animals and it can provide long term solutions.

A number of studies show that, the QTLs (Quantitative Traits Loci) for resistance to gastrointestinal nematodes in ruminants are located on chromosomes. The major histocompatibility complex (MHC) has been consistently associated with nematode resistance (Schwaiger *et al.*, 1995; Dukkipati *et al.*, 2006). The polymorphism in MHC class II genes is playing important role in resistance to helminths, in which *DRB* and *DQA locus* are important in small ruminants. They encode for beta chain and  $\alpha$ -chain of the DR and DQ molecules respectively found in high concentrations on the surface of antigen-presenting cells. MHC class II molecules are found on the surface of antigen-presenting cells (APC), mainly on B lymphocytes, macrophages and dendritic cells and are responsible for presenting exogenous antigens to CD4<sup>+</sup> T lymphocytes. A peptide binding groove is formed in between  $\alpha 1$  and  $\beta 1$  domains with a beta pleated floor and, the greatest polymorphic variability in the amino acids is in those facing the groove. A number of studies showed that the MHC region had a statistically significant association with gastrointestinal nematode parasite resistance (Schwaiger *et al.*, 1995; Outteridge *et al.*, 1996; Paterson *et al.*, 1998; Van Haeringen *et al.*, 1999). The MHC of goat (Cahi/GoLA/CLA) is located on chromosome 23 (Vaiman *et al.*, 1996).

## MATERIALS AND METHODS

### Animals for study

The experimental local hill goats used in present study belonged to Indian Veterinary Research Institute herd maintained at IVRI, Mukteshwar (Nainital, Uttarakhand) is located in the temperate Himalayan region of India at 29°28'20"N, 79°38'52"E, and has an average elevation of 2,171 metres (7,123 feet) above the mean sea level (msl).

### Blood sample collection

The Blood samples (5ml) were collected aseptically in plastic containers with anticoagulant (0.1% ethylene diamine tetraacetic acid (EDTA)) from the jugular vein from 60 local hill goats.



**Subramani et al.****Isolation of Genomic DNA**

The genomic DNA was isolated using the 'Genomic DNA Purification Kit' supplied by Qiagen using standard protocol. The isolated DNA was resolved by 0.8% agarose gel and checked for concentration and quality. The quality (ratio of  $A_{260}/A_{280}$ ) and quantity ( $\text{ng}\mu\text{l}^{-1}$ ) of genomic DNA was estimated using NanoDrop (NanoDrop 1000-Thermo Scientific Spectrophotometer, USA).

**Amplification of *cahi-DRB3* exon 2**

The PCR was standardised for amplification *cahi-DRB3* exon 2 from genomic DNA with primers described by Baxter *et al.*, (2008). The PCR was standardised with annealing temperature 58.5°C.

**Sequence based typing and analysis**

The sequences obtained from the forward and reverse reactions of each gene were analysed using GENE TOOL and Lasergene program (DNASTAR, Madison, WI) software as well as visually using chromogram of corresponding gene. The nucleotide sequences were compared with sequences in the GenEMBL database using the BLAST algorithm. The heterozygous positions were assigned ambiguity codes as recommended by the IUPAC-IUB Biochemical Nomenclature Commissions. A consensus of nucleotide sequence containing ambiguity codes at heterozygous positions was generated. The analysed sequences were compared with published sequences of Chinese indigenous goat and chegu goat.

**RESULTS AND DISCUSSION**

The PCR amplicons of *DRB3* revealed 319bp in agarose gel electrophoresis (Fig.1). The amplified products consisted of 267bp exon 2 and both side of exon flanked by introns. The PCR samples were purified and sequenced for exon 2.

**Sequence results**

The PCR was standardised with annealing temperature 58.5°C. The PCR amplicons of *DRB3* revealed 319bp in agarose gel electrophoresis. The amplified products consisted of 267bp exon 2 and both side of exon flanked by introns. The PCR samples were purified and sequenced for exon 2. It was found that *DRB3.2* gene is highly polymorphic. By comparing with the published deduced amino acid sequences, it was found that the glycosylation site (amino acid positions 19 to 21) was conserved and the codon positions from 51 to 55 of many of the animals were similar to amino sequence TELGR. The analysis of sequences revealed single band of 319bp including 267bp exon2 encoding 89 amino acid (Fig. 2). The analysis of the *DRB3.2* sequences of hill goats revealed existence of six alleles namely *Cahi DRB3.2*, *Caae DRB3.2*, *DRB\*01*, *DRB\*02*, *DRB\*03* and *DRB\*18*. The frequency distribution of different alleles is given in (Table. 2). The frequency of occurrence of *Cahi DRB3.2* allele is maximum with 45.2%, followed by *DRB\*02* (29%), *Caae 3.2* (12.9%), *DRB\*18* (6.5%) and others alleles with 3.2%. The nucleotide sequence alignment of *cahi-DRB3* with published alleles reveals variation in 71 nucleotide (Fig.2) and 55 amino acid positions. The nucleotide identity with published alleles was between 88-98%. (Fig.3) and phylogenetic analysis is given in Fig.4. The polymorphism in MHC gene is strongly influence the outcome of infection and lead to genetic resistance to infections. The main function of MHC molecule is the presentation of peptide antigen to T lymphocytes. Special attention is paid to the MHC class II molecules that induce the immune response in case of extracellular infection. The widest polymorphism among the MHC genes is found in *locus DRB*. This gene encodes the beta chain of the DR molecule, a protein found in high concentrations on the surface of antigen-presenting cells. The most frequently investigated fragment of the *DRB* gene covers exon 2, which codes the binding site for a foreign protein (Charon, 2003). The present study is designed to study the genetic diversity in hill goats of Uttarakhand with respect to MHC class *DRB3.2*, hitherto no one studied any of the genetic study in these goats. The important feature of MHC class II





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genes is the extensive polymorphism and this polymorphism is characterized by a large number of alleles at each locus and a large number of amino acid substitutions between alleles. Associations of alleles of the bovine major histocompatibility complex DRB3 exon 2 (BoLA DRB3\*02) with occurrence of disease and production traits have previously been documented (Sharif et al., 1998). However, little is known about the associations between CLA-DRB3\*02 alleles and the resistance to disease (nematode infection) and production traits (e.g. meat and milk) of goat.

In this study, the highly polymorphic nature of caprine DRB3 gene exon 2 has been demonstrated by PCR and sequence analyses. The extensive polymorphism observed at caprine DRB3.2 locus shows the hallmark typical of classical MHC genes, *i.e.* (1) multiple nucleotide and amino acid substitutions between alleles, and (2) a large number of alleles (Snibson *et al.*, 1998). The analysis of sequences revealed single band of 319bp including 267bp exon2 encoding 89 amino acid. Among the 267 nucleotides and 89 amino acid positions, 49 (18.35%) nucleotide positions and 34 (38.2%) amino acid residues were variable. It was found that *DRB3.2* gene is highly polymorphic. The analysed sequences were compared with published sequences of Chinese indigenous goat and chegu goat. By comparing with the published deduced amino acid sequences, we found that the glycosylation site (amino acid positions 19 to 21) was conserved and the results are similar to previous report (Meng Hua Li *et al.*, 2006). Also, the codon positions from 51 to 55 were similar to amino sequence TELGR of many of sequences, which was found to be highly conserved in primate DRB genes. This position is located at the transition between  $\beta$  sheet and  $\alpha$  helix and is encoded by a nucleotide sequence, which is believed to promote recombination both between different alleles and between different loci (Gyllensten *et al.*, 1991). The frequency distribution of both alleles and genotypes of hill goats at DRB3\*02 are deviated highly from homogeneity expectation among goat populations. The pattern of polymorphism in six DRB3\*02 alleles in the hill goats of Uttarakhand are matching to Chinese indigenous goats (Meng Hua Li *et al.*, 2006). In addition to highly polymorphic regions, the conserved structure present in the DRB3\*02 alleles in Uttarakhand goat populations suggested that there were antigens specific for infectious agents of goat. On the other hand, the high level of polymorphism found in CLA-DRB3\*02 corresponded to a wide range of antigens.

## REFERENCES

1. Perry, B.D and Randolph, T.F. 1999. Improving the assessment of the economic impact of parasitic diseases and of their control in production animals. *Vet. Parasitol.* 84:145–168
2. Sykes, A.R. 1994. Parasitism and production in farm ruminants. *Anim. Prod.* 59: 155– 172
3. Iqbal, Z., Akhtar, M., Khan, M. N. and Riaz, M. 1993. Prevalence and economic significance of haemonchosis in sheep and goats slaughtered at Faisalabad abattoir. *Pakistan J. Agric. Sci.* 30: 51–53
4. Perry, B.D., Randolph, R.F., McDermott, J.J, Sones, K.R. and Thornton, P.K. 2002. Investing in animal health research to alleviate poverty. International Livestock Research Institute (ILRI), Nairobi, Kenya. pp. 148.
5. Annual Report. 2010. Network programme on gastrointestinal parasitism. (ICAR funded project).
6. Smith, S.M., (2002). AGRI-FOCUS July 2002. Cooperative Extension, Washington State University, Grant and Adams Area, <http://grant-adams.wsu.edu>
7. Sacket, D., Holmes, P., Abbott, K., Jephcott, S. and Barber, M. 2006. Assessing the economic cost of endemic disease on the profitability of the Australian beef cattle and sheep producers. Final report, Meat and Livestock Australia, Sydney.
8. McLeod, R.S. 1995. Cost of major parasites to the Australian livestock industries. *Int. J. Parasitol.* 25:1363-1367.
9. Annual Report. 2011. Network programme on gastrointestinal parasitism. (ICAR funded project).
10. Baker, R.L., Mwamachi, D.M., Audho, J.O., Aduda, E.O. and Thorpe, W. 1999. Genetic resistance to gastrointestinal nematode parasites in Red Maasai, Dorper and Red Maasai x Dorper ewes in the sub-humid tropics. *Anim. Sci.* 69(2): 335-344.
11. Bishop, S.C. and Stear M.J. 1999. Genetic and epidemiological relationships between productivity and disease resistance: gastro-intestinal parasite infection in growing lambs. *Anim. Sci.* 69: 515-524.





**Subramani et al.**

12. Schwaiger, F.W., Gostomski, D., Stear, M.J., Duncan, J.L., McKellar, Q.A., Epplen, J.T. and Buitkamp, J. 1995. An ovine major histocompatibility complex DRB1 allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Int. J. Parasitol.* 25(7): 815-822.
13. Dukkupati, V.S.R., Blair, H.T., Garrick, D.J. and Murray, A. 2006. 'Ovar-Mhc' - ovine major histocompatibility complex: structure and gene polymorphisms *Genet. Mol. Res.* 5 (4): 581-608.
14. Outteridge, P.M., Andersson, Douch, P.G, Green, R.S, Gwakisa, P.S, Hohenhaus, M.A. and Mikko, S. 1996. The PCR typing of MHC-DRB genes in the sheep using primers for an intronic microsatellite: application to nematode parasite resistance. *Immunol. Cell Biol.* 74: 330-336
15. Paterson, S., Wilson, K. and Pemberton, J.M. 1998. Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. *Proc. Natl. Acad. Sci. USA.* 95: 3714-3719.
16. Van Haeringen, W.A., Gwakisa, P.S., Mikko, S., Eythorsdottir, E., Holm, L.E., Olsaker, I., Outteridge, P.M. and Anderson, L. 1999. Heterosigosity excess at the cattle DRB locus revealed by large scale genotyping of two closely linked microsatellites. *Anim. Genet.* 30: 169-176.
17. Vaiman, D., Schibler, L., Bourgeois, F., Oustry, A., Amigues, Y. and Cribeu, E.P. 1996. A genetic linkage map of the male goat genome. *Genetics.* 144(1): 279-305.
18. Baxter, R., Hastings, N., Law, A and Glass, E.J.(2008). A rapid and robust sequence based genotyping method for BOLA DRB3 alleles in large number of heterozygous cattle. *Anim. Genetics.* 39.pp.56-563.
19. Charon, K.M. 2003. Genes controlling resistance to gastrointestinal nematodes in ruminants. *Anim. sci. papers and reports.* 22(1): 135-139.
20. Sharif, S., Mallard, B.A., Wilkie, B.N., Sargeant, J.M., Scott, H.M., Dekkers, J.C.M. and Leslie, K.E. 1998. Associations of the bovine major histocompatibility complex DRB3 (BoLA-DRB3) alleles with occurrence of disease and milk somatic cell score in Canadian dairy cattle. *Anim. Genet.* 29: 185–193
21. Snibson, K.J., Maddox, J.F., Fabb, S.A. and Brandon, M.R. 1998. Allelic variation of ovine MHC class II DQA1 and DQA2 genes. *Anim. Genet.* 29: 356–362.
22. Meng-Hua Li, Kui Li, Juha Kantanen, Zheng Feng, Bin Fan. and Shu-Hong Zhao 2006. Allelic variations in exon 2 of caprine MHC class II DRB3 gene in Chinese indigenous goats. *Small Rumin. Res.* 66: 236–243.
23. Gyllensten, V.B., Sundvall. And Erlich, M.H.A. 1991. Allelic diversity is generated by intraexon sequence exchange at the DRB1 locus of primates. *Proc. Natl. Acad. Sci. U.S.A.* 88: 3686–3690.

**Table 1: Primers for the PCR amplification and sequencing of DRB3 Gene**

Orientation	Primer	Sequence (5' - 3')	Length(bases)
Sense	DRB3FRW	CGC TCC TGT GAY CAG ATC TAT CC	23
Antisense	DRB3REV	CAC CCC CGC GCT CAC C	16

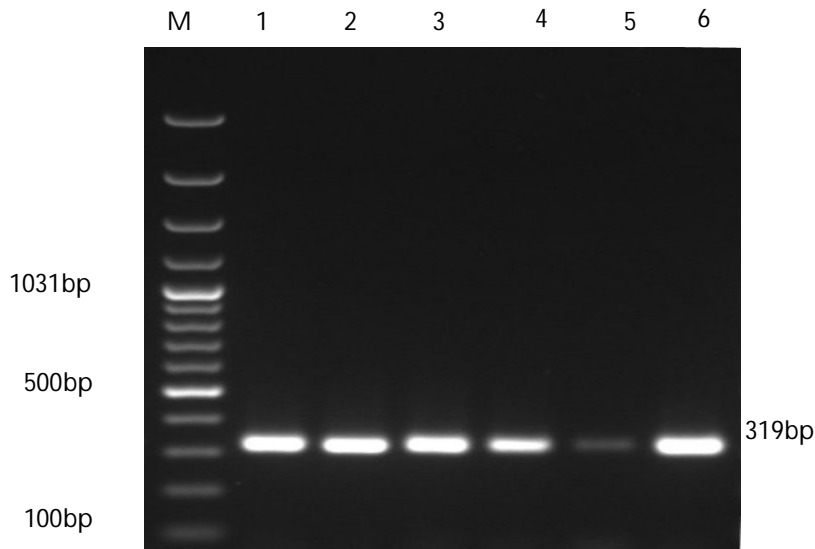
**Table.2.The frequency of different DRB3 alleles in Kumaon hill goats**

Allele	Frequency	Percent (%)
DRB*01	1	3.2
DRB*02	9	29.0
DRB*03	1	3.2
Cahi DRB3.2	14	45.2
Caae DRB3.2	4	12.9
DRB*18	2	6.5
<b>Total</b>	<b>31</b>	<b>100.0</b>

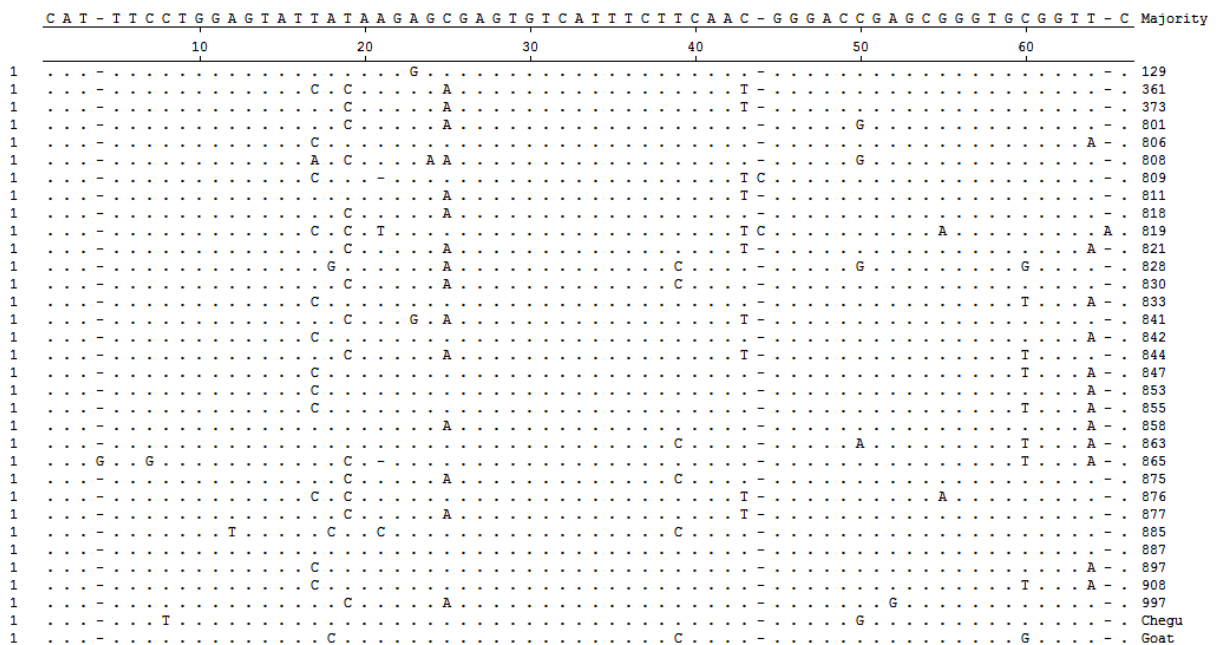




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**Fig.1.**The amplification of *Cahi-DRB3* exon 2 showing a band at 319bp in Kumaon Hill goats  
 Lane M: 100bp plus DNA ladder  
 Lane 1-6: 319bp PCR product of *DRB3* exon 2

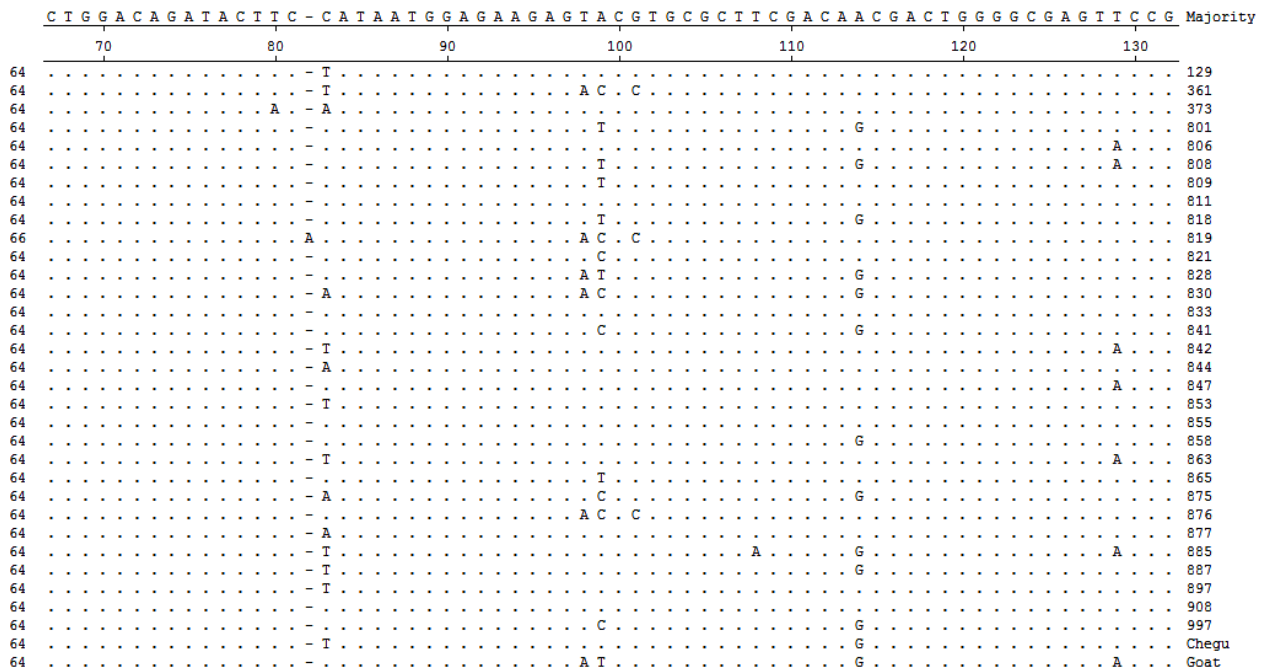


**Fig.2.**The Nucleotide sequence alignment of *cahi-DRB3* with published alleles (cont..)

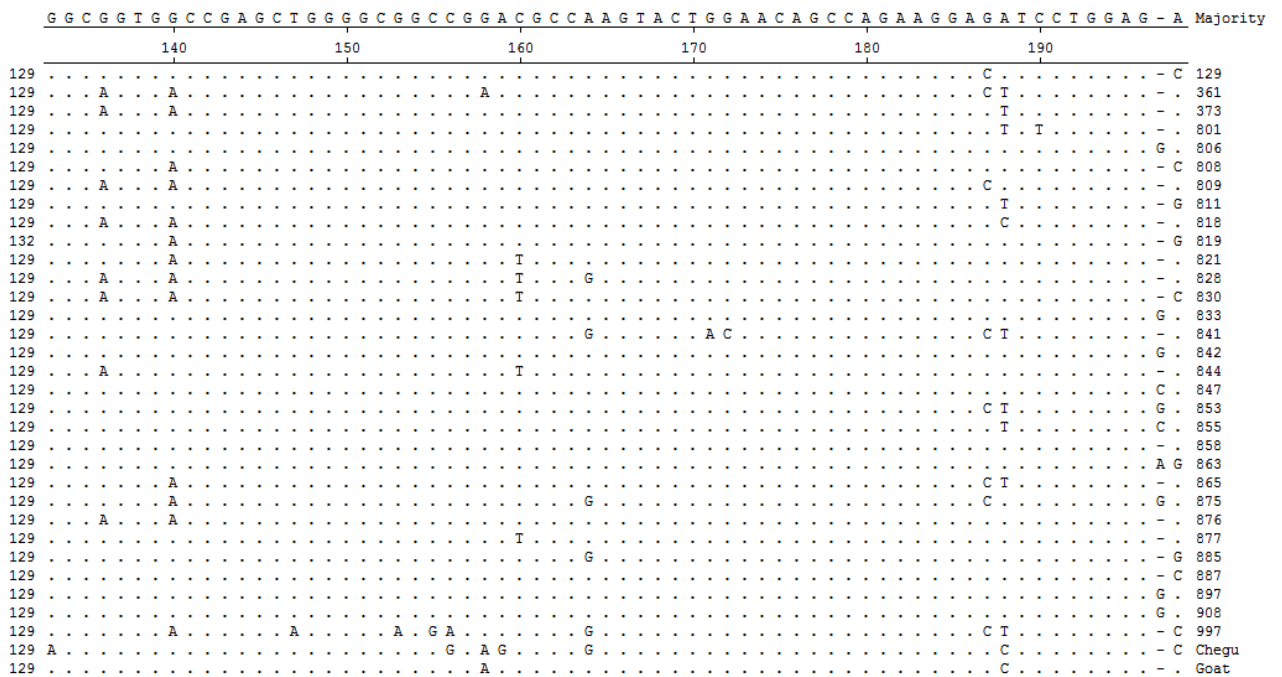




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**Fig.2. The Nucleotide sequence alignment of *cahi-DRB3* with published alleles**



**Fig.2. The Nucleotide sequence alignment of *cahi-DRB3* with published alleles.**







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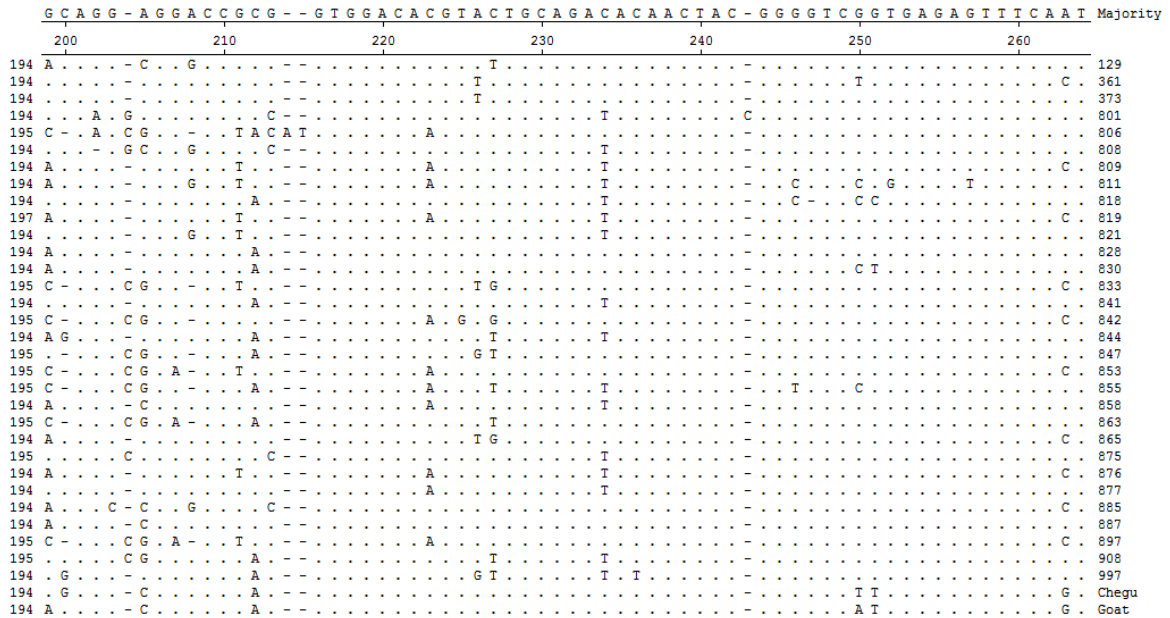


Fig.2.The Nucleotide sequence alignment of *cahi-DRB3* with published alleles (cont.)

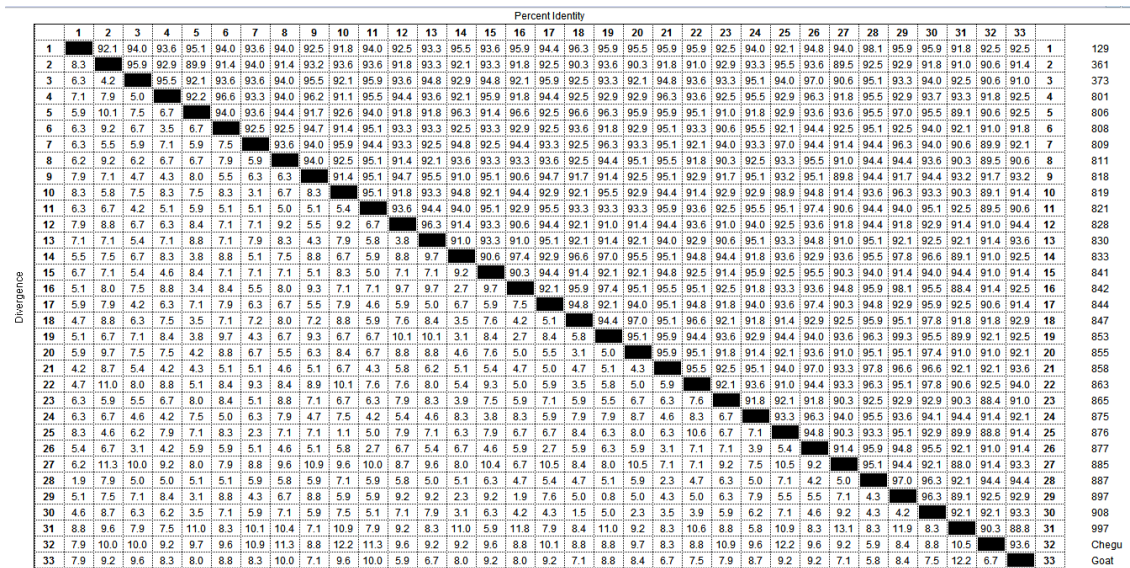


Fig.3.The nucleotide sequence homology of *cahi-DRB3* with published alleles





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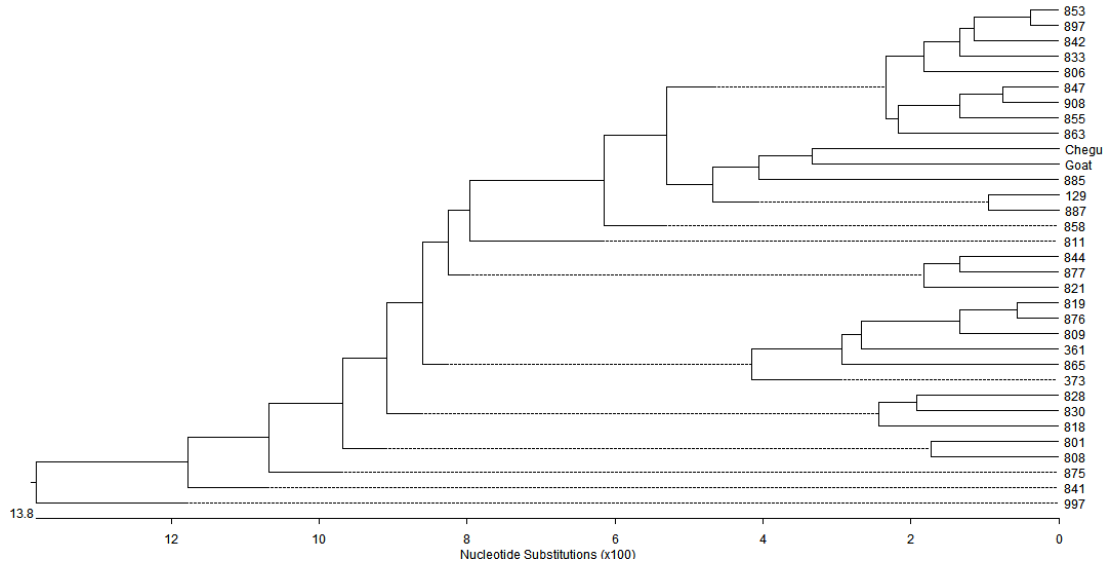


Fig.4.The phylogenetic analysis of *cahi-DRB3* with published alleles





## RESEARCH ARTICLE

## Microbial Quality of Broiler Meat Available in Retail Markets of Wayanad District

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### ABSTRACT

Present study was conducted to evaluate the bacterial quality of broiler meat available in 20 local markets in 6 different locations of Wayanad district, Kerala state. The bacterial colonies (Total viable count-TVC, Coliform count-CC, *E. coli* count- ECC, Faecal Streptococcal count-FSC) were counted after incubation at specified temperature. The counts per gram of the sample were estimated by multiplying the mean colony forming unit (CFU) count of the sample in duplicate plates with the dilution factor. A total of 40 samples (in duplicates) were collected from retail outlets of Wayanad district. The analysis of samples revealed the total viable count ranged from  $8.2 \times 10^3$  to  $1.86 \times 10^5$  cfu/ml, with lowest count from Kalpetta region. Further, the carcasses had counts at the level of 3, 2,  $2.0 \log_{10}$  cfu/mL for coliforms, generic *E. coli*, and faecal streptococcus, indicating significant contamination. Poultry meat contamination with microorganisms which cause deterioration in food quality, and foodborne diseases, is a major challenge for poultry industries, which can be prevented by adopting the use of modern slaughtering technologies and practicing preventive measures on poultry farms

**Key words:** Broiler meat, Microbial Quality, Kerala

### INTRODUCTION

Poultry is an important part of the animal food and the volume of their production, marketing and consumption is increasing to satisfy the public demand worldwide within the last decades (Bryan, 1980; Anand et al., 1989; Mead 2004). The presence of pathogenic and spoilage microorganisms in poultry meat and its by-products remains



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asignificant concern for suppliers, consumers and publichealth officials worldwide Food borne diseases associated with the consumption of poultry meat and its processed products are of public health significance worldwide (Chaiba et al.,2007). The consumption of poultry meat increased worldwide within last decade (Mead.2004). And Poultry meat is more popular in the consumer market because of advantages such as easy digestibilityand acceptance by the majority of people (Yashoda et al., 2001). However meat is most perishable of all staple foods since it contains sufficient nutrient needed to support the growth of microorganisms (Maripandi, A. and A.A. Al-Salamah, 2010). Contaminated raw meat is one of the main sources of food-borne illnesses (Bhandare et al.,2007). There are few surveys and lack of information on bacteriological status of poultry meat in the retail markets of Wayanad district. Since the chicken meat is directly sold on the same day at ambient temperature in open outlets, it seems important to investigate microbial quality such as total viable count, *E.coli*, faecal streptococci. Coliformcount. Therefore this study was conducted to investigate the microbial quality of raw broiler meat available in all common retail shops of 6 different places of Wayanad district.

**MATERIALS AND METHODS****Sampling of materials**

A total of 40 broiler meat samples (in duplicates) were collected from 20 retail outlets in 6 different locations of Wayanad district. 10-20 gm of meat sample is collected from each market in Sterile polythene covers. The collected samples of each market were labeled transported to laboratory in thermocool boxes with ice for further analysis.

**Microbiological assay**

**Preparation of dilutions;** 10 gm of meat sample is triturated with 90ml peptone water (0.5%) using sterile mortar and pestle (10 fold dilution). 1 ml of homogenised meat solution is then transferred in to 9 ml of 0.5% peptone water to form  $10^{-2}$  dilution, same was followed to get  $10^{-3}$  dilution.

**Total viable count;** one ml of each dilution ( $10^{-2}$  &  $10^{-3}$ ) was pipette in to sterile Petri plates and mixed with 25 ml of plate count agar at 40-45°C uniformly under sterile conditions. Solidified Plates were incubated upside down at 39°C. After 24 hours the number of all colonies on the plate (between 30-300) was counted for each dilution. Each colony forming unit represented a bacterium that was present in the diluted sample, therefore the concentration of viable bacteria per milliliter in initial sample can be calculated and expressed in CFU /ml.

**Fecal streptococcal count;** 0.1ml of each ( $10^{-2}$  &  $10^{-3}$ ) dilution was spread out on a sterile Petri-dish contained a solidified KF streptococcal agar using sterile L shaped glass rod. Then all plates incubated upside down at 37°C. After 24 hours the number of all colonies on the plate (between 30-300) was counted for each dilution.

**Coliform count;** using solidified VRBA plates coliforms counts were taken as described above for fecal streptococci.

**E.coli count;** using solidified EMB plates *E.coli* counts were taken as described above.

**RESULTS**

Meat and surface samples included in this study showed high viable bacterial counts as shown in table 1. The analysis of samples revealed the total viable count ranged from  $8.2 \times 10^3$  to  $1.86 \times 10^5$  cfu/g, with lowest count from Kalpetta region. Further, the carcasses had counts at the level of 3, 2,  $2.0 \log_{10}$  cfu/g for coliforms, generic *E. coli*, and faecal streptococcus, indicating significant contamination

**DISCUSSION AND CONCLUSION**

Observations showed heavy bacteriological load in meat with total viable counts ranging from  $8.2 \times 10^3$  to  $1.86 \times 10^5$  CFU/g. The broiler meat had counts at the level of 3, 2,  $2.0 \log_{10}$  cfu/g for coliforms, generic *E. coli*, and faecal





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streptococcus. Amara et al. (1994) reported TVC of chicken meat as high as 6.6-7.2 log cfu/g while Oumokhtar (2000) reported a mean TVC of 4.5 log cfu/g. These counts were lower than 6.18 log<sub>10</sub> cfu/g, 5.37 log<sub>10</sub> cfu/cm<sup>2</sup> and 6.14 log<sub>10</sub>cfu/g of coliforms, *E. coli* and faecal streptococci reported by Chaiba et al. (2007). The TVC were lower than 5 X 10<sup>5</sup> to 10<sup>7</sup> cfu/g as detected by Heetun et al. (2015) and *E. coli* counts were higher than 2 X 10<sup>5</sup> cfu/g reported by Heetun et al. (2015). Bhicoo (2011) who observed an average population of 5.6 log cfu/g of total coliforms for chicken sourced from markets which were higher than the present study findings. The higher microbial counts in broiler meat showed there is risk of health hazard in meat handlers and consumers. Routine examination of poultry meat and education of butchers and consumers regarding the importance of these pathogens and maintenance of hygienic conditions during slaughtering, processing need to be implemented to reduce the risk of infection.

## REFERENCES

1. Bryan F.L., 1980 Poultry and meat products. [Edited by Silliker J.H., Elliot R.P., BairdParker A.C., Bryan F.L., Christian J.H.B., Clark D.S., Olson J.C. and T.A. Roberts] In Microbiol. Ecology of Foods, 2: Food Commodities. (ICMSF) 410 – 458.
2. Anand S.K., Mahapatra C.M., Pandey N.K. and Verma S.S., 1989 Microbial changes on chicken carcasses during processing. *Indian J. Poult. Sci*, 24(3), 203 – 209.
3. Mead, G.C., 2004. Microbiological quality of poultry meat: a Review. *Brazilian J. Poultry Sci*. 6(3), 135 – 142.
4. Chaiba A, FF Rhazi, A Chahloai, BR Soulaymani and M Zerhouni., 2007. Microbiological quality of poultry meat on the Mekne's Market (Morocco). *Inter. J. Food Safety*, 9, 67-71.
5. Yashoda KP, NM Sachindra, PZ Sakhare and DN Rao, 2001. Microbiological quality of broiler chicken carcasses processed hygienically in a small scale poultry processing unit. *J. Food Quality*, 24, 249–259.
6. Maripandi, A. and A.A. Al-Salamah, 2010. Multiple-antibiotic resistance and plasmid profiles of *Salmonella enteritidis* isolated from retail chicken meats. *Am. J. Food Technol.*, 5, 260-268
7. Bhandari N, DB Nepali and S Paudyal, 2013. Assessment of bacterial load in broiler chicken meat from the retail meat shops in Chitwan, Nepal. *Int. J. Infect. Microbio.*, 2,99-104.
8. Amara A, Badoum M, Faid Mand Bouzoubaa K, 1994. Microbial contamination of poultry slaughtered in traditional shops in Morocco. *Microbiologie des Aliments de Nutrition*, 12, 323–327
9. Oumokhtar B, 2000. Qualité bactériologique de viandes, d'abats, de préparations carnées et d'huîtres commercialisées à Rabat. Thèse de Doctorat National, Université Chouaib Doukkali, Faculté des Sciences, El Jadida, Morocco
10. Heetun I., Goburdhun D, Neetoo H., 2015. Comparative Microbiological Evaluation of Raw Chicken from Markets and Chilled Outlets of Mauritius. *J. World's Poult. Res*, 5(1), 10-18.
11. Bhicoo J., 2011. Evaluation of food hygiene in central market of Port-Louis. BSc Thesis. Faculty of Agriculture, University of Mauritius.





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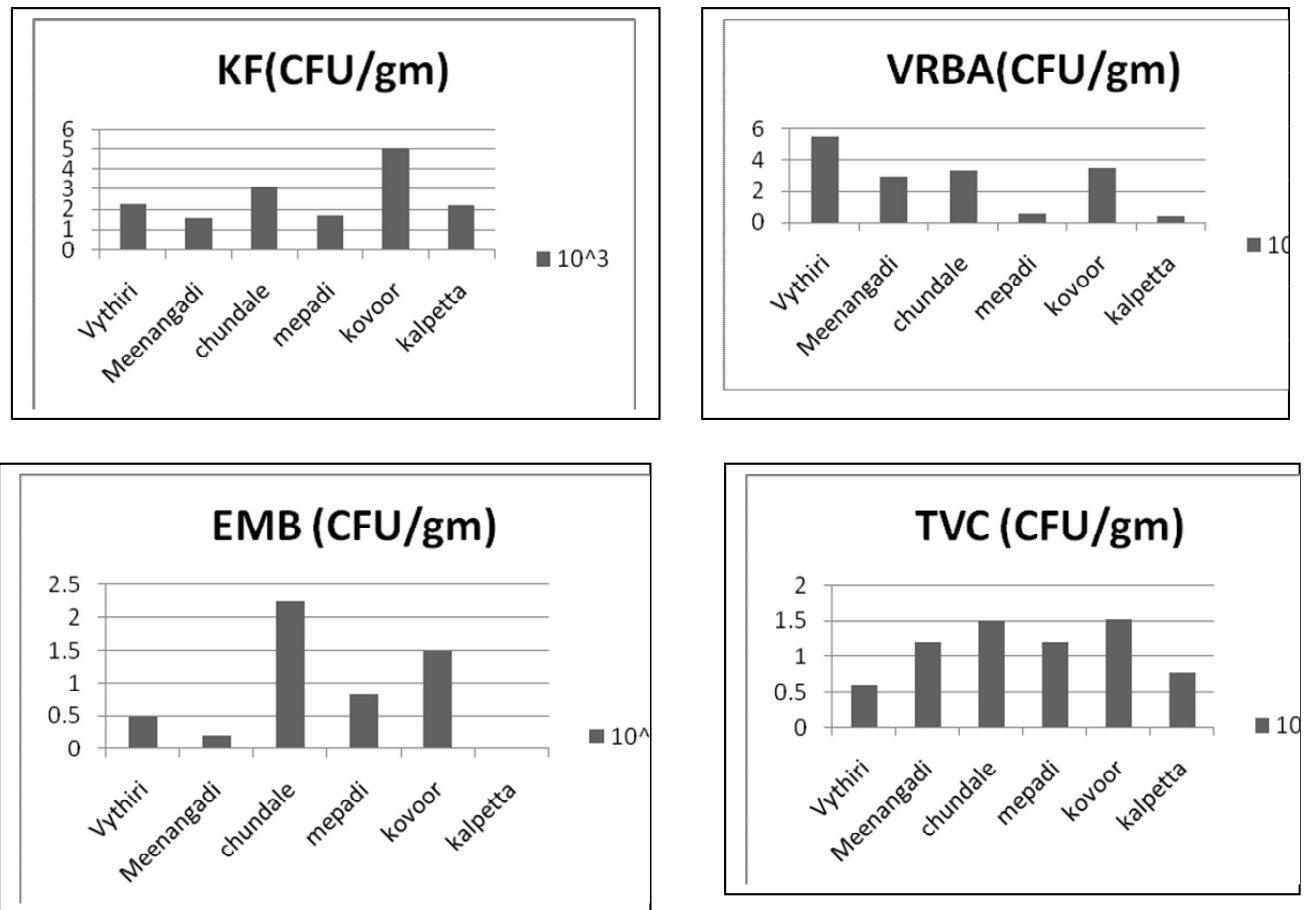


Fig.1. Graphs showing bacterial count in various media

Table 1. Bacterial counts of meat samples of retail outlets of Wayanad district (CFU/g in different media)

Location	Market no.	TVC	VRBA	EMB	KF
Vythiri	01	0.96x10 <sup>4</sup>	2.6 x10 <sup>3</sup>	-ve	3.1 x10 <sup>3</sup>
	02	1.09x10 <sup>4</sup>	8.2 x10 <sup>3</sup>	-ve	2.7 x10 <sup>3</sup>
	03	1.53x10 <sup>4</sup>	1.4 x10 <sup>3</sup>	0.3 x10 <sup>3</sup>	2.9 x10 <sup>3</sup>
	04	1.91x10 <sup>4</sup>	9.9 x10 <sup>3</sup>	1.7 x10 <sup>3</sup>	03 x10 <sup>2</sup>
Meenangadi	05	1.57x10 <sup>4</sup>	0.4 x10 <sup>3</sup>	-ve	1.7 x10 <sup>3</sup>
	06	3.30x10 <sup>4</sup>	2.2 x10 <sup>3</sup>	-ve	2.0 x10 <sup>3</sup>
	07	2.09x10 <sup>4</sup>	3.8 x10 <sup>3</sup>	0.5 x10 <sup>3</sup>	1.3 x10 <sup>3</sup>




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	08	3.07x10 <sup>4</sup>	5.6 x10 <sup>3</sup>	0.3 x10 <sup>3</sup>	1.2 x10 <sup>3</sup>
Chundale	09	2.67x10 <sup>4</sup>	2.6 x10 <sup>3</sup>	0.5 x10 <sup>3</sup>	3.9 x10 <sup>3</sup>
	10	2.08x10 <sup>4</sup>	7.4 x10 <sup>3</sup>	6.2 x10 <sup>3</sup>	3.8 x10 <sup>3</sup>
	11	2.80x10 <sup>4</sup>	0.7 x10 <sup>3</sup>	1.8 x10 <sup>3</sup>	2.3 x10 <sup>3</sup>
	12	2.83x10 <sup>4</sup>	2.6 x10 <sup>3</sup>	0.5 x10 <sup>3</sup>	2.4 x10 <sup>3</sup>
Mepadi	13	2.80x10 <sup>4</sup>	0.9 x10 <sup>3</sup>	-ve	2.5 x10 <sup>3</sup>
	14	1.05x10 <sup>4</sup>	-ve	2.5 x10 <sup>3</sup>	1.3 x10 <sup>3</sup>
	15	3.30x10 <sup>4</sup>	0.7 x10 <sup>3</sup>	-ve	1.2 x10 <sup>3</sup>
Kovoor	16	2.93 x10 <sup>4</sup>	1.9 x10 <sup>3</sup>	1.2 x10 <sup>3</sup>	7.3 x10 <sup>3</sup>
	17	2.34 x10 <sup>4</sup>	5.2 x10 <sup>3</sup>	1.8 x10 <sup>3</sup>	2.8 x10 <sup>3</sup>
	18	1.26 x10 <sup>4</sup>	-ve	-ve	0.4 x10 <sup>3</sup>
Kalpetta	19	0.82 x10 <sup>4</sup>	0.1 x10 <sup>3</sup>	-ve	0.8 x10 <sup>3</sup>
	20	2.40 x10 <sup>4</sup>	1.2 x10 <sup>3</sup>	-ve	1.7 x10 <sup>3</sup>





## Management of Obstructive Urolithiasis in a Calf using Tube Cystostomy Technique

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### ABSTRACT

A three month old male Jersey crossbred calf was presented to Veterinary dispensary, Manchikeri of Karwar district in Karnataka with a history of anuria for the past 36 hours. Physical examination was carried out to check the status of the urethra and urinary bladder. Lateral abdomen radiography revealed that there was no radiopaque calculus in the urinary system and bladder was found distended. Ultrasonography revealed an intact distended bladder. The condition was diagnosed as obstructive urolithiasis based on the history of anuria, clinical signs and physical examination. Tube cystostomy was performed under general anaesthesia with diazepam-ketamine combination to effect. Routinely post-operative antibiotic, analgesic and urinary acidifier ammonium chloride were given. The tube was removed on 10<sup>th</sup> post-operative day. Animal started normal urination and had an uneventful recovery.

**Key words:** Urolithiasis, Calf, Foley's catheter, Tube Cystostomy

### INTRODUCTION

Urolithiasis is the formation of urolith(s), which may lodge anywhere in the urinary system but most frequently at the distal end of sigmoid flexure in ruminants and causes obstruction to urine flow (Radostitset *al.*, 2000). Occurrence of urolithiasis is significantly more common in male ruminants compared to females due to their anatomical





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conformation of the urethral tract (Smith and Sherman, 1994). The female have short, wide, and straight urethra while the male has long, narrow and tortuous urethra which makes them more prone to urethral obstruction, particularly distal aspect of the sigmoid flexure in bovines and urethral process in sheep and goats. The decreased urethral orifice is a major predisposing factor for obstructive urolithiasis (Smith and Sherman, 1994). In addition, factors such as diet, age, breed, genetic makeup, season, soil, water, mineral, and urinary tract infections plays an important role in the genesis of urolithiasis (Udall and Chow, 1969). The clinical signs and physiological parameters of urolithiasis may vary with the degree of urethral obstruction, its duration, age and sex of the animals, and status of urinary bladder and urethra. Urethral obstruction in calves is a fatal disease that predisposes to high mortality rate unless the animal is subjected to emergency surgical treatment for correction of the obstruction. Medical treatment of obstructive urolithiasis in ruminants has generally been unrewarding. Multiple surgical techniques have been described for treatment of such affection including urethrotomy (Singh *et al.*, 2010), urethrostomy (Khan *et al.*, 2009), penile transaction with urethral fistulation (Misk and Semieka, 2003), tube cystostomy (Kushwaha *et al.*, 2007), bladder marsupialization (May *et al.*, 1998) and laparotomy and urethrotomy (Abdel and Sedeeq, 2005). In spite of all these surgical techniques, a frustrating situation arises when surgeon has to treat an affected calf, as the surgeon has to weigh advantages against disadvantages of each technique and the benefits against the cost of treatment, and to determine the effect of the technique on breeding capability of the calf. Currently the most successful method of treating obstructive urolithiasis both in cattle and small ruminants is surgical tube cystostomy. It diverts urine through a catheter placed from the urinary bladder exiting through the body wall. The catheter is then intermittently occluded to encourage urination through the urethra.

**MATERIALS AND METHODS**

A three month old male Jersey crossbred calf was presented to Veterinary dispensary, Manchikeri of Karwar district in Karnataka with a history of anuria, inappetance, dull and depression (Fig. 1). Physical examination revealed distended abdomen and urinary bladder. Lateral abdomen radiography revealed, there was no radiopaque calculi in the urinary system and bladder was distended. Ultrasonographic examination revealed an intact distended bladder. Physiological parameters like heart rate, rectal temperature, respiratory rate and capillary refill time were within the normal range. The condition was diagnosed as obstructive urolithiasis based on the history of anuria, clinical signs and physical examination. Since, exact site of obstruction was not known, surgical tube cystostomy was resorted to.

General anaesthesia was induced using diazepam at the rate of 0.2mg/kg body weight and ketamine hydrochloride at the rate of 2mg/kg body weight and were given intravenously and maintenance using diazepam and ketamine combination to effect. Local infiltration was done at the site of incision using 2% lignocaine. The animal was placed in right lateral recumbency. Left side of the abdomen near to inguinal region was prepared for aseptic surgery. A linear skin incision was made oblique to the inguinal area. After incising the skin, fascia, muscles and the peritoneum, bladder was identified. The bladder was intact and inflamed (Fig. 2). A subcutaneous tunnel parallel to the prepuce was made through which the Foley's catheter was passed with the pointed end towards the incision. Foley's catheter was passed from outside to the abdominal cavity where the catheter tip was held in mosquito forceps and directly stabbed the bladder at an avascular area and its bulb was inflated with sterile normal saline to fix the tube within the bladder and the catheter was secured within the lumen by tying the purse-string suture (Fig. 3). Peritoneum and muscles were sutured together with No.1 polyglactin 910 in continuous suture pattern. Subcutaneous tissue was sutured with No.0 polyglactin 910 in continuous suture pattern and skin was sutured using nylon No. 1 in horizontal mattress pattern. The Foley's catheter was sutured at multiple sites on the ventral abdomen (Fig. 4). Postoperatively Enrofloxacin antibiotic at the rate of 5mg/kg body weight was administered parentally for 5 days and analgesic meloxicam at the rate of 0.3 mg/kg body weight for 5 days. Advised the owner to give ammonium chloride at 200 mg/kg body weight, twice daily, orally for 30 days. Local antiseptic dressing was done with povidone iodine for eight days. The catheter was allowed to drain freely until normal urination resumed, after which it was clamped on every alternate day with infusion set flow regulating clamp to determine the urethral patency. Catheter was removed after normal urination resumed through urethra.



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## RESULTS AND DISCUSSION

Animal started normal urination after 10<sup>th</sup> day post-operatively and had an uneventful recovery without any complications. Obstructive urolithiasis causes economic loss to the farmer due to loss of animals and cost of treatment. Mortality rate in the cases of obstructive urolithiasis is very high which is mainly due to rupture of urethra or urinary bladder. It is more prevalent in the extreme winter and summer. Occurrence of urolithiasis in peak winter may be due to the decreased water intake and deficiency of vitamin- A, arising from lesser availability of green fodder (Radostitiset *al.*, 2000). Desquamated epithelial cells may be due to deficiency of vitamin A and infections (Jones and Miesner, 2009). Excess sunlight and vitamin D may play an important role in urolithiasis in summer. This may be related to water balance of animals, during winter animals will not take much water and produce concentrated urine (Kushwahaet *al.*, 2007). Conversely, during summer, urine may be more concentrated due to increased water loss in heat.

Although, urolithiasis equally affects male and female animals but obstruction occurs mainly in males due to presence of long and narrow urethra (Tamilmahaneet *al.*, 2014). The etiology of urinary calculi formation in ruminants is recognised to be multifactorial. Diet is considered to be a major factor. High phosphorous and low calcium are commonly used as concentrate rations which predispose the animal to phosphate uroliths (Funaba et al., 2001). Diet given (concentrate) and the changes brought about by weaning may be contributing factors for development of obstructive urolithiasis in young ruminants. It was found that feeding had more significant effect on urolith formation than castration, especially in buffalo calves. Treatments for obstructive urolithiasis include medical dissolution of calculi and surgical management. In general, less severe cases can be corrected with medical management. Some report says that medical treatment is not effective for long term and only provided temporary relief (Ewoldtet *al.*, 2006). In more severely obstructed cases surgery is the only option. Surgical tube cystostomy is the most optimistic procedure for obstructive urolithiasis in ruminants, especially with intact bladder. The procedure is relatively simple, requiring a short duration of anaesthesia and resulting in restoration of full urethral patency in successful cases (Fortier *et al.*, 2004). Its success in cases with concurrent urethral rupture has been reported. The free flow of urine through the external urethral orifice could be due to many factors. Such as, by giving anti-inflammatory drugs relived the spasm and inflammation of urethra, calculolytic agent like ammonium chloride and sodium chloride along with water reduced pH of urine and it promotes the dissolution of calculi, by passing of urine through the Foley's catheter may reduce the calculi size and frequent occlusion of catheter with clamp could bring urethral patency by flushing urethra of all debris and calculus material (Ewoldtet *al.*, 2006). Complication of tube cystostomy might be due to blockade of tube with blood or tissue debris, urethral rupture, tube dislodgement, and infection (Misk and Semieka, 2003). Different surgical treatments are available for obstructive urolithiasis but each operation having their own advantages and disadvantages. Tube cystostomy surgery provides alternatives to those operations.

## CONCLUSION

It is concluded that tube cystostomy is a quick, practicable, field-applicable, and reliable method for the management of obstructive urolithiasis in ruminants. Surgical management along with medical management provided better treatment options for obstructive urolithiasis in ruminants.

## REFERENCES

1. Abdel, F. M. and Sedeek, A. M. (2005). Dorsal versus ventral urethrotomy technique for treatment of obstructive urethrolithiasis in cattle calves. *Assiut. Vet. Med. J.* 51: 198-209.
2. Ewoldt, J. M., Anderson, D. E., Miesner, M. D. and Saville, W. J. (2006). Short- and Long -Term Outcome and Factors Predicting Survival after Surgical Tube Cystostomy for Treatment of Obstructive Urolithiasis in Small Ruminants. *Vet. Surg.* 35: 417-422.



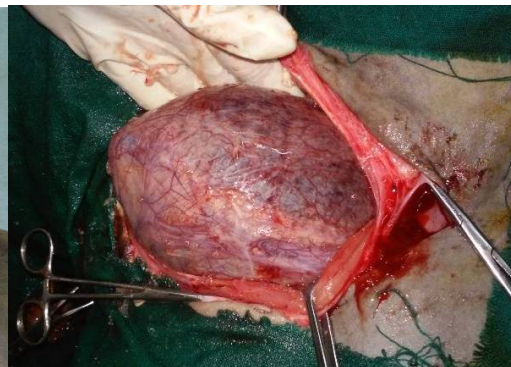


**Sharanabasav badami et al.**

3. Fortier, L. A., Gregg, A. J., Erb, H. N. and Fubini, S. C. (2004). Caprine obstructive urolithiasis: Requirement for 2nd surgical intervention and mortality after percutaneous 22 tube cystostomy, surgical tube cystostomy, or urinary bladder marsupialization. *Vet. Surg.* 33: 661–667.
4. Funaba, M., Yamate, T., Narukawa, Y., Gotoh, K., Iriki, T., Hatano, Y. and Abe, M. (2001). Effect of supplementation of dry cat food with D, L-methionine and ammonium chloride on struvite activity product and sediment in urine. *J. Vet. Med. Sci.* 63: 337-339.
5. Jones, M. L. and Miesner, M. D. (2009). Urolithiasis. In: Merchant T ed, *Food Animal Practice* 5th ed. St Louis: Saunders. 322-325.
6. Khan, S. A., Hassan, M. M., Hossain, M. F. and Epstein, J. H. (2009). Correction of urolithiasis in male calf by ischealurethrostomy. *IntasPolivet.* 10: 255-257.
7. Kushwaha, R. B., Amarpal, P. K., Thakur, K., Aithal, H. P., Pawde, A. M. and Pratap, K. (2007). Urine diversion technique using Foley's catheter for the management of obstructive urolithiasis in six buffalo calves: a clinical study. *Indian. J. Vet. Surg.* 28: 70-71.
8. May, K. A., Moll, H. D., Wallace, L. M., Pleasant, R. S. and Howard, R. D. (1998). Urinary Bladder Marsupialization for Treatment of Obstructive Urolithiasis in Male Goats. *Veterinary Surgery.* 27: 583-588.
9. Misk, N. A and Semieka, M. A. (2003). Clinical studies on obstructive urolithiasis in male cattle and buffaloes. *Assiut. Vet. Med. J.* 49: 258-274.
10. Parrah, J. D., Moulvi, B.A., Hussain, S.S., Bilal, S. and Ridwana. (2010). Comparative efficacy of tube cystostomy and cystostomy with indwelling urethral catheterization in the management of obstructive urolithiasis in bovines. *Indian. Vet. J.* 58: 23-26.
11. Radostitis, O.M., Blood, D.C., Gay, C.C. and Hinchcliff, K.W. (2000). *Veterinary Medicine: A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses.* BailliereTindall, London. 493-498.
12. Singh, T. P., Amarpal, Kinjavdekar, H. P. and Pawde, A. M. (2010). Comparison of four surgical techniques for the management of obstructive urolithiasis in male goats. *Indian. J. Vet. Surg.* 31: 15-20.
13. Smith, M. C. and Sherman, D. M. (1994). Urinary System. In: Cann. CG ed, *Goat Medicine.* Philadelphia: Lea &Febiger. 398-402.
14. Tamilmahan, P., Mohsina, A., Karthik, K., Gopi, M., Gugjoo, M. B., Rashmi and Zama M. M. S. (2014). Tube cystostomy for management of obstructive urolithiasis in ruminants. *Veterinary World.* 7: 234-239.
15. Udall, R. H. and Chow, F. H. (1969). The etiology and control of urolithiasis. *Advanced Veterinary Science & Comparative Medicine.* 13: 29-57



**Fig. 1: Animal presented with anuria, in appetite and distended abdomen**



**Fig. 2: Intact and inflamed bladder**





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**Fig. 3:**Foley's catheter was secured in bladder with purse-string suture



**Fig. 4:** The Foley's catheter was sutured at multiple sites on the ventral abdomen





## Report of Conjoined Twin Monster in a Buffalo.

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### ABSTRACT

A case of dicephalic monster in buffalo was reported and the dystocia was relieved by cesarean and obstetrical operations.

**Key words:-**dicephalic monster, buffalo, caesarean section, carboxy methyl cellulose.

### INTRODUCTION

Monstrosities are malformed fetuses, which are rare in buffaloes (Chauhan and Verma, 1995 and Bugalia *et al.*, 2001). Incidence among all calves seems to range from 0.2 to 3.0 percent with 40 to 50 percent born dead and only a small fraction of reported defects not being externally visible. Monstrosities are associated with either congenital defects or infectious disease (Arthur *et al.*, 2001) and may or may not interfere with birth. Abnormal duplication of the germinal area during embryogenesis of a monozygotic fetus will give rise partial duplication of body structures (Sharma *et al.*, 2010). Duplication of the cranial portion of the fetus is more common than the caudal portion (Roberts, 2004). Dystocia is common sequelae of fetal monstrosities. Fetotomy offers a good alternative to the caesarean for relieving a fetal monster causing dystocia (Vermunt, 2009). In the present study, a dicephalic fetal monster was relieved manually with proper positioning and lubricating with Carboxy-Methyl Cellulose(CMC).



**Doddamani et al.****Clinical Examination and Treatment**

Aneight-year-old Murrah buffalo in its first gestation was presented to veterinary dispensary Galataga (Chikkodi) with the history of straining, rupture of water bag since one hour without further progress but continued with abdominal straining. Specific obstetrical examination after proper restraint and epidural anesthesia revealed a live fetus in anterior longitudinal presentation. At the time of clinical examination, the buffalo was standing, restless and occasionally showed tenesmus. The vaginal examination revealed fully dilated cervix, both the forelimbs of foetus (up to carpus) had passed the cervix and fetal movements were present. Repulsion and deeper exploration beyond pelvic brim revealed the presence of two fetal heads joined at mandible (conjoined twin). The per vaginal delivery could not be facilitated due to large size of the conjoined heads of the fetus. The caesarean section was performed after restraint in lateral recumbancy under local infiltration with 2% Lignocaine hydrochloride. A 40 cm long oblique low flank (Young's approach) incision was taken and a live male monster calf was delivered through laparohysterotomy. The fetal membranes were carefully removed by separating cotyledons, six Ultraxboli were kept in the uterus and the uterine incision was closed with Cushing followed by Lambert suture pattern using chromic catgut No.1. The laparotomy incision was closed by standard technique. Supportive treatment was carried out with Calcium borogluconate, Rintose, Meloxicam and Chloropheneramine maleate. Antibiotic therapy with Amoxicillin + Cloxacillin along with intrauterine infusion of Ultrax was followed for 5 days. Daily antiseptic dressing was followed until wound healing. The buffalo was followed continuously and the normal feeding and water intake was resumed within two days after surgery. The uterus was involuted completely by 28 days after surgery. The animal exhibited first postpartum estrus after 45 days but discharge was not clear hence treated for endometritis and breeding was done after a gap of six months.

The fetus was a male and had two normal heads fused with each other at mandible and had one neck and four eyes, four ears, one trunk, one thorax, one pair of forelimbs, one pair of hind limbs, one sacrum and one tail (Fig 1 and Fig 2). As per Roberts (1971), the condition could be classified as dicephalic conjoined twins are non inherited teratological defect (Noden and Lahunta, 1984). Conjoined twins are monozygotic and monstrosities arise due to incomplete division of embryo usually at the primitive streak development stage. Duplication of cranial parts of fetus is more common than the caudal part. However, duplication can occur at both cranial and caudal end with the middle area of monster remaining single (Roberts, 1971). Similar to present report, Sloss and Dufty (1980) were also of the opinion that, fetotomy in such cases is difficult, often terminable task and hence caesarean operation be performed which likewise be difficult because of large size of conjoined twin.

**REFERENCES**

1. Arthur, G.H., D.E. Noakes, H. Pearson and T, J, Parkinson. 2001. Veterinary Reproduction And Obstetrics, 8<sup>th</sup> Ed. W.B. Saunders Co Ltd., London., United Kingdom. 118
2. Bugalians, Biswas, R.K. and Sharma, R.D., 2001. Diplopagus sternopagus Monster in Indian Water Buffalo (*Bubbalis bubalis*). *Indian Journal of Animal Reproduction*. 22 (2):102-104.
3. Chauhan, K.S. and H.K. Verma. 1995. A Case of Dystocia Due to Diplopagus Monster In Buffalo. *Indian Journal of Animal Reproduction*. 16(1); 75
4. Fisher, K.R.S., Partlow, G.D. and Walker, A.F. (1986). Clinical And Anatomical Observations of a Two Headed Lamb. *Anat Rec*. 214(4): 432-440.
5. Noden, D.M. and Lahunta, A.D. (1984). The Embryology of Domestic Animals – Developmental Mechanisms and Malformation. 1<sup>st</sup> Edn, Williams and Wilkins, Baltimore, London.
6. Roberts, S.J. (1971). Veterinary Obstetrics and Genital Diseases. Scientific Book Agency, Calcutta. India. P. 255.
7. Roberts, S.J. (2004). Veterinary Obstetrics and Genital Diseases. CBS Publishers and Distributors, Delhi. India. 73-74.
8. Sharma, A., Sharma, S and Vasishta, N.K. (2010) A Diprosopus Buffalo Neonate: Case Report. Buffalo. Bull. 29(1): 62-64.





**Doddamani et al.**

9. Sloss, V. and Dufty, J.H. (1980). Hand Book of Bovine Obstetrics. Williams and Wilkins. Baltimore, London.
10. Vermunt, J. (2009). Fetotomy. In Noakes, D. E., Parkinson, T. J. and England, G. C. W. (Eds) Veterinary Reproduction and Obstetrics, Saunders Elsevier, Oxford, Saunders Co Ltd, London, United Kingdom. Pp: 326-343.



**Fig. 1. Dicephalus monster in a buffalo.**





RESEARCH ARTICLE

## Effect of High Protein Diet on Blood Biochemical Profile during Late Pregnancy in Malabari Goats.

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### ABSTRACT

A study was conducted in Malabari goats to assess the effects of high protein diet on blood biochemical parameters in late pregnancy. A total of 24 pregnant Malabari goats, at fourth month of gestation were divided into two groups of 12 animals each. One group was used as the control fed with 18 per cent crude protein while the second group was fed with high protein diet of 20 per cent. Blood samples were collected at the day kidding and analysed for parameters like haemoglobin, total protein, blood urea nitrogen, glucose, calcium and phosphorus. Statistical analysis of the data revealed that feeding of high protein diet significantly increased the level of blood urea nitrogen (16.75 vs 19.41), total protein (6.42 vs 8.01) and phosphorus (7.92 vs 8.35) compared to goats that were fed with less protein diet.

**Keywords:** high protein diet, late pregnancy, kidding, total protein

### INTRODUCTION

Rearing of large animals has become an unsuccessful enterprise which is evident from the dwindling cattle population of the state. Goats are easy to manage and their small size makes them suitable for homestead farming of Kerala and also the demand for goat milk and meat is increasing nowadays. Plane of nutrition of does is important to achieve better birth weight and growth performance of kids. Foetus mainly develop during the last month of gestation. Thus nutrient supplementation especially protein to goats in last month of gestation is crucial for the growth of kids and their survivability. The elixir of life is blood and it is useful for assessing the health status, physiological, pathological conditions and can also be used for diagnostic and prognostic evaluation of various types of diseases and conditions in animals. It also helps in distinguishing state of stress, which can be maturational,





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environmental or physical (Aderemi, 2004). Examining blood for their constituents is used to monitor and evaluate health and nutritional status of animals. Therefore, the aim of the present study was to assess and determine changes in biochemical values in pregnant Malabari goats when supplemented with high protein diet in late pregnancy.

**MATERIALS AND METHODS**

A total of 24 pregnant Malabari goats were selected from University Goat and Sheep farm, Mannuthy, in their third month of gestation by abdominal palpation. Pregnancy was further confirmed by ultra sound scanning. Till fourth month of gestation all the animals were fed with a standard concentrate mixture of 18 per cent CP and 65 per cent TDN and offered fresh hybrid napier grass as the sole roughage *ad libitum*. In fourth month of gestation they were divided into two groups with 12 animals each, one control (T1) fed with 18 per cent CP and 65 per cent TDN and second experimental (T2) group were fed with high protein diet of 20 per cent CP and 65 per cent TDN. Blood samples were collected from jugular vein prior to breeding period and at the day of kidding. Within two hours of bleeding, blood was centrifuged to separate serum and stored at  $-20^{\circ}\text{C}$ . The samples were analysed for serum glucose level, blood urea nitrogen (BUN) (Kaneko *et al.*, 2008), serum calcium (Christian *et al.*, 1967) and serum phosphorus (Bernhart and Wreath, 1955). Blood haemoglobin was estimated by cyanomethaemoglobin method using reagents from Agappe diagnostics Ltd, Ernakulam, India. Plasma protein, blood urea nitrogen, serum calcium, serum phosphorus was determined using the blood analyzer (Mispa plus, SEAC radim group) and kits supplied by Agappe diagnostics, Ernakulam, India. All the animals were fed as per ICAR (1998) standards and maintained on the similar management conditions prevailing in the farm.

**RESULTS AND DISCUSSION**

The ingredient composition and proximate composition of the two concentrate mixture is given in Table 1 and Table 2 respectively. The average CP content of the control group concentrate mixture were 18.19 per cent and the treatment group was 20.16 per cent. The gross energy content of the control and treatment group concentrate mixture was  $3817.6 \pm 0.04$  and  $3804.97 \pm 0.10$  respectively, suggesting that they were maintained in a isocaloric diet. The data on the values of haemoglobin, total protein, blood urea nitrogen (BUN), blood glucose, plasma calcium and phosphorus content of the blood samples collected from animals of both control group and experimental group at the day of kidding are shown respectively in Table 3. Statistical analysis of the data ( $P < 0.01$ ) revealed that when high dietary protein was fed to does in last month of gestation significantly increased the blood total protein content which was in close agreement with Hamada *et al.*, (2013) who reported that total plasma protein of does supplemented with high protein was significantly higher than control group. Improvement in the total protein values may be due to the increased amino acid absorption from the dietary protein. Thus the increased dietary protein in late pregnancy improved the blood protein levels which further can lead to increased absorption of amino acids which in turn improves the better growth of kids.

Serum glucose levels were not significantly different between goats supplemented with high level of protein compared to control group during late pregnancy. Similar results were reported by Hamada *et al.*, (2013) during late pregnancy in Egyptian Goat and Sheep. The reason for non-significance may be related to increased glucose mobilisation during advanced stage of pregnancy as blood glucose level in pregnant animal is low due to foetal demand (Bell A.W. and D.E. Bauman, 1997). In the present study, level of serum phosphorus was significantly high in high protein fed Malabari goats than the control group (7.92 vs 8.35 mg/dl) and equivalently Jadalla *et al.* (2012) reported in their study on the effects of dietary protein level on goats of North Kordofan, Sudan that with different dietary crude protein (4.1 per cent, 7.6 per cent, 10 per cent and 11.3 per cent), high phosphorus level (4.2 mg/100 ml) were found in goats on 7.6 per cent crude protein followed by those fed on 10 per cent and 11.3 per cent protein. BUN levels were significantly higher in high protein fed group compared to control group and similarly Kumagai and Ngampongsai (2006) reported an increase in the blood urea nitrogen levels (32.2 mg/dl versus 26.7 mg/dl) with increase in the dietary CP. A significant increase in the BUN value is due to increased metabolism of





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protein which results in increased ammonia concentration which undergo metabolism in the liver and hence elevated BUN levels. These results are similar to the findings of Sarwar *et al.* (2010) who reported that there was linear increase in BUN level when the CP of feed was increased.

## CONCLUSION

From the results obtained it can be concluded that high protein diet (T<sub>1</sub>: 18% CP vs T<sub>2</sub>: 20%CP) in Malabari goats had a significant effect on serum total protein, BUN and serum phosphorous during late pregnancy and high protein fed groups had higher values than the control group. But there was no significant effect on haemoglobin, glucose and serum calcium.

## REFERENCES

1. Aderemi, F.A. 2004. Effects of replacement of wheat bran with cassava root sieviate supplemented of un-supplemented with enzyme on the Haematology and serum biochemistry of pullet chicks. *Trop. J. animal Sci.* 7:147-153.
2. Kaneko, J.J., Harvey, J.W. and Bruss, M.L. 2008. *Clinical Biochemistry of Domestic Animals*. (6thEd.) Academic Press, California, 351p.
3. Christian, G., Thin, A.T. and Patricia, A.T. 1967. Estimation of calcium and magnesium in serum and urine by atomic absorption spectrophotometry. *J. Clin. Pathol.* 20: 280-282.
4. Bernhart, D.N. and Wreath, A. 1955. Calorimetric determination of phosphorus by modified phosphomolybdate method. *Anal. Chem.* 27: 440-441.
5. Hamada D.H. Mahboub, Sameh G. A. Ramadan, Mohamed A.Y. Helal and Enas A.K. Azi. Effect of Maternal Feeding in Late Pregnancy on Behaviour and Performance of Egyptian Goat and Sheep and Their Offspring. *Global Veterinaria* 11 (2): 168-176, 201.
6. Bell. A. W and D.E. Bauman, 1997. Adaptation of glucose metabolism during pregnancy and lactation. *Journal of Mammary Gland Biology and Neoplasia.* 2: 265-78.
7. Jadalla, J. B., Ismail, I. A. and Mekki, D. M. 2012. Effects of Dietary Protein Level on milk yield, composition and some haematological parameters in dessert goats of North Kordofan, *Sudan. J. Anim. Prod. Adv.* 2: 379-388.
8. Kumagai, H. and Ngampongsai, W. 2006. Comparative studies on dry matter intake, digestibility and nitrogen metabolism between Thai native and Anglo Nubian \* TN bucks. *Small Rum. Res.* 66: 129-134.
9. Sarwar, M., Mukhtar, N., Muhammad, A.S. and Mahrun Nisa. 2010. Traditional versus high input feeding system. Impact on nutrient intake, blood dynamics, hormonal profile weight gain and economics in growing lambs. *Eg. J. of Sh. & G. Sci.* 5(1): 127 – 145.

**Table 1. Ingredient composition of the two experimental concentrate mixtures, (%)**

Ingredients	T <sub>1</sub>	T <sub>2</sub>
Yellow maize	15	10
Wheat bran	30	29
Soya bean meal	21	28
Deoiled rice bran	30	29
Calcite	1	1
Common salt	1	1
Mineral mixture*	2	2





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\*Ksheeramin – Mineral mixture for ruminants- Each kg contains elemental calcium, 240g, elemental phosphorus 120g, elemental magnesium 7g, elemental iron 5g, elemental zinc 4500mg, elemental manganese 1500mg, elemental copper 1200 mg, elemental iodine 275mg, elemental cobalt 150mg, elemental potassium 100mg.

**Table 2. Chemical composition of the two experimental concentrate mixtures and fodder (on dry matter basis), %**

Parameters	Concentrate mixtures	
	T <sub>1</sub>	T <sub>2</sub>
Dry matter	90.43±0.04	90.45± 0.16
Crude protein	18.19±0.07	20.16±0.03
Ether extract	2.60±0.03	2.85±0.16
Crude fibre	8.60±0.09	8.73±0.13
Total ash	7.83±0.25	8.00±0.12
NFE	62.79±0.20	60.25±0.26
AIA	4.18±0.07	4.87±0.09
Calcium	1.08±0.05	1.01±0.01
Phosphorus	1.28±0.02	1.28±0.16

**Table 3. Haematological parameters\* of the animals maintained on two experimental rations at the day of kidding**

Parameter	T <sub>1</sub>	T <sub>2</sub>	P value
Blood hemoglobin, g/dl	11.14±0.40	10.55±0.31	0.27
Total plasma protein, g/dl	6.42±0.22 <sup>a</sup>	8.01±0.01 <sup>b</sup>	0.01**
BUN, g/dl	16.75±0.48 <sup>a</sup>	19.41±0.22 <sup>b</sup>	0.01**
Glucose, mg/dl	59.44±2.25	60.29±1.82	0.91
Calcium, mg/dl	10.65±0.21	10.79±0.17	0.79
Phosphorus, mg/dl	7.92±0.42 <sup>a</sup>	8.35±0.21 <sup>b</sup>	0.01**

\*Average of six values

a,b,c - Means with different superscripts within the same row differ significantly

\*\* (P< 0.01)





RESEARCH ARTICLE

## Management of Esophageal Foreign Body Obstruction in a Puppy

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### ABSTRACT

Due to their indiscriminate feeding habits, incidence of esophageal foreign bodies are most common in dogs and cats.This paper discusses the medical management of esophageal foreign body obstruction in a puppy.A40 days old non-descript puppy was presented to Veterinary dispensary, Manchikeri of Karwar district in Karnatakawith the history of tapioca ingestion, absence of food and water intake and absence of urination and defecation from past 36 hours.Clinical examination revealed marked dehydration, depression, gagging, respiratory distress and repeated attempts to swallow.On physical palpation no foreign body could be detected. Plain radiographs of the neck and thorax revealed foreign body obstruction in esophageal lumen at the base of heart.After stabilizing the animal, oralglycerine liquid was given. After 5 min animal started nausea and regurgitation releasing part of tapioca. The relief of obstruction was confirmed by passing endotracheal tube into the stomach. The animal responded well to the treatment by next day and started taking food and water normally.Animal had an uneventful recovery.

**Key Words:** Puppy, foreign body, glycerine

### INTRODUCTION

Due to their indiscriminate eating habits, incidence of esophageal foreign bodies are most common in dogs and cats. Foreign bodies are the inanimate objects that may cause obstruction or partial obstruction of esophageal lumen. They



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can be bones, needles, fish hook, wood, raw hide, dental chew treats, balls, and others. Foreign bodies get lodged in esophagus because their large size and the sharp edges can cause that embedded in esophageal mucosa. Foreign bodies most commonly found at thoracic inlet or base of heart or epi-phrenic area because esophageal structure and limits esophageal dilatation at these sites (Theresa *et al.*, 2002). Esophagitis, mucosal laceration, esophageal stricture, esophageal diverticulum formation are potential complications (Cynthia *et al.*, 2010).

**MATERIALS AND METHODS**

A 40 days old non-descript puppy was presented to Veterinary dispensary, Manchikeri of Karwar district in Karnataka with the history of tapioca ingestion, absence of food and water intake and absence of urination and defecation from past 36 hours. Clinical examination revealed marked dehydration, depression, gagging, respiratory distress and repeated attempts to swallow. Rectal temperature was 101.8F. On physical palpation no foreign body could be detected. Plain radiographs of the neck and thorax revealed foreign body obstruction in esophageal lumen at the base of heart (Fig.1). Because of a clear history of tapioca ingestion and also less chances of an esophageal lumen perforation, confirmatory diagnosis done by using barium swallow contrast radiography (Fig.2).

The initial treatment was carried out using intravenous DNS and RL fluid combination @ 20ml/kg. Ceftriaxone given @ 25mg/kg i.v and meloxicam @ 0.2mg/kg s/c. Oral glycerine liquid was given @ 1-2 g/kg per orally. After 5 min animal started nausea and regurgitation releasing part of tapioca. The relief of obstruction was confirmed by passing endotracheal tube into the stomach. The animal responded well to the treatment by next day and started taking food and water normally (Fig.3).

**RESULTS AND DISCUSSION**

Esophageal foreign body is most common in dogs and cats. It may be complete or partial obstruction. The signs varies depends on location foreign body and duration of obstruction. Line of treatment includes patient stabilization and removal of foreign body as early as possible by surgically or endoscopically with grasping instruments and forceps or by using balloon catheter method (Theresa *et al.*, 2002). In the present case we tried medical lubrication with glycerine liquid which is having lubricant, humectant, emollient and soothing properties (Mario and Michele, 2008), which aids in easy passage of tapioca from esophagus to stomach (Schiller, 2001).

**CONCLUSION**

This study reports a case of tapioca obstruction in esophageal lumen was successfully relieved medically by using oral glycerine.

**REFERENCES**

1. Cynthia M. Khan, Scott Line and Susan E. Aiello. 2010. The Merck Veterinary Manual. (10<sup>th</sup> Ed.).344p.
2. Mario Pagliaro and Michele Rossi. 2008. The Future of Glycerol: New Uses of a Versatile Raw Material. 1:18.
3. Schiller, L.R. 2001. Review article: the therapy of constipation. *Aliment Pharmacol Ther.* **15**:749–763.
4. Theresa Welch Fossum, Cheryl S. Hedlund, Donald A. Hulse, Ann L. Johnson, Howard B. Seim 111, Michael D. Willard and Gwendolyn L. Carroll. 2002. *Small Animal Surgery.* (2<sup>nd</sup> Ed.). 318-321p.





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**Fig.1: Foreign body in esophageal lumen Fig. 2: Confirmatory diagnosis by contrast at base of heart radiography using barium.**



**Fig.2: Obstruction was out, confirmed by passing endotracheal tube into the stomach**





RESEARCH ARTICLE

## Histomorphology of Poison Gland in Russell Viper Snake (*Daboia russelii*)

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### ABSTRACT

Grossly poison gland in Russell viper was situated in the upper jaw just below the eye pit. It was divided into 2 lobes, anterior and posterior, which was light brownish in color. The anterior lobe was directly connected through a duct to the fang. Histologically, the gland was covered by a capsule which was more fibrous in nature, loosely arranged. Each gland was subdivided into various lobes, by interlobular connective tissue septas. In the interlobular septas large number of blood vessels was seen. Each lobule was further subdivided into smaller lobules with connective tissue and was lined by simple columnar epithelium. These simple columnar epithelial cells showed basal nuclei and apical secretory bleb like secretions. Within the cytoplasm number of vacuoles were visible indicating the presence of secretory material, as lipoidal in nature. Under H&E staining preparation there was typical arrangement of simple columnar cells resting on basement membrane, they were segregated with each other by wide gap, however at the apical end they were attached to each other by brush border with secretory blebs outside the brush border. The connective septa in the interlobular septas showed few melanin pigments also, Further, study on the histochemical nature of the secretions is to be made.

**Key words:** Russell viper, grossly poison gland, H&E staining, simple columnar cells



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## INTRODUCTION

Russell vipers are considered to be one among the big four poisonous snakes in India. The venom glands that secrete the zootoxins are a modification of the parotid salivary gland. Venom glands are located in the upper jaw below the orbit in most of the poisonous snakes. Even lizards like *heloderma* species have venom glands in the lower jaw. In some species of snakes like *maticoura*, Elapidae, Atractaspis, Viperidae have venom glands which rarely extend in the body to the level of heart [Holz PH, 1999]. Venom glands are present in the upper jaw and can rarely extend upto the level of heart. Exceptionally, coral snakes and brown water snakes being non-poisonous do have venom gland. The location of these glands and its gross morphological features, topography and its histomorphological features are not described in the literature for the viperidae family. Therefore, the present study was undertaken to know the morphological features and the gross features were needed to understand its details.

## MATERIALS AND METHODS

Two viper snakes found dead in the vicinity of college campus were collected and dissected in the head region to know the gross and histomorphological features of venom gland. The venom gland was collected from the site and was fixed in 10 %Neutral buffer formalin and processed by routine method [Singh and sulochana, 1996] and stained by Haematoxylin Eosin and Trichrome stain [Culling, 1974].

## RESULTS

In the present study viper gland was located in the upper jaw just below the eyeball which was surrounded by compressor muscle and connective tissue. Venom gland was elliptical in shape that was covered by compressor muscle (Fig.I). On dissecting the gland, the terminal end of the venom duct was surrounded by fang sheath (Fig. II ) On pressing the venom gland the fang at its opening showed the release of venom (Fig I). Histologically the venom gland was covered by connective tissue capsule which was more fibrous in nature and loosely arranged. Below the capsule the gland was divided into number of lobes separated by connective tissue septa (Fig. III). Each lobe was further subdivided into lobules with a thin layer of connective tissue septas (Fig. IV). The secretory material is also present within lumen of lobules along with secretory blebs (Fig. V). The lobules were lined by simple columnar epithelium, each columnar cell were distinctly separated from each other which is not a common feature in columnar epithelium. Each cell presented a distinct ciliary surface with apical bleb like secretory products. The nuclei with distinct heterochromatin and euchromatin material were located at the base of the cell. The cytoplasm showed vacuolation and certain granular network indicating that the secretory material could be lipoproteinous/glycoproteinous (Fig. VI & VIII). The thin layer of connective septa which separated the poison gland into different lobes, comprised mostly of collagenous type of connective fibers which was very distinct under special Trichrome stain (Fig VII). Further study is needed to explore its histochemical nature.

## DISCUSSION

Venom glands are commonly found in snakes and some of the other species like scorpion but the morphological features are quite different in two species. [Belal et al., 2013] scorpion showed compound tubular glands in the sting of scorpion, the secretory epithelium showed neutral mucopolysaccharides (PAS positive) with the absence of glycogen. However, in the present study the venom gland was also lobulated similar to the compound tubular glands and were lined by typical columnar cells with vacuolated and granular cytoplasm as evidenced in normal histological feature. However the histochemical nature is yet to be conducted. By histologically it can be thought the secretory material could be lipoproteinous/glycoproteinous/mix type. According to [Holz PH, 1999] mentioned that snake venom are quite complex and contain proteins with few aminoacids to high molecular weight. Among venom toxins RNAses, DNAses, phospholipases, proteolytic enzymes, thrombin like enzymes, hyaluronidases,





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lactatedehydrogenases, ACHnerases, nucleotidases and l-aminooxidase and others.Ten enzymes and more than two dozens of proteins are found in the all the snake venom [Russell FE,1983]. [Strydom DH, 1979] suggests that these enzymes evolved from digestive enzymes. However, these reports need to be evaluated further to understand the exact histochemical nature.

**CONCLUSION**

The venom gland in Russell Viper is located in the upper jaw surrounded by compressor muscle. The venom stored in the collective lumen is released during envenomating strike. During the strike, contractions of the compressor muscle pressurize the gland, forcing the venom to flow through the duct and enter the prey. Histologically, the venom gland is divided into number of lobes and lobules, lined by simple tall columnar epithelium. The epical surface of these cells has got secretory bleb and cytoplasm of these cells is lipoidal in nature indicating secretory in nature. The histochemical nature of these cells has to be studied further.

**REFERENCES**

1. Holz PH. The reptilian renal portal system: influence on therapy. In: Fowler ME, Miller RE editors. Zoo and wild animal medicine, current therapy 4, chapter snakes. Philadelphia: WB saunders; 1999. P.49.
2. Singh UB, Sulochana S.Handbook of Histological and Histochemical Techniques.2<sup>nd</sup> ed. Premier Publishing House: Hyderabad; 1996.
3. Culling CFA. Hand Book of Histological and Histochemical Techniques. 3rd ed. Butterworths: London; 1974.
4. Belal A, Soliman NM, Shoukry ME, Mohallal , Hamdy AW. Fetaih, Howayda S, khaled. The Fine structure of the stinger, histology and histochemistryof the venom gland in the scorpion *Androctonus amoreuxi* (Buthidae). Journal of Basic & Applied Zoology. 2013; 66:41-46.
5. Russell FE, 1983 snake venom poisoning. Great Neck, NY: Scholium International Inc; cited in Fowler and Miller 1999.
6. Strydom DH, 1979 evolution of toxins found in snake venoms. In: Lee C-Y editor. Snake venoms. Berlin: Springer-Verlag; cited in Fowler and Miller 1999.



**Fig: I. Showing the compressor muscle over the venom gland and release of venom when pressure is applied on the gland.**





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Fig. II. Showing the fang sheath , surrounding the venom duct on dissection

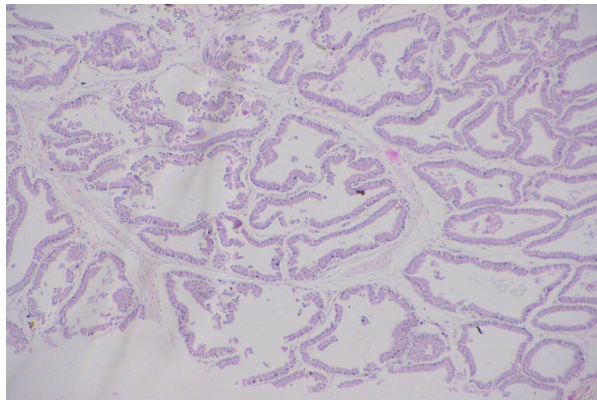


Fig. III. Venom gland is subdivided into various lobes, by interlobular connective tissue septas (H & E, stain 40x)

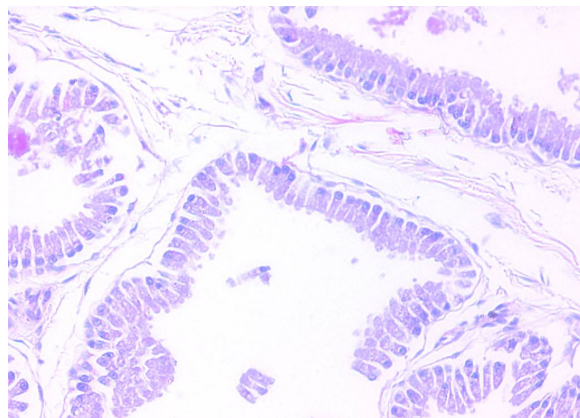


Fig. IV. Each lobule further subdivided into smaller lobules with connective tissue and was lined by simple columnar epithelium. (H & E stain 200x)





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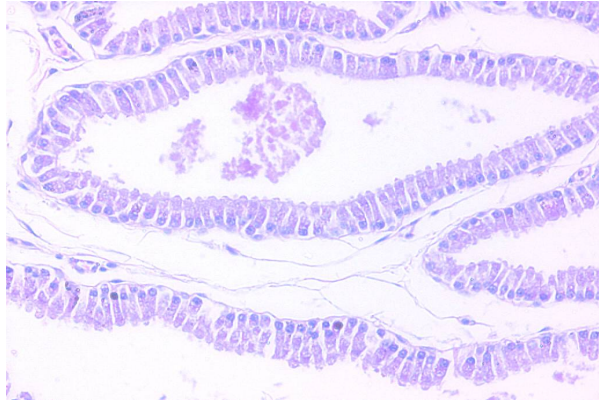


Fig. V. There is typical arrangement of simple columnar cells on basement membrane with the secretory material inside the lumen of lobule (H & E stain 400X)

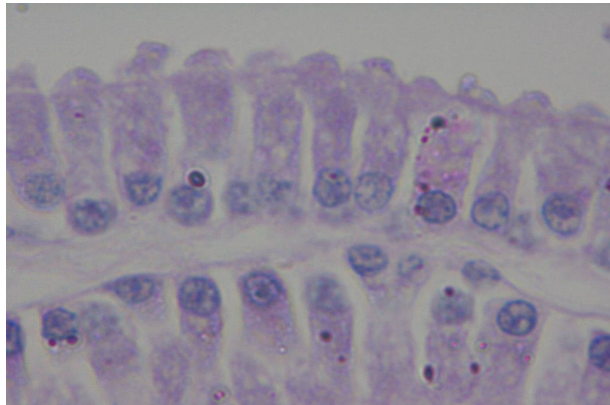


Fig. VI. Segregated simple columnar cells with secretory bleb, open phase nucleus at the bottom and vacuolated and granulated cytoplasm (H & E stain 1000x).

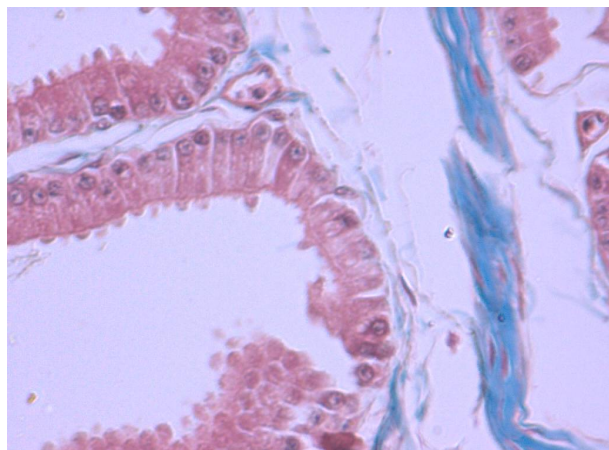
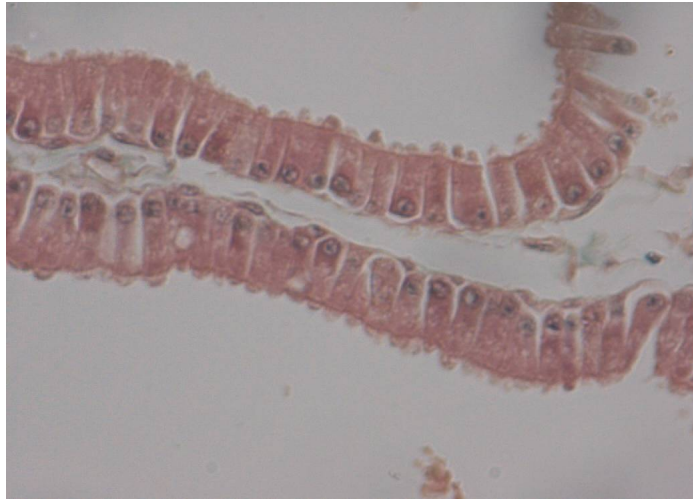


Fig. VII. Collagenous type of connective tissue separating gland into different lobes (trichrome stain. 200x)





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**Fig. VIII. Segregated simple columnar cells with secretory bleb, open phase nucleus at the bottom and Vacuolated and granulated cytoplasm (Trichrome stain 400X)**





RESEARCH ARTICLE

## Umbilical Hernia in a Puppy and its Surgical Management

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### ABSTRACT

Hernia is an abnormal protrusion of an organ or tissue through a normal body opening. Umbilical hernias occurs through umbilical ring. A two month old male German Shepherd dog was presented to the Veterinary dispensary, Manchikeri of Karwar district in Karnataka with the history of fluctuating swelling at the umbilicus which increased in size gradually. Physical examination revealed hernia contents and hernia ring, the case was diagnosed as umbilical hernia. General anaesthesia was induced using diazepam-propofol and maintenance done by isoflurane to effect. Herniorrhaphy was performed using monofilament polypropylene suture material. Routine postoperative care was given and the animal had an uneventful recovery. Post-operative observation of five months did not revealed recurrence of the condition.

**Key Words:** Umbilical hernia, Puppy, Polypropylene, Herniorrhaphy.

### INTRODUCTION

The umbilicus is the remnant of fetal-maternal connection. At birth, this structure consists of the paired umbilical arteries, a single umbilical vein and the urachus. Following a normal delivery, the smooth muscle that surrounds the umbilicus contracts in response to the stretching of the cord at parturition. Separation of the umbilical cord allows the umbilical arteries and urachus to retract into the abdomen, where they close by smooth muscle contraction (Rings, 1995). A hernia is a protrusion of the contents of a body cavity through a weak spot of the body wall. Umbilical hernia occurs in all domestic animals but more common in foals, calves and pups (Priesteret *al*, 1970). Umbilical hernia in



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dogs are more common in males than females (Waters et al, 1993). This may be from an accidental or a normal anatomical opening, which does not completely fulfil its physiological function. Hernia may be small at birth and gradually enlarge with age. Generally a hernia consists of hernial ring, sac and contents. The contents of an umbilical hernia are usually fat, omentum and, in some larger hernia, segments of small intestines are also present. Congenital umbilical hernias are of concern for heritability, although many cases of umbilical hernia are secondary to umbilical sepsis. Diagnosis is usually straightforward, especially if the hernia is manually reducible or use of diagnostic imaging techniques like ultrasonography. The present case reports about the successful surgical management of congenital umbilical hernia in German Shepherd puppy.

**MATERIALS AND METHODS**

A two month old male German Shepherd dog was presented to Veterinary dispensary, Manchikeri of Karwar district in Karnataka with the history of fluctuating swelling present at the ventral abdominal wall at the point of umbilicus (Fig. 1). History suggested that the swelling tends to increase in size as the pup gradually grows. Clinical parameters like heart rate, respiratory rate and rectal temperature were within the normal physiological limits. Palpation revealed the huge size of hernial ring and the hernial contents. It was a reducible type of hernia (Fig. 2). After recording the clinical history of the case, diagnosis was based on physical appearance and palpation of the defect in the umbilical orifice. Since, it was a reducible type of hernia, surgical repair was resorted to. The animal was fasted overnight and pre-medicated with glycopyrrolate at the rate of 0.01 mg/kg body weight intramuscularly and after 10 minutes, general anaesthesia was induced using diazepam at the rate of 0.2mg/kg body weight and propofol at the rate of 3.5mg/kg body weight were given intravenously "to effect". Endo tracheal tube intubation was done and the cuff inflated. General anaesthesia was maintained using 1.1 – 1.4% isoflurane in 100% oxygen. The animal was positioned on and surgical site was draped.

After aseptic preparation, animal was placed in dorsal recumbency and surgical incision was made over the skin of the herniated mass to cut through the hernial sac and expose the hernial contents. The hernial content consisted of omentum and intestines (Fig. 3). There were no adhesions and contents were replaced into the abdominal cavity. The hernial ring edges were freshened and herniorrhaphy was done using No. 0 monofilament polypropylene suture material in simple interrupted pattern (Fig. 4). Subcuticular sutures were applied by using No. 2-0 polyglactin 910 to avoid dead space. Skin incision was closed with nylon by horizontal mattress stitches. Tincture benzoin seal was applied to suture line. Routine postoperative care was given. On tenth postoperative day the stitches were removed and uneventful recovery was noticed. Post-operative observation of five months did not reveal recurrence of condition.

**RESULTS AND DISCUSSION**

The umbilical hernia was successfully reduced in the present case. The herniorrhaphy was done with an ease and bring about with no complications. The umbilicus in new-borns consists of the urachus (a tube that attaches the fetal bladder to the placental sac) and the remnants of the umbilical vessels that transport blood between the foetus and its mother. Normally, just after birth these structures shrink until only tiny remnants remain within the abdomen (belly). If the area in the body wall through which these structures passed remains open, abdominal contents can protrude through the defect resulting in an umbilical hernia (Dennis and Leipold, 1968). Hernia size varies depending on the extent of the umbilical defect and the amount of abdominal contents contained within it. The etiology of umbilical hernia is likely to have a genetic component (Distlet et al, 2002); however, excess traction on an oversized foetus or cutting the umbilical cord too close to the abdominal wall are other possible causes. Many of the umbilical hernial cases are secondary to the umbilical sepsis. Various methods have been described in literatures for the treatment of umbilical hernia including counter irritation, ligation of the hernial sac, clamping, transfixation sutures and even safety pins and commercially-available rubber bands. The most popular technique among them is the wooden or



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metal clamp technique. This method may result in infection, loss of clamp or premature necrosis of the hernial sac. The latter complication can lead to an open wound, and possibly to evisceration or formation of an enterocutaneous fistula. These methods are suitable only for reducible hernia and not for the strangulated or complicated ones. If the hernia ring is more than one finger in size or persists for more than 2 to 3 weeks, then surgical intervention is indicated (Pugh, 2002). Open method of herniorrhaphy is the most common method of veterinary treatment (O'Connor, 1980) and is always indicated for animal when adhesion or abscess which is commonly associated with umbilical hernia. If prompt diagnosis and treatment is not initiated, the conditions may lead to possible complications like adhesions and hydrocele of the hernial sac, incarceration, abscess and torsion (Venugopalan, 2007).

**CONCLUSION**

It is concluded that early diagnosis and treatment with surgical intervention is essential for a favourable outcome. Open method of Herniorrhaphy is relatively simple, quick and practically applicable procedure in field levels for the management of umbilical hernia in animals.

**REFERENCES**

1. Dennis, S. M. and Leipold, H. W. Congenital hernias in sheep. *J. Am. Vet. Med. Assoc.* **1968**,152: 999-1003.
2. Distl, O., Herrmann, R., Utz, J., Doll, K. and Rosenberger, E. Inheritance of congenital umbilical hernia in German Fleckvieh. *J. Anim. Breed Genet.* **2002**,119: 264-273.
3. O'Connor, J. J. *Dollar's Veterinary Surgery*. 4th ed. Bailliere Tindall & Cox, London, **1980**, 676.
4. Priester, W. A., Glass, A. G. and Waggoner, N. S. Congenital defects in domesticated animals: General consideration. *Am. J. Vet. Res.* **1970**, 31: 1871-79.
5. Pugh, D. G. *Sheep & Goat Medicine*. Saunders, Philadelphia. **2002**, 104-105.
6. Rings, D. M. Umbilical hernias, umbilical abscesses, and urachal fistulas. *Surgical considerations. Vet. Clin. North. Am. Food. Anim. Pract.* **1995**, 11: 137-148.
7. Venugopalan, A. *Essentials of Veterinary Surgery*. 8th Edn. Oxford and IBH Publishing Co. Pvt. Ltd. New Delhi. **2007**, 280.
8. Waters, D. J., Roy, R. G. and Stone, E. A. A Retrospective Study of Inguinal Hernia in 35 Dogs. *Vet. Surg.* **1993**, 22: 44-49.

**Fig. 1: Swelling at umbilical area****Fig. 2: Reducible type of hernia**



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**Fig. 3: Hernial contents**



**Fig. 4: Muscle suturing**







## Comparison of Superficial Keratectomy with BCG Vaccine for the Treatment of Initial Stages of Eye Cancer in Bullocks.

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### ABSTRACT

The study was conducted in 26 clinical cases of bullocks with small to moderate sized growths in the eye with partial loss of vision. The bullocks were divided into two groups. The bullocks (n=6) which had small sized ocular growths of less than 1cm were subjected to intralesional injection of BCG vaccine and bullocks (n=20) which had larger ocular growths or those which did not improve with four doses of BCG vaccine were subjected to superficial keratectomy and silver nitrate cauterization. The bullocks were evaluated on days 7, 15, 30 and 60 for clinical improvement. Haematobiochemical observations were made before treatment and after the treatment in both groups. Gross and histopathological examination revealed plaque, papilloma, non invasive carcinoma and invasive carcinoma in different animals of both the groups. In group I, no complications were found at the injection site of BCG vaccine. No significant difference was observed in haematological and biochemical values between the groups and between the intervals. It is concluded that intralesional injection of BCG is effective, if it is given in initial stages such as plaques and papilloma. Superficial keratectomy and cauterization of tumor bed with 1% silver nitrate can be employed as an effective treatment if the neoplasm is not involved entire cornea and vision is not lost.

**Key words:** Eye cancer, BCG vaccine, Bullocks



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## INTRODUCTION

Eye cancer is the most common neoplasm of cattle (Panchbhaiet *al.*, 1987) having great economic importance due to carcass condemnation of the affected animals at slaughter house (Williams and Gelatt, 1981). Superficial keratectomy (Bhaskaret *al.*, 2010), immunotherapy (Radhakrishnanet *al.*, 1991, Rajmaneet *al.*, 2007), radiotherapy (Banks and England, 1973) and chemotherapy are employed for the treatment of eye cancer in bovines. Radiotherapy and chemotherapy are costly and facilities are not available at field level in India. Panchbhaiet *al.* (1989), Klein *et al.*(1990), Rajmaneet *al.*(2007) reported complete regression of the lesion in 37.5% of cases treated with intralesional injection of BCG vaccine. Use of BCG vaccine may avoid surgical treatment and losses due to it. The present study was conducted to evaluate clinical efficacy of superficial keratectomy and silver nitrate application with BCG vaccine.

## MATERIALS AND METHODS

The present study was conducted on 26 bullocks presented for the treatment of smaller to moderate growths on cornea and limbus. The bullocks were divided into two groups as group I and II. In group I, six bullocks with small growths on cornea and limbus were subjected for intralesional injection of BCG vaccine. Whereas, in group II, 20 bullocks which had slightly larger growths on cornea and limbus were subjected to superficial keratectomy followed by cauterization of tumor bed with 1% silver nitrate. Eye ball was covered with third eyelid flap by membranoplasty. The programme of clinical study is shown in following table 1.

In group I, the bullocks were observed for one month for regression of the tumor. Biopsy was taken two weeks after BCG vaccine for histopathological examination. Whereas, in group II, excised tumor mass was collected in 10% formaline. The tissue was processed as per the standard technique. The paraffin sections were cut to size of 3.5microns thickness. The slides were stained in haematoxyllin and eosin for histopathological studies (Culling, 1974). Post-operatively, bullocks were administered with streptopenicillin injection 2.5g intramuscularly and melonex @ 0.2mg/kg body wti.m for 4 days. Eye drops consisting of ciprofloxacin and dexamethasone was administered 3 times a day for 7 days. Sutures of membranoplasty were removed on day 7. Clinical observations of bullocks of both the groups were made on days 0, 3, 7, 15, 30 and 60 after treatment with either BCG vaccine or superficial keratectomy. Gross appearance of the tumor bed, healing, extent of vision saved after the treatment were evaluated based on lacrimation, blepharospasm, inflammation at the tumor site of superficial keratectomy, regression of the tumor and reoccurrence if any. Physiological parameters such as rectal temperature, respiration rate, and heart rate were recorded on day 0, 15, 30 and 60. Haematological parameters such as total Erythrocyte count, total Leucocyte count, differential Leucocyte count and haemoglobin were evaluated on day 0, 15 and 30. Biochemical parameters such as AST and ALT on day 0, 15 and 30.All the values were analyzed statistically by different methods described by Snedecor and Cochron (1968).

## RESULTS AND DISCUSSION

In the present study, BCG was effective in four bullocks of group I. Out of six bullocks, two bullocks (33.33%) showed complete regression, two bullocks (33.33%) partial regression and two bullocks (33.33%) showed neither regression nor progression. Klein (1991), Panchbhaietal. (1989) and Rajmaneetal. (2007) reported complete regression in 37.5% of the total treated cases in bovines with eye cancer. Dhawanetal. (1987) reported BCG as a potent immunostimulator of B and T cells especially in the enrichment of natural killer cell activity. These natural killer cells are major producers of interferons and other cytokines in response to various stimuli. The antitumour activity of interferon includes activation and increase in antitumour activity of macrophages, induction of increase in natural killer cell activity and regulation of expression of histocompatibility antigen in cells thus increasing the immunity and interrupting the neoplastic process in benign precursor lesion of Bovine ocular squamous cell carcinoma. No complications were found at the injection site when observed on day 0, 7, 15, 30 and 60.In group II, on day 15, all the bullocks showed





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normal healing of corneal wound at the site of superficial keratectomy. Lacrimation and inflammatory changes were minimum. There was no recurrence of the tumor. The cornea was transparent which retained vision except at operated site. Slight cloudiness was seen on cornea around the operated site in two bullocks. On day 60, all 20 bullocks showed clear transparent cornea. The operated bed was completely healed with a small whitish scar. There was no recurrence of the tumor in 19 out of 20 bullocks. However, follow up after 3 months revealed recurrence of the growth in one bullock which was re operated and cauterized with 1% silver nitrate. Results of both the groups are shown in table 2. Bhaskar*et al.* (2010) reported similar findings after three month follow up.

Histopathological study of 26 tissue samples revealed plaques in six bullocks, papilloma in six, non invasive carcinoma in four and invasive carcinoma in 10 bullocks. Histologically plaques were characterized by acanthosis, thickening of mucous epithelium and greater thickening of prickle cell layer. Monlux*et al.* (1957), Bhaskar*et al.* (2010) observed hyperplasia and proliferation of prickle cell layer as distinctive histological feature of plaque in bovines. Papillomas were characterized by multiple papillary projections comprising of mature epithelial cells at the centre and immature epithelial cells at periphery, folding or ingrowth of epithelium to form inner connective tissue core (Monlux*et al.*, 1957, Gelatt and Williams, 1995). Non invasive carcinoma was characterized by islands of tumors with central keratinization or nest and increased mitotic figures. Similar findings were observed by Bhume*et al.* (1990). Invasive carcinoma were characterized by multiple islands of squamous epithelial cells forming cell nests, concentrically arranged keratin at the centre forming keratin pearls. Sharada*et al.* (1995), Das *et al.* (2004) and Bhaskar*et al.* (2010) observed similar findings in invasive carcinoma of eye in bovines. There was no significant difference in physiological parameters such as respiration rate, heart rate and rectal temperature before and after treatment in bullocks of both the groups. Haematologically, haemoglobin, total erythrocyte count, total leucocyte count and differential leucocyte counts were within the normal limit before and after treatment in both the groups. Biochemical observations such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were within the normal range before and after the treatment. It is concluded that intralesional injection of BCG is effective, if it is given in initial stages such as plaques and papilloma. Superficial keratectomy and cauterization of tumor bed with 1% silver nitrate can be employed as an effective treatment if the neoplasm is not involved entire cornea and vision is not lost.

### REFERENCES

1. Bhume, R.I. (1990). Bovine ocular squamous cell carcinoma with special reference to different therapies. Unpublished M.V.Sc. Thesis, MAU, Parbhani.
2. CULLING, C. F., 1974. Handbook of Histopathological and Histochemical Techniques. 3rd edition, Butterworth and Co, London., pp 193-197.
3. Dhawan, B.N.; R.K. Moneswar and N.B. Singh (1987). Interferons in biomedical research. 1st edn. Central drug Research Institute, Lucknow (India) 54:115.
4. Klein, W.R. (1990). Immunotherapy of squamous cell carcinoma of the bovine eye and of equine sarcoid. Tijdschr Diergeneeskd., 115(24):1149- 1155.
5. Klein, W.R.; E.J. Ruitenber; P.A. Steerenber; W.H. Jong de; W. Kruijzinga; W. Misdorp; J. Bier; R.H. Tiesjema; J.G. Kreeftenberg; J.S. Teppema and H.J. Rapp (1982). Immunotherapy by intralesional injection of BCG cell walls or live BCG in bovine ocular squamous cell carcinoma : A preliminary report. J. Natl. Cancer Inst., 69:1095-1101.
6. Panchbhai, V. S., Kulkarni, P.E., Pargaonkar, D. R., Deshpande, B. B, Bakshi, S. A. and Kalbande, V. H., 1989. Comparative evaluation of different therapies in management of ocular squamous cell carcinoma in cattle. *Indian Vet. J.*, 66(1): 65-68.
7. Panchbhai, V.S.; P.E. Kulkarni and B.B. Deshpande (1987). Some observations on ocular growths in cattle. *Indian Vet. J.*, 64:29-32.
8. Radhakrishnan, C., W.B. Justin, S. Dharmaceelan and L. Nagarajan (1999). A successful treatment of bovine ocular squamous cell carcinoma by surgery and immunotherapy – A report of two cases. *Indian Vet. J.*, 76:245-246.





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9. Rajmane, S. U., V. D. Aher., Bapatand Karpe, A. G., 2007. Immunotherapy and surgical treatment of eye cancer in bovines. *Intaspolivet*, 8(1):182-184.
10. RajuSharda., Namrata-Sharda. and Ghosh, R. C., 1995. Squamous cell carcinoma of eye in Jersey cows: A case report. *Indian J. Vet. Surg.*, 16(2): 127.
11. R.P. Bhaskar., Shivaprakash, B.V., D. Dilipkumar, and S. M. Usturge., 2010. Vision saving techniques for eye cancer involving a portion of cornea in bullocks. Large animal surgery session, proceedings of XXXIV Annual congress of ISVS and international symposium pp: 123.
12. Snedecor, G.W. and Cochran, W.G., 1968, Statistical Methods, Edn 8<sup>th</sup>, Iowa state university press, Ames, Iowa-500010.
13. Williams, L.W. and K.N. Gelatt (1981). Ocular squamous cell carcinoma in Veterinary Ophthalmology, 2nd edn. Lea and Febiger, Philadelphia : 622-632.



**Fig.1. Bullock showing neoplastic growth on limbus of eye.**



**Fig.2. Same bullock with complete regression of the lesion 60 days after intralesional injection of BCG vaccine.**





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**Table 1.Design of technical programme of clinical study**

S.No.	Groups	No of animals	Method of treatment
1.	Group I	06	BCG vaccine intralesionally
2.	Group II	20	Superficial keratectomy followed by cauterization of tumor bed with silver nitrate

**Table. 2 Results of treatment of eye cancer with BCG vaccine and superficial keratectomy.**

SI.No.	Groups	Treatment given	Animals	Results	Remarks
1.	Group I	Intralesional injection of BCG vaccine	2	Complete regression	No recurrence
			2	Partial regression	–
			2	Neither regression nor progression	–
2.	Group II	Superficial keratectomy and silver nitrate application	19	Cured	No recurrence
			1	–	Recurrence after 3 months and reoperated

