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RESEARCH ARTICLE

Unlocking the Potential of Rainfed Agriculture: A Case of North Western Regions of Tamil Nadu,India

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ABSTRACT

The paper assesses the process of structural transformation of rainfed agriculture in north-western zone (Krishnagiri district) in Tamil Nadu. Based on pattern of rainfall along with the availability of irrigation water in Krishngiri district, ragi and horsegram are the dominate crops under rainfed condition was selected for the present study. The result has shown that ragi and horsegram occupied about 14.65 per cent and 9.11 per cent of the total area respectively in 2013-2014. The study also registered an increasing trend in area, production and productivity of ragi and horsegram (1984-2013). The growth rates in ragi and horsegram had primarily been driven by yield improvement. The growth rate of area under ragi and horsegram had registered negative and significant. Expect for ragi production and yield, the instability in area, production and productivity of both crops had increased during the period of analysis. Nevertheless, the status of ragi and horsegram cultivation is still poor and concerted efforts are required to bring about further impressive growth in these rainfed crops.

Keywords : Rainfed agriculture, Agro-climatic regions, Krishnagiri district, Cropping pattern, Growth and instability.

INTRODUCTION

Rainfed agriculture is now emerging as a major opportunity in raising overall agricultural growth. Even after achieving the full irrigation potential, nearly 50 per cent of net cultivated area will remain dependent on rainfall. Rainfed agriculture supports nearly 40 per cent of Indian population in 2013. India ranks first among the rainfed countries in the world in terms of rainfed areas, but ranks among the lowest in rainfall yield (less than 1tonnes/ha).

Indeed, due to continuous erosion, the productivity of all lands and crops yield decreased, while the demand for agricultural produce increased. This is mainly due to the climatic impact faced by the farmers. Since, rainfall over the periods is increasing but production was not sustained (Bhatia. M.S, 2005). For the sustainable could occur only due to developments in agricultural research and effective dissemination of research output.

This paper has highlighted the percentage of gross cropped area under two major rainfed crops and some of the fundamental issues on trends in area, production and yield of major rainfed crops for making them sustainable in their area, production and yield of major crops grown in selected districts mainly to maintaining food security in future is a challenging task demanding intensive and extensive Research and Development efforts to meet the targets and to make rainfed agriculture viable. This makes agriculture quite a challenge in the state and yet some regions in the state have come up as models for agriculture in rainfed areas. Further, this paper also focused to looks into the growth and instability in area, production and yield of major rainfed crops in the district. This is due to instability in agricultural production, for any reason, results in unpredictable behavior and decision making from the population engaged in primary sector which is passed on to the economy as a whole.

MATERIALS AND METHODS

The Study Area

Tamil Nadu, which is one of the states of peninsular India, is blessed with a variety of seven agro-climatic zones enabling cultivation of crops under rainfed condition. The magnitude of rainfed agriculture varies significantly across the agro-climatic zones in Tamil Nadu as shown in Table 1. The percentage of rainfed area to net area sown is very high north western zone being 28.73 per cent. However, share of rainfed area in the net sown area was 16 to 25 per cent in the southern, western and north-eastern zone of Tamil Nadu. Indeed, North western zone consisting six districts, namely Dharmapuri, Krishnagiri, Salem, Namakkal, Perambalur and Ariyalur districts in which share of rainfed area was found to be maximum in Krishnagiri district.From the Table 2, it is obvious that Krishnagiri district trends maximum area under rainfed in Tamil Nadu state. Thus, Krishnagiri district belonging to north western zone was selected for the present study to known the growth rates of major rainfed crops under cultivation. Based on pattern of rainfall along with availability of irrigation water rainfed crops was cultivated.

Time Series Data on Rainfall in Krishnagiri Districts (in mm)

The time series data regarding rainfall for the last ten years (2004-05 to 2013-14) in the Krishnagiri district are presented in Table 3. While observing the actual rainfall, it shows a wide variation ranging from the minimum of 600 mm to the maximum of 1200 mm, whereas the normal rainfall ranging from of 850 mm to 865 mm. The deviation in the actual rainfall was 158.55 mm. The coefficient of variation was found to be 17.76 in the study area.

Cropping Pattern under Rainfed Agriculture in Krishnagiri District

The cropping pattern is largely influenced by the magnitude and pattern of rainfall along with the availability of irrigation water in Krishngiri district. The shifts in cropping pattern with the extension of irrigation towards crops are well recognized. Under rainfed agriculture when rainfall is higher and rainfall season is relatively longer as in Krishnagiri district less water duty crop like ragi and horsegram could also be cultivated under rainfed conditions as shown in Table 4. Thus, present study much focused on growth and instability of ragi and horsegram which comes under rainfed condition.

The Data

The study was mainly based on secondary data. The requisite secondary data were collected from the season and crop report from 1984-2013.

Analytical Tools

The data were processed using tabular analysis, growth and instability in production, area, and yield for major crops is examined at regional level. To analyze the growth and instability of area, production and yield of ragi and horsegram have been taken into account as mentioned above.

For estimating Compound Growth Rate, the data were seleted for the period from 1984-2013. Growth rates were used to measure the performance of the economic variables. The growth in area, production, yield of ragi and horsegram were found out by using the exponential growth function of the form

$Y = a\, b^t\, e_t$

Where,

Y = Dependent variable (Area. Production, Yield)

t = time variable

e_t = Error term

a and b are unknown constants to be estimated

The unknown constants a and b were found by applying methods of least square by transforming the equation into logarithmic form

$\ln Y = \ln a + t \ln b$

Where

ln Y is natural logarithm of Y, ln a and ln b are similarly defined.

The compound growth rate 'r' was computed by using the relationship

r = (Antilog of (ln b) – 1) x 100

$$\text{Where, } \ln b = \frac{\Sigma (t \ \ln Y) - (\Sigma t \ \Sigma \ln Y) / n}{\Sigma t^2 - (\Sigma t)^2 / n}$$

and n is number of time points

The significance of ln b was tested by t-ratio.

$$t = \left| \frac{\ln b}{SE(\ln b)} \right|$$

$$\text{Where } SE(\ln b) = (SS_{\ln Y} - (\ln b)^2 SS_t) / ((n - 2) SS_t)$$

$$\text{where, } SS_{\ln Y} = \Sigma (\ln Y)^2 - (\Sigma \ln Y)^2 / n$$

$$\text{and } \quad SS_t = \Sigma t^2 - (\Sigma t)^2 / n$$

The critical value is t- table value for n~ 2 degrees of freedom.To examine the extent of variability in the area, production, yield, the Cuddy-Della Valle Index is used (Cuddy and Della Valle 1978). The simple coefficient of variation overestimates the level of instability in time-series data characterized by long term trends whereas the Cuddy-Della Valle index corrects the coefficient of variation.The instability in area, production and yield of major crops is measured in relative term using Cuddy-Della Valle Index (IX).

$IX = CV \sqrt{1 - R\ Squared}$

Where,

IX = Instability Index

CV = Coefficient of variation (in per cent)

R squared = Coefficient of determination from a time-trend regression adjusted by the number of degrees of freedom.

The simple coefficient of variation over estimates the level of instability in time-series data characterized by long term trends whereas the Cuddy-Della Valle index corrects the coefficient of variation.

RESULTS AND DISCUSSION

Percentage of gross cropped area under important crops in Krishnagiri district

The agro-climatic variations in Krishnagiri district are large and hence, district is bestowed more with foodgrains. The fact remains that crop pattern in Krishnagiri district was dominated by food grains, which occupied 70.69 per cent of Gross Cropped Area (GCA) in 1990. The share of food grains dropped to 59.65 per cent in 2013. Information presented in Table 5 suggests that main rainfed crops occupy major share of area and rest of GCA is devoted to other crops.Ragi and horsegram dominate the crop pattern since there was gradually increasing from 1990-2013. The proportion of area under horsegram increased from 8.52 per cent to 11.90 per cent during 2010-2013 while ragi has indicated a gradual decline of around 2 per cent. This shift could be attributed to expanding due to rainfall condition in Krishnagiri district.

Area, Production and Yield of Rainfed Crops

Trends in area under rainfed crops in Krishnagiri district

An examination of area under important crops in Krishnagiri district during the triennium ending (TE) 2000-01 and 2013-14 in Table 6 indicates that ragi and horsegram was the dominant crop which occupied 23 per cent of ragi and 18 per cent of horsegram to the total rainfed acreage during TE 2000-01. Acreage allocation under these crops in TE 2013-14 indicates incremental increase in acreage under ragi with 37.05 per cent, whereas, horsegram had sustain growth (18.83 per cent). This trend could be due to minimum requires irrigation for ragi and horsegram crops.

Trends in production under rainfed crops in Krishnagiri district

The pattern of production of important crops in rainfed condition in Krishnagiri district is depicted in Table 7. The maximum positive change in production could be noticed for ragi. The share of ragi increased from 28.76 per cent to 44.03 per cent between TE 2000-01 and 2013-14. In case of horsegram, it turned out less than 4.61 per cent during TE 2013-14. The proportion of horsegram production was found to be 5.91 per cent during TE 2000-01. The share of proportion in horsegram production does not show perceptible decline due to significant gains experienced by ragi.

Trends in yield under rainfed crops in Krishnagiri district

Trends in yield rates depicted in Table 8 indicate that yield rates of ragi increased significantly. It jumped from around average of 1910 kg/ha in TE 2000-01 to 2396 kg/ha in TE 2013-14. Average yield of horsegram was declined from 497 kg/ha to 489 kg/ha between TE 2000-01 and 2013-14. It could be due to easy availability of inputs and infrastructure.

Growth analysis of Rainfed Crops in Krishnagiri District

Compound Annual Growth Rates of Rainfed Crops

After providing details of area, production and yield of important crops grown in rainfed condition in TE 2000-014 and 2013-14, it would be worthwhile to understand the annual growth rate of these above mentioned crops in the selected sub-periods (Phase I: 1984 to 1999 and Phase II: 1999 to 2013) and overall (1984 to 2013). It can be seen from the table that growth in area, production and productivity of ragi and horsegram crops was quite impressive during the 1999 to 2013. Area under ragi and horsegram has showed negative and significant growth with 2.25 per cent and 4.70 per cent, respectively. The yield has exhibited a sharp increase. This is contributed by expansion in acreage and growth in yield but former has played a much bigger role.

Instability in Area, Production and Yield of Rainfed Crops

The instability measured using the co-efficient of variation of area, production and yield of rainfed crops in the two sub-phases has shown a general increase in instability (Table 10). The technology practice adopted by the farmers

brought up the variability in ragi and horsegram cultivation. A similar trend has been seen in the case of instability in horsegram. Expect for ragi production and yield, the instability in area, production and yield was increased.The instability in production and yield of horsegram was found to be more stable during second sub-phase (1999 to 2013). The spread of irrigation and improvement in varietal technology have contributed to the stable in productivity.

Major Findings and Policy Implications to Promote Rainfed Agriculture

The agro-climatic variations (north-west region) in Krishnagiri district of Tamil Nadu are largely under rainfed condition and therefore a variety of crops are grown in the district. At present, crop pattern in Krishnagiri district is highly skewed towards foodgrains crops with the area allocation of 59.65 per cent of GCA. In addition, ragi and horsegram are grown on 14.65 per cent and 9.11 per cent of total area. An examination of area, production and yield of rainfed crops in Krishnagiri district of Tamil Nadu at TE 2000-01 and 2013-14 indicates that acreage under ragi has increased from 23.40 per cent to 37.05 per cent between 2000-01 and 2013-14 despite a sustain in acreage under horsegram with 8 per cent of total rainfed area between TE 2000-01 and 2013-14. The production and productivity also followed the same trend.

Growth rates of area and yield of ragi and horsegram had slow increase over the entire study period was predicted denoted ragi and horsegram were over-protected prior to recent year mainly to maintain their production efficiency. A general increase in instability of area, production and productivity of ragi as well as horsegram was observed. The policy of operationalization of several developmental schemes for their crops should implement for impressive performance in the domestic markets. It was noted that area and production of ragi and horsegram in Krishnagiri district increasing over the years. There is a need to stabilize the production of these crops by arresting the decline in area in order to take advantage of the nutraceutical properties of ragi and horsegram.

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Table1. Extent of Rainfed Agriculture in Agro-Climatic zone of Tamil Nadu (hectare)

S. No.	Agro-climatic Zones	Net area sown	Net Irrigated Area	Rainfed area	Per cent
1	North Eastern Zone	1086564	778326	308238	16.18
2	North Western Zone	875952	328734	547218	28.73
3	Western Zone	813006	498585	314421	16.51
4	Cauvery Delta Zone	628318	509091	119227	6.26
5	Southern Zone	989449	495476	493973	25.94
6	High Rainfall	76081	29103	46978	2.47
7	Hilly Zone	74870	385	74485	3.91
Total Rainfed area				**1904540**	**100.00**

Source: Season and Crop Report, 2013-2014.

Table 2. Share of Rainfed area in North-western zone of Tamil Nadu (hectare)

S. No.	Agro-climatic Zones	Districts covered	Net area sown	Net Irrigated Area	Rainfed area	Per cent
1.	North Western Zone	Dharmapuri	145253	53114	92139	16.84
		Krishnagiri *	184607	50527	134080	24.50
		Salem	208274	107242	101032	18.47
		Namakkal	147833	64165	83668	15.29
		Perambalur	97364	26591	70773	12.93
		Ariyalur	92621	27095	65526	11.97
			875952	**328734**	**547218**	**100.00**

Note: * Selected district; Source: Season and Crop Report, 2013-2014.

Table 3. Time Series Data on Rainfall in Krishnagiri Districts (mm)

Year	Actual	Normal
2004-05	872.7	855.9
2005-06	1185.6	855.9
2006-07	637.1	863.5
2007-08	984.9	863.5
2008-09	971.8	863.5
2009-10	920.5	863.5
2010-11	1000.8	856.8
2011-12	838.5	850.7
2012-13	695.1	850.7
2013-14	818.4	850.7

SD	**158.55**	**5.66**
CV	**17.76**	**0.66**

Source: Season and Crop Report for respective years.

Table 4. Cropping Pattern under Rainfed Agriculture in Krishnagiri district (Hectare)

Sl. No.	Crops	Area	Per cent
1	Paddy	-	0.00
2	Cholam	4941	1.48
3	Cumbu	198	0.06
4	Ragi *	48838	14.65
5	Maize	75	0.02
I	Total Cereals	55451	16.63
6	Red gram	8445	2.53
7	Black gram	3955	1.19
8	Green gram	1886	0.57
9	Horsegram *	30358	9.11
II	Total pulses	55002	16.50
III	Total food grains	110453	33.13
10	Cotton	1382	0.41
11	Groundnut	11310	3.39
12	Gingelly	599	0.18
13	Coconut	486	0.15
	Total Area	**333379**	**100.00**

Note: * Selected Crops; Source: Season and Crop Report, 2013-2014.

Table 5. Percentage of Gross Cropped Area under Rainfed Crops in Krishnagiri District

Year	GCA* (ha)	Ragi	Horsegram	Total foodgrain
1990-1991	519913	16.34	13.34	70.69
2000-2001	485837	14.49	11.89	56.99
2010-2011	170286	23.66	8.52	53.28
2013-2014	255019	22.21	11.90	59.65

Note: * Gross Cropped Area; Source: Season and Crop Report of Tamil Nadu, 1970-2013.

Table 6. Trends in Area under Rainfed Crops in Krishnagiri District (TE 2000-01 and TE 2013-14) (hectares)

Year	Ragi	Horsegram	Total area under rainfed
1998-1999	58393	40808	266629
1999-2000	64598	54573	261130
2000-2001	70410	57782	298888
Average	64467	51054	275549
Share in total rainfed area	23.40	18.53	100.00
2011-2012	44379	21562	130769
2012-2013	44309	21937	134080
2013-2014	56649	30358	127378
Average	48446	24619	130742
Share in total rainfed area	37.05	18.83	100.00

Source: Season and Crop Report, 2013-2014.

Table 7. Trends in Production under Rainfed Crops in Krishnagiri District (TE 2000-01 and TE 2013-14) (tonnes)

Year	Ragi	Horsegram	Total Foodgrain Production
1998-1999	101780	19690	436800
1999-2000	130080	28630	422160
2000-2001	138910	27910	430342
Average	123590	25410	429767
Share in total foodgrain production	28.76	5.91	100.00
2011-2012	117968	11087	249633
2012-2013	80401	8068	186574
2013-2014	153811	17755	363688
Average	117393	12303	266632
Share in total foodgrain production	44.03	4.61	100.00

Source: Season and Crop Report, 2013-2014.

Table 8. Trends in Yield under Rainfed Crops in Krishnagiri District (TE 2000-01 and TE 2013-14) (kg/ha)

Year	Ragi	Horsegram	Total foodgrains Yield
1998-1999	1743	483	1816
1999-2000	2014	525	1662
2000-2001	1973	483	1554
Average	1910	497	1678
2011-2012	2658	514	2330

2012-2013	1815	368	1603
2013-2014	2715	585	2391
Average	2396	489	2108

Table 9. Compound Annual Growth Rates of Area, Production and Yield of Rainfed Crops (Per cent)

Year	Area	Production	Yield
	Ragi		
1984-1985 to 1998-1999	-2.63 * (-4.72)	-3.48 NS (-0.92)	0.88 NS (-0.25)
1999-2000 to 2013-2014	-3.69 * (-4.31)	-0.39 NS (-0.20)	3.43 ** (2.14)
1984-1985 to 2013-2014	-2.25 * (-8.30)	-1.21 NS (-1.16)	1.06 NS (1.10)
	Horsegram		
1984-1985 to 1998-1999	-1.81 NS (-1.45)	1.20 NS (0.50)	3.07 NS (1.94)
1999-2000 to 2013-2014	-7.70 *** (-3.16)	-5.43 NS (-1.51)	2.46 NS (1.07)
1984-1985 to 2013-2014	- 4.70* (-6.51)	-4.38 * (-3.96)	0.34 NS (0.48)

Note: The figures within the parentheses are t-values.

* Significant at 1% level; ** Significant at 5% level; *** Significant at 10% level; NS – Non Significant

Table 10. Instability of Area, Production and Yield of Rainfed Crops

Year	Area	Production	Yield
	Ragi		
1984-1985 to 1998-1999	8.62	35.63	34.19
1999-2000 to 2013-2014	14.35	30.66	24.96
1984-1985 to 2013-2014	12.13	35.16	30.65
	Horsegram		
1984-1985 to 1998-1999	19.76	36.51	22.03
1999-2000 to 2013-2014	43.62	64.72	30.80
1984-1985 to 2013-2014	28.56	45.78	28.12

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RESEARCH ARTICLE

Standardization of Bioassay Techniques to Study the Insecticide Resistance in Cotton Jassid, *Amrasca biguttula*

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ABSTRACT

Chemical control is the main method for controlling the cotton jassid, *Amrasca biguttula.*Reported techniques for the evaluation of insecticide toxicity to jassid are not in agreement with field conditions and do not allow us to verify whether doses used in the field are efficient for control. Thus, the objective of this work was to develop a bioassay methodology to study the toxicity of insecticide formulations to cotton jassid that represent field conditions for fast-acting insecticides and slow-acting insecticides. The leaf-dip method was the most efficient method for toxicity studies of insecticides formulations to *Amrasca biguttula*. We verified that bioassays with fast-acting insecticides should be performed with Petiole dip method with exposure time of 24 hours in the agar medium. Conversely, the modified bioassay method is essential for effective detection of resistance.

Keywords : Cotton Jassid, Bioassay Methods, Petiole Tip Method.

INTRODUCTION

Cotton fibre has exercised a profound influence on humans from time immemorial. With a history going back to antiquity, the fibre has maintained its pristine purity and importance to this day. Even in modern times, cotton is a vital crop of commerce in India and is popularly known as 'Whitegold'. With over 2500 textile mill units, about 1.5 million power-looms, 4 million hand-looms and thousands of garment, hosiery and processing units, the textile industry has grown up as the largest agro based industry in the country. Cotton ecosystems throughout the world harbor a wide variety of insects. The number of species found in the crop may range from a few hundred to more

than a thousand. The vast majority of these species are parasitoids and predators of phytophagous species. Estimates of the number of pests species range from 20 to 60 but significant damage is caused by 5-10 key pests in most production systems (Luttrell *et.al.,*1994). Cotton jassid is the most destructive sucking pest of American cotton in the North Zone and is present throughout the country. Besides cotton it also feeds on okra, potato, brinjal and some wild plants. Adults are about 3 mm long and greenish yellow during the summer, acquiring a reddish tinge in the winter.Insecticide efficiency evaluated by toxicity bioassays on the insects. The standard technique used in toxicity bioassay is leaf immersion.

MATERIALS AND METHODS

The investigations were carried out in Insect Toxicology Laboratory of the Department of Entomology, Punjab Agricultural University, Ludhiana.

Biological materials

Amrasca biguttula (Ihida) (Cicadellidae Homoptera) was collected from Punjab Agricultural University Research Farm and various places of Punjab. The test insect was subsequently maintained in the screen house at Entomological Research Farm and was used for all experiments as and when required Freshly emerged adults of the test insect were used in the studies.

Standardization of bioassay method

In leaf disc method, the already employed method of Dittrich and Ernst (1983) was used. Leaf discs of 5 cm diameter were cut from centre of leaves of cotton and dipped in distilled water for 20 seconds. After air drying at room temperature, each leaf disc was placed upside down on a bed of agar medium. The mortality was observed after 24 hours.Pree et al (2000) method was employed as dry film method. The distilled water was added to the bottom of Petridish with a tight fitting lid. The lid was snapped in place after addition of distilled water and the fluid in the dish swirled briefly in both upright and inverted positions. Excess solution was poured off and the dishes were air dried for an hour and cotton jassid was transferred for mortality count. In direct spray method, FAO (1970) recommended method was compared. Leaf disc of 5cm diameter was kept upside down in agar medium and distilled water was sprayed by the Potter tower. The adult jassids were released and mortality was observed after24 hours.Cahill et al (1996 b) method was followed in Petiole dip method. Fully expanded leaves were cut from cotton along with Petiole and immediately immersed into the distilled water for 18 hours at approximately 32^0C. Leaf discs were cut from these treated leaves and placed on the Petridish for observation of mortality after 24 hours.

RESULTS AND DISCUSSION

Standardization of bioassay techniques

Comparison of different bioassay methods

The mortality of A *biguttula* adults subjected to different bioassay methods viz. leaf disc, direct spray, dry film, and petiole dip are given in Table 1. The highest percent mortality (80%) was observed in dry film method, while the lowest percent mortality (40%) was found in petiole dip method. The levels of per cent mortality of leaf disc and direct spray were 45 and 60 per cent, respectively. Although the per cent mortality observed in all the four methods is very high, the petiole dip caused minimum mortality among the four methods.

Influence of exposure time with different bioassay methods

The results showing the percent mortality of *Amrasca biguttula* with the varying exposure time viz. 12, 24, 36 and 48 hours are given in Table 2-7.

Influence of exposure time (12 hours) with different bioassay methods

Percent mortality, after 12 hours of exposure of test insect are given in Table 2. The highest percent mortality (25%) was obtained in the dry film method, while the lowest percent mortality (5%) was found in both leaf disc and Petiole dip methods. The levels of percent mortality in direct spray was 15 percent. The results suggest leaf disc and petiole dip caused low control mortality but their exposure period was considered to be short.

Influence of exposure time (24 hours) with different bioassay methods

The morality number and percent mortality after 24 hours of *Amrasca biguttula* collected from Entomological Research Farm against different bioassay methods are given in Table 3. The highest percent mortality (85%) was obtained against dry film method, while the lowest percent mortality (40%) was found in Petiole dip method. The percent mortality of leaf disc, direct spray, dry film and petiole dip were respectively in the order of 45, 55, 85 and 40 percent against the exposure time of 24hours.The data presented in Table 3 revealed that, the methods leaf disc and petiole dip were found lesser mortality per cent than dry film and direct spray methods.

Influence of exposure time (36 hours) with different bioassay methods

The data presented in Table 4 shows the mortality number and percent mortality after 36 hours of exposure of *Amrasca biguttula* collected from Entomological Research Farm. The highest mortality (95%) was obtained from dry film method and least mortality was found in petiole dip method. The levels of per cent mortality of leaf disc, direct spray, dry film and petiole dip were respectively in the order of 55, 65, 95 and 50 percent against the exposure time of 36hours.The results revealed that, the percent mortality was high when the exposure period is 36 hours than 24 hours in all bioassay methods by 10 per cent.

Influence of exposure time (48 hours) with different bioassay methods

The mortality number and percent mortality after 48 hours of exposure time of *Amrasca biguttula* collected from Entomological Research Farm against different bioassay methods are given in Table 5. The highest percent (100%) was obtained against dry film method, while the lowest per cent mortality (50%) was found in petiole dip method. The percent mortality of leaf disc, direct spray, dry film and petiole dip were respectively in the order of 60, 75, 100 and 50 percent against the exposure time of 48hours.The presented data revealed that, the percent mortality was increased by 5 percent after 48 hours of exposure compared to 36 hours of exposure, except petiole dip method. The Petiole dip method gave constant percent mortality than other methods. Mortality observed in petiole dip method was 5, 40, 40 and 50 per cent respectively after an exposure period of 12, 24, 36 and 48 hours. The mortality was observed after 12 hours but the exposure period was considered too short, however, same mortality was observed after 24 and 36 hours of exposure period (Table 7).

Influence of medium on mortality

Leaf disc method

Mortality and percent mortality after 24 hours of *Amrasca biguttula* collected from Entomological Research Farm against different mediums viz. Agar, foam, cotton and filter paper are given in Table 8. The leaf disc and petiole dip methods were compared. In leaf disc method, the highest per cent mortality (75%) was obtained when filter paper was used as medium and lowest mortality was found in Agar as medium. The level of percent mortality using the medium of Agar, Foam, cotton and filter paper were respectively in the order of 45, 60, 65 and75 per cent after 24 hours of exposure time. The presented data revealed that, the percent mortality was low (45%) in agar medium than other mediums.

Petiole dip method

Mortality and percent mortality of *Amrasca biguttula* against different mediums viz. Agar, Foam, cotton and filter paper with petiole dip method are given in Table 9. The highest mortality (75%) was obtained in the medium of filter

paper and lowest (35%) in Agar. The level of percent mortality using the medium of Agar, Foam, cotton and filter paper were respectively in the order of 35, 50, 50 and 75 percent after 24 hours of exposure time.The presented data revealed that, the percent mortality was low (35%) in Agar medium than other mediums.

Influence of Agar concentration on mortality

Leaf disc method

The influence of Agar concentration viz. 1.0, 1.5, 2.0, 2.5 and 3.0 percent on mortality of *Amrasca biguttula* collected from Entomological Research Farm. The highest mortality (60%) was obtained when Agar 3.0 percent was used and lowest mortality was found when agar 2.0% was used. The level of mortality using different agar concentrations 1, 1.5, 2.0, 2.5 and 3.0 percent were respectively in the order of 55, 50, 40, 55 and 60 percent after 24 hours of exposure time.The presented data revealed that the percent mortality was low (40%) at 2.0% agar concentration than others.

Petiole dip method

The influence of agar concentration viz. 1.0, 1.5, 2.0, 2.5 and 3.0 percent on mortality of Amrasca biguttula collected from Entomological Research Farm. The highest mortality (55%) was obtained when agar concentration 3.0 percent was used, and the lowest percent mortality (35%) was found in 2.0% agar concentration. The level of percent mortality using different concentrations viz. 1, 1.5, 2.0, 2.5 and 3.0 percent were respectively in the order of 45, 40, 35, 50, 55 percent after 24 hours of exposure time.The presented data revealed that, the percent mortality was low (35%) at 2.0 percent agar concentration than others.

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Table 1: Comparison with different bioassay methods

S.No	Method	No.of insects released	Mortality after 24 hours	% mortality
1	Leaf disc	80	36	45
2	Direct spray	80	48	60
3	Dry film	80	64	80
4	Petiole dip	80	32	40

Table 2: Influence of exposure time with different bioassay methods (12hrs)

S.No.	Method	No.of insects released	Mortality after 12 hours	% mortality
1	Leaf disc	80	4	5
2	Direct spray	80	12	15
3	Dry film	80	20	25
4	Petiole dip	80	4	5

Table 3: Influence of exposure time with different bioassay methods (24hrs)

S.No.	Method	No.of insects released	Mortality after 24 hours	% mortality
1	Leaf disc	80	36	45
2	Direct spray	80	44	55
3	Dry film	80	68	85
4	Petiole dip	80	32	40

Table 4: Influence of exposure time with different bioassay methods (36hrs)

S.No.	Method	No.of insects released	Mortality after 36 hours	% mortality
1	Leaf disc	80	44	55
2	Direct spray	80	52	65
3	Dry film	80	76	95
4	Petiole dip	80	40	50

Table 5: Influence of exposure time with different bioassay methods (48hrs)

S.No.	Method	No.of insects released	Mortality after 48hrs	% mortality
1	Leaf disc	80	48	60
2	Direct spray	80	60	75
3	Dry film	80	80	100
4	Petiole dip	80	40	50

Table 6: Influence of medium on mortality (Leaf disc method)

S.No.	Method	No.of insects released	Mortality after 24hrs	% mortality
1	Agar	80	36	45
2	Foam	80	48	60
3	Cotton	80	52	65
4	Filter paper	80	60	75

Table 7: Influence of medium on mortality (Petiole dip)

S.No	Method	No.of insects released	Mortality after 24hrs	% mortality
1	Agar	80	28	35
2	Foam	80	40	50
3	Cotton	80	40	50
4	Filter paper	80	52	75

Table 8: Influence of Agar concentration on mortality (Leaf disc)

S.No	Agar concentration	No.of insects released	Mortality after 24hrs	% mortality
1	1.0%	80	44	55
2	1.5%	80	40	50
3	2.0%	80	32	40
4	2.5%	80	44	55
5	3.0%	80	48	60

Table 9: Influence of Agar concentration on mortality (Petiole dip)

S.No	Agar concentration	No.of insects released	Mortality after 24hrs	% mortality
1	1.0%	80	36	45
2	1.5%	80	32	40
3	2.0%	80	28	35
4	2.5%	80	40	50
5	3.0%	80	44	55

REVIEW ARTICLE

Fatty Acid Metabolism in *Leptospira* a Key to its Pathogenicity and Evasion from Host Immune Response Leading to Prolonged Survival of the Organism

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ABSTRACT

Leptospirosis is found to be the widest spread neglected zoonotic disease in the world. Leptospirosis is caused by *Leptospira* which is a gram negative spirochete, obligate aerobe. The fatality rate of leptospirosis is as high as 5%. The pathogenic serovar *Leptospira interrogans* is able to infect wide range of hosts from rodents to humans and are highly virulent, one of the main factors responsible for this heightened virulence of *Leptospira* is the ability of the organism to evade the immunogenic response of the host by hiding in proximal tubule of the kidney, liver which act as a safe abode for the survival of the organism. *Leptospira* has the exceptional ability to utilize toxins produced against it as terminal electron acceptor by metabolizing the toxins in such a way that it does not produce free radicals or ions which can harm *Leptospira*. The unusual sustenance of *Leptospira* against host immune response also lies in the fact that *Leptospira* feed on fatty acids as their only source of carbon and energy even though they are capable of utilizing glucose, when they have the genes required for glucose metabolism. This makes the study of Life cycle of *Leptospira* and metabolism in *Leptospira* intriguing.

Keywords : *Leptospira,* Zoonotic Disease, Immunogenic Response, Glucose Metabolism.

INTRODUCTION

Leptospirosis is a zoonotic disease caused by a spirochete *Leptospira*, which is a gram negative spherically coiled organism which comprise both pathogenic and saprophytic strains of bacteria with thousands of serovars (Nascimento, 2004; R. C. Johnson, 1970)*Leptospira interrogans* is an obligate aerobe spirochete with periplasmic flagella(Guerra, 2009; Levett, 2001). It usually resembles a question mark when viewed through a light microscope, which gives the species its name *Leptospira*(Cameron, 2015). *Canicola, Icterohemmorhagia*e and *Australis* are some of the pathogenic serovars of the genus *Leptospira*(Hanson, 1982).Serovars are nothing but distinct variations within a species of bacteria. serovars are further classified together based on their cell surface antigens, which makes the epidemiological classification of organisms to sub-species level(B. d. l. P. M. Adler, A., 2010; Samir, 2015). A group of serovars possessing common cell surface antigens is known as serogroup(Ueta, 1972).

Pathogenic strains also comprise hundreds of different serovars of *Leptospira*. Pathogenic *Leptospira* are classified as single species known as *Leptospira interrogans* which includes 223 serovars arranged in 23 groups(Murray, 2015; Thiermann, 1984).The major difference between different serovars of *Leptospira* is the variations present in carbohydrates of Leptospiral lipopolysaccharides. (Nascimento, 2004; Ueta, 1972).Leptospirosis is considered as one of the wide spread disease worldwide due to wast number of serovars and animal hosts. Pathogenic and saprophytic Leptospires have diverse life cycle based on their habitat and their surrounding environment(Goncalves-de-Albuquerque, 2012; Levett, 2001). Almost all leptospires are hydrophilic in nature and are favored by humid conditions and neutral pH (6.9-7.4) for their survival. *Leptospira* usually harbor natural habitats like shallow lakes, ponds, rivers and streams due to the presence of high humidity(Hanson, 1982; Joseph. Staneck, 1973). Water gets contaminated due to the presence of *Leptospira* in these water bodies, when animals and humans come in contact with these waterbodies, get infected(Samir, 2015).

Leptospires usually enter the body of animals and humans through cuts, bruises, oral transmission by consumption of contaminated water, and by feeding on contaminated pastures and also through conductive of eyes, nasal cavity and vagina through venereal transmission.*Leptospira interrogans* often infects wild and domestic animals such as Dogs and cattle, Humans are the accidental hosts of *Leptospira*(Dupouey, 2014). *Leptospira* is found to survive in neutral or slightly alkaline pH for around three months or even longer. *Leptospira interrogans* is usually transmitted to humans through direct contact with infected animal fluids such as urine, saliva, and blood. When these body fluids which possess *Leptospira interrogans* in high amount get mixed with the water bodies pose a threat to large number of population dependent on the water source, as *Leptospira interrogans* has the ability to invade through bruises, cuts and burns due to damaged skin and enter the blood stream(Hanson, 1982).

On infection the Leptospires replicate at exponential rates in the blood of the host resulting in Leptospiral toxicity and consequently lead to sessions and capillary damage due to septicemia. The infection by Leptospires lead to activation of humoral immunity in the host resulting in increased amount of circulating antibodies in the blood, to escape the immune response of the body Upon entering the blood stream, *Leptospira* replicate at an exponential rate and make Liver and Kidney its abode(B. Adler, 2014; Goncalves-de-Albuquerque, 2012; Murray, 2015; Vijayachari, 2008; B. Wasinski, 2011; B. D. Wasinski, J., 2013; Xue, 2010). *Leptospira* hide in proximal real tubules of kidney and in female genital tract (in females), the hooks present at the ends of *Leptospira* aid the bacteria to adhere to the walls of the organs and makes the organisms capable to overcome the flushing actions seen in kidney and genital tract. In case of females the infection may persist in mammary gland, which has been confirmed by isolation of organisms from the milk especially in cattle(Joseph. Staneck, 1973).

Based on the host - serovar adaptation these organisms might localize in such specialized organs like kidney for long term or even for entire life time of host. Resulting in shedding of these organisms in urine and genital fluids which intern contaminate water and vegetation. Since *Leptospira* are viable even after six months outside host under favorable conditions, the cycle continues. This capacity of *Leptospira* to stay viable for 6 months outside host makes

the organism more threatening and hard to eradicate.*Leptospira interrogans* infection leads to a disease condition, commonly known as Leptospirosis, *Leptospira* infection can be asymptomatic with a mild non-specific illness or may result in a more severe chronic condition like Liver damage and renal failure resulting in multi organ failure and ultimately death, such chronic leptospiral infection is referred to as Weil's Syndrome. There are several other common names used to refer Leptospirosis, which include Canicola fever, Haemorrhagic Jaundice, Mud fever and Swine herd disease(B. d. l. P. M. Adler, A., 2010).The infection by *Leptospira* results in two stages, Bacteremia stage wherein the organism replicates in the blood and the typical symptoms of flu are seen, these symptoms last for 3-7days. After 7 days the immune phase sets in wherein the infected host is symptom free for upto 1 month. But even during this phase meningitis can be commonly seen. After this period of 1 month symptoms start appearing(Thiermann, 1984). The symptoms of the disease are found to be common and confusing, the symptoms include flu, headache, myalgia, Jaundice, nephritis, hay fever, etc.,(B. Adler, 2014; B. d. l. P. M. Adler, A., 2010; Guerra, 2009; Levett, 2001).

Clinical illness due to *Leptospira* generally occur in two stages, namely: Febrile and Immune, these two stages can last for a few days to three weeks or longer.

Febrile stage

The febrile stage begins with flu like symptoms, which are fever, chills, myalgia and intense headache. This first phase of leptospirosis normally resolves after four to nine days and the patient is briefly asymptomatic for a few days and before the second phase begins.

Immune Stage

Immune stage is characterised by meningitis, liver damage, jaundice and renal failure, due to these wide range of symptoms the infection is often wrongly diagnosed as meningitis, encephalitis or even influenza.The severity of the *Leptospira* infection depends on the infecting serovar and the host immune response, this is found to be true as the same serovar can cause mild disease with one host but severe disease condition in other hosts. Even though the case fatality is low normally, with advancing age the case fatality rate increases and may reach 20% or more in patients with Jaundice or kidney damage(B. Adler, 2014; Cinco, 2010; Goncalves-de-Albuquerque, 2012; Guerra, 2009; Levett, 2001).

Epidemiology

Leptospirosis which is commonly transmitted by the urine of infected animals, occurs worldwide but is most commonly seen in temperate or tropical climates. *Leptospira* species have been found in cattle, pigs, horses, dogs, rodents and wild animals(Dupouey, 2014; Hanson, 1982; Vijayachari, 2008; B. Wasinski, 2011).Humans become infected through contact with water, food or soil contaminated with urine from infected animals. This may happen by swallowing contaminated food or water, through contact of skin or through contact with mucosal surface such as the eyes or nose. Outbreaks are usually caused by exposure to contaminated water through cuts, bruises oral transmission and by feeding on contaminated pastures and also through conjunctiva of eyes, nasal cavity and also through vagina by venereal transmission. The efficiency of *Leptospira* to infect wide range of hosts makes it essential to have a closer look towards the metabolism in *Leptospira*(B. D. Wasinski, J., 2013).

Metabolism in *Leptospira*

Major source of energy and carbon for the metabolic requirements of *Leptospira* is obtained by fatty acids of the host cell(Cox, 1969b; Henneberry, 1970; Joseph. Staneck, 1973). *Leptospira* utilize the fatty acids of the host cell for their energy requirements by catabolizing fatty acids through βoxidation of fatty acids. The major source of fatty acids for β oxidation are phospholipids, hydrolysis of which yields fatty acids(YasutakeYanagihara, 1964). Since phospholipids are abundant in host cells, it makes the host cell optimal growth medium for the growth of

Leptospira(N. Stern, 1968).To provide the optimal growth condition for Leptospiral growth tween is added in the culture media as a source of fatty acid in Invitro cultures. β oxidation of fatty acids was thought to be the only source of energy and carbon in fatty acid in case of *Leptospira*.

This hypothesis was largely due to lack of phosphofructokinase gene which indicates incomplete glycolytic pathway, that is Phosphokinase gene which is a essential gene responsible for synthesis of enzymes like phosphofructokinase which forms the key enzyme responsible in initiation phase of Glycolysis by catalysis of phosphorylation of fructose to fructose 6 phosphate. Indicating that glucose does not act as a source of carbon and energy(Shuang-Xi Ren, 2003). Later with the discovery of the gene responsible for the synthesis of glucokinase which is a alternative gene for the possibility of glycolytic pathway and glucose acting as a source of carbon re-emerged(Nascimento, 2004).It is also found that *Leptospira* posses a pyrophosphate fructose 6 phosphate 1 phosphotransferase instead of phosphofructokinase. This was further supported by the discovery of Glucose-sodium symporter which is the only glucose uptake system present in *Leptospira*. But ABC transporter is found to be absent in *Leptospira*, with incomplete phosphoenol pyruvate protein phosphotransferase system, with no B or C sugar permease components(Nascimento, 2004).

It is intriguing to know that glucose uptake through glucose-sodium symporter does not involve phosphorylation of glucose. In such case the function or the presence of glucokinase becomes a key in phosphorylation of glucose to glucose 6 phosphate after the uptake(Y. K. Yanagihara, S.Mifuchi, I., 1984).So it is evident from the above facts that *Leptospira* posses a functional glucose uptake and metabolism pathway of glycolysis, but it still remains a mystery as to why *Leptospira* do not depend on glucose as their primary source of energy and carbon to an extent where *Leptospira* fail to utilize glucose in the normal growth conditions(Y. K. Yanagihara, K.Takeda, K.Mifuchi, I.Azuma, I., 1983; Y. K. Yanagihara, S.Mifuchi, I., 1984).

Leptospira interrogans has an exceptionally good defence mechanism to guard against the host immunogenic attack caused due to *Leptospira* infection. *Leptospira* evades the toxic hydrogen peroxide burst by the host cell with its di-haem cytochrome C peroxidase, which converts hydrogen peroxide to water without producing oxygen free radicals, there by escaping the toxic attack, not only does *Leptospira* escape the toxic attack by neutralizing hydrogen peroxide but also utilizes it in its favour by using it as terminal acceptor of reducing power for respiration, leading to generation of ATP through F_o-F_1 type ATPase that is encoded in a single operon, atpBEFHAGDC which has a structure similar to that of Eubacteria(B. Adler, 2014; B. d. l. P. M. Adler, A., 2010; Cameron, 2015; Cinco, 2010; R. E. B. Corin, E. Cox, C. D., 1978; R. E. C. Corin, C. D., 1980; Cox, 1969c; Goncalves-de-Albuquerque, 2012; Murray, 2015; P. J. Rao, 1964; Samir, 2015; Thiermann, 1984; Xue, 2010).

Fatty acid composition of *Leptospira*

Leptospira obtain fatty acids from their host by the activity of the enzymes esterase's and lipase, which hydrolyze phospholipids to fatty acids and glycerol, generally Leptospires use Phosphotidyl ethanolamine (PE) for this purpose(R. C. Johnson, 1970),rarely Phosphatidyl glycerol (PG) and diphosphatidyl glycerol (DPG) are used. On hydrolysis of PE the resulting fatty acid is used as both carbon and energy source by *Leptospira*.Most of the *Leptospira* depend on long chain fatty acids for their nutrition, as there are no enzymes present in Leptospires which can be used for chain elongation of fatty acids.The fatty acids obtain from host are converted to unsaturated fatty acids by the action of desaturase enzyme which are present inLeptospires. Usually incorporation of double bond is seen at ∆9 or ∆11 position of the fatty acids based on chain length of fatty acid based on chain length of fatty acid and also depending on the type of desaturase enzyme acting on the fatty acid(R. C. Johnson, 1970). These unsaturated fatty acids are likely to be incorporated in the plasma membrane, conferring fluidity to plasma membrane(N. Stern, 1968).
But there are few exceptions like *Icterohemmorhagiae*and *ballum* are unable to desaturase fatty acids, such organisms solely depend on unsaturated fatty acids present in the host or culture medium for unsaturated fatty acids(Vanaja, 2001).

Growth requirements of *Leptospira* at Human body temperature

It is found that the toxicity of free fatty acids produced by hydrolysis of PE, PG and DPG is neutralized by the presence of pyruvate, this might be due to reduction of peroxide by pyruvate by formation of acetate, CO_2 and water (Joseph. Staneck, 1973; R. C. Johnson, 1970).There is a varied response to peroxide by different serovars of *Leptospira* depending on their ability to produce peroxidase. There are few well known serovars like *Icterohemmorhagiae* and *Copenhagnii* which reduce peroxides produced, by the action of the peroxidase enzyme produced by these organisms and are capable to use peroxides as the final electron acceptors in ETC (Nascimento, 2004), whereas the fatty acid toxicity is seen in case of serovars which are unable to produce peroxidase and unable to utilize peroxides in their favor resulting in fatty acid toxicity due to peroxide sensitivity in these organisms, this includes serovars like *canicola,Pomona, etc.(Joseph. Staneck, 1973)*.

Proteins which bind to fatty acids are also found to be capable of reducing fatty acid toxicity, hence these serovars which do not produce peroxidase enzyme also grow in the human body due to the shield provided by the host body by decreasing the fatty acid toxicity with the action of pyruvate and proteins. The toxicity is reduced by protein either due to presence of biotin in it or due to the biotin mimicking action of protein(Joseph. Staneck, 1973).It is found that in addition to peroxidase, *Leptospira* also possess catalase, hence even the oxidative burst by the host immune system is easily tackled by *Leptospira*(R. E. C. Corin, C. D., 1980). The activity of catalase to neutralize H_2O_2 is found to be more rapid and efficient (around 50 times greater) than the peroxidase activity to neutralize H_2O_2. This might be one of the factors using which *Leptospira* lacking peroxidase can infect and survive in the host even after exposure to oxidative burst in host body.

The virulent strains of *Leptospira* utilize higher concentrations of Fe^{2+}, Mg^{2+} and K^+ which are present in human body, to increase fatty acid transport into the cell, stabilization of plasma membrane and also to reduce peroxide toxicity by the oxidation-reduction actions of Fe^{2+}(Joseph. Staneck, 1973), Iron is also found to be a cofactor in peroxidase enzyme in serovars which produce peroxidase enzyme.It has been found that *Leptospira* being strictly aerobic in nature possess fully functional ETC (Cox, 1969a; Nascimento, 2004) with a slight difference from human ETC in which cytochrome c is replaced by electron transport protein which posses 2 heme prosthetic groups instead of one heme prosthetic group (Nascimento, 2004).*Leptospira* lack kinase enzyme required to phosphorylate pyruvate, ribose, acetate, glycerol and hence is unable to use these substances as substrates. Even though the complete set of genes required to produce enzymes of glycolysis, there is no certainty that glycolysis occurs in *Leptospira*, but it is found that *Leptospira* possess transaldolase activity with fructose 6 phosphate and erythrose 4 phosphate, by which *Leptospira* synthesize pentoses(Cox, 1969b).

The pentose phosphate pathway in *Leptospira* can be the reason for restricted glycolysis even in presence of complete set of enzymes required for Glycolysis (Nascimento, 2004). The fact that Leptospires possess more unsaturated even numbered, long chain fatty acids supports the function of desaturase active in *Leptospira*(Cox, 1969b; R. C. Johnson, 1970).Sugars like arabinose, rhamnose, xylose, mannose, galactose are present in more concentration than glucose, and also pentoses are more abundant than hexoses in *Leptospira*. The absence of deoxyribose in pentose backs the fact that the pentoses like ribose obtained are not by degradation of RNA but are due to functional pentose phosphate pathway (xiR. C. Johnson, 1970; Shuang-Xi Ren, 2003).One of the remarkable finding incase of *Leptospira* is that the fatty acids present in them are straight chain fatty acids and no branched chain fatty acids are found. Which is in favor of evidence of β oxidation as branched chain fatty acids undergo ω oxidation and α oxidation but there are no evidences for ω oxidation and α oxidation of fatty acids.*Leptospira* are capable of converting saturated fatty acids to unsaturated fatty acids by the action of desaturase enzyme, but only monounsaturated fatty acids can be produced, not polyunsaturated fatty acids, polyunsaturated fatty acids can be obtained through the transport of polyunsaturated fatty acids only from host or growth medium into the cell (Ueta, 1972).

DISCUSSION

Metabolism in *Leptospira* is complex and requires a lot of facts to be unraveled to understand the mechanisms involved, some of which are: the reason for absence of glycolysis even in presence of all the genes required to produce enzymes required for glycolysis, and the mechanism by which catalase and peroxidase enzymes of *Leptospira* neutralize the magnitude of oxidative burst by the host immunity. The pentose phosphate pathway in *Leptospira* need to be further deciphered as the entire pathway is not yet clear. The reason for selection of fatty acid over carbohydrate as energy and carbon source is yet to be found.

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RESEARCH ARTICLE

A Sociological Studies of Cybercrime and Cyber Security Guidelines

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ABSTRACT

Technology has become a part and parcel of present society. Man has attempted to gain control over nature by means of technology. Great success is achieved and man has obtained large degrees of control over nature, time and distance. However, the control and order exercised by technology seem to extend over man himself. It's as if man has lost control over his own instruments. It is the dynamics of technology because humans have engrossed themselves in this all powerful social fact. Indiscriminate use of modern technology has alienated man from himself and people around him .Man has himself become an object or material in the organization of modern technology leading to powerlessness; an aspect of new technological culture that has deprived man from face-to-face relations. Ritual world poses many challenges for the society. Even though many types of Cyber-crimes such as cyber bullying, cyber defamation and cyber blackmailing occur in virtual environment but they do have an effect in real life. However, it is very difficult to control these online crimes in physical world because of lack of adequate knowledge and expertise which is required to deal with online crime.

Keywords : Cybercrime, Security, Online, Technology, People, World.

INTRODUCTION

The internet provides the means to link up the many and diverse networks already in existence. Since commercialization of the internet during the mid-1990s, it has grown manifold .Even though majority of worldwide total internet connections are located in developed countries; the fact is that these are growing at a very fast rate in developing countries too. An unequal access also follows along existing lines of social exclusion within individual countries and factors such as employment, income, education, ethnic disability are reflected in the patterns of internet use. These inequalities point out the social characteristics behind the emergence of Cyber Crime and cybercriminals.

However it is worthwhile that crime and deviance cannot always be strictly separated in criminology. The boundaries between the criminal and deviant are socially negotiated and have become a recurrent feature of contemporary developments around the internet. Some criminologists argue that cybercrime is not a new type of crime but is same as non-virtual crime; it just uses new tools and techniques, while some others say that Cybercrime is radically different and focuses on social structural features of the environment.

Cybercrime

Cyber-crime is a crime involving, using or relating to computers especially the internet. Crimes involving use of information technology or usage of electronic means in furtherance of crime are covered under the scope of Cyber-crime. The ambit of the term includes all kinds of objectionable or unlawful activities, misuse or abuse taking place in cyber world, through or against the computer, internet, and telecommunication networks run with computer system or technology. The scope of Cyber-crime is bound to increases in view of the ever increasing technological advancement in the area.The Act and Punishments of Cybercrimes is shown in Table 1.

Cyber Law

Cyber law is important because it touches almost all aspects of transactions and activities on concerning the Internet, the World Wide Web and Cyberspace. Initially it may seem that Cyber laws are a very technical field and that it does not have any bearing to most activities in Cyberspace. But the actual truth is that nothing could be further than the truth. Whether we realize it or not, every action and every reaction in Cyberspace has legal and Cyber legal perspectives.

Cyber Security

Cyber security means protecting information, equipment, devices computer, computer resource, communication device and information stored therein from un authorized access, use, disclosure, disruption, modification or destruction. Security is essential for the protection of all types of assets. Information stored in a computer is a valuable asset for the person who stores it. In order to ensure the confidentiality of data, unauthorized access and disclosure of information should be prevented. The integrity of the data should be protected by preventing unauthorized modification of information. Cyber Security means measures taken to protect a computer or computer system (as on the Internet) against unauthorized access or attack. The definition of cyber security has been added by the Information Technology Amendment Act 2008. According to section 2(1) (nb) of the I.T.Act.

Cyber Security of the India Government

The Government has an important role to play in cyber security assurance in the form of long-term strategies. A cyber security strategy has been outlined by Department of Information Technology, Government of India, to address the strategic objectives for securing country's cyber space and is being implemented through the following major initiatives. It focuses on creation, establishment and operation of cyber security assurance framework aimed at assisting government, critical infrastructure organizations and other key users of nation's economy. Protection of Critical Information Infrastructure, augmentation of facilities at CERT-In, creation of sect oral CERTs, enhancing global co-operation among security agencies, promoting national awareness programs and training on cyber security, security research and development are some of the strategies adopted by the Government. It also focuses on creation of national cyber alert system for rapid identification and response to security incidents and information exchange to reduce the risk of cyber threat and resultant effects.

Information Security and National Interest

There may arise situations where information security conflicts with the interest of the nation as a whole. In such situations Section 69 of the Information Technology Act empowers the Central Government or a State Government to direct any agency of the Government to intercept, monitor or decrypt any information stored in any computer

resource. The reasons for doing so should be recorded in writing. The following are the grounds of Interception, monitoring, decryption or blocking of information. (a) in the interest of the sovereignty or integrity of India, (b) defense of India, (c) security of the State, (d) friendly relations with foreign States, (e) public order, (f) for preventing incitement to the commission of any cognizable offence relating to above, (g) for investigation of any offence. On the same grounds except for investigation of offences, directions can be given by the Central government under section 69-A of the IT Act, for blocking from public accesses any information through computer resource. The procedure and safeguards subject to which interception or monitoring or decryption or blocking may be carried out, is prescribed by the Government.

Mandatory duty is imposed on the subscriber or intermediary or any person in-charge of the computer resource to extend all facilities and technical assistance to the government or the authorized officer to (a) provide access to or secure access to the computer resource generating transmitting, receiving or storing such information (b) intercept, monitor, or decrypt the information or (c) provide information stored in computer resource. If they fail to do so the prescribed punishment is imprisonment up to seven years and also fine. Same punishment is to be given for failure to comply with the directions of the Central government for blocking from public access of information through computer resource.

Protection of Critical Information Infrastructure

Many of the critical services that are essential to the well-being of the economy are increasingly becoming dependent on IT. It is necessary to secure the information resources belonging to Government as well as those in the critical sectors. The critical sectors include Defense, Finance, Energy, Transportation and Telecommunications. Any computer resource which directly or indirectly affects the facility of Critical Infrastructure can be declared as a protected system. The Central Government is competent to prescribe the information security practices and procedures for such protected system. The Central Government may, by notification, designate any organization of the Government as the National Nodal Agency to protect Critical Information Infrastructure. The agency is to be responsible for all measures including Research and Development relating to protection of Critical Information Infrastructure.

Web Server Security

A secure web server provides a foundation for the organization's hosting environment. It enables an organization to publish information. However, if an organization is not cautious in configuring and operating its web site, it may be vulnerable to a variety of security threats. Malicious intruders may change the contents of the web pages, may cause monetary losses or spoil the reputation of the organization. Web Server Security Guidelines issued by CERT-In on 17th August, 2004 discussed the overall methodology to be adopted in creating a Secure Web Infrastructure. It puts together the best practices along with other references to be followed in implementing the same. It tries to cover all broad aspects including strategies like defense in depth using various security controls at network, operating system and application levels. Guidelines for securing web server were also issued in 2005 and 2006. Web Server Security guidelines issued on 18th November 2010 recommend the security practices for designing, implementing and operating publicly accessible Microsoft Internet Information Services(IIS) Web servers. The guidelines are designed to mitigate the risks associated with public Web Servers and may be used by the organizations to reduce web-related security incidents.

Security Guidelines for Organizations

Schedule 2 of the Information Technology (Certifying Authorities) Rules, 2000 lays down certain guidelines for ensuring information security. A mandatory duty is imposed on organizations to develop internal processes in compliance with these guidelines. The general principle is that the person who is generating the information is also responsible for its security. The custodian of information is responsible for the proper implementation of security guidelines and making the information available to the users on a need to know basis. The rules insist that the site

where information is stored should not be in locations that are prone to natural or man-made disasters, like flood, fire, chemical contamination and explosions.In order to ensure protection from fire, the combustible materials shall not be stored within hundred meters of the operational site. Personnel at the operational site should be trained to monitor and control the various equipment and devices installed at the operational site for the purpose of fire and environmental protection. In order to ensure information security, each organization should designate a properly trained System Administrator who will ensure that the protective security measure of the system is functional and who will maintain its security posture. Highly sensitive information assets shall be stored on secure removable media and should be in an encrypted format to avoid compromise by unauthorized persons. Prevention, detection, and deterrence measures shall be implemented to safeguard the security of computers and computer information from misuse. Security controls shall be installed and maintained on each computer system or computer node to prevent unauthorized users from gaining entry to the information system and to prevent unauthorized access data.

Access control software and system software security features shall be implemented to protect resources. Control features shall be implemented for passwords. Responsibilities and duties shall be assigned to ensure that all file servers and personal computers are equipped with up-to-date virus protection and detection software. All sensitive information on the network shall be protected by using appropriate techniques. Intelligent devices generally known as “Firewalls” shall be used to isolate organization’s data network with the external network Emergency response procedures for all activities connected with computer operation shall be developed and documented. These procedures should be reviewed periodically.

Security Guidelines for Certifying Authorities

The information Technology (Certifying Authorities) Rules, 2000 provide that the Certifying Authorities Should have the sole responsibility of integrity, confidentiality and protection of information and information assets employed in its operation, considering classification, labeling, storage, access and destruction of information assets according to their value, sensitivity and importance of operation. Security guidelines for certifying authorities have been laid down in Schedule III of the Rules. The guidelines impose a mandatory duty on Certifying Authorities to ensure that all personnel performing duties with respect to its operation must receive comprehensive training in relevant aspects of the Information Technology Security Policy and Security Guidelines framed by the Certifying Authorities. Procedures and security controls to protect the privacy and confidentiality of the subscriber’s data under the Certifying Authority’s custody should be implemented. Confidential information provided by the subscriber must not be disclosed to a third party without the subscribers’ consent, unless the information is required to be disclosed under the law or a court order.

The Information Technology (Certifying Authority) Regulations 2001 also imposes a mandatory duty on certifying authorities to maintain secure and reliable records and logs for activities that are core to its operations. The Certifying Authority should also assure the confidentiality of subscriber information. The Certifying Authority as approved, in respect of security and risk management controls, should continuously ensure that security policies and safeguards are in place. Such controls include personnel security and incident handling measures to prevent fraud and security breaches.

Guidelines for Home Computers

Security guidelines issued by the Indian Computer Emergency Response Team (CERT-In) on 31st December, 2005 prescribe basic guidelines to the users of home computer working with computer systems running Windows Operating System. The basic purpose is to create awareness about computer security issues among home computer users and suggest them the tasks to be performed to secure their computer systems to protect their information assets. The guidelines give an overall picture of different types of cyber-attacks and the security safeguards that should be adopted to protect the system from such attacks. The traditional method of defense in depth strategy is suggested as the best method to secure data from attack by cyber criminals.

Physical Security

Physical Security is essential for protecting valuable information stored in computers. Hardware is more visible than software largely because it is composed of physical objects. Hardware attacks are done by adding devices hooked to the system, changing them, removing them, intercepting the traffic to them and flooding them with traffic until they can no longer function. Other physical attacks where computers have been drenched with water, burned, frozen, gassed and electrocuted with power surges do occur. Negligent users may spill soft drinks, beer, corn chips and other food items on computing devices. Particles of dust and ash from cigarette smoke may spoil the precisely engineered parts of computers.

Goals of Cyber Security

In the virtual world of computer and internet, cyber security offers confidentiality, integrity and availability to information assets. Confidentiality means the concealment of information resources by restricting access to information through access control mechanisms. Sensitive information should be kept secret so as to prevent the misuse. Integrity refers to the trustworthiness of data or resources by preventing unauthorized alteration of data. The term availability refers to the ability to use the information or resource desired. The goal of cyber security is protection of information by preventing, detecting, and responding to cyber-attacks. Prevention involves implementation of mechanisms which prevents the attacker from accessing and changing data. Detection is most useful when an attack cannot be prevented, but indicates the effectiveness of preventive measures. Detection mechanism will report that an attack has occurred. Responding to attack means stop the attack, assess and recover from attack by repairing the damage caused by that attack. Prevention of cyber-attacks can be achieved by offering physical security to computer system and also by adopting suitable software for securing the system.

CONCLUSION

The developments in the field of Information Technology have made drastic changes in our way of life. Electronic commerce and e-governance has considerably eliminated paper based transactions. The growth of international trade through e-commerce, necessitated framing of rules for validating e-commerce transactions. It was also necessary to give proper guidance to national legislators to frame law to promote electronic commerce in the international sphere.The use of internet has established online communities which have brought about new kind of social relationships. Relationships on networking sites also turn real when people are involved. It is through the use of technology that people learn more about worldly affairs. Use of mobiles and internet for rise to a new tyrimeie Cybercrime. Online crime can be conducted from anywhere and at any time with a computer and a network connection. It leads to easy victimization. Pornography has degraded social and moral values of youngsters and even children. Here the studies have suggested that the unique features of Cyber Crime Cyber law for developing information security awareness is a powerful technique device.

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Table 1.Act and Punishments of Cybercrimes

Cybercrimes	**Act**	**Punishments**
Impersonation (fake) profiles, in community networks, fake e-mail Ids etc…)	66 D of IT Act 419 IPC	3 years and fine up to 1 Lakh
Cheating (lottery scam using e mails and SMS, fake websites, mail asking financial helps etc…)	66 A, 66A (b) (c), 66 D 420 IPC	3 Years and fine
Software theft (using the source code of software and creating another).	66 A r/w 43 (j)	3 years or fine up to 5 lakhs or both
Morphing (editing the photo with nude photo and publishing)	66 C r/w 43 (i), 66 A(b),509 IPC	3 years or fine up to five lakh or both
Cyber terrorism (crimes against the Nations by means of computer /mobile/internet etc.	66 F of IT Act	Up to life imprisonment.
Spoofing (hiding identity and using others identity)	66D of IT Act	3 years and fine up to 1 lakh
Website Hacking	66C r/w 43(a)	3 years or fine up to 5 lakh or both
Email Hacking (methods are phishing, new website.Account registration) etc	66 E r/ w 43(a)	3 years or fine up to 5 lakh or both
Pornography (makes people especially children leading psychic states that cause rape, child molestation, sex, exhibitionism, sexual violence etc…)	67,and 67A of IT Act	5(7) years and fine up to 10 lakh
Child pornography(hidden in pornographic sites)	67 B of IT Act	5(7) years and fine
Credit / ATM card Frauds (using scanner and camera)	420 IPC 66 D 0F IT ACT	3 years and fine up to 1 lakh
Taking video /photo of women or spreading it	119 (b) of KP ACT	3 years or RS.10,000 or both

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RESEARCH ARTICLE

Detection and Identification of *Xanthomonas perforans* by Direct Sequencing and *in silico* Analysis of *hrpB* Gene

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ABSTRACT

Xanthomonas is a large genus of gram-negative bacteria that cause disease in hundreds of plant hosts including many thriftily important crops. *hrp* genes, basic hypersensitivity pathogenecity genes of the tomato pathogen *Xanthomonas perforans*, are regulated dependent on environmental conditions. PCR-based methods propose advantages over more time-honored diagnostic tests, in that organisms do not have to be cultured prior to their detection and protocols are highly sensitive and rapid. As a result, there is a budge in research towards DNA. This study presents the development of *in silico* and *in vitro* PCR methods for specific detection of *Xanthomonas perforans*, causal agent of bacterial spot of tomato and characterization of *hrpB* gene product using *in silico* analysis tools. Our findings have significant implications for the development of a new tool for the identification of *Xanthomonas perforans*. Our exertion is aimed at evaluating whether the sequence polymorphism of diagnostic PCR products could be extended to identify the infecting *X. perforans* in the atmosphere. Detection of *X. perforans* associated with infected plant material/seed sample was achieved by amplification of a DNA fragment of bacteria by polymerase chain reaction. Primers used in this reaction were specific for hypersensitive reaction and pathogenecity (*hrp*B) gene cluster. The molecular and bioinformatics approaches were described to identify *X. perforans.* In the current study, partial sequencing of *hrpB* gene was carried out and sequence

was deposited to NCBI data base. This study also proved to distinguish *X. perforans* along with closely related species.

Keywords : Polymerase chain reaction, *hrp*B gene, *Xanthomonas perforans*, *in silico* analysis, Phylogenetic analysis.

INTRODUCTION

Vegetable production can be adopted as a strategy for improving livelihood and alleviating the nutritional status of the people. *Lycopersicon esculentum* Mill is one the most important crop grown all over India. Every year, heavy yield losses in the agricultural production of many countries are attributed to phytopathogenic bacteria. Moreover, with the globalization of trade, the worldwide import and export of food crops facilitates the risk of the rapid spreading of such bacteria. Therefore, efficient and rapid quarantine procedures are required, not only to prevent pathogen spreading, but also to manage the already infected areas (Schaad *et al.,* 2003).Gram-negative bacterium *Xanthomonas perforans* (ex Doidge, 1920) Vauterin *et al.* (1995) is the causal agent of bacterial spot of tomato (*Lycopersicon esculentum* Mill.). The disease causes considerable losses in productivity and fruit quality especially in areas of warmer and humid climate (Jones *et al.,* 1998; Al-Dahmani *et al.,* 2003). The genus *Xanthomonas* comprises many phytopathogenic species (Ryan *et al.,* 2011) and a total of thirteen genus members are considered as quarantine organisms by EPPO (European and Mediterranean Plant Protection Organization) (Albuquerque *et al.,* 2012).

The taxonomy of the causal agents was subjected to many reclassifications but now the pathogens belong to four species *Xanthomonas euvesicatoria*, *Xanthomonas vesicatoria*, and *Xanthomonas gardneri* and *Xanthomonas perforans* (Jones *et al.,* 2004; EPPO, 2013).redicting and ranking potential invasive species present significant challenges to researchers and biosecurity agencies (Paini *et al.,* 2010). The pathogen is transported principally on seeds of tomato or capsicum, young seedlings of solanaceous weeds and also nearly all agents passing through infested fields (including insects, tools, soil) (Bashan 1986; Tamir-Ariel *et al.,* 2012). Moreover, the genus *Xanthomonas* comprises several subgroups with each separately composed of various genetically and phenotypically distinct species (Jones *et al.,* 1998) which makes their quarantine more complex. Control of *Xanthomonas* sp. relies principally on the use of pathogen-free seeds, fruits and transplants to prevent spread at an early stage (Paret *et al.,* 2012).

Up to the mid-90's, the classification of *Xanthomonas* species and isolates was based on phenotypic data. The main criteria for the creation of new species rested on host specificity. The taxa "pathovar" was added to distinguish *Xanthomonas* species at the infra sub-specific level. Some species, e.g. *X. perforans*, *X. axonopodis, X. transluscens* or *X. campestris*, comprised more than ten, 40 and 125 pathovars, respectively (Vauterin *et al.,* 1990). Several methods were used in an attempt to classify *Xanthomonas* species. Identification and classification of plant pathogenic bacteria are historically based on phenotypic characteristics such as symptoms caused, presence of specific antigens for serology analysis (ELISA), biochemical characteristics, substrate utilization profiles (BIOLOG), fatty acid composition (FAME) and multilocus enzyme electrophoresis profiles (MLEE) (Louws *et al*., 1999). Biochemical tests are labor intensive and time consuming while serological methods have high development costs and at times less sensitive. Considering the economical losses of tomato and pepper production caused by *X. vesicatoria* and limited options of the pathogen suppression in later stages of the disease, rapid and accurate detection of the pathogen is vital for the tomato and pepper production

Nucleic acid-based techniques have also been applied to the detection and identification of phytopathogenic bacteria, including some members of the Xanthomonads. The techniques developed for detection and identification of Xanthomonads were based on random probes (Lazo *et al.,* 1987) or on plasmid DNA fragments specific for a few pathovars of *X. campestris* or even for a group of strains (Garde and Bender, 1981). Highly conserved regions in the bacterial genome of phytopathogenic bacteria could be more useful for the selection of specific DNA probes for

detection and identification of a larger number of *Xanthomonas* strains, pathovars, or species. The *hrp* gene clusters that determine hypersensitivity and pathogenicity may be appropriate for selection of probes for detection and identification of phytopathogenic bacteria. The hrp gene cluster is required by bacterial plant pathogens to produce symptoms on susceptible hosts and a hypersensitive reaction on resistant hosts or on nonhosts and has been found in several phytopathogenic bacteria, such as *Erwinia amylovora*, *Pseudomonas solanacearum*, *P. syringae* pv. *phaseolicola* , and *X. campestris* pv. *vesicatoria*. Furthermore, *hrp* functions seem to be highly conserved among a number of phytopathogenic bacteria. The *hrp* genes of phytopathogenic bacteria are also very similar at the protein level to genes that are involved in the secretion of pathogenecity factors by bacterial pathogens of mammals. By contrast, nonpathogenic bacteria are unable to produce symptoms on susceptible hosts and hypersensitive reaction on nonhosts, and apparently they do not possess DNA sequences similar to *hrp* genes. Physically and functionally similar *hrp* sequences occur among several pathovars of *X. campestris* but not in opportunistic Xanthomonads (Leite *et al.,* 1994)

The *hrpB* genes of *X. perforans*, encompasses 6kb and encodes eight putative proteins subunit has shown to be a useful and more discriminating alternative to the 16S rRNA gene for inferring phylogenetic relationship. Comparison of *hrpB* sequences has been used for phylogenetic analysis among some members of domains. Previous work clearly illustrates that *hrpB* sequence analysis is a powerful tool for the identification of members of the family Xanthomonas. In order to investigate the possible characterization of the genus *X. perforans* through *hrpB* gene sequence, a 517 bp portion of this gene from 16 distinct strains was determined and further analyzed. Phylogenetic analysis has also been used to characterize the *Xanthomoans* species. In many regions and countries; however, genetic analysis of *Xanthomonas* species in Karnataka, India is still limited. Therefore the main objective of this study were to detect *Xanthomonas perforans* by using *hrp* gene by PCR, and to determine the *Xanthomonas* genotypes in these regions based on *hrp* gene region amplification and sequencing. This study also examines sequences of the *hrpB* genes of *X. perforans* for their use in detection and identification of phytopathogenic Xanthomonads. Oligonucleotide primers specific for *hrpB* genes were tested for their suitability for identification of these Xanthomonads by PCR.

MATERIALS AND METHODS

Isolation of *Xanthomonas perforans*

The phytopathogenic bacteria were isolated from infected plant material/seeds collected from farmers' field and local seed agencies, and were subjected to screening in the laboratory following different seed health testing methods like direct plating method and liquid assay method (ISTA, 2005).Direct plating method: Collected plant material/seed samples were surface-sterilized with 2% sodium hypochlorite followed by repeated washing with distilled water and blot dried, then plated directly on to the semi-selective medium like Tween B (McGuire *et al.,* 1986), Plates were incubated at 28±2^{o} C for 24–48 h. The yellow colonies with hydrolytic zones around the piece of plant material and seeds were observed for *X. perforans.*

Liquid assay method: The collected plant material/seeds were surface-sterilized as mentioned above. After that, they were macerated using sterile mortar and pestle in 10 ml of sterile distilled water. One milliliter of the supernatant was mixed with 9 ml of sterile distilled water to obtain a dilution of 10^{-1}, and the similar serial dilutions were done up to 10^{-5}. Fifty microliters of each dilution was placed on semi-selective media using Drigalski spreaders in triplicates. The plates were incubated at 28+28^{o}C for 24–48 h and observed for typical colonies of *X. perforans.*

Characterization of the Pathogen

Characterization of the pathogens was done by subjecting the isolated bacterial colonies to various biochemical tests like Gram staining, KOH solubility test, starch hydrolysis, lipase activity, gelatin hydrolysis, and oxidative/fermentative metabolism of glucose. The strains were also subjected to hypersensitive test using tobacco

(*Nicotiana tobaccum*), and pathogenecity test was carried out by using known susceptible host cultivars as per (Lelliot and Stead, 1987), with appropriate reference isolates.

PCR Studies

DNA extraction: Bacterial DNA was isolated using bacterial genomic DNA isolation kit from BangaloreGeNei (Bangalore, India) according to the manufacturer's instruction. The isolated phytopathogenic bacteria were subjected to molecular characterization.

In silico primer designing

Primers for the hrpb gene were specifically designed by using *in silico* primer designing tool of FastPCR software (Primerdigital Ver. 6.0). The sequence of the hrpb gene was retrieved from Genbank database, copied to FastPCR workspace and specifications required for the designed PCR primer with respect to the sequence such as melting temperature (Tm), linguistic complexity control, the secondary (non-specific) binding test and Circular DNA (bacterial gene present in circular DNA) were specified, followed with a run command. After a successful run the tool provided a list of primers feasible to run PCR, out of which two primers (forward and reverse) were selected based on our polymerase enzyme , percent coverage of the primers, and quality score of the primer, etc.,

In silico PCR

Before running a PCR *in vitro* the efficiency of the selected primers (Fig. 2)were crosschecked by using *in silico* PCR tool of FastPCR (Primerdigital ver. 6.0) where the forward and reverse primers selected from the list of primers were copied and paste into the additional sequence or predesigned primers (probe) list, followed by specifying specifications for the PCR run such as maximal product size, PCR product prediction, Circular Sequence (sequence to be amplified) and alignment options followed by run command, after the successful *In silico* PCR run, the results report section of the tool specifies the binding sites of the given primer set and also the length of the product obtained after amplification, in case of both linear DNA and Circular DNA respectively. After a satisfactory result is obtained through *in silico* PCR (Fig. 3), the primers selected for the *in silico* PCR run are actually synthesized and used for *in vitro* PCR amplification.

In vitro PCR

DNA was amplified in 25 µl of reaction mixture which was prepared in PCR tubes (25 µl) containing 1x PCR buffer (2.5 µl), 3U/ml *Taq* polymerase (0.5 µl), 1mM dNTPs (2.0 µl), 10 pmol/µl of primer (2.0 µl) and 100 ng of genomic DNA/µl (1.0 µl) of reaction mixture. Reactions were run for 25 cycles, each consisting of 60 s at 94° C, 60s 58° C, and 60s 72° C, with initial denaturation of 5 min at 94° C and final extension of 10 min at 72° C. Products were analyzed by separation in 1.2 % agarose gels and were stained with ethidium bromide and visualized under UV light.

Gel Elution

The PCR products were eluted using eppendorf QIAquick gel extraction kit (QIAGEN, Germany). The amplified products in the samples positive for PCR were subjected to DNA sequencing. After the confirmation of the isolates, all the products were purified and were sent for nucleotide sequencing of both strands to a commercial sequencing agency (Europhins Pvt. Ltd, Bangalore, Karnataka, India). The products were sequenced using ABI-Prism (Applied Biosystems, USA) genetic analyzer that works based on the principle of Sangers' dideoxy termination method. The sequences were then analyzed by DNA star sequence alignment software and BLAST analysis was done to confirm the sequenced data with the standard strains and was percent homology determine (www.ncbi.nlm.nih.gov/BLAST).

BLAST Analysis

The sequence was subjected to BLAST analysis to confirm the sequence data with the standard strains of *Xanthomonas* and also to determine the percentage identity of the gene sequence with other genes in the organisms of same genus,

to further predict the function if protein product of the *hrpB* gene presently sequenced. Out of the list of sequences which were found to posses significant identical sequence with *hrpB* gene presently sequenced, hrpB6 gene of *Xanthomonas citri* was selected as the protein product of it was already known, making it easy for the comparative study.

Phylogenetic Analysis

To determine the degree of relatedness and alo to determine the conserved regions in the given *hrpB* gene sequence of *Xanthomonas vasicatoria,* the *hrpB* gene sequences of *Xanthomonas* species of sixteen strains were retrieved from GenBank. Second, the *hrpB* gene nucleotide were aligned using ClustalW.Sequences were aligned with ClustalW on the EMBL-EBI web server (http://www.ebi.ac.uk/clustalW/). Phylogenetic tree was obtained from DNA sequences by the maximum likelihood method using the Mega 7.0v software (http://www.megasoftware.net). Evolutionary distances were estimated and the DNA sequences of all the isolates have been deposited in the GenBank database with the accession number as shown in table (Table 1). Branch supporting values were evaluated with 1000 bootstrap replications.

In silico Prediction of Synthesized Protein

To determine the function of the sequenced *hrpB* gene, it is essential to find out the translated protein sequence of the gene, hence to obtain the translated sequence, the sequence of the DNA sequence of *hrpB* gene was translated using Expasy translate tool where the DNA sequence is pasted and output format and genetic codes were selected, and command to translate was given.

In silico Protein Structure Prediction

Out the six open reading frames predicted by the translate tool the ORF with reasonably satisfactory read length was selected and the ORF sequence selected. Homology model of the selected ORF sequence was predicted using Expasy SWISS model workspace, where the *in silico* structure of the selected protein product is predicted through homology modeling. To obtain a better insight into the function of *hrpB* gene sequenced in the present study, structure of hrpB6 gene of *Xanthomonas citri* which was found to have 96 percent identity with *hrpB* gene sequenced in the present study, was also predicted through Expasy SWISS model workspace through homology modeling, this also provides the function of the predicted model through its structure. The predicted structures and functions of the proteins of *hrpB* of *Xanthomonas perforans* and hrpB6 gene of *Xanthomonas citri* were compared, to understand the significance and activity of hrpB gene in *Xanthomonas perforans.*

RESULTS AND DISCUSSION

Isolation of plant pathogenic *X. perforans*

Out of the 10 tomato cultivars screened, six tomato cultivars showed the presence of *X. perforans*. *X. perforans* colonies exhibited typical morphological characteristics such as yellow colonies with hydrolytic zones. These pathogens were further purified by restreking them on their respective semi-selective media. Different isolates of *X. perforans* were further subjected to biochemical/physiological assays along with hypersensitive and pathogenecity tests to confirm the identity of *X. perforans* (Table 1).

Biochemical characterization of bacterial isolates

The isolated bacterial strains showed Gram-negative characteristic by both Gram staining and also KOH solubility test. The isolates of *X. perforans* did not show clear zone of hydrolysis, which indicated negative reaction for starch hydrolysis. The inoculated *X. perforans* on Tween 80 agar plates showed the presence of white precipitate around the colonies of the bacteria, hence both the bacteria were positive for lipase activity. After 7 days of incubation, *X. perforans* did not liquefy gelatin media when compared with the control, hence they were negative for gelatin

hydrolysis, The isolated *X. perforans*, changed the color from green to yellow indicating positive for oxidation test, but in the fermentation test no color change from green to yellow was observed, thereby indicating a negative test. Necrosis was evident in tobacco plants within 48 h of infiltration with bacterial cells, whereas sterile distilled water infiltrated leaf regions did not show any change in leaf color. Tomato plants inoculated with *X. perforans* showed bacterial spot symptoms. Control plants inoculated with distilled water did not show any symptoms (Table 2).

PCR studies and phylogentic analysis

In silico primers designed

The primers selected from the list of primers predicted based on our sequence length (Fig. 1), DNA polymerase and other physicochemical parameters were:
hrpB Forward Primer 5'-ATGCTGGCTGAGACGCCCCTG-3' and
hrpB Reverse Primer 5'- AGCGAGTCCATCACCAGCAGC -3'.

In silico PCR analysis

In silico PCR analysis using the *in silico* primers designed (Fig.2) resulted in a product size of 786 bp in case of linear DNA (Fig.3), which is considerably a good amplification, hence these primers were synthesized and used for *in vitro* PCR.

In vitro PCR

hrpB primers designed *in silico* were used to amplify the tested bacterial DNA in all the respective positive isolates confirm the pathogens. The PCR product of 786 bp was obtained in all the isolates and negative control which lacks the template DNA did not show any band (Fig. 4). *Xanthomonas perforans* collected from the infected plant materials/seed samples were characterized by both biochemical and molecular detection. The *hrpB* gene clusters that determine hypersensitivity and pathogenecity may be appropriate for selection of probes for detection and identification of phytopathogenic bacteria.

BLAST analysis

BLAST of *hrpB* gene sequenced in the present study resulted in significant amount of identity with several *Xanthomonas* species with over 94 percent identity.

Phylogenetic analysis

16 Xanthomoans species, collected were nucleotide sequenced, alignment of nucleotide of *hrpB* region with all the strains of this study (Table 1). Phylogenetic construction was performed from the aligned sequence using maximum likelihood method using the Mega 7.0v software (http://www.megasoftware.net).This phylogenetic analysis could segregate the strains into four groups (cluster I to IV) in maximum likelihood with two major clusters (I and IV).In maximum likelihood phylogenetic tree, our isolates have clustered with other country isolates. The Indian isolates are divergent, on the phylogentic tree the Indian isolates are placed separately with Germany, France, Columbia and China isolates.

There is a formation of two major cluster in that isolate, KF 495204 has formed cluster with Colombia isolate, KF495202 clustered with France and KF 495203 forward cluster with China isolate in cluster I. where in, cluster II, KF 495205 formed cluster with France isolate and interestingly, Isolate KF495306, KF495307 from our study has formed cluster with China isolates in Cluster II. The phylogenetic tree (Fig. 5), it can be seen that this cluster shows higher divergence. The branches were widely separated in *hrp* gene sequence; we were able to distinguish all sixteen isolates of Xanthomonas species using the sequence analysis. It was possible to separate the sixteen isolates and place them under different clusters. The result obtained from phylogenetic tree dendogram showed a diversity of polymorphism between *X. perforans* strains of different origin.

In silico protein prediction

Translation of the gene using Expasy translate tool resulted in six open reading frames out of which the first ORF 5'3'Frame 1 had 271 amino acid sequence and was most likely to be considered to form protein as there were no intermediate nonsense codons in the sequence, hence this frame was selected for protein structure prediction. Since the protein sequence of the gene was already known there was no need to translate *hrpB6* gene.

In silico prediction of protein structure

The SWISS model workspace of Expasy predicted two possible homology models (structures) for the ORF 5'3' Frame 1 of *hrpB* gene and one precise model for protein product of *hrpB6* gene of *Xanthomonas citri* which was used for comparative study, when the available protein sequence of *hrpB6* gene was used (Fig. 6).PCR – mediated *In silico* methods provide faster and competent results in designing primers and running the *In silico* PCR helps in finding efficiency of the designed primers, which saves a lot of time and resource when compared to conventional trial and error method, *in vitro* PCR comprises a DNA-based approach to assess the diversity of pathogen populations to detect pathogens and diagnose disease, construction of precise genetic diversity maps will provide a better framework for addressing important plant disease problems related to detection of pathogens, diagnosis of disease and ultimately management of disease risk.

Xanthomonas is a major genus of gram negative phytopathogenic bacteria that infect almost all crop plants and is responsible for major economic losses. Although disease symptoms caused by the bacteria are very diverse, pathogens of all genera share a common feature; they contain a large cluster of basic pathogensity genes, the *hrp* genes. Its 23-kb *hrp* cluster comprises six *hrp* loci, *hrpA* to *hrpF,* which are required for full pathogenecity (Bonas *et al.,* 1991).The species *X. perforans* has been described as being exclusively plant pathogenic and shows a high degree of host specificity. This finding has led to division of this species into many pathovars, according to the host ranges of strains. *X. perforans* is the causal agent of bacterial spot disease of pepper and tomato plants and has served as a model system to study the molecular basis of pathogenesis. The use of oligonucleotide primers provides a sensitive and specific tool for detection of DNA by amplification.

The complexity of the genus and resource requirements for comprehensive pairwise analyses required for DNA–DNA hybridization analysis make this technique suitable for routine identification in most diagnostic laboratories. The morphological characteristics on Tween B medium, physiological, biochemical characterization results, hypersensitive response in tobacco plant leaves and pathogenicity test results confirmed that the pathogen indeed was *X. perforans* (Bradbury, 1986; McGuire *et al*., 1986; Kavitha and Umesha, 2007). The present investigation showed that for detection of *X. perforans* on the seeds, the direct plating method can be routinely used.Direct plating method was carried out by plating the infected plant materials and seed samples on their respective semi-selective media such as Tween B for *X. perforans*, which resulted in the growth of yellow colonies with hydrolytic zones, typical mucoid creamy white colonies around the plant material.

Colony morphology, biochemical/physiological tests, hypersensitivity test, and pathogenecity tests were included for the identification and confirmation of the isolated pathogen as *X. perforans* (Kavitha and Umesha, 2007). Biochemical tests are labor intensive and time consuming while serological methods have high development costs and at times less sensitive. Early and accurate detection and identification of new cases of *Xanthomonas* diseases as provided by molecular based methods (Louws *et al*., 1999; Lopez *et al*., 2006; Narayanasamy, 2011) will assist in speeding up their management. Compared to conventional diagnostic methods, PCR offers several advantages, because organisms do not need to be cultured prior to detection; moreover it is highly sensitive, relatively simple and fast to perform. There has been a shift towards DNA-based protocols developed for diagnostic purposes as well as for etiological or epidemiological studies, as reported by several reviews published over the past fifteen years (Henson and French, 1993; Palacio-Bielsa *et al.,* 2009).

Molecular identification of *Xanthomonas* species including the tomato bacterial spot causing agents have been performed by PCR followed by restriction enzyme analyses (Leite *et al.,* 1994, 1995) and by rep-PCR (Louws *et al.,* 1995). Specific primers are often used as molecular tools for the detection of various plant pathogens (Robene-Soustrade *et al.,* 2010; Lopez *et al.,* 2012), including the tomato bacterial spot species complex (Koenraadt *et al.,* 2009). Most recently, phylogenies have been developed that encompass species within higher-level taxa, including the genus *Pseudomonas* and the family *Enterobacteriaceae* (Ait Tayeb *et al.,* 2005; Paradis *et al.,* 2005). Both these studies revealed good correlation between established reference species and their phylogenetic identification. In contrast to the narrow spectrum of oligonucleotide primers previously used for detection and identification of only certain strains of *X. campestris* (Hartung *et al.,* 1993), the hrp-specific primer pairs designed *in silico* seem very useful for the identification of a large range of phytopathogenic Xanthomonads.

This is perhaps not surprising, because the *hrp* region seem highly conserved among different phytopathogenic Xanthomonads as determined by Southern hybridization studies carried out by Bonas *et al.,* 1991, Stall, R. E., and G. V. Minsavage, 1990, Rui *et al.,* 1994, which was similar to our studies. We previously showed that analysis of 16S rDNA using PCR-SSCP and Colony PCR-SSCP methods were useful in detection of plant pathogenic bacteria of the genus *Xanthomonas* (Chandrashekar *et al.,* 2012; Umesha *et al.,* 2012). Different secretion systems and their effectors have been shown to contribute to the virulence of plant pathogens. The type III secretion system (T3SS) encoded by the hrp (Hypersensitive Response and Pathogenecity) gene cluster (Bonas *et al.,* 1991, Kim *et al.,* 2003) and type III secreted effectors have been widely studied for their role in hypersensitivity and pathogenicity. Effectors common between strains are believed to be responsible for conserved virulence function and avoidance of host defense (Potnis *et al.,* 2011).

Obradovic *et al.* (2004) have designed a set of primers for the bacterial spot pathogen based on an *hrp* sequence that is highly conserved among the pathogenic Xanthomonads; restriction enzyme analysis of the amplicon produced with these primers has been used to differentiate the four bacterial spot-causing Xanthomonad groups.Leite *et al.* (1995) assessed variation within the hrp gene cluster of representative *X. campestris* pv. *vesicatoria* A and B group strains. Genomic DNA was amplified using oligonucleotide primers specific for different regions of the hrp gene cluster, and the PCR products were digested with restriction enzymes. Phylogenetic analysis placed the A and C strains in a cluster distant from the B group strain (Leite, 1994). *X. gardneri* was distantly related to the A, B, and C groups but had a high degree of similarity to *X. campestris* pv. *taraxaci* and several other pathovars. Parkinson *et al.* (2007) constructed a phylogram from alignment of *gyrase* B (*gyrB*) sequences for all Xanthomonad species, both to indicate inter-species relatedness and as an aid for rapid and accurate species-level identification, Which was similar to our studies.

In conclusion, the results presented here indicate that phytopathogenic strains of *X. campestris* and related Xanthomonad species can be detected and may be identified by analysis of DNA fragments amplified with hrp gene-specific primers. The conservation of the hrp DNA sequence among a large number of pathovars of *X. perforans*, as well as in related *Xanthomonas* spp., but lack of the *hrp* DNA sequence among non phytopathogenic bacteria, makes this method a useful tool for detection and identification of many plant pathogens. Consequently, *hrp* oligonucleotide primers may be also useful to determine the pathogenic nature of unknown Xanthomonads.

This is particularly significant for assessing the complex population of phytopathogenic and nonpathogenic Xanthomonads associated with plants and plant parts. The presence of phytopathogenic strains in such samples may be determined by amplification of the *hrp* fragments without the need for the troublesome methods of isolation of the organism and inoculation into potential host plants. Moreover, restriction fragment length polymorphisms detected in the genomes of different strains seem valuable. The genetic methods of analyzing populations of bacteria will provide valuable additional information for taxonomic, ecological, and epidemiological studies of phytopathogenic Xanthomonads. Primer sets able to amplify targeted gene fragments from collection of related pathogens can also be a viable strategy to detect pathogens and for disease diagnosis. Leite *et al.* (2004) have designed primers that amplify

hrp gene fragments from many Xanthomonad. Such an assay would be useful for disease diagnosis and pathogen detection on seed or plant material.A positive result would indicate the presence of *Xanthomonas* strains and in many cases the species or pathovar could subsequently be deduced based on the host with which it was associated. It may be sufficient to develop primers to genes such as *hrpB* genes and rely on the known biology or ecology of the pathogen to reach specific conclusion about the pathogen identity.

Protein function predicted after the homology models were built, the SWISS model workspace automatically predicted the function of the protein based on its structure and it was found that hrpB6 gene codes for flagellum-specific ATP synthase, whereas in case of protein product of *hrpB* gene out of the two models predicted model one codes for Flagellum-specific ATP synthase just like hrpB6 gene of *Xanthomonas citri*, and model two codes for ATP synthase subunit alpha, but model one is found to exist more likely in nature due to higher sequence identity as well as considerable coverage, whereas model two had significantly less sequence coverage when compared to model one. It is also found that the model one of *hrpB* gene had significant similarity with hrpb6 protein model. Indicating that *hrpB* gene codes for Flagellum-specific ATP synthase which plays a critical role in virulence. Use of *in silico* protein structure and function prediction can lead to a rapid growth in the knowledge regarding several pathogenic gene products and also the virulence patterns of various pathogens.

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Table 1. Strains used in the study for partial *hrp* gene sequencing and phylogenetic tree construction

Genera	Species	Pathovars/Strain	GenBank accession no.
Xanthomonas	smithii	smithii Hrp gene	DQ643828
	axonopodis	manihotis	EU121376
	citri	mangiferaeindicae	KF019739
	citri	mangiferaeindicae	KF019738
	smithii	citri	DQ286697
	citri	mangiferaeindicae	KF019735
	campestris	vesicatoria	XCU33548
	campestris	vesicatoria	DQ286694
	campestris	vesicatoria	DQ286699

	campestris	vesicatoria	XCU45888
	perforans	perforans	KF495202
	perforans	perforans	KF495203
	perforans	perforans	KF495204
	perforans	perforans	KF495205
	perforans	perforans	KF495306
	perforans	perforans	KF495307

Table 2. Screening of different tomato cultivars for *X. perforans* by direct plating method

Cultivars	Results
Abhinav	+
Safal	+
Indam	+
Mrytunjaya	+
Rasi	+
Allrounder	+
Naveen	+
Vignesh	+
Valley	+

'+' indicate the presence and absence of phytopathogenic bacteria, respectively.

Table 3 Biochemical characterization of *X. perforans*

Biochemical tests	Results
	X. perforans
Gram's staining	-
KOH solubility	+
Starch hydrolysis	-
Lipase activity	+
Gelatin hydrolysis	-
O/F test	+/-
Hypersensitivity test	+
Pathogenecity test	+

*All the tests were conducted in four replicates and were repeated twice. + indicates positive reaction, - indicates negative reaction.

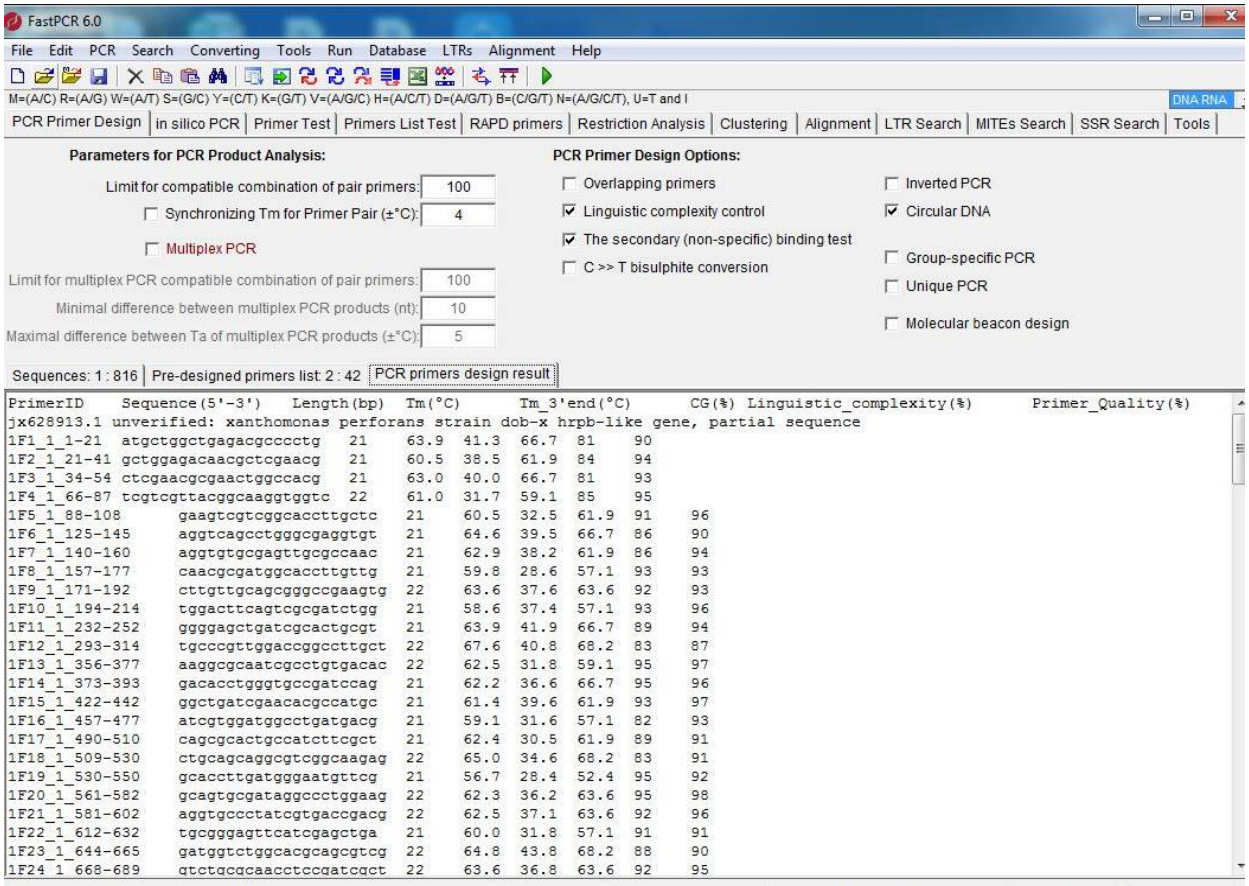

Fig.1. FastPCR primer design workflow showing list of primers designed for *hrpB* gene. Primers for the *hrpB* gene were specifically designed by using *in silico* primer designing tool of FastPCR software (Primerdigital Ver. 6.0).

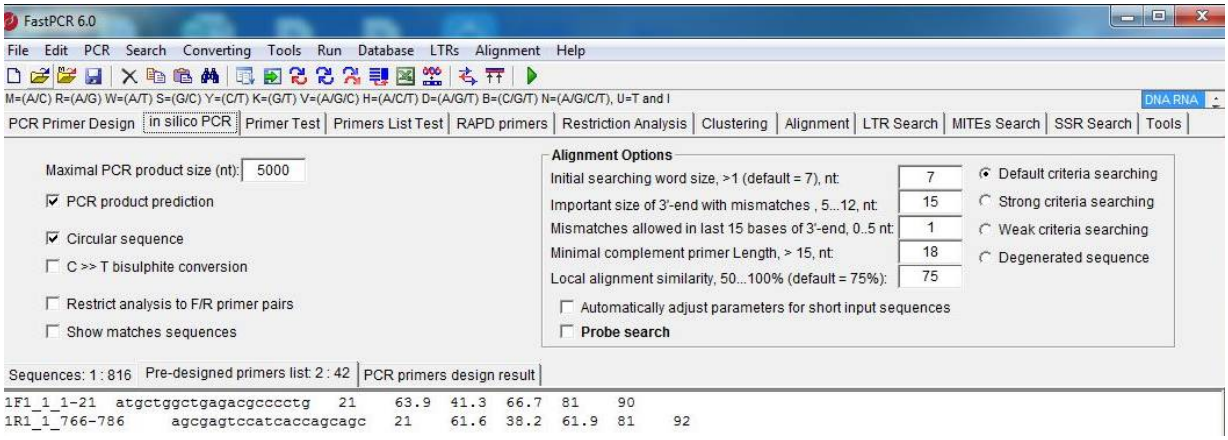

Fig. 2. FastPCR dialogue box showing the pair of primers (forward and reverse) selected and used for *in silico* PCR amplification

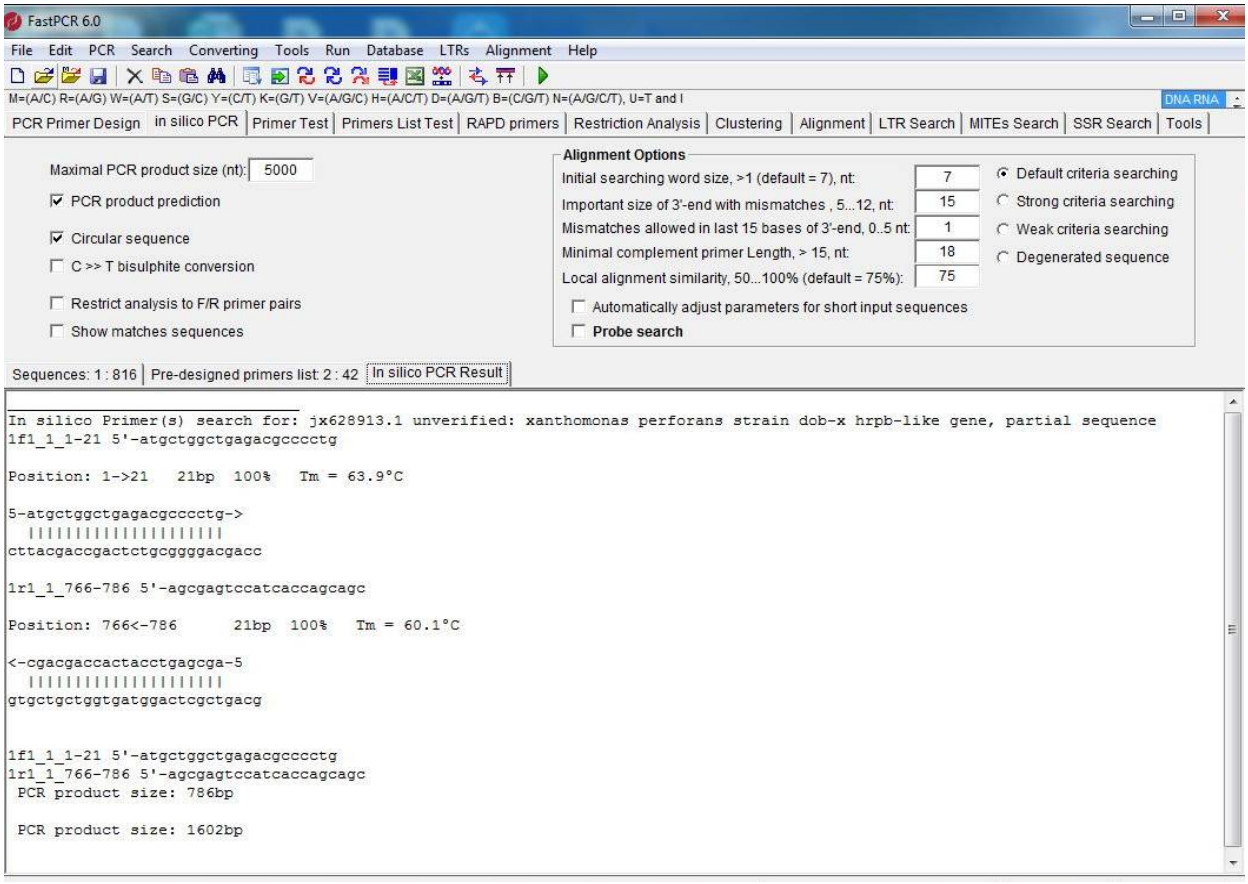

Fig. 3. *In silico* PCR result for amplification of *hrpB* gene using designed primers with product size of 786 bp for linear DNA. The primers selected for the *in silico* PCR run are actually synthesized and used for *in vitro* PCR amplification.

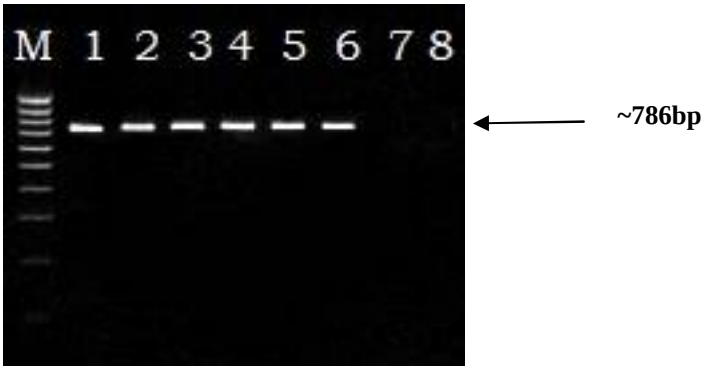

Fig. 4. Electrophoresis in 2% agarose gel showing amplification for *hrp* gene of *X. perforans* species. Analysis products amplified, Lanes 1-6- positive PCR product of *X. perforans* samples, Lane 7- Negative control, Lane 8- Non-relevant bacteria, M- 100bp Gene Ruler.

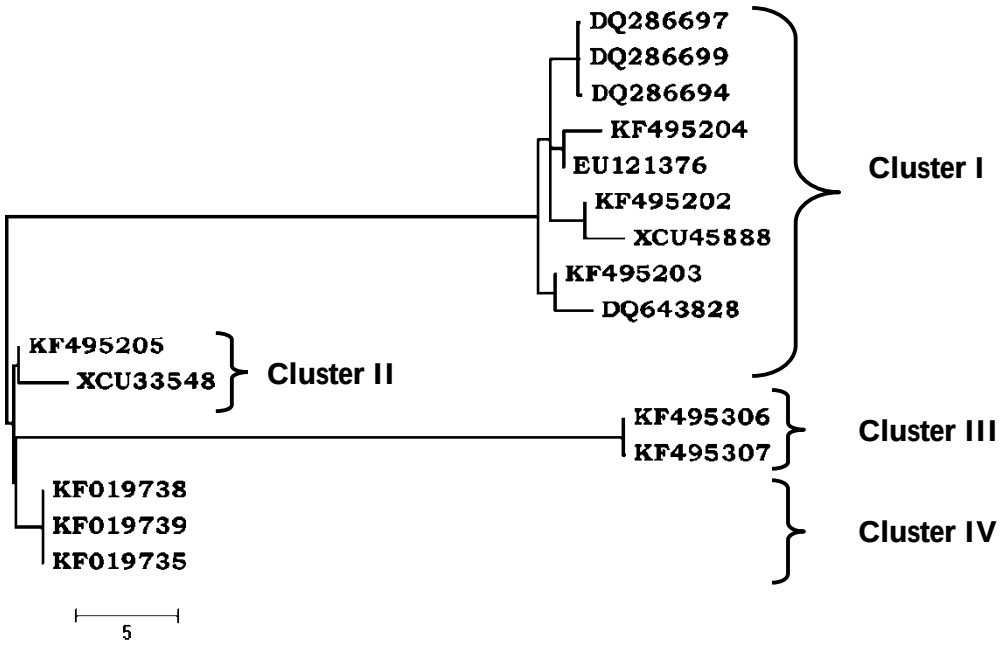

Fig. 5. Maximum-likelihood phylogenetic tree of *X. perforans* isolates based on *hrp* gene sequences. Percentages with cluster designations are *hrp* gene sequence similarity. Bootstrap values obtained from 1,000 resampling of the dataset, are given at the nodes of the tree. Clusters based on *hrp* gene sequence analysis are numbered consistently. Bars: 0.05 substitutions per nucleotide.

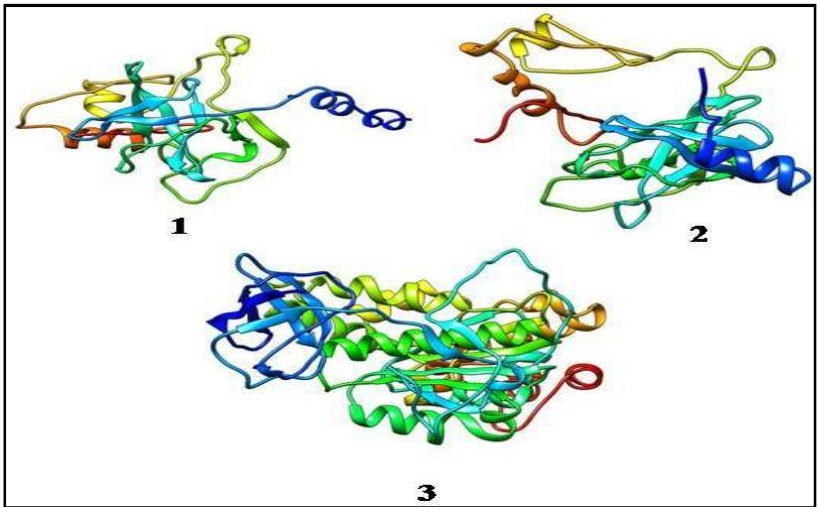

Fig. 6. *In silico* prediction of protein structure- 1. Model 1 of *hrpB* gene obtained through homology modeling using SWISS model workspace, which codes for Flagellum-specific ATP synthase; **2.** Model 2 of *hrpB* gene with lesser sequence identity than model 1 which codes for ATP synthase subunit alpha; **3.** Protein structure of *hrpB*6 gene product coded by *Xanthomonas citri* through homology modeling using SWISS model workspace of Expasy, which codes for Flagellum-specific ATP synthase.

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RESEARCH ARTICLE

GC-MS Analysis and Antifungal Potential of *Coriandrum sativum* L. Seed Essential Oil against Pathogenic Fungi

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ABSTRACT

The coriander seed essential oil was isolated from the powdered seeds by hydro-distillation method. GC-MS analysis of coriander seed oil revealed the presence of forty-four different compounds out of which forty-one were identified constituting approximately 98% of the coriander seed essential oil. The major compounds identified were linalool (75.2%), geraniol (5.24%), geranyl acetate (4.98%), camphor (1.76%), a-pinene (1.67), limonene (1.49%) etc. The antifungal activity of the coriander seed oil was tested using poisoned food technique against phytopathogenic fungi *Ganoderma lucidum, Pleurotus florida* and *Alternaria alternata*. Coriander oil was found to be more fungitoxic against *P. florida* with ED_{50} value of 0.458 mg/ml than *G. lucidum* and *A. alternata* with ED_{50} values of 0.548 and 0.578 mg/ml respectively.

Keywords : *Coriandrum sativum*, wood decaying fungi, essential oil, hydro-distillation, antifungal activity.

INTRODUCTION

Availability of quality planting material, disease free management of plantations and preservation of wood under storage and use are amongst the key elements required for enhanced and improved forest productivity. Management of pathogenic and wood decay fungi is one of the serious concerns in Indian forestry. In case of wood, 45% losses in sawn timbers are attributed to diseases and 73% of this loss is due to fungal decay. Tree decay is the major worldwide cause of damage to timber than all other destructive agents combined, resulting in 20-80% of annual loss (Agrow 2007). Fungal infection in the trees was observed due to various reasons like mechanical injuries in the stem, pruning of the branches, through the inflorescences, attack of Ambrosia beetle and termites. The inflorescences are attacked by various fungi species like *Alternaria* sp., *Chetomium* sp., *Aspergillus* sp., etc. Fungal

mycelia gradually invade the xylem tissues from the top of the branches and spread basipetally ultimately causing the death of the infected branches. *Ganoderma lucidum* and *Pleurotus florida* are wood decaying fungi causing white rot disease (Rajputa and Rao, 2007; Deepatharshini and Elango, 2015) while *Alternaria alternata* causes blight disease (Sharma *et al*. 1999, Singh *et al*. 2011) to various trees.

A huge amount of synthetic pesticides for biomass production are used the world over. The total value of world's agrochemical market is estimated between the US $31-35 billion of which herbicides account for 48% followed by insecticides (25%) and fungicides (22%) (Agrow 2007). The use of synthetic fungicides and wood preservatives has put human and animal health at risk as well as contaminating the environment owing to the toxicity of the synthetic chemicals to the non-target organisms, resistance/cross resistance, and non-biodegradable nature. Efforts are being made to replace these synthetic chemicals with alternatives which are safer and obviously less harmful to the environment (Zanie *et al*. 2008; Seyran *et al*. 2010; Yangui *et al*. 2010). Within this context is the utilisation of aromatic plants as natural sources of antifungal agents (Chang and Cheng 2002; Chang *et al*. 2001; Wang *et al*. 2005).

Coriander is a glabrous, aromatic, herbaceous annual herb belonging to the family Apiaceae (Duke *et al*., 2002). It is commonly known as *Dhaniya* in Hindi, *Dhanya* in Sanskrit and *Kotthamalli* in Tamil. It is native to the Mediterranean and Middle Eastern regions and has been known in Asian countries for thousands of years. The seeds of coriander are one of the most important spices in the world and are regularly used by the Indian cuisine (Bhandari and Gupta, 1991; Ravi, Prakash and Bhat, 2007). It is the most widely consumed popular ingredient in the world as a domestic spice, a traditional medicine, and a flavoring agent (Gupta, 2010). Coriander available throughout the year provides a fragrant flavor that is reminiscent of both citrus peel and sage. It's essential oil is also used in pharmaceutical recipes and as a fragrance in cosmetics (Al-Mofleh *et al*., 2006; Millam *et al*., 1997).The antimicrobial, antifungal, antioxidant and radical-scavenging properties of essential oils have been reported (Sokovic & Griensven, 2006). The use of coriander seed essential oil against wood decaying fungi has not been exploited yet.

MATERIALS AND METHODS

Plant material

Coriander seeds (500g) were crushed, powdered and dipped in distilled water overnight. The hydrodistillation of powdered material was carried out using Dean and Stark apparatus. The contents were refluxed for 10 hours. The essential oil layers were collected separately in a conical flask. The essential oil layers were partitioned using separatory funnel thrice using diethyl ether (3x100 ml). The ethyl ether layer was dried over sodium sulphate to remove traces of moisture present, if any Evaporation of ether yielded 0.18% essential oil which was stored in air-tight sealed glass vials covered with aluminium foil at 4°C for further use.

Chemical composition of coriander seed essential oil

Coriander seed essential oil was analyzed using GC-MS (QP2010 Plus, Shimadzu, Japan), equipped with a Rtx-5 MS capillary column (30.0 m x 0.20 mm i.d., 0.25 μm film thickness) for the separation of the components of coriander seed essential oil. The injector was maintained at 250 °C and operated in split injection mode with the split valve closed for 1 min. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50 °C for 2 min, raised to 180 °C at 3 °C/min, then to 280 °C at 10 °C/min. The interface temperature was 260 °C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 600 m/z. Data acquisition was started 3.0 min after injection. MS parameters used were; Ionization Voltage (EI) 70 eV, peak width 2 s, mass range 40–600 amu and detector voltage 1.5 V. Peak identification was carried out by comparison of the mass spectra with mass spectra data available on database of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries.

Cultures

Pure cultures of *G. lucidum*, *P. florida* and *A. alternata* were obtained from Department of Microbiology Laboratory and Department of Plant Pathology, PAU, Ludhiana (India). The isolates were maintained on Potato Dextrose Agar (PDA) at 4 ˚C.

Antifungal Assay

The antifungal activity against the test pathogens was determined by the poisoned food technique (Grover and Moore, 1962). In poisoned food technique, 20 ml of Potato Dextrose Agar (PDA) was poured into sterilized Petri dishes and measured amount of coriander seed essential oil was added to get the required concentrations 0.02, 0.04, 0.06, 0.08, 0.1, 0.12 g/100 ml sterile molten PDA. The test fungi were inoculated with 5 mm mycelial bits from 7-days-old cultures and the Petri dishes were wrapped with parafilm along the rim to check the release of the volatile components, inverted and incubated for 7 days at 25 ±2 ˚C. The radial growth of the mycelium was recorded and results were expressed as percentage mycelium growth inhibition by the formula:

$$\text{Mycelial growth inhibition (\%)} = \frac{\text{Mycelial growth in control- Mycelial growth in treatment}}{\text{Mycelial growth in control}} \times 100$$

RESULTS AND DISCUSSION

Coriander seed oil composition

The major constituents of the essential oil are shown in Table 1. Forty-four different components were detected constituting approximately 98% of the oil. The major components were linalool (75.2%), geraniol (5.24%), geranyl acetate (4.98%), camphor (1.76%), a-pinene (1.67), limonene (1.49%) etc. Linalool mainly contributes to the aromatic odour of the essential oil. The compositions of *C. sativum* significantly varied from the other studies reported earlier. Yield of essential oil was found to be less while linalool content was more than that reported from Pakistan and Bangladesh (Anwar *et al* 2011, Bhuiyan *et al* 2009).

Antifungal activity

The antifungal activity of *C. sativum* essential oil against wood decaying fungi has not been reported earlier. The antifungal activity of coriander seed oil was evaluated against *G. lucidum*, *P. florida* and *A. alternata* at various concentrations (Table 2). The coriander seed oil was not effective at the lowest concentration tested i.e. 0.2 mg/ml concentration against any tested fungi whereas at 0.4mg/ml concentration the coriander seed oil showed 17.5 and 31.11% inhibition against *G. lucidum* and *A. alternata* respectively, but no effect was observed against *P. florida*. At 0.5mg/ ml concentration of coriander oil showed 86.67% inhibition against *P. florida* while against *G. lucidum* and *A. alternata* inhibition was 32.25 and 42.22%. The coriander seed oil at 0.6 mg/ml concentration significantly reduced the colonial growth of tested fungi *G. lucidum* and *A. alternata* by 68.75 and 52.22% whereas *P. florida* was completely inhibited.Coriander seed essential oil was found to be more fungitoxic against *P. florida* than *G. lucidum* and *A. alternata.* Coriander seed essential oil is more effective against wood decaying fungi. More than 50% inhibition against all fungi was achieved at 0.6mg/ml. ED_{50} and ED_{90} values of coriander seed essential oil are presented in Table 3.

CONCLUSION

This study confirmed that coriander seed essential oil possessed *in vitro* antifungal potential against pathogenic fungi of trees. The study showed that the maximum activity was observed at 1.2 mg/ml concentration. Linalool, the major component (75%) of coriander seed essential oil, had been reported for various biological activities. Thus linalool may be responsible for its activity of essential oil against wood decaying fungi. Further study are required to identify the

bioactive principles responsible.These finding suggested that coriander seed oil can be exploited as an alternative source of natural fungicide for the management of wood decaying fungi.

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Table 1: GC-MS data of Coriander seed essential oil

Name	Retention Time	Area (%)	Name	Retention Time	Area (%)
α-Pinene	7.730	1.67	n-Undecanal	24.095	0.08
Camphene	8.267	0.10	4-vinyl-Guaiacol	24.385	0.20
β-Pinene	9.348	0.24	Myrtenyl acetate	24.899	0.05
Myrcene	9.966	0.22	Citronellyl acetate	26.111	0.09
p-Cymene	11.342	0.66	Neryl acetate	26.599	0.06
Limonene	11.516	1.45	Geranyl acetate	27.472	4.98
γ-Terpinene	12.854	0.49	Dodecanal	28.441	0.12
Linalool oxide	13.470	0.65	(E)-Caryophyllene	28.862	0.18
1-Octanol	13.776	0.21	Dodec-(2e)-enal	30.790	0.29
-	14.178	0.74	trans-2-Dodecen-1-ol	31.002	0.13
Linalool	15.300	75.02	2,6-Di-tert-butyl-4-methylphenol	32.696	0.09
Camphor	16.797	1.76	-	35.937	1.25
Borneol	17.747	0.86	-	37.557	0.04
1-Nonanol	18.047	0.09	Trans-tetradec-2-enal	38.685	0.10
Terpin-4-ol	18.259	0.24	4-Nonyl-phenol	40.290	0.04
α-Terpineol	18.875	0.63	(z)-9-Octadecenoic acid	41.954	0.07
(-)-Myrtenol	19.162	0.10	n-Tetradecanal	43.792	0.08
Decanal	19.574	0.12	Phytone	44.753	0.28
β-Citronellol	20.606	0.72	n-Texadecanoic acid	48.386	0.17
Geraniol	21.870	5.24	n-Eicosane	50.804	0.03
Geranial	22.505	0.18	(z)-9-Octadecenal	50.926	0.05
2-n-Octylfuran	23.508	0.11	Ethyl octadec-9-enoate	51.766	0.16

Table. 2: Percentage inhibition at different concentrations of coriander seed essential oil

Concentration (mg/ml)	Percentage Inhibition of Mycelial growth		
	G. lucidum	***P.florida***	***A. alternate***
0.2	0	0	0
0.4	17.5	0	31.11
0.5	32.5	86.67	42.22
0.6	68.75	100	52.22
0.8	83.75	100	61.11
1.0	100	100	78.85
1.2	100	100	100

Table 3: ED_{50} and ED_{90} values of coriander seed essential oil against tested fungi

Sr.no.	Test Fungus	ED_{50} (µg/ml)	ED_{90} (µg/ml)
1.	*G.lucidum*	0.548	0.876
2.	*P.lucidum*	0.458	0.525
3.	*A.alternata*	0.578	1.105

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RESEARCH ARTICLE

Flow – Injection Spectrophotometric Determination of Calcium using Bromopyrogallol Red (BPR) as a Color Agent

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ABSTRACT

This study investigates FI spectrophotometric determination of calcium using bromopyrogallol red has been developed. A calcium solution injected into KCl-NaOH buffer (pH 11.6) and then merged with the aqueous BPR (11.00 x 10^{-4} M) and continuously monitored sample. The linear range for the determination of Ca obtained under the optimum conditions was 1–10 mg Ca L^{-1}. The average sampling rate was 240 samples/hr. The precision is in between 1.7-2.6% RSD and the accuracy (average recovery) of Ca is 102%, indicating that the method is sensitive, precise, accurate, and rapid.The proposed procedure has been validated by using reference materials and comparing the results with the standard methods. The "t"-exp values are found to be less than the "t"-table, indicating that the present method has a good validity and then applied to blood serum, natural waters, milk, and yogurt.

Keywords : Calcium, Bromopyrogallol red (BPR), Flow injection (FI), Spectrophotometry, Blood serum analysis, Waters analysis, Milk and Yogurt analysis.

INTRODUCTION

Calcium compounds are of interest to several industries, such as pharmaceutical, cement, pulp and paper, metallurgy and mining [1]. Flow injection procedures for the determination of calcium have been reported and some other techniques have also incorporated FIA such as flame AAS, flame photometry, ICP-AES, ISE, fluorimetry and enthalpimetry [2-8]. Flow injection spectrophotometry has gained most attention owing to its simplicity. A number of color reagents has been employed for FIA determination of Ca which included complexon, chlorophosphonazoIII, o-cresolphthalein, 4-(2-pyridylazo)-resorcinol(PAR), arsenazo I, emodin, 3,3-bis(carboxymethyl)-o-cresolphthalein,

methylthymol-blue, alizarin red, and arsenazo III [9-20]. Some reported FIA procedures are based on chelatometric titration for calcium using EDTA and a color indicator such as calmagite and murexide [21,22,23]. None of the proposed reagents satisfies the requirements of FIA-photometric method and with limited applications.In the present work the developed photometric batch method assays of Ca using BPR is adapted to Flow injection direct spectrophotometric determination of Ca using BPR as a color agent. The proposed procedure was applied to the estimation of Ca in serum, milk, yogurt, and natural waters satisfactorily.

MATERIALS AND METHODS

Apparatus and flow manifold

A schematic diagram of the flow injection system for determination of Ca using BPR as a color agent is shown in Fig.(1). The manifold consists of a peristaltic pump (P) of the type (Desaga Heidel berg England) with six channels.Generally, peristaltic pumps driven by a constant speed motor and are capable of delivering remarkable reproducible volumes of liquid. The flow rate range of the pump is 0-10 ml min-1. A sample injection via a rotary valve (S) type (Rheodyne U.S.A.) with a sample loop of 80Ml capacity was used. The valve was made of PTFE with a good resistance against the corrosion of chemicals. Teflon tubes of 0.7 mm i.d., a mixing device (MD) consisting of a single-string glass beads in a column of glass tubing (3 mm i.d.), and a home-made (Y) piece (Perspex) to mix and connect the lines.A spectrophotometric detector (D) type 6300 visible spectrophotometer (JENWAY) with a flow-through cell was used (60Ml Hellma, QS, 1 cm). An automatic AC-voltage regulator (Stabic-Japan) as an associated electronics and a single pen xy/xy-yt recorder (R) type (SERVGOR 790, LEM) were used. pH measurement made 9420 digital pH meter with Philips PW.

Chemicals and reagents

Chemicals used are of the analytical grade, analar or the highest purity available. All glassware were rinsed with a solution of the tetra sodium salt of EDTA (0.001M) and then with double distilled water, just before use [23].

Preliminary Investigations

The reaction of Ca with BPR reagent at pH about 12 produces violet colored complex with a maximum absorption at 540 nm (Fig.2).The effects of various parameters related to the behavior of the color reaction in the FI technique have been investigated and suitable conditions are selected for the procedure. The proposed FIA system is applied to various samples. Each sample is injected three times and the average peak height in (mm) is presented.

RESULTS AND DISCUSSION

Chemical optimization

Order of addition

The effect of variations in FI manifold on the absorption peak height has been studied with different reaction manifolds. The best sensitivity and reproducibility of sample peaks, gained with FI manifold depicted in Fig (1). For subsequent experiments the sample introduced directly into the pH 12 buffer carrier stream and then was mixed with the reagent solution of BPR, using glass bead column as a mixing device.The effect of different parameters on the absorption peak height, using 5 ml/25 ml (4.99×10^{-4} M) BPR, 8 ppm Ca and pH-12 buffer was examined. The system was operated at flow rate of 3ml. min^{-1}, sample volume 80 μl and GBC of 5 cm length, at room temperature.

Effect of BPR reagent

The effect of BPR reagent concentration was investigated by measuring the peak heights recorded at 540 nm. The peak height increases with increasing BPR concentration up to 1.397×10^{-4} M. However, a 1.00×10^{-4} M BPR

concentration is recommended for the subsequent experiments because of good base-line stability and good reproducibility.

Effect of pH

The effect of pH was investigated by measuring the peak heights due to the Ca-BPR colored system at different pH values in the range of 10-12.5, using optimized BPR concentration. The best peak height was found at pH 11.6, which used as optimum pH in the system.

Physical optimization

Sample volume

Using the selected FI manifold, optimum concentration of BPR (1.00×10^{-4}M), pH 11.6, and 8 ppm Ca injected sample. Different sample volumes(loops)(20-140μl) are tested. Though a slight increase in sensitivity is observed with an increasing sample volume, a 80μL injecting sample provides good correlation in the calibration range of 0-10 ppm Ca. Higher volumes cause doublet peaks, possibly due to the fact that the reagent did not sufficiently disperse into the middle of the sample zone.

Effect of mixing devices

The glass bead column (GBC) mixing device provides better mixing of sample and reagent than mixing coils alone [2]. The effect of 5, 10, and 15cm GBC length was studied, the doublet peak profiles disappeared, 5cm GBC as mixing device was more suitable to be used for subsequent experiments, because of a shorter residence time and higher sensitivity.

Flow rate

Under the optimized conditions, the selected FI manifold is operated at different flow-rates (2-8ml.min^{-1}). It was found that 3ml.min^{-1} provides best sensitivity. So it was recommended as the optimum flow-rate for the subsequent experiments.The optimum working conditions as a result of above chemical and physical optimizations are given in Table 1.

Calibration Graph

A calibration graph was constructed under the optimum conditions, illustrated in Table 1. Using the FI manifold (Fig. 1), by injecting a series of Ca standard solutions in the linear range of 1-10 ppm Ca. The calibration plot and its statistical data are given in Fig. 3.

Precision and accuracy

The precision and accuracy for the determination of Ca by spectrophotometric FIA using BPR as a color agent was studied under the optimum conditions.Table 2 shows that the relative standard deviation (RSD) from five injection of Ca standard solutions containing (2, 4 and 8 ppm) concentration was in between 1.7-2.6%. The accuracy of the method was tested by the recoveries of Ca amount in the three injected concentrations. The results obtained in the Table 2, show that the mean recovery of Ca is 102%.

Interference

The seriously interfering ions in the batch methods (part A) has been re-investigated here using the optimum conditions described in the Table (1) solutions of 8 ppm Ca, containing varying concentrations of divers ions were injected. No remarkable increasing over the tolerance limit of the tested ions was observed over those obtained with the batch system.

Analytical applications

The FI spectrophotometric method which has been developed for determination of Ca, using BPR as a color agent, was applied to various samples satisfactorily. The determination of calcium was carried out by the present method in aqueous solution was applied to the analysis of calcium in various samples, with further dilution as needed for each sample, and the assay for Ca was conducted from the calibration graph by injecting samples under the optimized conditions, using the FIA manifold. The results obtained by the present FIA method and those obtained by AAS and standard EDTA titrimetric methods are given in the Table (3) indicating reasonable comparison.

Validity of the Method

In order to test the results for the existence of a systematic error for the present method the "t" test for assay of Ca in baby milk powder (Guicoze-2) was used [24]. The results of the present methods were compared with AAS method.The "t"-exp is 1.89. This value is less than 2.75 ("t"-test from table) at 95% confidence level for five degrees of freedom. This indicates that there is no systematic error, no significant difference between the two methods and that the present method has good validity.

CONCLUSION

The FIA spectrophotometric method which has been developed for calcium using a BPR as a color agent, allows fast determination at low operating cost and simple. The linear range for the determination of Ca obtained under the optimum conditions given in the Table (1) was 1–10 mg Ca L^{-1}. The average sampling rate was 240 samples / hr. The precision is in between 1.7-2.6% RSD and the accuracy (average recovery) of Ca is 102%, indicating that the method is sensitive, precise, accurate and rapid. The proposed FIA method can be applied satisfactorily for the assay of Ca in, serum, natural waters, milk, and yogurt. The result obtained is compared favorably with those obtained by AAS and standard EDTA titrimetric methods. The "t"-exp. Values were found to be less than the "t"-table, indicating that the present method has a good validity.

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Table 1: Optimum working conditions for spectrophotometric FIA system of Ca determination.

Parameters	Value
pH	11.6
BPR concentration	1.00 x 10^{-4}M
Wave length selected	540 nm
Sample volume	80μL
GBC length	5 cm
Flow rate	3ml. min^{-1}
Reaction tubing diameter	0.7mm i.d.
Temperature	25°C(room temp.)
Residence time	15 sec.

Table 2: Precision and accuracy of the method.

Injected calcium	2 ppm		4 ppm		8 ppm	
No.	h(mm)	Found	h(mm)	Found	h(mm)	Found
1	32	2.1	62	4.15	117	7.85
2	31	2.0	60	4.0	115	7.75
3	32	2.1	62	4.15	116	7.80
4	33	2.15	61	4.10	120	8.10
5	32	2.1	63	4.20	117	7.85
X	32	2.09	61.6	4.12	117	7.87
R%	104.5		103		98.4	
SD	0.055		0.076		0.135	
RSD %	2.6		1.8		1.7	

Table 3: Determination of Ca (ppm) in various samples using FIA method.

Sample	Present		EDTA		AAS	
	ppm Ca	D.F.	ppm Ca	D.F.	ppm Ca	D.F.
Tap water	64	10	65	-	65	10
Tigris-water	43	5	42.5	-	43	10
Guicoze-2	68.4	10	66	-	67.1	10
Ulker-milk	22.5	10	22.4	-	24.3	10
Yogurt(natural)	30.4	6	30	-	29.2	10
serum	90	20	89	-	93	10

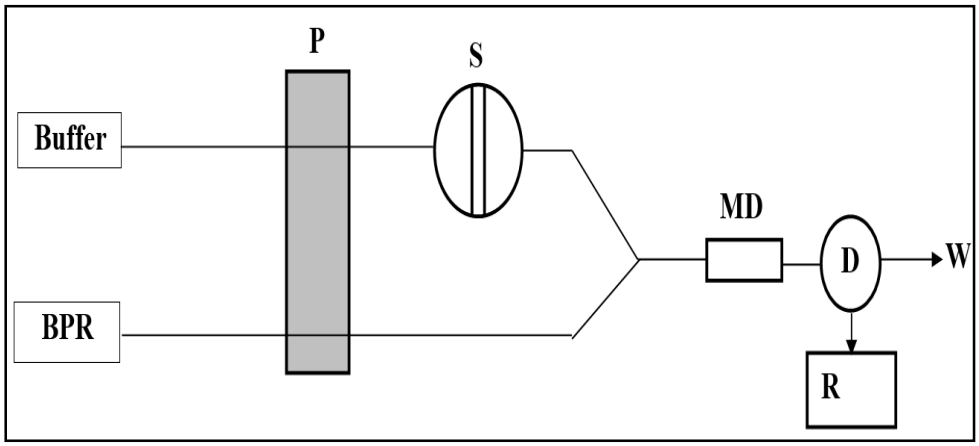

Fig. 1: Proposed schematic diagrams of the FI manifold for determination of calcium

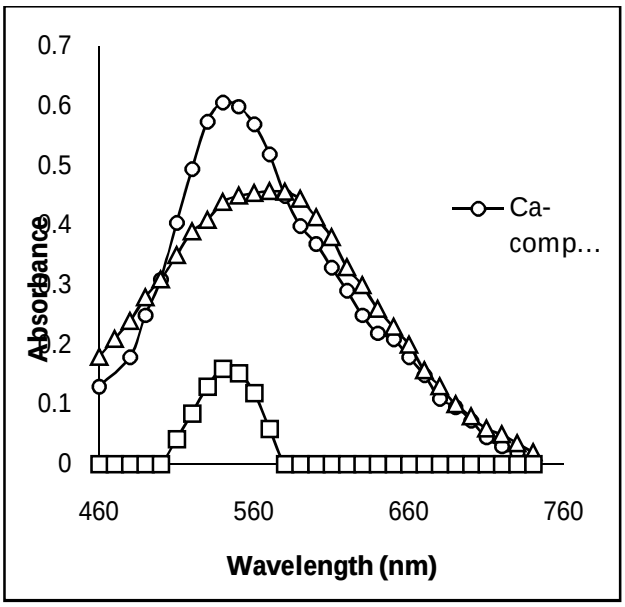

Fig. 2: Absorption spectra of 15 mg Ca/25 ml measured against blank, distilled water and blank against distilled water.

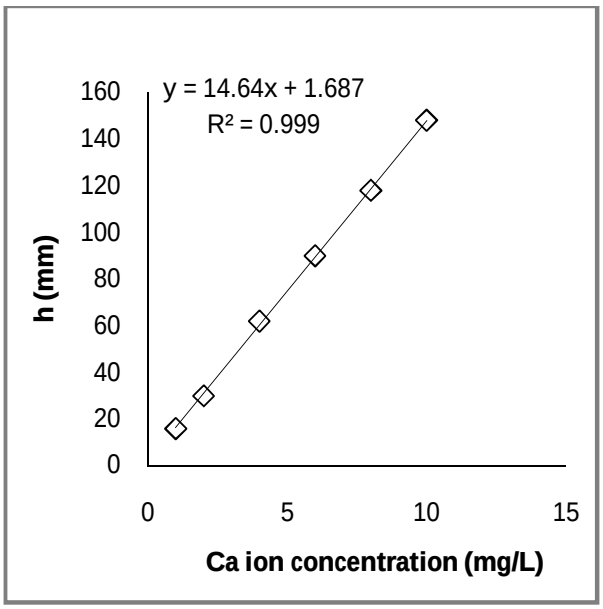

Fig. 3: FIA-calibration curve for the determination of calcium with BPR.

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RESEARCH ARTICLE

Assessment of Water Quality and Organic Pesticides in Gomti River Water at Lucknow, Uttar Pradesh

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ABSTRACT

Gomti river originate from Madhogang Tanda village in Pilibhit district, Uttar Pradesh. It covers several districts including Shahjahanpur, Kheri, Hardoi, Sitapur, Lucknow, Barabanki, and Sultanpur and finally merged with Ganga river at Jaunpur. Now a day's entire world is severely facing problems of air, water and soil pollution. Gomti river receive huge amount of untreated sewage; agricultural runoff with pesticides and fertilizer; street washout with oil, sediment and heavy metals. The present study is focused to evaluate physicochemical quality and pesticide concentration of Gomti water at Lucknow. Water samples were collected from five selected sampling sites *viz* Kukrail drainage (site-I), Gomti barrage (site-II), Central Drug Research Institute (site-III), Gaughat (site-IV) and Talkatora industrial area (site-V). Water samples were analyzed for pH, temperature, free carbon dioxide (CO_2), dissolved oxygen, biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, chloride and fluorides from January 2015 to December 2015. Most of the parameters were below the desirable limits of Bureau of Indian Standards (BIS) while dissolved oxygen and nitrates were comparatively higher than BIS. Sample collected from site-I and site-V were highest polluted while site-IV was least polluted. Except these parameters concentrations of pesticides (α-HCH, β-HCH, □-HCH, p,p′-DDT, p,p′-DDE, p,p′-DDD, α-Endosulfan, β-Endosulfan and Methyl parathion) were also analyzed.

Keywords : Gomti River, Water Pollution, Pesticides, Physicochemical Parameters.

INTRODUCTION

Gomti river is one of the many transcendental rivers in India. A massive amount of untreated sewage, industrial pollutant, agricultural runoff, pesticides, fertilizer and heavy metals are discharged in Gomti every year. Wastes are directly poured from distillery, oil, vegetable, carbon and dairy industries (Singh et al., 2005). Analysis of physicochemical parameter showed that the quality of water is getting toxic for flora and fauna (Preety Singh 2014). Discharge of organic wastes, sewage wastes, human excreta, municipal garbage and toxic discharge from factories increased day by day (Pramod et al., 2014). Besides these many pesticides of different chemical nature are mixed to river as agricultural runoff. These pesticides are detected in various environmental matrices; in water, soil and air. Among different class of pesticides organochlorine and organophosphorous are most common. Organochlorine pesticides resists biodegradation thus concentrated and produce a significant magnification at the end of the food chains. Several developed countries banned many organochlorine pesticides because toxicity to human beings and their ecosystem. Several neurological disorders and cancers are being reported due to contamination of pesticides in drinking waters in side by areas of Gomti river (Annual health report by CSIR-IITR, Lucknow, 2012).

Water pollution is a major global problem today. Several health related complications are being increased due to bad quality of water. Fishes has bioaccumulation of harmful pesticides and heavy metals which finally reached to human body. In India, it is estimated that more than 14,000 people die every day due to water pollution (Khare & Khare 2012). Therefore, water quality program is necessary for the protection of fresh water resources since drinking water quality is a critical issue at present and future (Singh et al., 2005). Hydroelectric dams changes ecology of aquatic life, both the upstream and downstream of river and adversely effects water quality. Poor water quality may cause disturbance to the natural ecosystem, affecting food chain and population of aquatic life and wildlife. Thus, it is crucial to keep the health of the river at an acceptable level. Increasing human populations and development of industries along river and coastal areas have increased pollutant inputs and deteriorate water quality of the surrounding areas (Jindal and Sharma 2011; Sanchez et al., 2007; Suratman et al., 2009).

Water quality index (WQI) is helpful in assessing suitability of river water for agriculture, aquaculture and domestic use. It relates a group of parameters to a common scale and combining them into a single number. WQI is one of the most effective tools to provide feedback on the quality of water to the policy makers and environmentalists (APHA 1999; Apipathi and Puttaiah 2006). It determines overall water quality status at a certain time and location. There are several water quality indexes developed to evaluate river water quality all over the world. These indexes use various numbers of water quality parameters. Meher et al used total 14 parameters such as pH, alkalinity, conductivity, turbidity, total dissolved solids (TDS), dissolved oxygen (DO) and other parameters for developing WQI for different sections of the Ganga river (Meher et al., 2015). Al-Shujairi proposed a WQI formula for seven water quality parameters (TDS, total hardness, pH, DO, biochemical oxygen demand (BOD), nitrate and phosphate to evaluate water quality in the Tigris and Euphrates rivers in Iraq (Al-Shujairi 2013). The present study is focused to evaluate physicochemical quality and pesticides concentration of Gomti water at Lucknow. Better WQI analysis in time to time will help in designing the policies to safeguards the quality of life. Hence regular assessment is needed in order to know the current scenario of water quality.

MATERIALS AND METHODS

Sampling

Five sampling sites were selected in Gomti river at Lucknow city. These are Kukrail drainage (site-I), Gomti barrage (site-II), Industrial waste water at CDRI (Site-III), Gaughat (site-IV) and Talkatora industrial area (site-V). Samples were collected at monthly intervals during the first week of each month from January to December 2015 between 9:00 AM to 11:00 AM. The water samples (3 replicates) were collected in clean two liter container. Analysis was done for determination of physicochemical parameters *viz* temperature, pH, dissolved oxygen, free CO_2, biochemical oxygen

demand (BOD), chemical oxygen demand (COD), nitrate, chlorides and fluorides as per the standard methods of American Public Health Association (APHA,1998).Pesticides Analysis was done by using liquid-liquid extraction method prescribed by APHA (1998). In this method n-hexane was used as solvent. One liter water sample was filtered and kept in 2 liter separating funnel. After adding n-hexane, separating funnel was shaken gently for proper mixing. Now separating funnel was kept for 15 minutes to separate two phases. The lower oily layer was collected in a small funnel having cotton and anhydrous sulfate on its opening to remove remaining water molecule. The above extraction process repeated three times. The extracts were combined, filtered and concentrated to 2ml. Finally extract was transferred into air tight GC vials for pesticides analysis. The pesticides were analyzed by gas chromatography-electron capture detector (GC-ECD) with Varian-6800. The condition were used are given in Table 1.

RESULTS AND DISCUSSION

Temperature, pH, Free CO_2 and Dissolved Oxygen

Results of the various physicochemical parameters of river Gomti at different sampling sites from January to December 2015 are summarized in Table 2 and 3. River water temperature depends on seasons, geographic locations, sampling time and temperature of the effluent entering into the water stream (Apipathy& Puttajah 2006). Water temperature directly or indirectly influences both biotic and abiotic part of aquatic system and their metabolic as well as physiological activity (Singh et al., 2014; Pramod et al 2014; Srivastava & Srivastava, 2011). In this study, temperature of Gomti water was recorded as 15.2 °C in January at site-V while in June it was 33.4 °C at site-III.The pH is a measure of the hydrogen ion concentration in water and indicates whether water is acidic or alkaline. The result showed no significant difference in pH values throughout the year at all the sampling sites. Gomti water was slightly alkaline and varies between pH 8.37 in June at site-V to pH 7.16 in November at site-IV.

The maximum pH in June at site-V is might be due to receipt of sewage and industrial effluents containing high amount of carbonates and bicarbonates. Additionally, immense evaporation of water in summer leads to rise in carbonate and bicarbonate concentration. Beside these, variation in pH during different season of the year are due to removal of CO_2 by photosynthesis, dilution of waste with fresh water, reduction in salinity and temperature and decomposition of organic matter (Shafi et al., 2013). CO_2 react with water to form carbonic acid which dissociates in carbonate and bicarbonate thus changes pH of water. Free CO_2 in Gomti water varied from 73.3 to 35.1 mg/L. Highest value (73.3mg/L) was reported in May while lowest value (35.1mg/L) was in December. The increase in CO_2 level during summer is because of decay, decomposition and mineralization of organic matter.Dissolved oxygen (DO) is an important parameter in water quality assessment and biological processes prevailing in water (Basavaraddi et al., 2012). Temperature significantly effects the characteristics of an aquatic system affecting the dissolved oxygen levels.

The average dissolved oxygen concentrations are always higher in low water temperatures since low water temperature promotes oxygen solubility (Rajendran & Mansiya 2015; Schmidt et al., 2016). Several ponds and lakes have low dissolved oxygen in summer as decomposition of organic matter depleting oxygen. This is the most common cause of fish kills, especially in summer when warm water holds less oxygen (Varsha et al., 2013; Serajuddin 2013). Maximum value of dissolved oxygen in water of Gomti river was recorded 12.28 mg/L at site-IV in December and minimum value was 4.51 mg/L at site-II in July 2015. Similar result was reported by Mishra and Prakash, in Gomti water at Sultanpur, Uttar Pradesh (Mishra 2014).

BOD and COD

BOD is an important parameter for surface water quality, show level of organic contamination. It is the amount of oxygen consumed by bacteria in the decomposition of organic material. High BOD illustrate lesser DO which is potentially unhealthy for the biodiversity of river. Elevated BOD demand resulted from high organic pollution since biodegradation of organic materials exerts oxygen tension in a water body (Abida & Harikrishna 2008). A huge amount of organic waste when added into the river augments the microbial activity of the aquatic system resulting

escalation of BOD and depletion of DO. The values of BOD were lesser during winter, increased gradually in summer. In this study, BOD ranges from 16.4 to 3.7 mg/L. Maximum BOD was 16.4 mg/L at site-I in June and minimum 3.7 mg/L at site-IV in December 2015. Similar observation was also reported in Sai river Uttar Pradesh (Vineeta & Girdhari 2015).COD is the amount of oxygen required for the chemical degradation of carbonaceous and nitrogenous contaminants in water. In surface water, if COD level is too high than the standard limit, the water can't used for drinking purposes and also harmful for aquatic organism (Singh 2006). The maximum value of COD was recorded 34.9 mg/L at site-I in the month of June while the minimum value was 14.2 mg/L at site-IV in December 2015. It means the COD values were lower during rainy season and it gradually increases in summer and reaches maximum in the first month of rainy season (Sikander 1986; Singh 2013; Mishra & Mishra 2008).

Nitrate, Chloride and Fluoride

Organic wastes are converted into ammonia by the action of microbes, which further oxidized into nitrite ($NO2^-$) and nitrate ($NO3^-$). Both nitrate and nitrite are part of nitrogen cycle. Since nitrite is easily oxidized to nitrate, the predominant product of decomposed domestic wastes is nitrate (Lalitha 2003). Nitrate should not be increased to desirable limit in drinking water because excess nitrate can cause methemoglobinemia or "blue baby" disease. Methhemoglobin loses its ability to carry molecular oxygen. It has been reported that nitrate may causing cancer in animals and human beings. It react with amino acids to form nitrosamines, which have been reported to cause cancer (Barrett et al., 1998).The maximum desirable value of nitrate in drinking water is 45mg/L(Adam 1980). The concentration of nitrate in Gomti water was varied from maximum 82.15mg/L at site-V to minimum 40.26 mg/L at site-IV.Chlorides are widely distributed in nature as salts of sodium (NaCl), potassium (KCl) and calcium ($CaCl_2$). Sodium chloride is extensively used in the production of industrial chemicals(Brooker 1984).Chlorides are essential elements of life but high levels in freshwater can harm aquatic organisms by interfering with osmoregulation. It alter reproduction rates, increased species mortality and changing the characteristics of the entire local ecosystem(Hart 1991).The recommended limit chloride is 250 mg/L.Excessive chlorides over 500 mg/mL give the water salty taste.

High concentration of chloride in river water indicates pollution due to organic waste. The chloride concentration in Gomti water was found in the range of 14.5 to 29.0 mg/L which is lesser than the permissible limit (250 mg/L) as per BIS standard.Fluoride is widely distributed in nature, exists in combination with other elements as fluoride compounds. It enters to human body through drinking water, river and groundwater. It is toxic when exceeds in drinking water beyond the maximum permissible limit of 1.5 ppm (WHO, 1984). High concentration of fluoride cause health problem not only in human beings but also in diverse species of domestic animals. Dental caries reduction, mottled enamel, osteoscleorosis, crippling fluorosis, kidney changes, musculoskeletal and nervous disorder are diseases may cause due to fluoride at different concentrations(Choubisa 2013; 2014). Very high concentrations of fluoride prevent the accumulation of chlorophyll 'a' and 'b' and photochlorophyll in plants may affect photosynthesis. Variation of fluoride ion at different sites in Gomti is varied from 0.12 to 0.54 mg/L. These values indicate less fluoride concentration as compared to BIS permissible limit. The highest concentration of fluoride was found at site-I.

Pesticides

The concentration of organochlorine and organophosphorous pesticides (HCH-isomers, DDT and its metabolites, Endosulfan-isomers and methyl parathion) in water of Gomti river are summarized in Table 3 and 4. Among different isomers, HCH and α-HCH was extensively distributed with concentration ranging from 16.5 to 120.0 ng/L. However, β-HCH (684.3 to 1369.0 ng/L) was comparatively higher than other isomers. □-HCH ranges from 36.8 to 283.7 ng/L. There was no seasonal variation in the distribution of pesticides. The wide distribution of α-HCH is because of tendency of α-HCH for long distance transport while higher level of β-HCH associated with resistance to environmental degradation (Willet et al., 1998). DDT is an organochlorine insecticide almost insoluble in water. The concentration of p,p′-DDT, p,p′-DDE and p,p′-DDD was ranged from 27.4 to 725.4; 27.0 to 364.8 and 2.4 to 104.3 ng/L, respectively. These compounds have higher affinity to the particulate thus river and marine sediments are thought to

be the major sink for them (Doong et al., 2002). DDT and its metabolites persists very long time in the environmental due to slow degradation (Kim et al., 2007, Singh et al., 2012, David et al., 2011, Harriet et al 2012). Endosulfan is one of the most toxic organochlorine pesticides causing many fatal poisoning. It is a xenoestrogen an endocrine disruptor leads to reproductive and developmental damage in animals and humans. It is highly toxic for aquatic organisms and bio-accumulated in fishes.

The solubility of endosulfan is 0.3 mg/L with a half-life of five weeks in water but β-isomer has 150 days in neutral condition. Though, endosulfan is banned in many countries but extensively used in India with different trade name such as Endocel excel, Endofil 45, Endum. In this study, concentration of α-endosulfan was observed 13.5 to 456.1 ng/L and β-endosulfan from 26.8 to 316.8 ng/L. Concentration of endosulfan was maximum at site-V due to extensive use of these pesticides in agriculture as well as discharge of industrial effluents. These findings are supported by several previous studies (Singh et al., 2012; Haldar et al., 1987). Methyl parathion is an organophosphorous pesticides, used for agriculture and pest control purposes. It has a solubility of 24 mg/L with a half-life of 10 days to two months. This pesticide was absent in Gomti river water during entire study. This finding was supported by Singh et al while measuring pesticide concentration in water and sediment of river Ganga at selected sites in middle Ganga plain (Singh et al., 2012).

CONCLUSION

Water pollution is not only an aesthetic problem but it is a serious economic and public health issue. So periodical monitoring of the water quality is required to assess the condition of surface water. In the present study, we observed some physicochemical parameters are acceptable level according to BIS guideline while others display notable increase from the desirable limit. The water quality data obtained from this study revealed that Gomti river found highly polluted due to discharge of domestic and industrial waste and not safe for aquatic flora and fauna. To improve quality of Gomti water, immediate action must be taken for better management. Quality of river water can be restored either by considerable decrease in pollution load from incoming drains or maintaining a substantial flow of water in the river. Artificial aeration and flow augmentation must be incorporated to achieve the standards.

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Table 1: Column specification and operating condition for gas chromatography

Detector	Electron Capture Detector (ECD)
Column specification	DB-5 column of 30m length, 0.25mm inner diameter, film thickness of 0.25µm
Injection port temperature	260°C
Detector temperature	300°C
Column programming	180°C hold for 2min@3°C/min 210°C hold for 2min@30°C/min 260°C hold for 5 min Total retention time 20.33 min
Carrier gas	Helium
Split ratio	9:1
Carrier gas flow rate	2 ml/min
Back up flow, back up gas nitrogen	30 ml/min
Injection volume	2µl
	Total volume of sample water: 2ml

Table 2: Monthly variation in physicochemical parameters of Gomti water at Lucknow from January 2015 to December 2015

Parameters	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
1.pH												
Site-I	7.82	7.81	7.85	7.87	8.10	7.18	7.19	7.54	7.80	7.55	7.46	7.65
Site-II	7.77	7.47	7.93	7.94	7.99	7.86	7.26	7.84	7.36	7.66	7.32	7.54
Site-III	7.64	7.77	7.78	7.43	7.31	7.55	7.34	7.56	7.32	7.38	7.24	7.38
Site-IV	7.56	7.55	8.10	7.32	7.48	7.43	7.18	8.14	7.86	7.75	7.16	7.87
Site-V	7.34	7.12	7.32	7.79	7.89	8.37	7.24	7.36	7.64	7.22	7.42	7.45
2.Temperature(°C)												
Site-I	18.2	21.2	24.1	27.9	30.0	32.2	31.5	28.6	27.4	27.1	26.5	19.2
Site-II	17.6	20.4	23.6	28.2	31.2	31.9	29.3	29.1	27.7	27.4	27.2	18.9
Site-III	16.8	20.0	22.8	29.6	31.8	33.4	31.0	28.8	28.4	23.2	24.6	16.7
Site-IV	15.8	21.5	23.9	28.5	29.8	31.7	31.5	31.2	30.2	28.8	23.3	17.2
Site-V	15.2	20.6	22.4	26.7	31.6	32.8	31.6	27.8	28.7	24.6	21.1	16.4
3. Free CO_2 (mg/L)												
Site-I	53.1	58.2	62.8	67.1	68.3	57.4	55.8	52.6	50.9	51.7	50.9	51.4
Site-II	46.3	47.5	48.1	51.9	53.8	51.6	47.0	44.2	43.6	42.4	43.5	42.6
Site-III	47.5	49.0	51.4	52.1	54.3	55.8	53.2	49.0	45.9	42.1	43.2	44.9
Site-IV	36.7	39.0	42.5	47.3	48.2	46.7	44.3	40.6	38.5	35.6	34.8	35.1
Site-V	55.2	59.2	63.7	68.6	73.3	71.8	67.4	58.3	53.9	47.8	49.7	52.2
4. DO (mg/L)												
Site-I	6.9	7.2	6.1	9.2	8.4	7.2	4.5	5.3	4.8	5.5	5.6	6.5
Site-II	10.4	11.9	9.7	8.1	7.5	6.2	9.4	9.8	9.1	10.4	10.5	11.2
Site-III	10.5	9.7	7.2	6.6	6.3	8.5	10.8	9.3	9.8	10.4	10.1	11.5
Site-IV	11.5	10.1	8.8	7.9	8.1	8.3	9.2	9.9	10.5	10.7	11.2	12.8
Site-V	7.67	6.98	5.44	6.27	5.62	4.66	5.36	6.57	7.14	8.80	8.25	7.52

Table 3: Monthly variation in physicochemical parameters of Gomti water at Lucknow from January 2015 to December 2015

Parameters	Jan	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
5. BOD (mg/L)												
Site-I	14.5	14.6	15.3	15.5	15.9	16.4	15.5	15.2	14.6	13.5	12.7	11.3
Site-II	9.2	9.84	10.6	10.8	11.2	12.0	11.9	10.8	10.3	9.6	9.4	8.5
Site-III	8.7	10.5	11.1	11.5	11.6	12	12.6	11.5	11.1	10.5	9.9	9.2
Site-IV	5.6	5.8	5.7	6.2	6.2	6.5	5.9	5.2	4.7	4.4	4.2	3.7
Site-V	14.6	14.7	15.2	15.4	15.8	16.2	14.8	13.7	12.8	11.6	10.6	9.2
6.COD (mg/L)												
Site-I	24.6	25.2	25.6	32.7	34.3	34.9	33.4	31.5	28.3	25.7	22.8	20.4
Site-II	19.5	20.6	21.1	22.4	23	23.4	22.7	20.9	19.4	18.2	17.6	15.6

Site-III	21.4	22.8	23	22.3	24.5	26.7	25.8	24.4	22.5	19.7	18.3	16.3
Site-IV	17.8	18.5	19.4	20.1	21.2	23.2	21	20.6	19.7	18.4	16.7	14.2
Site-V	28.7	30.9	31.3	31.6	33.8	34.6	33.7	31.6	31.9	30.6	28.2	24.6
7.Nitrate (mg/L)												
Site-I	66.2	66.8	69.6	71.4	73.5	72.5	70.7	70.2	69.6	66.5	61.2	63.8
Site-II	64.5	62.5	55.9	66.2	67.6	68.8	66.2	62.5	60.1	62.5	59.8	62.5
Site-III	77.8	68.2	72.5	70.8	70.5	73.0	71.9	68.2	66.8	64.5	66.7	68.7
Site-IV	45.7	45.1	46.3	46.8	47.9	47.6	45.6	43.5	40.9	40.2	42.1	43.8
Site-V	76.4	74.1	75.5	77.8	80.6	82.1	80.1	79.3	80.0	78.1	76.1	77.5
8.Chloride (mg/L)												
Site-I	18.3	18.9	21.3	22.7	24.2	28.9	27.4	25.8	23.4	21.2	20.4	18.1
Site-II	15.8	13.4	14.5	16.3	18.7	21.7	19.9	19.3	17.8	18.3	17.8	17.3
Site-III	18.9	20.2	19.8	20.1	20.6	22.3	21.6	20.4	18.3	16.5	16.2	15.8
Site-IV	14.6	15.6	15.9	16.6	16.3	16.4	15.8	16.6	17.5	17.9	16.8	16.4
Site-V	19.2	21.0	24.7	23.5	24.5	29.0	27.4	24.2	22.9	20.7	19.5	18.7
9. Fluoride (mg/L)												
Site-I	0.43	0.19	0.23	0.16	0.13	0.25	0.34	0.54	0.32	0.28	0.15	0.57
Site-II	0.24	0.12	0.18	0.13	0.43	0.26	0.21	0.16	0.19	0.43	0.32	0.29
Site-III	0.20	0.18	0.15	0.16	0.23	0.19	0.22	0.16	0.17	0.23	0.21	0.13
Site-IV	0.19	0.16	0.13	0.25	0.17	0.15	0.14	0.16	0.20	0.18	0.12	0.15
Site-V	0.23	0.19	0.20	0.43	0.32	0.18	0.14	0.22	0.16	0.13	0.15	0.20

Table 4: Concentration of pesticides in Gomti water at various sites in the month of January 2015

Pesticides and its metabolites	Concentration of pesticides in water (ng/L)				
	Site-I	Site-II	Site-III	Site-IV	Site-V
α-HCH	16.5	38.0	57.2	32.4	78.0
β-HCH	1050.0	1005.0	754.0	1250.0	953.6
ϒ-HCH	36.8	283.7	65.7	57.3	48.4
p,p′-DDT	BDL	BDL	27.4	36.9	56.0
p,p′-DDE	BDL	BDL	50.0	ND	2.4
p,p′-DDD	BDL	57.6	BDL	ND	BDL
α-Endosulfan	56.0	230.2	140.7	BDL	140.6
β-Endosulfan	120.2	BDL	320.0	ND	207.3
Methyl parathion	ND	ND	ND	ND	ND

ND = Not detected, BDL = below Detectable Limit

Table 5: Concentration of pesticides in Gomti water at various sites in the month of June 2015

Pesticides and its metabolites	Concentration of pesticides in water (ng/L)				
	Site-I	**Site-II**	**Site-III**	**Site-IV**	**Site-V**
α-HCH	120.0	93.6	62.0	43.7	96.8
β-HCH	1230.0	1320.0	684.3	1369.0	1004.2
Υ-HCH	58.6	55.0	68.3	42.9	38.4
p,p′-DDT	56.8	540.1	125.6	284.4	725.4
p,p′-DDE	104.3	BDL	193.3	106.2	214.7
p,p′-DDD	27.0	56.9	105.7	BDL	364.8
α-Endosulfan	13.5	34.7	152.6	15.2	456.1
β-Endosulfan	26.8	36.3	270.4	BDL	316.8
Methyl parathion	ND	ND	ND	ND	ND

ND = Not detected, BDL = below Detectable Limit

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RESEARCH ARTICLE

Studies on Extraction of Juice from Noni Fruits (*Morinda citrifolia* Linn.)

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ABSTRACT

Noni (*Morinda citrifolia* Linn.) has been used in folk remedies by Polynesians for over 2000 years, and is reported to have a broad range of therapeutic effects. The noni juice was extracted by hot and cold techniques. In the hot process, noni fruits were steam blanched for 2,4,6,8 and 10 minutes respectively and the juice was extracted. In the cold process, noni fruits were frozen for 6,12,18,24 and 30 hours respectively, thawed and the juice was extracted. The percentage recovery of juice yield and physico-chemical properties were analysed for the treatments carried out the extracted juice. Results showed that, hot processed juice samples showed a significant ($p<0.05$) change in juice yield, pH, acidity, total soluble solids (TSS) and colour values. In the cold process method, the freezing for 24 hours and thawing the noni fruit sample was found to be the best in terms of juice yield (46.935%), colour values (L* 95.93, a* -0.83 and b*-19.40) and the chemical parameters pH, TSS and acidity (3.67, 13.15°Brix and 1.664 %) respectively. Based on the juice yield and substantial physico-chemical properties of noni products, the 24 hours freezed and thawed treated noni juice was recommended for further study.

Keywords : Noni fruits, Extraction, Juice yield, Physico-chemical characteristics.

INTRODUCTION

Noni is called for Indian mulberry (*Morinda citrifolia* Linn.). It originates from Southeast Asia such as Indonesia and Australia [1]. In 2002, noni juice was accepted in the European Union as a novel food [2].There are 160 phytochemical compounds have been identified in the noni plant the majority of the micro-nutrients are phenolic compounds, organic acids and alkaloids [3].Particularly, the fruit juice from noni is in high demand in alternative medicine for different kinds of illnesses such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems, and drug addiction [4][3].Mostly noni is consumed as extracts from its fruits although leaves, flowers, bark and roots were also used [5][6].The use of concentrates of this juice, preserved juice drinks, encapsulated freeze-dried fruit juice, concentrated extracts, powders, tinctures, and fruit leather form has become massive due to the medicinal properties attributed to noni, such as being anti-microbial, anti-carcinogenic, analgesic and anti-inflammatory [7].

Many of these noni products are made with ripe or sub ripe fruit that is processed without decaying or adding water. The fruit may be chipped and dried in commercial farming operations for later rehydration and juice extraction in distant factories [6].For reasons that noni is so much valued, uses and demand of noni is not only high in its producing countries but also in other countries such as United States, Japan, India and Europe [7].Commercially noni juice was traditionally made by fermentation of ripened noni fruits in sealed containers for 2 months at ambient temperature. Some noni juice was made by boiling of noni fruits for hours and many Pacific islanders use fermentation to make noni juice at home by placing noni fruits in jars in outdoors under the sun for months [1]. In these processing, storage, light, temperature and oxygen can promote the undesirable chemical reactions that can reduce the medicinal components of the juice was obtained by gradually seep out from fermented noni fruits [9].

The aim of this study was to investigate the suitable technique for the extraction of noni juice to meet the increasing demand and improve the nutrient composition of noni juice.

MATERIALS AND METHODS

Fresh noni fruits used were obtained from Horticultural College and Research Institute, Periyakulam, Tamil Nadu. The fruits were selected at maturity stage 4 (hard white) [7].The fruits were allowed for ripen at room temperature (30±2ºC) for 2 days under closed condition before treatments. The weighed ripened fruits were washed using tap water and subjected to hot process technique, where on steam blanching (B) was done at 100ºC for 2, 4, 6, 8 and 10 min respectively, and then cooled at room condition for 5 min. Another technique was cold process method, the washed ripened noni fruits were kept for freezing treatment (F) at -18ºC for 6, 12, 18, 24, 30 hours respectively and thawed (T) at room temperature for before evaluation. After pretreatments the juice was extracted by pressing and passing in nylon net. The sample was analyzed for percentage recovery of juice yield, colour values, pH, TSS and total titratable acidity.

Percentage recovery of juice yield

Juice yield, J_Y in %, was calculated as the ratio of the weight of extracted juice to the total weight extracted juice and the residual products after extraction [10].

$$Jy = \frac{100Q_P}{Q_P+Q_R}$$

Where Q_P and Q_R is weight of juice extracted and residual product respectively in g.

Colour Values

The color values of samples were measured using a Lovi bond tinto meter (RT 100). The instrument was calibrated by placing black and white standard plates. The deviation of the colour of the samples was observed with the reference on distilled water in the computed interface. Results were expressed in the L*a*b* scale with an accuracy of 0.1%, where L* is the lightness, a* represents the green-red axis (redness) and b* the blue-yellow axis (yellowness), as recommended by the International Commission on Illumination in1976.

pH value

The pH value was measured using a pH meter (OAKLON pH 700, EUTECH Instruments, Singapore). The pH meter was calibrated using pH 4 and pH 7 buffers. Measurement of pH value was done at room temperature using 10 ml of sample.

Total Soluble Solids (TSS)

Total soluble solid was found out by using a hand refractometer on 0 to 45º Brix (Erma, Japan). A drop of extracted sample was kept on the hand refractometer and the Brix was noted (ºBrix).

Total Titratable Acidity

Total titratable acidity of the extracted sample was determined according to the method by Ranganna [11]. The 5ml of sample was mixed with distilled water made up to 50 ml and filtered. Known aliquots were titrated against 0.1N NaOH using phenolphthalein as indicator. The total titratable acidity was expressed as percentage citric acid.

$$\% \text{ acid} = \frac{V \times 64g \times N \times V_M \times 100}{V_S \times W \times 1000}$$

Where V, N, V_M, W and Vs are mole of 0.1 N NaOH used, normality, quantity of volume made up, weight of sample and volume of sample used, respectively.

Statistical analysis

For each treatment, three replications were performed. Data were analyzed by ANOVA and FCRD using statistical software (SAS version 9.2). Differences were reported as significant at $p<0.05$.

RESULTS AND DISCUSSION

Percentage Recovery of Juice Yield

Several studies have reported on the use of heat processing in the preparation of noni extract such as boiling [9]. Results (Table 1) showed that the extraction percentage of juice yield significantly ($p<0.05$) changed in hot and cold process techniques after 24 hours F/T and 4 min B process has produced highest percentage of juice yield for 46.935 and 46.030 % respectively. This result was highly significant ($p<0.05$) differed from control and 2 min B treated sample. The percentage of juice yield on 6, 12, 18 and 30 hours by F/T process showed the value was 42.895, 41.800, 44.740 and 41.550% respectively, significant difference than control and 10 min B extracted samples and it had a low significant change at 24 hours F/T and 4 min B treated samples. Physical changes can affect the production of juice extraction in frozen fruits due to stresses in cell volume, mechanical damage and freeze cracking [12]. The 4 min B extracted juice was significant ($p<0.05$) with other process on control, 2, 8 and 10 min B treated sample, juice and yield was 39.170, 35.320, 43.115 and 41.240 % respectively.

However heat treatment affects the physico-chemical properties of the extracted juice. In processing, the plant cell wall and cell membranes are intact and in functional form, they provide a barrier that is permeable only to certain small molecules, including water. If this barrier is somehow damaged, molecular movements through the barrier

become much easier, the most of this damage caused by heat treatment, such as might be applied in blanching [12]. Higher heating temperature has affected the significant reduction of clarity, viscosity and total polyphenol content of juice during processing [13].

Colour Values

Changes of colour, representative of important darkening or browning of extracted samples, was significant on hot and cold processing samples (Table 1), as a consequence of control. In the case of hot process, the lightness, yellowness significantly ($p<0.05$) decreased, and the redness increased after each blanching treatments. L*, a* and b* values were significantly changed in control to 10 min B extracted samples resulted for 91.29 to 82.23, -0.835 to -0.650 and -18.855 to -13.825 respectively. The oxidized chemical compounds formed in hot processing. Thus, the chemical changes would affect the colour variation due to thermal degradation, maillard reactions or oxidation induced by heating [14] [15]. In the case of cold process, extracted samples have increased significant change lightness, from 91.29 to 95.39 and yellowness, from -18.855 to -19.425 and decreased significant ($p<0.05$) changes occurred in redness from -0.835 to -0.832 on 30 hour F/T extracted sample.

pH Values

Figure 1 shows the effect of extraction process on pH value. The juice extracted by cold process sample had showed a non-significant difference between control and hot process samples pH value. Extending the cold treatment beyond 30 hours F/T did not result any significant difference in pH between control and other cold treatments. On 10 min B sample had a pH of 3.515, which showed a significant ($p<0.05$) reduction in pH compared to 6 and 8 min of B juice that accounted for 3.555 and 3.545 respectively. Heat generation during blanching might have contributed to the reduction of pH value. During fruit juice processing, changes and/or losses of certain compounds are likely to occur. The decreased pH may have been caused by chemical changes due to thermal degradation, Maillard reactions or oxidation induced by heating [14] [15]. pH value for 3.675 in control sample showed a significantly ($p<0.05$) higher pH compared to hot extracted juice did not show any significant difference when compared to cold pressed extracted juices for 6, 12, 18, 24 and 30 hours F/T process. Whatever the fruit and the freezing protocols, pH and total acidity did not make it possible to discriminate the fresh state from the F/T state [16].

Total Soluble Solids (TSS)

The effect of hot and cold processing on TSS content in the samples are shown in Figure 2. The TSS of the control sample was 13.0ºBrix. The TSS level, increased significantly ($p<0.05$) in all hot, 18, 24 and 30 hour F/T treated samples and did not change in 6 and 12 hours F/T treated samples. A higher maturity of the apples can significantly increase on soluble solids variations in the effect of freezing protocol [16]. During 10 min B extracted sample was having a TSS level for 14.2ºBrix, this sample have a highly significant ($p<0.05$) from all other treatments and control samples. In the hot processing samples, TSS was significantly ($p<0.05$) increases for increasing the blanching time of 2, 4, 6 and 8 min and accounted for 13.2, 13.3, 13.7 and 13.9ºBrix respectively. The TSS continually increased due to water loss on continuous heating, the solute concentration increase that leads to increase in TSS [17]. The probable reasons for increase in TSS can be the conversion of organic acids to sugars through gluconeogenesis [18].

Total Titratable Acidity

Titratable acidity values for the all treated samples are given in Figure 3. The result showed that, on 24 and 30 hours F/T extracted sample had similar acid content (1.664 %) and statistically no significant difference was observed with control and lower significant ($p<0.05$) changes on other cold processing samples. There was not much change occurred for total acidity in apples and mango during after freezing [16]. In hot process, the samples having acidity were decreased from 1.28 to 0.896 % in 2 to 10 min B time. On 2 min B extracted sample presented for 1.28 % acidity, which showed a significant ($p<0.05$) reduction in further hot processed samples on 4, 6, 8 and 10 min B resulted for 1.184, 1.088, 0.992 and 0.896 % correspondingly. At high temperature, the acidity trend to decrease in certain orange

cultivars due to the organic acids are converted to sugars or utilized for energy synthesis, the titratable acidity decreases [19].

CONCLUSION

Among the various extraction techniques experimented on noni juice, the control sample had a low percentage of juice yield compared to other processing methods. In hot processing, samples has a significant effect on physico-chemical properties, that resulted in a highly significant ($p<0.05$) change in percentage recovery of juice yield, pH, acidity, TSS and colour values with control and cold processing samples. Percentage recovery of juice yield (46.935%), colour values (L* 95.93, a* -0.83 and b*-19.40) and the chemical parameters pH, TSS and acidity (3.67, 13.15°Brix and 1.664 %) respectively on 24 hours F/T extracted juice has an extremely significant with control, hot and other cold processing samples. For maximum potential health benefits and the production of noni products to consumers, extraction of noni juice by cold processing on 24 hours F/T is strongly recommended.

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Table 1. Percentage Recovery of Juice Yield and Colour Values of Noni Juice from Different Processing Techniques

Extracted noni juice		Recovery of juice yield	Colour values		
		%	Lightness (L*)	Redness (a*)	Yellowness (b*)
Control		39.175*	91.29±2.49	-0.835±0.132	-18.855±0.921
Hot process	2 min B	35.320*	86.77±1.68	-0.780±0.119	-16.955±0.824
	4 min B	46.030**	85.94±1.92	-0.754±0.101	-15.312±0.836
	6 min B	44.270**	85.06±1.75	-0.710±0.121	-14.835±0.798
	8 min B	43.115	83.03±2.08	-0.672±0.105	-13.065±0.761
	10 min B	41.240	82.23±1.15	-0.650±0.098	-13.825±0.771
Cold process	6 hour F/T	42.895	92.49±1.32	-0.725±0.182	-19.155±0.931
	12 hour F/T	41.800	93.85±1.42	-0.694±0.139	-19.218±0.842
	18 hour F/T	44.740**	94.86±1.54	-0.781±0.111	-20.442±0.855
	24 hour F/T	46.935**	95.93±1.32	-0.832±0.154	-19.438±0.879
	30 hour F/T	41.550	95.39±1.71	-0.837±0.161	-19.425±0.854

*significant ($p<0.05$) B-Blanching
**Highly significant ($p<0.05$) F/T- Freezing and thawing

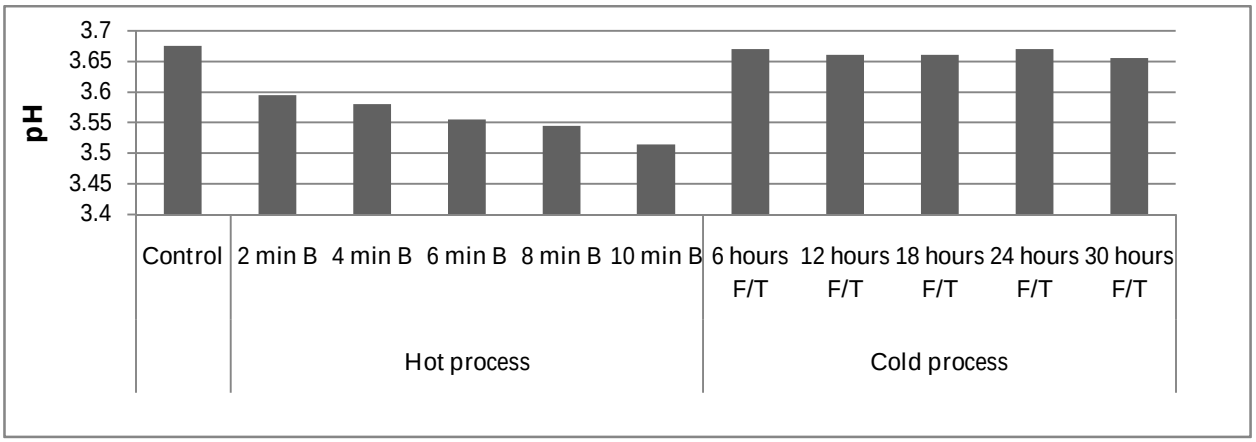

Figure 1.pH of Extracted Noni Juice
B-Blanching F/T- Freezing and thawing

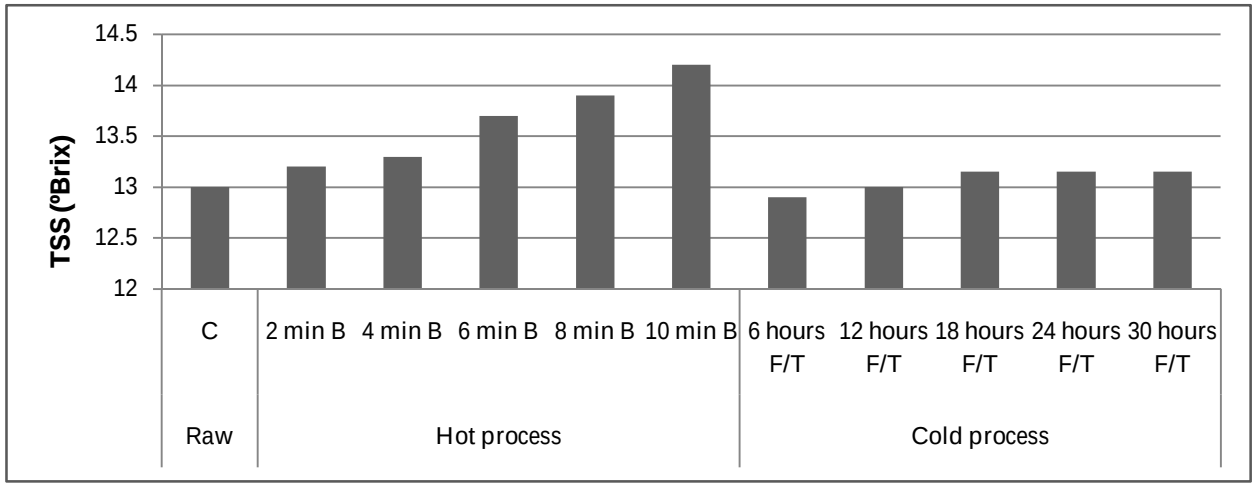

Figure 2.TSS (ºBrix) Content of Extracted Noni Juice

B-Blanching F/T- Freezing and thawing

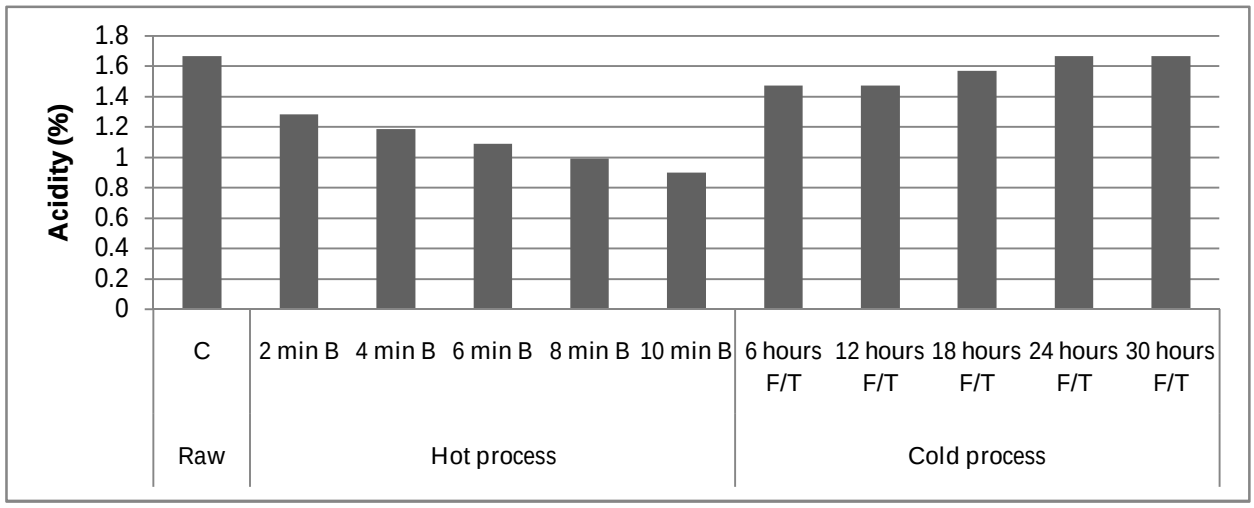

Figure 3.Total Titratable Acidity (%) Content of Extracted Noni Juice

B-Blanching F/T- Freezing and thawing

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RESEARCH ARTICLE

Use Geo Accumulation Index and Enrichment Factor in Assessing Pollution in Iraqi Tidal Flats of Some Heavy Metals

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ABSTRACT

Assessment of heavy metals as toxic pollutants has significant importance in environmental pollution studies. Surficial sediments of water resources have a high potential in releasing heavy metals to the upper water environment, hence sediment analysis presents guidelines to the establishment for monitoring the environmental systems. In this study, total concentrations of four heavy metals (Cd, Cu, Fe and Pb) were investigated along different sites of tidal flats in Basra South Iraq using Atomic absorption for analysis. Geo accumulation index (Igeo) and Enrichment Factor (EF) were computed and compared in different sites in this study too. The range of the concentration in the sediments areas as follows :(Cd: 7.05-15.98 mg/kg), (Cu: 21.07-109.2 mg/kg), (Fe: 1103.32-1147.38 mg/kg) and (Pb: 31.31-125.24 mg/kg). Total concentration of metals in sediment samples found to be in this order: Fe>Pb>Cu> Cd. The guidelines results show that the coastal is high to very high degree of Cd contamination on the other hand unpolluted to moderately polluted degree of Pb but unpolluted for the other studied metals.

Keywords : Heavy Metals, Pollution, Igeo., EF., Iraqi Tidal Flats.

INTRODUCTION

Pollution of the natural environment by heavy metals is a worldwide problem as these metals are indestructible and have toxic effects on living organisms when they exceed a certain concentration limit [MacFarlane and Burchett

2000]. The impact of anthropogenic alarm is most strongly felt by estuarine and coastal environments adjacent to bans areas [Nouri et al., 2008].Sediments are a mixture of several components including different mineral species and organic debris. It represents one of the final sinks for heavy metals discharged into the environment [Hassan,2007,Hassan, et al.,2010, Bettinetti et al., 2003, Hollert et al., 2003].Over the last decades, the study of assessment sediment has shown to be an excellent tool for establishing the effects of anthropogenic and natural processes on depositional environments [Vinodhini and Narayanan, 2008, Nadia, 2009].The assessment of sediment enrichment with elements can be carried out in many ways. The most common ones are the index of geo-accumulation index (Igeo).

Concentrations of metals in sediment of Iraqi costal have been documented by [Hassan, 2007, Al-Sabah, 2007, Al-jaberi, 2013, Al-Kuziea, 2015, Abdulnabi et al.,2016]. Al-Jaberi and Al-Dabbas (2014) shows that the Igeo in the studied of heavy metals have relatively values of (class 0 and 1) in the studied sites reflect unpolluted to slightly polluted with Cd and Pb. The indication of slightly to moderately pollution of sediments in the studied area may be as a result of anthropogenic activities, oil spilling and daily toxicity wastes that flow from the main rivers in Basra city[Abdulnabi,2016].In recent years, human impacts on the environment have been leading to eutrophication in coastal and marine areas, and interaction between human activities and natural force has had a major impact on the entire ecosystem. In this work Cd, Cu, Fe and Pb, total concentrations were determined for the surface sediment of 16 stations of Iraqi tidal flats, to do a field survey and a database on the concentrations of elements in these areas, which is not studied very much. Index of geo-accumulation and Enrichment factor was calculated to assess sediment contamination and determine if the concentrations of metals represent background levels for the North West of the Arabian Gulf.

MATERIALS AND METHODS

Samples were taken from 16 typical sections of the Surface sediment sample from tidal flats at the depth of 20 -30cm on July 2015(Fig.1).The samples were collected from each site using a plastic spade and put into an auto sealed polyethylene plastic bag. After sampling, sediments were transported to the laboratory for analysis. Samples were air dried and sieved to prepare for analysis. The dried sediments were digested in a mixture of HF,$HClO_4$,HNO3 [Sparks, 1996] and brought into solution in 0.5M HCl (50 ml). Total concentration in samples was analyzed for four heavy metals (Cd, Cu, Fe and Pb) on an AAS [Phonex, 985]. The data were statistically analyzed using the software SPSS v-19.0.

RESULTS AND DISCUSSION

Metal Concentration

In order to assess the metal content in the sediments, it's important to establish the natural levels of these metals. Apart from natural contribution, heavy metals may be released into the system from anthropogenic sources such as solid and liquid waste of industries, the oil spill in to the sea water in the studied areas that are considered as important commercial lines of the world oil transportation [Abdulnabi,2016].The data in mean table (1) show the mean, mini., maxi., Std. Deviation and median of the measured heavy metals concentrations (Cd, Cu, Fe and Pb) in the surface sediment of the studied area.

Cadmium

Cadmium is a non-essential element that causes kidney damage in humans and negatively affects plant growth and development. It is released into the environment by power stations, heating systems and metal working industries or urban traffic. It is also used in electroplating, pigment, plastic stabilizers, and Nickel- Cadmium batteries. The common source of contaminants is due to corrosion of galvanized pipes, erosion of natural deposits, discharge from metal refineries, run-off from waste batteries and paints. The mean value of Cd in sediments was 9.09 mg/kg ranged

between 7.63 mg/kg in station 9 and 12.69 mg/kg in station 7 (Fig 2). This was above the probable effect level of 0.15 mg/kg (0.1-0.2) [Kabata Pendias,2011].

Copper

Copper reaches the aquatic environment through wet and dry depositions, mining activities, and storm water run-offs, industrial, domestic, and agricultural waste disposal. Among industrial sources include copper plating, pulp and paper mills, e-waste, sewage and other forms of waste waters. In this study it was found that the mini. Cu concentration was 23.46 mg/kg in station 10 and the maxi. was 83.3 mg/kg in station 3 Fig.3.Copper (Cu) forms several minerals of which the common primary minerals are simple and complex sulfides. These minerals are quite easily soluble in weathering processes and release Cu ions, especially in acid environments [Hawks and Web, 1962]. The mean Cu in the sediment was 38.24 mg/kg, this value is above the mean Cu concentration in crust 26 mg/kg. The Cu concentration in surface sediment reflects the bioaccumulation of the metal and also recent anthropogenic sources of the element, the relative enrichment in copper content that could be due to environmental contamination, [Kabta Pendias, 2011].

Iron (Fe)

It was found in this study that the mini. Fe concentration was 1112.46 mg/kg in station 9 and the maxi. was 1143.58 mg/kg in Station 1 (Fig4). Such high values of the studied heavy metals is believed may be due to high contamination of clay percentages in the studied sediments as well as may be due to the oil and gasoline spill to the sea water in the studied areas that are considered as important commercial lines to the world ships that are passing in the north and Northwest of the Arabian Gulf [Al- Khion 2012]. This finding is in accordance with Al-Jaberi (2013) results.

Lead (Pb)

The principal source of Pb in the marine environment appears to be the exhaust of vehicles which run with leaded fuels [Heba, et al., 2004]. Also, lead reaches the sea by rain and wind blowing dust [Williams et al., 1978,Hassan et al.2016]. As a natural source for trace metals introduction to the Northern Arabian Gulf there are airborne and waterborne particulates. Dissolved and absorbed metal pollutants derived from the urban, industrial and agricultural centers of Iraq, Iran and Kuwait may be entering to the Northern Arabian Gulf [Abdulnabi,2016]. The majority of the suspended particulate load of Shatt Al-Arab is expected to be deposited in its estuary before they reach the Gulf in which absorbed trace metals are released there [Al-Khafaji, 1996, Abdulnabi,2016]. The high concentration of trace metals in the sediments of areas close to highly dense cities could be arising from sewage discharge as well as industrial pollution, ship wrecks, oil enrichment and transportation, Moreover, high concentrations of lead (Pb) is found everywhere which associated with high traffic density of automobile running by leaded gasoline [Al-Khafaji,1996, Riley and Chester, 1981].

It was found in this study that the mini. Pb concentration was 31.31 mg/kg in station 16 and the maxi. was 109.58 mg/kg in station 3 (Fig5). The anthropogenic source of Pb is leaded gasoline. The average abundance of Pb in the earth's crust is 14.5 (14-15) mg/kg. The accumulation of Pb in the aqueous environments is exposed to various pollution sources and it is of great ecological significance because this metal is known to greatly affect the biological activity in aqueous environments, [Kabta Pendias, 2011]. Lead is positively correlated with Fe, Zn, and Ni probably suggesting some association with the fertilizers, clay and heavy minerals [Hassan 2007, Al-Jaberi, 2013].

Assessment of Contamination

There are many sediments pollution indices that can be used to assess the level of contamination by heavy metals. For this purpose and to meet the objectives of this study, two indices were selected to evaluate the contamination level of Cd, Cu, Fe and Pb, in the sediments of Iraqi coastline. These are Geo accumulation index (Igeo) and Enrichment factor (E.F) (Tables 2 and 3).

Geo- Accumulation Index (Igeo)

The index of Geo accumulation (Igeo) means the assessment of contamination by comparing the levels of heavy metal obtained to a background level originally used with bottom sediments [Müller, 1969]. It was widely used by many authors [Gowd et al., 2010, Al-Kuziea, 2015, Hussan, 2007, Al-Sabah, 2007] in Iraqi sediment.The I geo for the metals studied were calculated using the Müller (1979)

I geo = log2 [Cn/1.5Bn]

Where: Cn is the measured concentration of the examined metal 'n' in sediments and
Bn is the background concentration of the same metal.

It is very difficult to establish Bn values for sediments in the Iraqi tidal flat owing to geochemical variability of various areas and different anthropogenic impacts [Al-Sultan et al., 2013]. In this work, Bn value has been taken equal to metal concentrations determined by Kabta Pandieas (2011). Based on the I-geo data and Müller's I -geo, the contamination level with respect to each metal at 16 stations is ranged in Table (3). Lu et al (2007) defined the constant 1.5 as a constant introduced to minimize the effect of possible variations in the background values which may be attributed to litho logic variations in the sediments. Müller (1969) designed a classification for the Igeo. This application was considered by many researchers like Hussan (2007), Al-Sabaah (2007), Huu et al. (2010) Al-kuziea 2015 and Al-Hujaaj (2016).The Igeo of Cd was found positive in all the stations, ranging from 5.085 to 5.817.with the mean value 5.322 ± 0.223 These results are of (class 6) which indicates that the concentrations of Cd in the sediments of these sites are extremely contaminated (Fig 6).

The Igeo of Cu concentrations was found positive in the stations 1, 2, 3, 4, 13, 15 ranging between (0.010-1.103), these results are of (class 1and 2) which indicates that the concentrations of Cu in the sediments of this sites are uncontaminated to moderately contaminated. While Cu in the other sites of the study area had negative values ranging between -0.732 to -0.099 with the mean value -0.410± 0.528 .These results are of (class 0) which indicates that the concentrations of Cu in the sediments of these sites are unpolluted and lower than the background (Fig 7).The Igeo of Fe was found negative in all station ranging from -6.075 to –6.035 , with the mean value –6.05± 0.015 these results are of (class 0) which indicates that the concentrations of Fe in the sediments of these sites are unpolluted and lower than the background (Fig 8).The Igeo of Pb had positive values in all station ranging between 0.476 to 2.284. These results are of (class 1, 2 and 3) which indicates that the concentrations of Pb in the sediments of these sites are (uncontaminated to moderately contaminated) to moderately contaminated and moderately to strongly contaminated. (Fig 9).

Enrichment Factor (EF)

Enrichment factor (EF) was calculated to determine if levels of metals in sediments of Iraq and its surrounding marine environment were of anthropogenic origins (e.g., contamination). To identify abnormal metal concentration, geochemical normalization of the heavy metals data to a conservative element, such as Al, Fe, and Si was employed. Several authors have successfully used iron to normalize heavy metals contaminants [Schiff and Weisberg, 1999, Baptista Neto et al., 2000, Mucha et al., 2003, Hussan 2010, Al-Kuziea 2015]. In this study, iron was also used as a conservative tracer to differentiate natural from anthropogenic components. According to Ergin et al., (1991), the metal EF is defined as follows:

EF = (X/Fe) sediment/(X/Fe) crust

Where, X/Fe is the ratio of the concentration heavy metal (X) to the Fe concentration.

EF values were taken as suggested by Sutherland (2000) for the metals studied with respect to crust average 5% [Kabta pandieas 2011]. EF < 1 indicates no enrichment, EF < 3 is minor enrichment, EF = 3–5 is moderate enrichment, EF = 5–10 is moderate to severe enrichment, EF = 10–25 is severe enrichment, EF = 25–50 is very severe enrichment, and EF > 50 is extremely severe enrichment. The mean mini. and maxi., std. deviation EF were calculated for all analyzed metals in this study(Table 4).

The EF values for Cd were fill in >50 (2288.44 to 3698.88) in all stations (Fig .10)With mean value 2670.63 (EF)which indicates the extremely severe enrichment degree of Cd contamination (very severe enrichment). These high values may revert to the low iron concentration in the study areas, compared with its concentration in the Earth's crust so it's better to choose another element for comparison instead of iron. The extremely severe enrichment cadmium content in the sediments may be due to the disposal of cadmium containing wastewater from factories and hospitals in Basra city to Shatt Al-Arab and Shatt Al Basra rivers caused to increase of cadmium content in the sediments especially in station 7. Increase in Cd concentration in the recent few years due to the close proximity of this site to industrial discharges.

Copper showing E.F ranging from very severe enrichment, to extremely severe enrichment (40.56 to 140.94) With mean value 64.50± 0.289 (at all station, as shown in Fig 11). In all the studied sites classified as class 5 and 6 representing is very severe enrichment, and is extremely severe enrichment. Pb had the second highest EF values 46.90 to 159.70 with mean value 81.70 ± 0.636 suggesting very severe enrichment, to extremely severe enrichment among the metals studied. Lead is known to come from the use of leaded gasoline .The principal source of Pb in the marine environment appears to be the exhaust of vehicles which run with leaded fuels (Heba, et al., 2004). Also, lead reaches the sea by rain and wind blow dust (Williams, et al., 1978). As a natural sources for trace metals introduction to Northern Arabian Gulf are airborne and waterborne particulates. Dissolved and adsorbed metal pollutants derived from the urban, industrial and agricultural centers of Iraq, Iran and Kuwait may be entering to the Northern Arabian Gulf.

CONCLUSION

Among four metals studied, the I-geo of Cd was ranged from strong to very strong class (I geo class = 4-5) for sediments in all stations. Whereas I geo of Pb (second abundance metal in the tidal flat of Iraqi sediment) was ranged from (0.476-2.284) for sediments in all stations .Practically Uncontaminated to moderately contaminated, moderately contaminated and moderately to strongly contaminated.While the tidal flat of Iraqi sediment where practically unpolluted- Background sample with Cu Uncontaminated to moderately contaminated, moderately contaminated and Uncontaminated with Fe. This might indicate that the tidal flat of Iraqi sediment has heavy accumulations of Cd and Pb metals, which apparently come from sewers that include industrial wastes in addition to the outside source.

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Table1: The mean, mini., maxi., Std. Deviation and median of the measured heavy metals concentrations(mg/kg)

Element	Mean	Mini.	Maxi.	Std.Deviation	Median
Cd	9.09	7.05	15.98	0.813	4.465
Cu	38.254	21.074	109.198	8.633	17.721
Fe	1132.2	1100.33	1147.38	5.702	569.138
Pb	56.308	31.31	125.24	10.856	27.396

Table 2: Igeo Classification [Müller 1979]

I geo	Class geo	Description of sediment quality
<0	0	Uncontaminated
0-1	1	Uncontaminated to moderately contaminated
1-2	2	Moderately contaminated
2-3	3	Moderately to strongly contaminated
3-4	4	Strongly contaminated
4-5	5	Strongly to extremely strongly contaminated
>5	6	Extremely contaminated

Table 3: The I geo values in the sediments of study area

Element	Mean	Mini.	Maxi.	Std.Deviation	Median
Cd	5.322	5.085	5.817	0.223	4.311
Cu	-0.12	-1.103	-0.732	0.528	-1.138
Fe	-6.05	-6.075	-6.035	0.015	-0.398
Pb	1.233	0.476	2.284	0.507	0.284

Table 4: Heavy metal enrichment factor (EF)in Iraqi sediments

Element	Mean	Mini.	Maxi.	Std.Deviation	Median
Cd	2670.63	2288.44	3698.88	4.779	26.151
Cu	64.50	40.56	140.94	.289	.599
Pb	81.704	46.9	159.709	.636	1.600

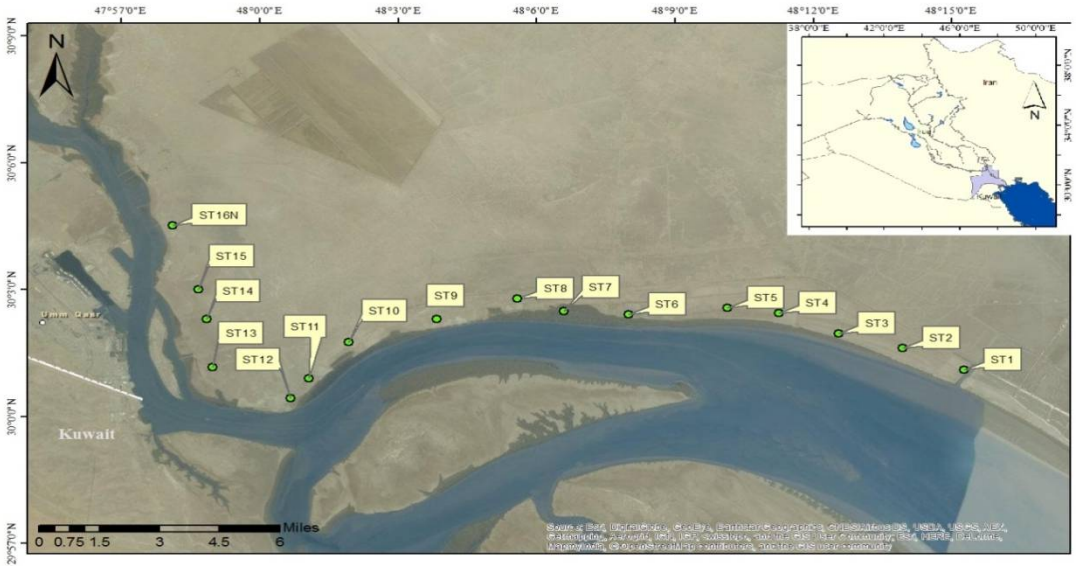

Fig. 1: Map showing the sampling station

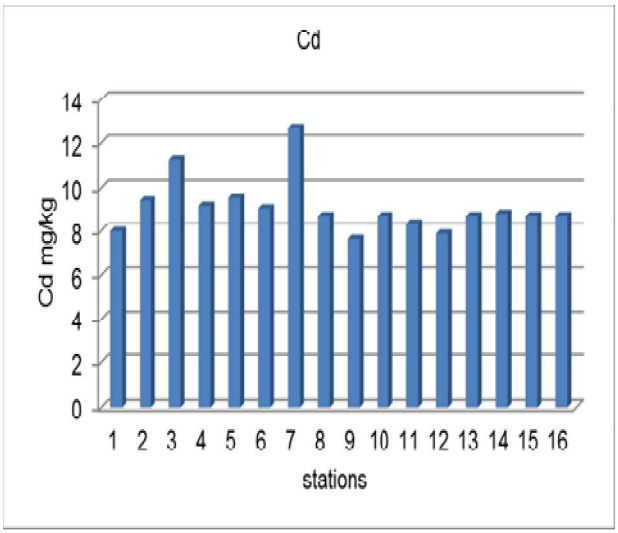

Fig.(2): The mean value of Cd in sediments stations

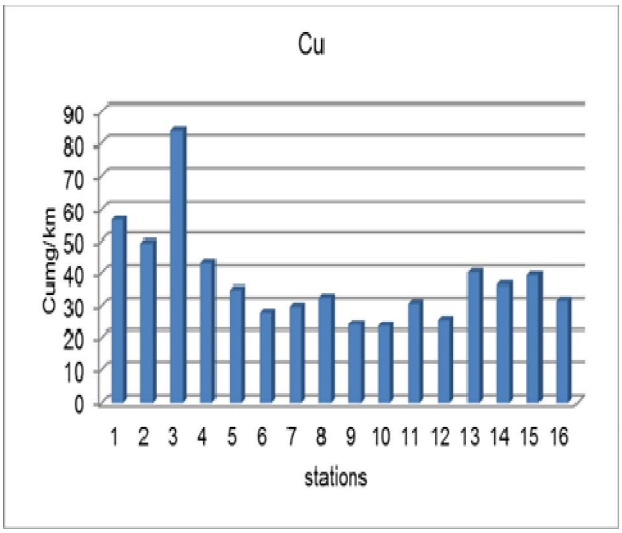

Fig.(3): The mean value of Cu in sediments stations

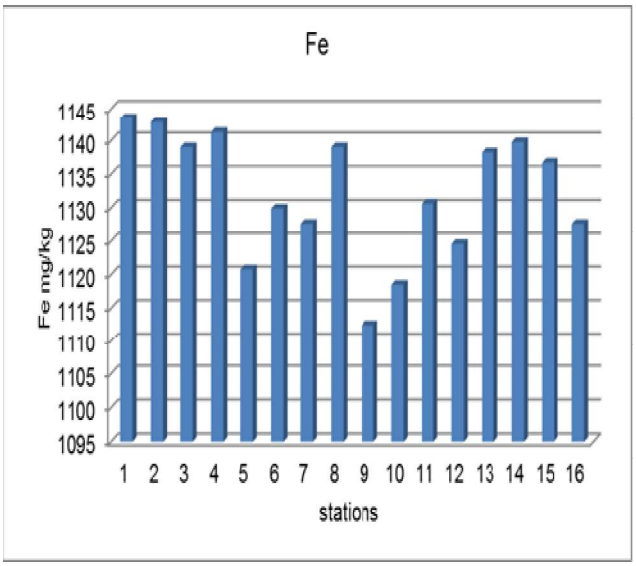

Fig.(4): The mean value of Fe in sediments stations

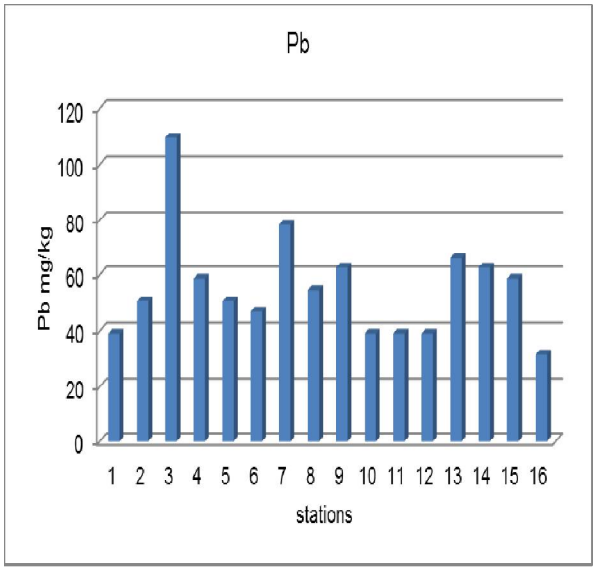

Fig.(5): The mean value of Pb in sediments stations

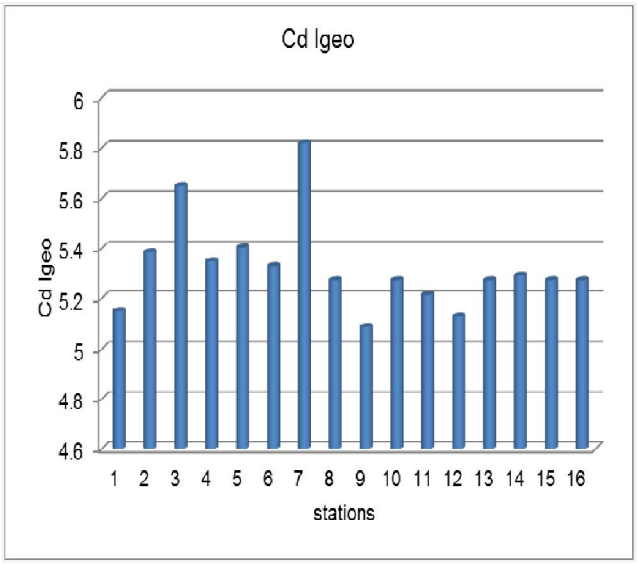

Fig.6: the value of Cd Igeo in the sediments stations

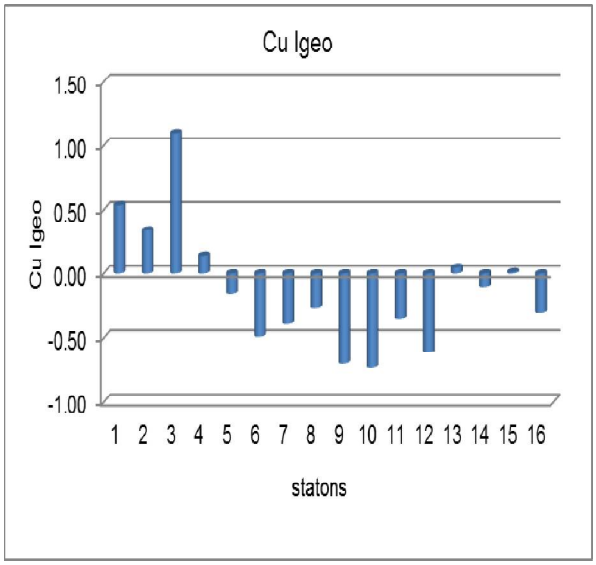

Fig.7: the value of Cu I geo in the sediments stations

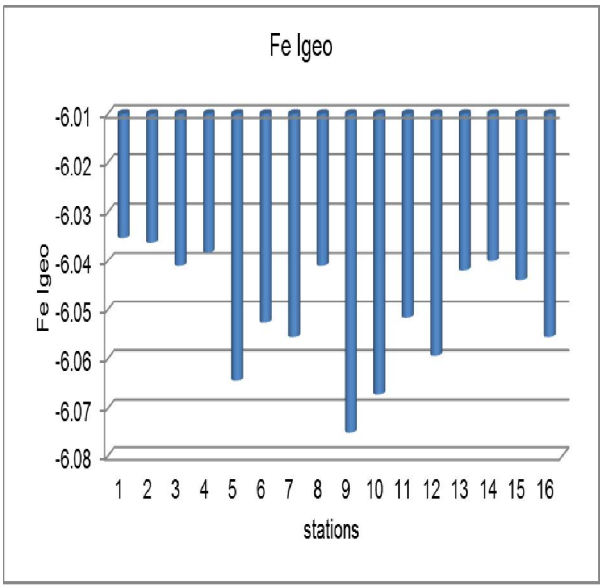

Fig.8: the value of Fe Igeo in the sediments stations

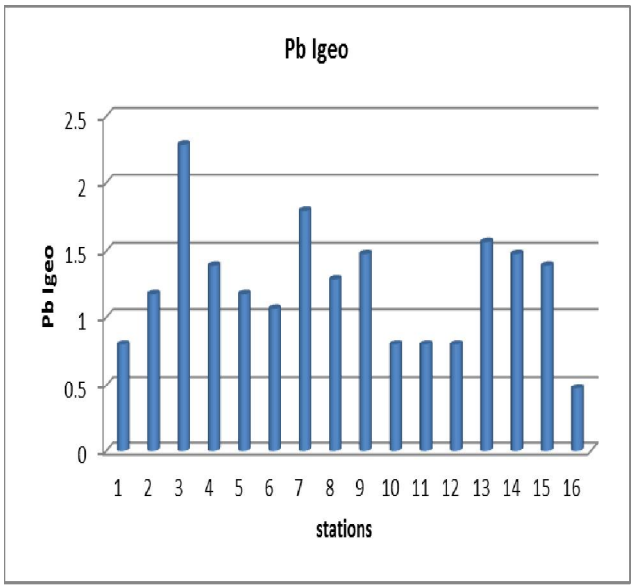

Fig.9: The value of Pb Igeo in the sediments stations

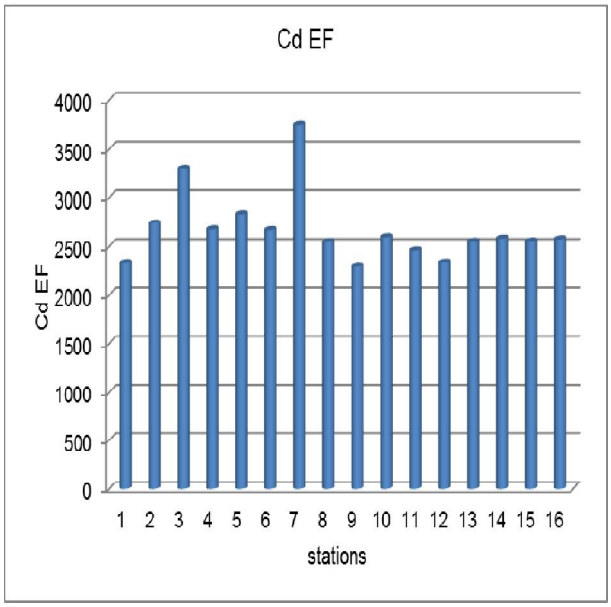

Fig.10:The value of Cd EF in the sediments stations

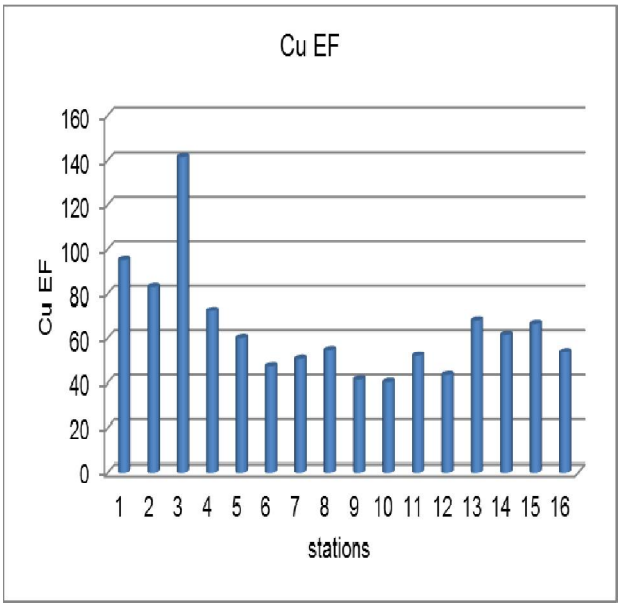

Fig.11:The value of Cu EF in the sediments stations

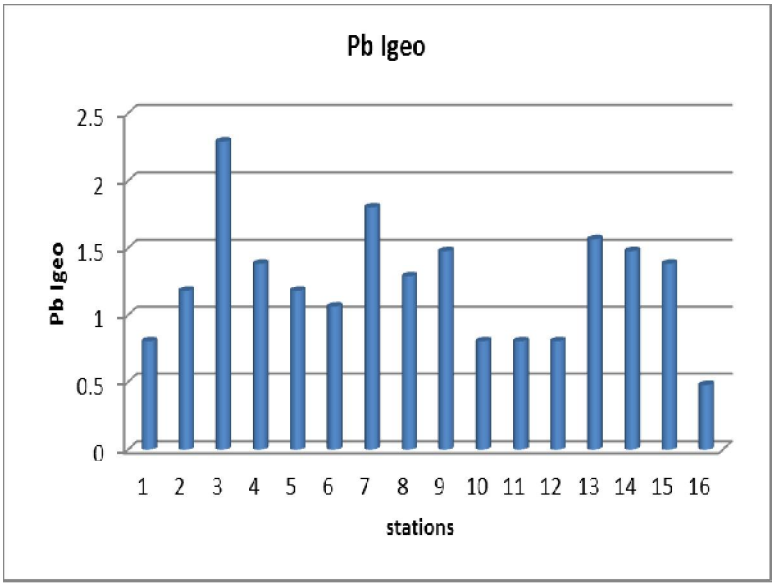

Fig.12 :The value of Pb EF in the sediments stations

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RESEARCH ARTICLE

Efficacy of Various Antibacterial Chemical against Bacterial Blight of Cluster Bean *In vivo* Condition

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ABSTRACT

An attempt was made to find out the efficacy of antibiotic (streptocycline), a fungicide (Copper oxychloride) alone and in their combinations as seed treatment as well as foliar sprays was tested against bacterial blight of clusterbean. The results indicate that average minimum disease intensity i.e. 16.0 per cent was observed in treatment T_5 (seed treatment with streptocycline +3 sprays of streptocycline) and yield was 8.48 q ha-1. This was followed by treatment T_4 (seed treatment with streptocycline + 2 sprays of streptocycline) where disease intensity was 16.30 per cent and yield was 8.15 q ha-1.

Keywords: Streptocycline, Clusterbean , *Xanthomonas axonopodis* pv. *cyamopsidis* .

INTRODUCTION

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub] popularly known by its vernacular name guar, is an important leguminous crop of India. It is cultivated mainly under arid and semi-arid conditions during *Kharif* season. It is known for its suitability for areas having light textured soils and low and erratic rainfall. It is very hardy and drought tolerant crop. Its deep penetrating roots enable the plants to utilize available moisture from deeper layer of soil more efficiently and thus offer better scope for rainfed cropping. The crop survives even at moderate salinity and alkalinity. It is especially suited for soil and climatic conditions of Rajasthan. In India, this crop occupies 26 lakh ha with annual production of 8.57 lakh tonnes of seeds and having average productivity of 3 q ha-1(Anonymous, 2003-04). The crop is mostly grown in India, Pakistan, USA, Italy, Mexico and South Africa. India is a leading country (83% area) of this crop in the world. Guar is mostly grown in Rajasthan, Gujarat, Haryana, Punjab and Uttar Pradesh. Rajasthan ranks first in respect of area and production, where the crop occupies an area of 16.56 lakh ha with a production of 4.81 lakh tonnes and productivity is 3.44 q ha-1.Guar crop suffers from a number of fungal, bacterial

and viral diseases. Among them bacterial blight caused by *Xanthomonas axonopodis* pv. *cyamopsidis* (Patel *et al*., 1953), is the most important and wide spread disease. It appears all most every year and may cause considerable damage depending upon the variety and environment conditions. In case of heavy infection, it may cause 58-68 per cent losses in grain yield (Srivastava and Rao, 1963; Gupta 2003 ; Gandhi and Chand, 1985).

MATERIALS AND METHODS

Two antibacterial chemicals i.e. streptocycline and copper oxychloride were taken alone or in combinations for controlling bacterial blight under field conditions. The treatments were as follows :

T_1 Seed treatment with streptocycline @ 1.0 g/kg seed
T_2 Three sprays of streptocycline @ 150 ppm
T_3 Three sprays of copper oxychloride @ 0.2%
T_4 Seed treatment with streptocycline (1.0 g/kg seed) + 2 sprays of streptocycline @ 150 ppm
T_5 Seed treatment with streptocycline (1.0 g/kg seed) + 3 sprays of streptocycline @ 150 ppm
T_6 Seed treatment with streptocycline (1.0 g/kg seed) + 2 sprays of copper oxychloride @ 0.2%
T_7 Seed treatment with streptocycline (1.0 g/kg seed) + 1 spray of streptocycline @ 150 ppm and copper oxychloride @ 0.2%
T_8 Control

RESULTS AND DISCUSSION

A field experiment was carried out at Agricultural Research Station, Bikaner against bacterial blight of clusterbean. For this experiment an antibiotic (streptocycline), a fungicide (Copper oxychloride) alone and in their combinations as seed treatment as well as foliar sprays were tested.The first disease symptoms were observed on 4th Sept., 2006 in some plants, therefore, first spray of antibacterial chemicals (fungicide and antibiotic), neem based products and cowdung suspension was given on 5th September, 2006 i.e. after the appearance of first symptoms. Subsequently two sprays of chemicals, neem products and cow dung suspension were repeated at an interval of 10 days i.e. 15 September, 2006 and 25 September, 2006. Final data on disease intensity were recorded before harvesting of the crop. The grain yield was recorded in each treatment.The results presented in Table 1 showed that average minimum disease intensity i.e. 16.0 per cent was observed in treatment T_5 (seed treatment with streptocycline + 3 sprays of streptocycline) and yield was 8.48 q ha-1. This was followed by treatment T_4 (seed treatment with streptocycline + 2 sprays of streptocycline) where disease intensity was 16.30 per cent and yield was 8.15 q ha-1. Treatments T_6 and T_7 gave satisfactory results where disease intensities were 29.30 and 26.60 per cent and yield obtained were 5.68 and 7.13 q ha-1, respectively.

The treatments T_1, T_2 and T_3 where seed treatments and sprays were given with streptocycline and copper oxychloride were less effective with disease intensity 45.30, 32.30 and 37.00 per cent and yields were 3.57, 4.70 and 3.80 q ha-1,respectively. In control the disease intensity was 76.0 per cent and yield was 2.22 q ha-1. Statistical analysis showed that all treatment were significant with respect to control. In present investigation an antibiotic and a fungicide *viz.* streptocycline and copper oxychloride (Blitox-50) alone and in combinations were tested against bacterial blight under field condition. Results showed that seed treatment with streptocycline @ 1.0 g /kg seed followed by 3 sprays of streptocycline @ 150 ppm (T_5) was most effective in controlling the disease as compared to other treatments where the disease intensity was 16 per cent and yield was 8.48 q ha-1. This was followed by seed treatment with streptocycline @ 1.0 g/kg seed and 2 sprays of streptocycline @ 150 ppm with disease intensity 16.30 per cent and yield 8.15 q ha-1,which showed at per results with treatment T_5 (seed treatment with streptocycline @ 1.0 g/kg seed and 3 sprays of streptocycline @ 150 ppm. Gupta (1977) reported that streptocycline and agrimycin 100 ppm were the best in controlling the bacterial blight disease. Lodha and Ram (1993) also reported that spray of

streptocycline was most effective for controlling bacterial blight of clusterbean. Our results were also similar to these findings. In our experiment the disease was reduced about 60 per cent as compared to control.

CONCLUSION

The studies revealed that seed treatment with streptocycline @ 1.0 g /kg seed followed by 3 sprays of streptocycline @ 150 ppm was most effective in controlling the disease where disease intensity was 16.00 per cent and yield was 8.48 q ha-1. The next best treatment was seed treatment with streptocycline (1.0 g/kg seed) and 2 sprays of streptocycline (150 ppm) with disease intensity 16.30 per cent and yield 8.15 q ha-1.

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Table 1 : Effect of chemicals on bacterial blight intensity and grain yield of clusterbean

Treatment		Average disease intensity (%)	Yield (q ha^{-1})
T_1 :	Seed treatment with streptocycline @ 1.0 g/kg seed	45.30 (42.30)	3.57
T_2 :	Three sprays of streptocycline @ 150 ppm	32.30 (34.63)	4.70
T_3 :	Three sprays of copper oxychloride @ 0.2%	37.00 (37.46)	3.80
T_4 :	Seed treatment with streptocycline @ 1.0 g/kg seed) and 2 sprays of streptocycline @ 150 ppm	16.30 (23.81)	8.15
T_5 :	Seed treatment with streptocycline @ 1.0 g/kg seed) and 3 sprays of streptocycline @ 150 ppm	16.00 (23.58)	8.48
T_6 :	Seed treatment with streptocycline @ 1.0 g/kg seed) and 2 sprays of copper oxy chloride @ 0.2%	29.30 (32.77)	5.68
T_7 :	Seed treatment with streptocycline (1.0 g/kg seed) and 1 spray of streptocycline @ 150 ppm + copper oxychloride @ 0.2%	26.60 (31.05)	7.13
T_8 :	Control (without seed treatment and spray)	76.00 (60.67)	2.22
General Mean		35.06 (35.78)	5.47
S.Em ±		(0.70)	0.28
CD (P=0.05)		(2.11)	0.86
C.V. (%)		3.37	9.02

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RESEARCH ARTICLE

Antibacterial Studies of Fruits Extract of *Lantana camara* L. (Verbenaceae)

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ABSTRACT

The aim of this research study is to evaluate the efficacy of antimicrobial activities of crude extracts from the fruits of *Lantana camara* L. (Verbenaceae). Dried and powdered fruit material was subjected to sequential solvent extraction with petroleum ether, chloroform, acetone and methanol by using maceration technique to prepare the crude extract and antibacterial activities was carried out by using *in vitro* method against standard bacterial strains, Gram positive (*Staphylococcus aureus*), Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella thyphimurium*). The influence of ultrasound radiation on the yield of crude extract was also studied. Among the four crude extracts, chloroform extract was recorded highest antibacterial activity.

Keywords: *Lantana camara* Linn., Gram Strains, Solvent Extraction, Ultra Sound Radiation.

INTRODUCTION

Nature possess the diversified bioactive molecules and has been a source of drugs for thousands of years [1,2] it play an important role in pharmaceutical industry [3] and in health care. Majority of the world's population still relying mainly on traditional medicines for comfort livelihood [4,5]. Secondary metabolites synthesized in all parts of the plant body and are mainly attributed due to their pharmacological actions [6], act as drug, as starting materials to produce synthetic drugs either by total synthesis [7] or by semi synthetic modification [8] of functional groups. Medicinal plants are usually screened for phytochemicals and characterization of active principle and used as drug [9].In Ethiopia medicinal plants play major role to the community and the traditional health care is culturally deep rooted with oral and written pharmacopoeias. Ethiopian plants have shown very effective medicinal value and the knowledge of their use provide a vital contribution to the community[10,11].Literature report shows that *L. camara* L.

is one of the major weed in agricultural areas and exotic species, which is invasive in dry lands of Ethiopia. According to folklore knowledge this plant known for having many medicinal uses[12] and all parts of this plant have been used for the treatment of several ailments.

Fruits are edible, people of Northern region (Gonder) and Southern region (Wonago) consume it as food as well as medicine in treating diarrhea [13]. Leaves of *L. camara* L. were used as an antitumeral, antibacterial, antihypertensive, antiseptic agent and used for the treatment of cough, root part were used for the treatment of malaria, rheumatism, and skin rashes, the fruits are useful in treating fistula, pustules, tumors, tuberculosis[14,15] asthma, high blood pressure, whole plant parts effectively used for the treatment of tetanus, rheumatism, malaria and ataxia of abdominal viscera[16], tea prepared from the leaves and flowers was taken against fever, influenza and stomach-ache, leaf extract has been said to possess the wound healing and antidiabetic property[17,18]. The fresh unripe fruits of *L. camara* L. possesses the antioxidant substance which may be responsible for the treatment of cancer and tumors there is much scope for fruits and more number of studies can be undertaken like oxidative stress, hepatoprotetive, nephroprotective, anticancer, skin disorders, rheumatism and asthma[19].The current study implies that fruits of *L. camara* L. investigate completely to better understand their properties, safety, efficacy and used as potential source to serve the community.

MATERIALS AND METHODS

Petroleum ether, chloroform, acetone and methanol for gradient extraction, petroleum ether, hexane and ethyl acetate for column chromatography, silica gel (60-120mm and 230-420 mm mesh size), precoated TLC plates were used for detection of spots. Dimethyl Sulfoxide (DMSO), Mueller Hinton agar, nutrient broth and standard drug gentamicine were used for antibacterial activity. All the chemicals and reagents of analytical grade were used. The following list of apparatus was used for the current research, rotary shaker (HY-5A Manoeuvre style vibrator), rotary evaporator (Heidolph, UK), UV (254 & 365 nm) and I_2 chamber.

Collection and preparation of plant material

Fresh fruit of *L camara* L. were collected from Oromia region, around Jimma University Kito furdisa campus during February 2015. The collected plant fruits were washed with distilled water and shade dried in laboratory at room temperature, further grounded with electrical grinder so as to enhance effective contact with solvent, to make the sample homogeneous, to increase the surface area, and facilitate the penetration of solvents into cells of the plant powder.

Sequential extraction of *Lantana camara* L. fruits[20]

Extraction was carried out at room temperature under normal condition by maceration technique also called as cold percolation method. The dry powder was weighed accurately and subjected to extraction in rotary shaker apparatus using the solvent in sequential order of polarity as petroleum ether, chloroform, acetone and methanol successively for 72 hrs with constant continuous shaking. Before proceed the extraction with next solvent the powder was air dried to remove the adhering solvent. The percentage yields of the extract were shown (Table 1).

Procedure for the preparation of crude extract:

Seven hundred fifty grams of fine powder was extracted twice with petroleum ether. The combined petroleum ether extracts were filtered and evaporated by means of a rotary evaporator to yield 6.72 g (0.896%) of extract. The residue obtained from filtration of the petroleum ether extract was then extracted two times with chloroform and the combined extracts were filtered and evaporated to yield 5.15 g (0.687%) of extract. The residue obtained from filtration of the chloroform extract was then extracted two times with acetone and the combined extracts were filtered and evaporated to yield 15.77 g (2.103%) extract. The methanol extract was prepared in the same way as chloroform

and acetone extract. The yield of methanol extract was 12.73 g (1.697%). The yield of acetone and methanol crude extract is more compare with petroleum ether and chloroform extract.

Comparison of the yield of crude extract by maceration, sonication & microwave method

The influence of ultrasound and microwave radiation on the yield of extraction with various solvents was studied, sonication method gave good yield in compare with other methods, microwave method result in bumping and violent eruption even under power of fewer watts, low intensity. The results (weight of the crude extract and percent yield) were given in the Table 2.

Procedure

Fifty grams of dry powder was extracted with 300 ml of petroleum ether by maceration, microwave and sonication method. The residue obtained from filtration of the petroleum ether crude extract was further sequentially extracted with chloroform acetone and methanol. It is quite evident that influence of ultrasound on the weight and % yield of extract.

RESULTS AND DISCUSSION

Evaluation of antibacterial activities of crude extracts from *L. camara* L. fruits

The results of antibacterial activities of four different extracts of *L. camara* L. fruits against human pathogenic bacteria was presented in Table 3. Among the four extracts, chloroform extract was recorded good antibacterial activity followed by acetone and petroleum ether extracts, whereas methanol extract does not show any significant antibacterial activity, the standard gentamicine was recorded maximum antibacterial activity compared to four extracts. The chloroform extract exhibited antibacterial activity with zone of inhibition from 12 mm to 16 mm at 100mg/ml concentration depending upon bacterial species. The most susceptible organism in the present investigation was *Pseudomonas aeruginosa* followed by *Salmonella thyphimurium* and *Staphylococcus aureus*, whereas *Escherichia coli* were found to be most resistant bacteria against all the extracts tested.

The differences in the susceptibility of the test organisms to the different extracts might be due to the variation in the rate at which active ingredients penetrate the cell wall and cell membrane structures[21,22,23,24]. The results are consistent with previous report of antimicrobial activities of leaves of *L. camara* L. chloroform extract record 15 mm, 13 mm and 12mm zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella thyphimurium*, whereas petroleum ether extract record13 mm, 11 mm, and 9 mm zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* respectively[25]. Thus, the observed antibacterial activities of crude extracts could justify the traditional use of plant for treatment of different bacterial and fungal infections.

CONCLUSION

The overall results of this study provide evidence that *L. camara* L. fruit extract as well as the isolated compounds shown promising antibacterial activity against both Gram negative and Gram positive pathogens. Thus, the observed antibacterial activities of the crude extracts and the isolated compounds could justify the traditional use of *L. camara* L. for the treatment of different bacterial infections. Further isolation and characterization of bioactive lead molecules is under progress and the results will be published in due course of the time.

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Table 1: Percentage yields of *L. camara* L. fruits extract

Solvent	Mass extracted (g)	% Yield
Petroleum ether	6.72	0.896
Chloroform	5.15	0.687
Acetone	15.77	2.103
Methanol	12.73	1.697

Table 2: Comparison of the Extract Yield by using Maceration, Microwave and Sonication method

Solvent	Maceration method (Cold percolation)		Microwave method		Sonication method	
	Extract (Wt) g	% Yield	Extract (Wt) g	% Yield	Extract (Wt) g	% Yield
Petroleum ether	0.450	0.90	Violent eruption	--------	0.86	1.72
Chloroform	0.352	0.70	Violent eruption	--------	0.94	1.88
Acetone	1.10	2.2	0.42	0.84	1.52	3.04
Methanol	0.91	1.8	0.73	1.46	1.30	2.60

Table 3: Antibacterial activity of crude extracts of *L. camara* L. fruit against bacterial strains

Bacterial strain	Conc. mg/ml	Diameter of zones of inhibition in mm					
		Pet.ether extract	Chloroform extract	Acetone extract	Methanol extract	Gentamicine	DMSO
Esch. Coli	100	9	12	12	8	22	NI
Staph.aureus	100	10	13	10	9	22	NI
Salmo.typhimur.	100	12	14	13	11	23	NI
Pseudo. aerug.	100	12	16	14	12	24	NI

Note: NI = No Inhibition

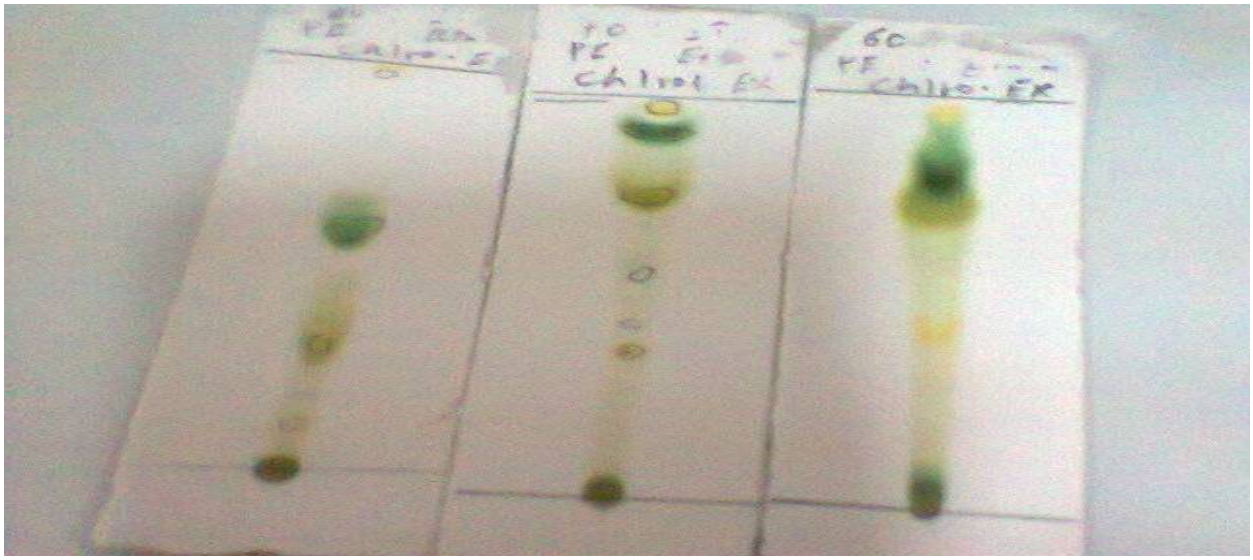
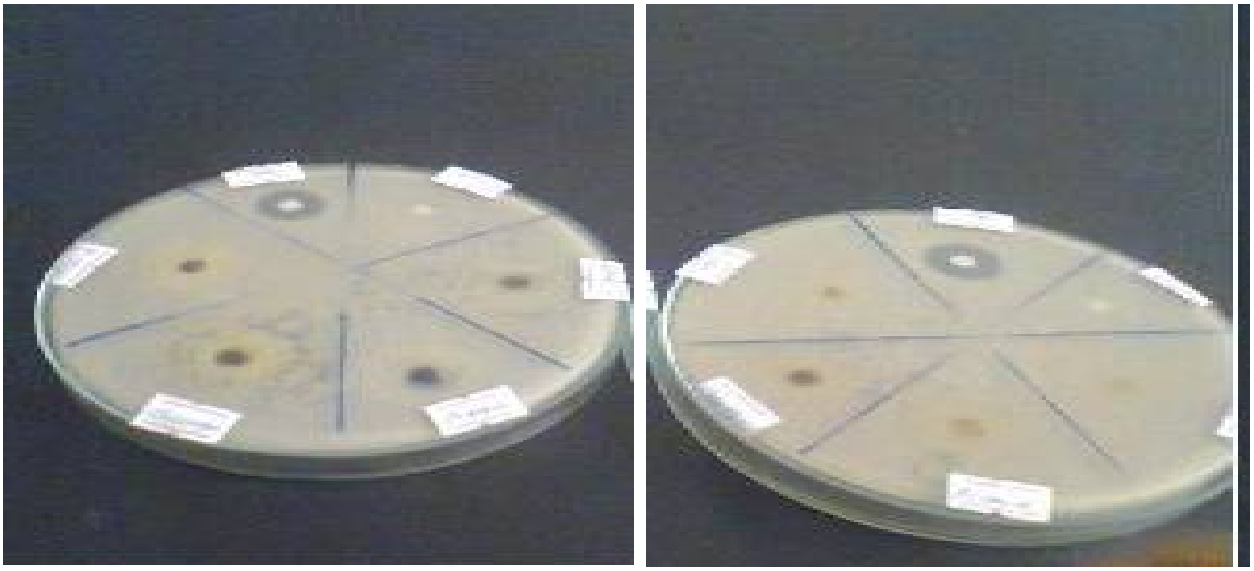
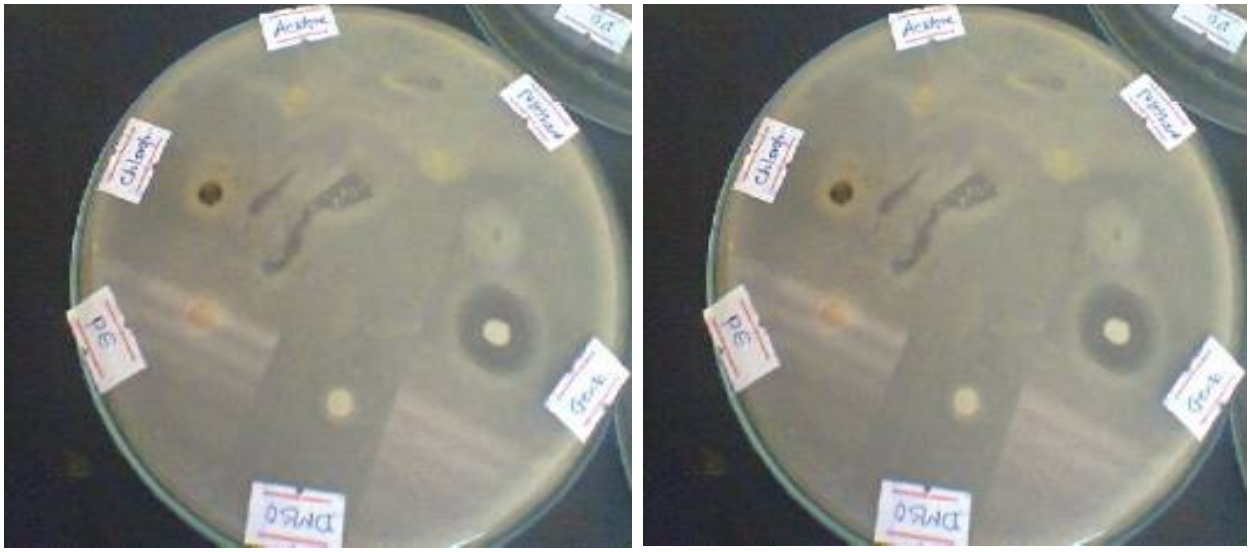


Fig.1 TLC profile of Chloroform crude extract in Eluent: Pet.ether & Ethyl acetate (different %)



(a) **(b)**



(c) **(d)**

Fig.2 Evaluation of antibacterial activity of crude extracts by disc diffusion:The efficacy of four crude extracts (Pet.ethetr, Chloroform, Acetone& Methanol) are evaluated against four microbial pathogens.
(a)*Pseudomonas aeruginosa*
(b) *SalmoneIlathyphimurium*
(c) *Staphylococcus aureus*
(d) *Escherichia coli*

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RESEARCH ARTICLE

Effect of Various Neem Based Products and Fresh Cow Dung Suspension against Bacterial Blight of Cluster Bean *In vivo* Conditions

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ABSTRACT

An attempt was made to find out the efficacy of three neem based products (nimbecidine, neem leaf extract and neem seed kernel powder) and cow dung suspension as seed treatment as well as foliar sprays was tested against bacterial blight of clusterbean. The average minimum disease intensity i.e. 28.30 per cent was observed in treatment T_6 (seed treatment with cow dung @ 20 g l-1 + 2 sprays of cow dung @ 20 g l-1) and yield was 6.04 q ha-1, followed by in treatment T_2 [3 sprays of nimbecidine (3 ml/l)] where disease intensity was 31.20 and yield 5.75 q ha-1.

Keywords: Streptocycline, Clusterbean , *Xanthomonas axonopodis* pv. c*yamopsidis.*

INTRODUCTION

Clusterbean [*Cyamopsis tetragonoloba* (L.) Taub] popularly known by its vernacular name guar, is an important leguminous crop of India. It is cultivated mainly under arid and semi-arid conditions during *Kharif* season. It is known for its suitability for areas having light textured soils and low and erratic rainfall. It is very hardy and drought tolerant crop. Its deep penetrating roots enable the plants to utilize available moisture from deeper layer of soil more efficiently and thus offer better scope for rainfed cropping. The crop survives even at moderate salinity and alkalinity. It is especially suited for soil and climatic conditions of Rajasthan. In India, this crop occupies 26 lakh ha with annual production of 8.57 lakh tonnes of seeds and having average productivity of 3 q ha-1 (Anonymous, 2003-04). The crop is mostly grown in India, Pakistan, USA, Italy, Mexico and South Africa. India is a leading country (83% area) of this crop in the world. Guar is mostly grown in Rajasthan, Gujarat, Haryana, Punjab and Uttar Pradesh. Rajasthan ranks first in respect of area and production, where the crop occupies an area of 16.56 lakh ha with a production of 4.81 lakh tonnes and productivity is 3.44 q ha-1.Guar crop suffers from a number of fungal, bacterial and viral diseases. Among them bacterial blight caused by *Xanthomonas axonopodis* pv. *cyamopsidis* (Patel *et al*., 1953),

is the most important and wide spread disease. It appears all most every year and may cause considerable damage depending upon the variety and environment conditions. In case of heavy infection, it may cause 58-68 per cent losses in grain yield (Srivastava and Rao, 1963; Gupta 2003 ; Gandhi and Chand, 1985).

MATERIALS AND METHODS

An experiment was conducted by using neem based products and cow dung suspension against bacterial blight of clusterbean under field conditions. The treatment were as follows:

T_1 Three sprays of nimbecidine (1.5 ml/l)
T_2 Three sprays of nimbecidine (3.0 ml/l)
T_3 Three sprays of neem leaf extract (3%)
T_4 Three sprays of neem seed kernel powder (5%)
T_5 Seed treatment with cow dung suspension @ 20 g/litre
T_6 Seed treatment with cow dung suspension @ 20 g/litre + 2 spray of cow dung @ 20 g/litre
T_7 Seed treatment with cow dung suspension (20 g/l) + neem leaf extract (30g/l)
T_8 Seed treatment with cow dung suspension (20 g/l) + neem leaf extract (30g/l) and 1 spray of neem leaf extract (30 g/l)

RESULTS AND DISCUSSION

A field experiment was carried out at Agricultural Research Station, Bikaner against bacterial blight of clusterbean. In this experiment using three neem based products (nimbecidine, neem leaf extract and neem seed kernel powder) and cow dung suspension as seed treatment as well as foliar sprays.The first disease symptoms were observed on 4th Sept., 2006 in some plants, therefore, first spray of neem based products and cowdung suspension was given on 5th September, 2006 i.e. after the appearance of first symptoms. Subsequently two sprays of chemicals, neem products and cow dung suspension were repeated at an interval of 10 days i.e. 15 September, 2006 and 25 September, 2006. Final data on disease intensity were recorded before harvesting of the crop. The grain yield was recorded in each treatment.The data presented in Table 1 showed that average minimum disease intensity i.e. 28.30 per cent was observed in treatment T_6 (seed treatment with cow dung @ 20 g l-1 + 2 sprays of cow dung @ 20 g l-1) and yield was 6.04 q ha-1, followed by in treatment T_2 [3 sprays of nimbecidine (3 ml/l)] where disease intensity was 31.20 and yield 5.75 q ha-1. Treatment T_3 (three sprays of neem leaf extract (3%)) and T_8 (seed treatment with cow dung suspension @ 20 g l-1 + neem leaf extract @ 30 g l-1 and one spray of neem leaf extract @ 30 g l-1) also gave satisfactory results where disease intensities were 32.70 and 34.00 per cent and yield obtained was 4.98 and 5.20 q ha-1, respectively.

The treatments T_1 [Three sprays of nimbecidine (1.5 ml/l)], T_5 (Seed treatment with cow dung suspension @ 20 g/litre) and T_7 [Seed treatment with cow dung suspension (20 g/l) + neem leaf extract (30g/l)] were less effective and disease intensity were 37.50, 46.30 and 40.00 per cent and yields were 3.64, 3.38 and 3.61 q ha-1, respectively. In control, the disease intensity was 72.30 per cent and yield was 2.98 q ha-1. Statistical analysis showed that all treatments were significant with respect to control.In another experiment, we had undertaken some neem based products *viz.* nimbecidine, neem leaf extract, neem seed kernel powder and fresh cow dung suspension for controlling bacterial blight of clusterbean. Results showed that treatment T_6 (seed treatment with cow dung suspension @ 20 g/l with 2 sprays of fresh cow dung suspension @ 20 g /l) was most effective in controlling the disease where disease intensity was 28.30 per cent and yield was 6.04 q ha-1. It was followed by treatment T_2 [three sprays of nimbecidine (3 ml/l)] where disease intensity was 31.20 per cent and yield was 5.75 q ha-1. In control disease intensity was 72.30 per cent and yield was 2.98 q ha-1 (Table 1). Kumar *et al.* (2001) used the leaf extract of neem (*Azadirachita indica*), bhang (*Canabis sativa*), datura (*Datura stramonium*), garlic cloved (*Allium sativum*), oak (*Calotrpis proceva)* against bacterial blight of clusterbean at different concentrations and found that these were effective in controlling the disease. Nutrient broth containing leaf extracts of aonla and eucalyptus at 1:1 and 1:4 ratio did not allow *Xanthomonas axonopodis* pv. *cyamopsidis* to grow (Kamalpreet *et al.,* 2005).

CONCLUSION

In this experiment, we had undertaken some neem based products *viz.* nimbecidine, neem leaf extract, neem seed kernel powder and fresh cow dung suspension for controlling of bacterial blight of clusterbean. Results showed that seed treatment with fresh cow dung suspension @ 20 g/litre with 2 sprays of cow dung suspension @ 20 g /lit was found most effective in controlling the disease as compared to control where disease intensity was 28.30 per cent and yield was 6.04 q ha-1. This was followed by three sprays of nimbecidine (3 ml/l) where disease intensity was 31.20 per cent and yield was 5.75 q ha-1.

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Table 1: Effect of neem based products and cow dung suspension on bacterial blight intensity and grain yield of clusterbean

Treatment		Average disease intensity (%)	Yield (q ha-1)
T_1 :	Three sprays of nimbecidine (1.5 ml/l)	37.50 (37.76)	3.64
T_2 :	Three sprays of nimbecidine (3 ml/l)	31.20 (33.96)	5.75
T_3 :	Three sprays of neem leaf extract (3%)	32.70 (34.88)	4.98
T_4 :	Three sprays of neem seed kernel powder (5%)	35.00 (36.27)	4.64
T_5 :	Seed treatment with cow dung suspension @ 20 g/litre	46.30 (42.88)	3.38
T_6 :	Seed treatment with cow dung suspension @ 20 g/litre and 2 sprays of cow dung suspension @ 20 g/litre	28.30 (32.14)	6.04
T_7 :	Seed treatment with cow dung suspension (20 g/l) + neem leaf extract (30g/l)	40.00 (39.23)	3.61
T_8 :	Seed treatment with cow dung suspension (20 g/l) + neem leaf extract (30g/l) and one spray of neem leaf extract (30 g/l)	34.00 (35.67)	5.20
T_9 :	Control (without seed treatment and spray)	72.30 (58.24)	2.98
General Mean		(39.70) 39.00	4.47
S.Em ±		(0.87)	0.12
CD (P=0.05)		(2.60)	0.37
C.V. (%)		3.85	4.79

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RESEARCH ARTICLE

Effect of Newer Insecticides on Coccinellids Population in Cauliflower Ecosystem

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ABSTRACT

The effect of newer insecticides was tested on coccinellids at CSAUA&T, Kanpurduring *Rabi* season 2014-15 in cauliflower field. The highest mortality of *Coccinella septempunctata* and *Coccinella transversalis* was noticed in fipronil 5 SC @ 1.0 ml/litre plot followed by imidacloprid 17.8 SL @ 0.2 g/litre and neem oil 2% @ 2.0 ml/litre. The safest chemical was found chlorantriniliprole 18.5 SC @ 0.3 g/litre and flubendiamide 48 SC @ 0.3 ml/litre which was followed by spinosad 45 SC @ 0.5ml/litre and emamectin benzoate 5 SG @ 0.2 g/litre in cauliflower against *C. septempunctata* and *C. transversalis*.

Keywords: Cauliflower, Coccinellids, Insecticides.

INTRODUCTION

Cauliflower (*Brassica oleracea var. botrytis* L.) is one of the most important winter vegetable cole crops in our country. This is an important cruciferous vegetable subjected to attack by a large number of insect-pests throughout its growth phase, which act as limiting factor in the profitable cultivation of these crops. The insect pests viz., aphid (*Lipaphis erysimi* Kalt. and *Brevicoryne brassicae* L.), diamond back moth, (*Plutella xylostella* L.), cabbage borer (*Hellula undalis* Fabr.), cabbage looper (*Trichoplusia ni* Hb.), leaf webber (*Crocidolomia binotalis* Zell.), painted bug (*Bagrada cruciferarum* Kirk.) and cabbage butterfly (*Pieris brassicae* L.), tobbaco caterpillar (*Spodoptera litura* Fab.) etc., are having more significance on cauliflower which affect the yield and quality throughout the country.Natural enemies such as predators and parasitoids provide the essential ecosystem service of conservation through biological control (Gardiner *et al*., 2011).Coccinellids has attracted considerable attention as biological control agent because of its potential to control many soft-bodied insect pests particularly the aphids. The use of agrochemicals, particularly pesticides, can hamper the effectiveness of natural enemies; causing disruption of the ecosystem services they

provide (Desneux*et al*., 2007, Stark *et al*., 2007, Stavrinides and Mills, 2009). Natural enemies are highly sensitive to the application of pesticides, and it has been shown that pesticide use patterns influence the abundance and species composition of beneficial species in agro-ecosystems (Zhang *et al*., 2007, Lu *et al*., 2012).

New chemistry has replaced several broad spectrum pesticides due to their disruptive effects on various bio-control agents (BCA's) viz., parasitoids and predators. A greater emphasis is being laid on more selective new chemicals which is more compatible in conserving the wide diversity of BCA's. Substances with lower molecular weights have greater capacity to penetrate in the insect cuticle (Stock and Holloway, 1993).In this context, the efficacy of few newer insecticides viz., Flubendiamide, Chlorantriniliprole, Emamectin benzoate, Fipronil, Imidacloprid, Spinosad and Neem oil etc., were evaluated under field condition for their comparative effect on natural enemies.

MATERIALS AND METHODS

The present study was carried out to evaluate the effect on abundance of coccinellids population. The trail was conducted at Student Instructional Farm, Chandra Shekhar Azad university of Agriculture and Technology, Kanpur (UP) during *Rabi* season 2014-15 on cauliflower. The field experiment was laid out in RBD with 8 treatments including control (untreated check). Impact of insecticidal applications on the coccinellids population (*Coccinella septempunctata* and *Coccinella transversalis*) was recorded in the field. Coccinellids were recorded from randomly 5 selected plants in each plot and same expressed as numbers of coccinellid population/ 5 plants. All the leaves of each plant in a sampling area were observed to record the coccinellids population. Pre-treatment counts of coccinellids were taken one day prior in all the plots at each time just before the application of insecticides. Post-treatment counts of coccinellids were taken after 7^{th} and 15^{th} days of application of treatments. The average percent reduction over control coccinellid population was worked out for each treatment. The data obtained was corrected by using Abbott's formula (1925) and transformed (Gomez and Gomez, 1976). The data were then subjected to statistical analysis by using analysis of variance.The insecticides used are listed in Table 1.

RESULTS AND DISCUSSION

The effect of insecticides was assessed on the basis of population reduction of coccinellids at different intervals after their application during *Rabi*, 2014-15 the results are in (Table-1).The day before spraying (DBS) mean population of coccinellids varied from 4.27 to 4.97 per 5 plants without any significant difference. After application of insecticides it was observed that all the treatments were found significantly toxic over untreated control. The minimum population of coccinellids was recorded in the treatment of fipronil 5 SC @ 1.0 ml/litre which provided to 24.68 average percent reduction over untreated control, followed by imidacloprid 17.8 SL @ 0.2 g/litre and neem oil 2% @ 2.0 ml/litre which provided 22.15 and 19.70 average percent reduction over untreated control and was somewhat toxic and statistically the treatments were at par in their toxicity. The maximum population of coccinellids was recorded in the treatment of chlorantriniliprole 18.5 SC @ 0.3 g/litre with 7.74 average percent reductions over untreated control, followed by flubendiamide 48 SC @ 0.3 ml/litre, spinosad 45 SC @ 0.5ml/litre and emamectin benzoate 5 SG @ 0.2 g/litre which provided 8.81, 13.20 and 16.45 average percent reduction over untreated control, respectively. They were proved least toxic and at par with each other in their toxicity.

The results clearly indicated that most of the newer insecticides or bio-rational pesticides were safer to natural enemies than conventional insecticides. The present findings are in accordance to work done by Ameta and Bunkar (2007) who found that flubendiamide, indoxacarb and spinosad did not cause adverse effect on the population of natural enemies. Muthukumar*et al.,* (2007) also reported similar results which show that besides spinosad, biolep, and emamectin benzoate and neem oil proved safer to natural enemies in the cauliflower ecosystem. Venkateswarlu *et al.,* (2011) reported similar results had as neem baan, spinosad, emamectin benzoate and chlorantriniliprole were proved safer to natural enemies in cabbage. Jadhav and Shukla (2013) studied relative toxicity of insecticides and

found that imidacloprid 0.005% was least toxic to the larvae and adults of *Coccinella transversalis* (F.). Balikai and Mallapur (2015) also tested the safety of the new anthranillicdiamide group of insecticides to cocccinellid population.

CONCLUSION

From the ongoing discussion, it can be seen that to reduce risk, newer insecticides have been found more appropriate as compared to conventional broad spectrum high dose insecticides as they enables to conserve natural enemy fauna and minimize residual effect in vegetables and environment.

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Table 1. Effect of newer insecticides on abundance of coccinellid population in cauliflower during *Rabi*, 2014-15

No. of Coccinellid / 5 Plant												
		First spray						Second spray				
S. No.	Treatment	Dose	DBS	7 DAS	% Reduction over control	15 DAS	% Reduction over control	7 DAS	% Reduction over control	15 DAS	% Reduction over control	Average % Reduction over control
1	Fipronil 5 SC	1.0 ml/l	4.97 (2.34)*	3.77 (2.07)	27.12	3.90 (2.10)	26.54	3.03 (1.88)	23.67	3.07 (1.89)	21.37	24.68
2	Chlorantriniliprole 18.5 SC	0.3 g/l	4.53 (2.24)	4.17 (2.16)	11.67	4.47 (2.23)	7.83	4.23 (2.18)	6.99	4.27 (2.18)	4.48	7.74
3	Flubendiamide 48 SC	0.3 ml/l	4.57 (2.25)	4.13 (2.15)	13.02	4.43 (2.22)	9.18	4.17 (2.16)	7.77	4.20 (2.17)	5.26	8.81
4	Emamectin benzoate 5 SG	0.2 g/l	4.87 (2.32)	4.07 (2.14)	19.70	4.30 (2.19)	17.34	3.73 (2.06)	14.80	3.70 (2.05)	13.95	16.45
5	Neem oil 2%	2.0 ml/l	4.27 (2.18)	3.47 (1.99)	21.92	3.60 (2.02)	21.07	2.97 (1.86)	19.13	3.00 (1.87)	16.67	19.70
6	Imidacloprid 17.8 SL	0.2 g/l	4.53 (2.24)	3.60 (2.02)	23.68	3.67 (2.04)	24.34	2.93 (1.85)	21.49	2.97 (1.86)	19.09	22.15
7	Spinosad 45 SC	0.5 ml/l	4.83 (2.31)	4.23 (2.18)	15.83	4.40 (2.21)	14.84	3.97 (2.11)	11.53	3.93 (2.11)	10.61	13.20
8	Untreated control	-	4.93 (2.33)	5.13 (2.37)		5.27 (2.40)		5.37 (2.42)		5.27 (2.40)		
	SE (m) ±	-	0.019	0.018		0.017		0.021		0.014		
	CD (P=0.05)	-	0.059	0.055		0.052		0.065		0.042		

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REVIEW ARTICLE

Organic Meat: A Review

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ABSTRACT

Organic foods are produced without using of any synthetic substances and are free from pesticide and antibiotic residues. Organic foods include fruits, vegetables, milk, meat, spices, dry fruits, cereals, millets etc. Due to the increasing health problems, people are slowly getting attracted towards organic products as it is helpful for leading a healthy lifestyle. Many researchers observed lower fat and cholesterol contents, higher levels of n-3 fatty acids and CLA (Conjugated Linoleic Acid) in organic meat in comparison to the conventional meat. The production of organic meat largely depends on the availability of organic fodder and feed stuff. Currently, though India is among the top ten countries in the world in terms of cultivable land under organic certification, but we are facing constraints like lack of knowledge, scale of operation, traceability and zoonotic diseases. Apart from this, the different organic certification bodies have their own labeling guidelines which are sometimes difficult to comply with. In view of the growing demand for organic livestock products and increased opportunities in organic livestock farming, appropriate policies and plans should devised by government to strengthen the organic farmers by educating them and by announcing production incentives.

Keywords: Organic Meat, Organic Certification, Constraints, Policies.

INTRODUCTION

India stands first in production of milk, pulses, okra, ginger and safflower, fruits like mango, banana, lemon, papaya etc. and second in rice, wheat and black pepper production and third in egg production. However, production processes for the above food items utilize various chemical fertilizers, pesticides and insecticides, whose residues appear in the products. One Government report showed high levels of pesticides from 509 food samples such as veggies, fruits, spices, wheat, rice and other food items in 2013-14 (Economictimes, 2016). The levels were above the

maximum residue levels (MRL) prescribed by Food Safety and Standards Authority and by Codex Alimentarius. Presence of unapproved antibiotics, antifungal chemicals etc. can lead to alteration of microflora and resistance to these drugs. Residues of heavy metals and toxins like dioxins, polychlorobiphenyls (PCBs) etc. are powerful hormone-disrupting chemicals linked to diabetes, obesity, cancer and reproductive problems. Pesticide residues in food act as endocrine disruptors causing immunosuppression, reproductive abnormalities and cancers (Aktar *et al*., 2009).

India is also a major exporter of spices, fruits, vegetables, cereal grains and pulses in the world. There are around 60 different varieties of spices which include pepper, cardamom, ginger, chilli, turmeric, garlic etc. produced and exported by India. USA, Germany and Netherlands are the major countries to which Indian spices are exported and rejection of these spices is due to contamination of salmonella, pesticide residue etc. Even though India has exported 2,62,158 tonnes of fresh vegetables and 48,591 tonnes of fruits to UAE in the year 2015-16 (APEDA, 2016), from May 2016 onwards UAE has tightened the norms of importing chilli pepper, mangoes and cucumber from India, making it mandatory for Indian exporters to produce a pesticide residue analysis report for each consignment of shipping. UAE has communicated that "fruits and vegetables coming from India showed high levels of pesticide residues exceeding the permitted limits of in accordance with international standards adopted by Codex Alimentarius Commission" (Economictimes, 2016).In the year 2014, ten consignments of shrimps originating from Andhra Pradesh and shipped from Odisha were rejected by the European Union (EU), stating high levels of banned antibiotics like chloramphenicol and nitrofuran (http://www.business-standard.com/article/companies).

Food safety is an important issue discussed more strongly now than ever before. The consumers are extremely conscious about their health and want to consume safe and quality food. As a result, many consumers are seeking alternatives to conventionally produced agricultural and animal products and organically produced plant and livestock products are the best alternatives.

Organic Foods

Organic foods are the ones which are produced without making use of any chemical component in the form of preservatives, pesticides, insecticides etc or without making use of any synthetic substances. Organic food is produced according to standards that take into account environmental and animal welfare. They are produced using methods that do not involve the use of synthetic pesticides or chemical fertilizers, do not contain genetically modified organisms, and are not processed using irradiation or chemical food additives.

The major benefits of organic food are that they are safe, healthy to consume and contain high amount of nutrients. Organic tomatoes are 50% higher in vitamin C content than conventional tomatoes as reported by Aurelice *et al*. (2013) whereas, organic strawberries are higher in fiber and total phenols (Abu-Zahra *et al*., 2007). Eating organic fruits and vegetables increases our antioxidant intake by 20-40% when compared to non-organic or conventional food products (Organic-center, Oct 12, 2014). This is mainly because these foods are grown in healthier soil without adding any chemical fertilizers and no pesticides or insecticides are applied. The major types of organic foods are organic vegetables, fruits, fish, spices, milk, meat etc. Among these, vegetables and fruits are the most common and widely available organic food products in the market. The total area under organic vegetable production is 2,90,137 hectares which is 0.5% of the total area of vegetables grown in the world, 57 million hectares (FAOSTAT, 2013).

Organic Meat

Organic meat is defined as the meat obtained from the animals raised under organic management. The demand for ‘organic meat’ is greatly increasing in the developed countries and people are ready to buy this meat even at a higher price.Organic meat production is supposed to use ecological resources, such as natural grasslands and by-products with low alternative value together with fodder that is grown without artificial fertilisers and pesticides. Organic

animals are given the possibility of more natural behaviour, for example, they stay outdoors all year in nature and if housed, simple sheds are used. They are restricted minimally and are allowed to express and behave most naturally.

Advantages of Organic Meat

The most acceptable theory of Bovine Spongy Encephalopathy (BSE) outbreak in United Kingdom was transfer of 'scrapie' from sheep to cattle via meat and bone meal which was widely used as a source of protein in livestock feed (Bear, 1997; Watson and Redman, 1999). 'Organic meat production' helps in reducing potential health hazards by preventing the use of harmones, antibiotics and pesticides which have carcinogenic, immunosuppressive and teratogenic impact on human health (Lee *et al*., 2001). And prohibition of antibiotics use could result in reduced contamination of the organic meat by antibiotic resistant bacteria like *E. coli* 0157: H7 infection. Apart from this, strict use of antibiotics on individual sick animals would also result in reduced antibiotic residues (Redman and Holden, 1994). Thus, organically produced animal products have lower levels of residues of veterinary drugs and pesticides. The 'organic' label provides the assurance that no food ingredient is subjected to irradiation and that genetically modified organisms have been excluded (Kouba, 2001). In addition, the use of herbs and berries result in new meat products with novel and interesting flavour variants (Haugaard *et al*., 2014). Addition of nitrite into organic products is against the general organic principles and also it has carcinogenic effect. So, to avoid such adverse effects, natural herbs and berries were added as they have antimicrobial activity and imparts flavour to the organic products.

According to Benbrook *et al*. (2008) **p**oultry and livestock that consume animal feeds and pastures grown using organic methods produce meat, milk, and eggs that have modestly higher levels of protein, more of some vitamins and minerals, and elevated levels of heart-healthy n-3 fatty acids and CLA**.** Angood *et al*. (2008) compared the composition of fatty acids and organoleptic quality of organic and conventional lamb, offered on the British market and observed that organic meat had a higher content of n-3 polyunsaturated fatty acids and showed a better organoleptic quality in terms of juiciness, tastiness and general acceptability compared to the lamb available on the market. Kamihiro *et al*. (2015) also reported a higher level of conjugated linolenic acid, its precursor vaccenic acid and omega 3 fatty acids and a lower ratio of omega 6 to omega 3 fatty acids in meat of organically reared beef when compared to meat of conventional beef. Miotello *et al.* (2009) compared meat characteristics of veal from organic and conventional farms and observed significantly lower fat and cholesterol contents, higher content of n-3 acids, reduced n-6:n-3 ratio and higher conjugated linoleic acid (CLA) level in organically reared veal. Higher content of CLA in organic lamb, higher level of PUFA and lower content of saturated fatty acids in organic pork and lower level of thigh fat in organically reared poultry meat had also been reported (Pariza *et al*., 2001; Hansen et al., 2000; Nilzen et al., 2001 and Castellini *et al*., 2002). However, Husak *et al.* (2008) observed that organic chicken thighs were less tender and chewier than thighs of conventionally reared chicken.

Organic Food Production in India

India is one of the potential suppliers in the global organic market. Various categories of organic food products are exported under organic certification from India. India produced around 1.35 million MT (2015-16) of certified organic products which includes all varieties of food products namely sugarcane, oil seeds, cereals and millets, cotton, pulses, medicinal plants, tea, fruits, spices, dry fruits, vegetables, coffee etc. Not only food products, but products like organic cotton fibre, functional food products etc. are also produced in India. The total volume of export during 2015-16 was 263687 MT and **o**rganic products are exported to European Union, US, Canada, Switzerland, Korea, Australia, New Zealand, South East Asian countries, Middle East, South Africa etc.In the US, the National Organic Program (NOP), was enacted as federal legislation in October 2002 to regulate organic food production and certification is handled by state, non-profit and private agencies that have been approved by the US Department of Agriculture (USDA).

In India, National Standards for Organic Production developed by Ministry of Commerce and Industry, Government of India, provides guidelines for organic production. Currently, India ranks 10th in the world for having cultivable

land under organic certification. The certified area includes 15% cultivable area with 0.72 million Hectare and rest 85% (3.99 million Hectare) includes forest and wild area for collection of minor forest produces. The total area under organic certification was 4.72 million Hectares in 2013-14. Currently in India the largest area under organic certification is covered by Madhya Pradesh followed by Himachal Pradesh and Rajasthan (APEDA, 2016).

NSOP Regulations for Organic Livestock Production in India as Follows

Origin of Animals

All animals intended for final sale as organic meat or meat products must be born and raised on an organic farm. "Organic farming is a system which avoids or largely excludes the use of synthetic inputs (such as fertilizers, pesticides, hormones, feed additives etc) and to the maximum extent feasible rely upon crop rotations, crop residues, animal manures, off-farm organic waste, mineral grade rock additives and biological system of nutrient mobilization and plant protection".When organic livestock is not available, certification programme shall allow brought-in-conventional animals according to the following age limits.

- Two- day old chickens for meat production.
- 18- week old hens for egg production.
- Piglets up to six weeks and after weaning.
- Calves up to 4 weeks old that have received colostrum and have been fed mainly milk diet. Breeding stock may be brought-in from conventional farms but maximum replacement rate will be 10 percent.

Breeds and Breeding

- Breeds which are adapted to local conditions should be chosen.
- Reproduction techniques should be natural. Natural mating and AI with semen from conventional bulls are mostly followed.
- Embryo transfer techniques are not allowed.
- Hormonal treatments for inducing heat in female animals are not allowed.
- Uses of GMO (Genetically Modified Organisms) are not allowed.

Feeding

- The livestock should be fed 100% organically grown feed.
- More than 50% of the feed shall come from the farm unit itself or shall be produced within the region.
- However, in some cases 15-20% of total feed could be obtained from conventional farms.
- The use of synthetic growth promoter substance, synthetic appetizers, preservatives, artificial colouring agents, urea, animal byproducts to ruminants, solvent extracted oilcakes, pure amino acids, genetically engineered organisms or products thereof, are not allowed.

Animal Health

An important objective of organic livestock husbandry is the avoidance of reliance upon routine and/or prophylactic use of conventional veterinary medicines.

- Natural medicines and methods, including homeopathy, ayurvedic medicine and acupuncture, shall be emphasized.
- The use of conventional veterinary medicines are allowed when no other non-allopathic alternative is available and where these are used, the withholding period shall be twice the legally required period.
- Vaccines shall be used only when diseases are known or expected to be a problem in the region of the farm and where these diseases cannot be controlled by other management techniques.
  However, genetically engineered vaccines are prohibited. Apart from the above-mentioned standards, there are several other standards concerning mutilation, record keeping, transport and slaughter.

Organic Certification

Organic certification is necessary for maintaining the quality and safety of the organic products before dispatching them to the market. Organic labeling could also give safe feeling to the consumers while purchasing the product. Products completely prepared with certified organic ingredients can be labeled as "100% organic". Products developed with 95% of organic ingredients can be labeled using the word "organic". A label having the word "made with organic ingredients" can be used when the product contains minimum 70% of organic ingredients in it. Apart from this, logo of the certification body may display or printed on the labeled product. Finally products having less than 70% organic ingredients cannot allow in the market. Some of the organic foods certifying bodies are mentioned below:

i. Codex Alimentarius Commission
ii. International Federation of Organic Agriculture Movement (IFOAM)
iii. APEDA- India and the certification logo is shown in Figure 1.
iv. USDA'S NationalOrganic Programme (NOP) –further devided into 4 classes as follows;100% organic, 95% organic, 70-75% organic and less than 70% organic.

Constraints in Organic Food Production

- ***Lack of knowledge*** - Majority of the farmers in the world are illiterate and are less aware regarding organic production practices, organic standards, organic certification procedure and organic marketing. Most of the information on organic farming is available through internet But, farmers are least exposed to such electronic media.
- ***Small farms -*** In tropical countries, especially in Asia and Africa, smallscale farmers depend on livestock production for their livelihood. However, the landless animal husbandry system, which is common in India, is not allowed under organic systems of livestock production. Small farms are generally not suitable for the development of organic livestock production, especially for exports.
- ***Problems in livestock feeding -*** In organic farming animals should be raised on pasture but, fodder scarcity is one of the most drawbacks in livestock farming. During summer, there is no enough supply of water and farmers are unable to produce required quantity of fodder. An important requirement of the EU regulation is that: 'animals must obtain a minimum of 30% dry matter intake from grazing pasture during the grazing season'
- ***Sanitary regulations -*** Governments of tropical countries are more concentrated towards the production of highly sanitized organic products by following the guidelines from good manufacturing practices(GMP), hazard analysis and critical control points(HACCP), International Organization for Standardization(ISO) certification etc. But, these practices are not followed in all organic sectors. Strong efforts are needed to establish disease control measures, mainly during the production, processing and packaging stages. So, government should help the organic farmers to implement these hygienic practices at the rural areas too.
- ***Traceability -*** This means identifying the original source of the organic product which is being marketed. As there are numerous small organic farm sources, traceability of product is difficult in developing countries. Traceability tools that are both cost effective and suitable for mixed farming conditions in tropical countries need to be developed.
- ***Existence of Diseases -*** Prevalence of infectious/zoonotic diseases also adversely affects trade in livestock products. In case of organic livestock production, animals must be free from diseases like FMD, swine fever and Rift valley fever. Products from such diseased animals are rejected during exportation.

Opportunities for Organic Farming

- Demand for organic livestock products is growing in the USA, EU, Japan, Argentina and Brazil.
- EU is a major importer of organic beef, sheep and goat meat from African countries like Namibia
- Consumers are ready to pay a large price premium for organic food in Austria, Belgium, Germany and UK.
- In 2001, 40% of beef and 16% of broiler meat imported into the UK came from developing countries, such as Argentina and Brazil who export organic livestock products to the EU.

CONCLUSION

In the present scenario, people are more attracted towards organic food including vegetables, fruits, spices, cereals, milk, eggs and meat products as they do not have problems of pesticide or antibiotic residues. The price for organic food products is more than conventionally produced products. However, consumers due to the awareness of the dangers of antibiotic and pesticide residues are willing to pay higher price for safe and good quality food products. Hence the demand for organic foods is more and we have to take steps to increase production of organic foods and for efficient marketing of these. By adopting the organic farming, animals could get freedom from hunger & thirst, discomfort, pain, injury or disease and fear. And the meat obtained from such organically raised animals may be of good or high quality. Access to open pasture is one of the major criterions for organic meat production. Rearing of different livestock species practiced in order to dilute the disease challenge to susceptible stock.

India is leading in buffalo meat export and the meat produced in India is lean and considered almost organic since most of the meat is procured from animals reared on grass, and not fed on antibiotics or growth promoters. Not only for local market, but also for export purposes, farmers should be assisted in rearing animals, especially, buffaloes, sheep and goats in an organic way so that their meat can be sold at a higher price. Supermarkets are the places where more than half of the organic meats get sold out in the world. The farmers should be educated about organic farming practices, certification and marketing of their produce so that better economic returns can be achieved. There are farmers at the village level who are rearing different species of animals for their daily income and such farmers should be given training on organic livestock farming for the more production of organic meat. Apart from this, the Government should recognize such organic farms and support them economically. Thus, the demand of organic meat from the market could be met and helps in extension of organic meat production.

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Figure 1. APEDA- India and the certification logo

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RESEARCH ARTICLE

Variability in Seeds of *Argania spinosa* according to the Shape and the Geographic Origin of the Fruit

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ABSTRACT

The fruit of *Argania spinosa* (Sapotaceae) is naturally characterized by three main forms: fusiform, round and oval. The fruit seed contains one to three kernels used in the extraction of argan oil renowned for its nutritional and cosmetic virtues. In order to characterize the phenotypic variability of the argan tree fruit in the Southwest of Morocco, a comparative study based on the shape and the geographical origin was carried out. Six characters were measured on 1,020 fruits: weight, length and width of the seed, kernel weight, number of kernels per carpel and seed. The results showed very highly significant differences in terms of both the shape and the area of origin. There were marked differences between all phenotypes especially almonds level weight, seed weight and kernel weight compared to the weight of the seed. Indeed, the fruits of the Amskroud region contained seeds and almonds with the largest average in weight and size. Moreover, the ratio of almond weight / seed weight, considered as criterion of argan oil production, was significantly higher in the Smimou region compared with the other provenances. Furthermore, the number and weight of kernels are significantly larger in the oval fruit and smaller in those rounded. The correlation coefficients showed that the weight was positively correlated with the characters related to the dimensions of the seed, the number and the weight of kernel. The width of the seeds was positively correlated with the number and the weight of the kernel. The results also revealed a large morphometric variability of the argan tree fruit under different environments. They would allow a combined improvement of different traits in order to select the most interesting morphotypes for the domestication and the conservation of the argan tree.

Keywords: *Argania spinosa*, Fruit, Region, Shape, Variability.

INTRODUCTION

The argan tree, *Argania spinosa* L. (Skeels) naturally occupies vast areas of the southwest of Morocco including arid, semi-arid and Saharan regions. The altitude ranges from sea level to 1700 meters on the Anti-Atlas and the southern slope of the western High Atlas [1].Annual rainfall of less than 100 mm in the Saharan areas, reaches 400 mm in semi-arid regions [2]. Concerning temperature, the argan tree can withstand hot periods (over 40 ° C) and cold periods whose the minimum values are sometimes less than - 2.6 ° C in winter [2]. In addition to its ecological role (barrier against desertification, soil protection, diversity of fauna and flora that it houses), the argan tree is of great economic importance to the rural populations of southern Morocco. Indeed, the harvested fruit is used for the extraction of argan oil from almonds that it contains [2].The argan oil has a specific chemical composition and has a growing interest in nutrition and cosmetics [3, 4]. Dried pulp of fruit and cake resulting from the extraction of oil, are used as feed for livestock [5].

Despite its environmental and socio-economic interest, the argan forest is in clear regression because of territory planning, land use and overgrazing. The reforestation programs and domestication of the argan tree requires the selection of performing trees especially regarding oil production. In fact, previous studies reported significant differences of the extraction yield and chemical composition of the oil [6,7].These variations may be due to some factors, mainly genetic variability and geographical origin. Thus, the literature reported the existence of six different kinds of fruit; fusiform, round, oval, oval apiculate, drop and spherical with a clear dominance of the first three forms [8,9]. The changes of temperature and rainfall had a great impact on flowering, fruiting and yield of fruit trees and variability between trees regarding the percentage of the different components of the fruit such as seed, nuts, almonds and oil content [6,8,10]. However, these studies concerned geographically limited areas and did not emphasize the combined effects of fruit shape and environment on the characteristics of the different compounds of the seed, especially those related to oil extraction yield. Our work is in this context and assigns the objective of studying the biometric characteristics variations of the argan fruit components, considering the three main forms (fusiform, oval and round) and six geographical regions with different climates.

MATERIALS AND METHODS

Plant Material

Fruit sampling was conducted in six locations of the southwest of Morocco: Aday, Admine, Argana, Amskroud, Smimou and Tamanar (Figure 1 and Table 1). The regions extend mainly over the provinces of Essaouira, Agadir, Taroudant and Tiznit. The climate of these regions is semi-arid to arid, influenced by the relief, the ocean coast and the Sahara. The degree of aridity increases from west to east. The altitude varies from 23 to 1132 m, the average temperature from 13.4 to 18 °C and annual rainfall from 145 to 445 mm.For each location, the fruits were picked at random on the floor under ten adult trees. Once in the laboratory, the fruits of each locality were dried and pulped to keep the seeds. These were divided into three lots according to their form: Fusiform, oval and round (Figure 2). Thereafter, the weight (SW), the length (Lg) and the width (Swi) of each seed was measured and the Lg /Swi ratio was calculated. The seed was then crushed to determine the number of carpels (CN) and almonds (AN). These were also weighed to determine their weight (AW) and AW/SW ratio.

Statistical Analysis

All statistical analyses of the data were carried out using Statistica version 8.0 (StatSoft, Inc. 2007). Analysis of variance was performed using the general linear model (GLM) after testing for normality and homogeneity of variance. Differences between means were determined using Duncan's and Newman-Keuls post hoc tests. Correlation analyses were conducted using Pearson correlation coefficient to determine the relationship among all the traits.The following statistical model (GLM) was employed to analyse the data:

$Y_{ij} = m + A_i + B_j + E_{ij}$

Y_{ij}, m, A_i, B_j and E_{ij} represented respectively the observed value, the mean value, the effect of provenance, the effect of seed shape and the random error.

RESULTS AND DISCUSSION

The descriptive statistical analysis of the quantified parameters (mean, standard deviation, coefficient of variation) made on 1020 fruits is presented in Table 2. The fruits coming from different localities (Aday, Admine, Argana, Amskroud, Smimou and Tamanar) were characterized by three distinct forms (fusiform, round and oval). The results showed that the morphological traits measured in these fruits namely length of the seed (SL), width of the seed (Swi), seed weight (SW), number of carpels per seed (CN), number of almonds per seed (AN) and kernel weight (AW) exhibited significant variations depending on the specific character. The values of the coefficient of variation (CV) are the lowest in the order of 16 and 17.62 % respectively for the character Swi and the SL/Swi ratio. In contrast, the highest values were noted for the characters AW and SW with 41.67 and 38.99 % respectively. In addition, the SW showed large variations and ranged from 0.70 to 6.88 g with an average value equal to 2.52 g. The SL varied from 1.05 to 3.90 mm with a mean value of 2.28 mm.The analysis of variance using the general linear model (Table 3) revealed very highly significant differences between the six regions for all measured variables ($P < 0.001$). Similarly, very highly significant differences were observed in the form of fruit (fusiform, oval and round) for SW, SL and Swi. A significant difference was noted for the SL/Swi ratio. This shows that the characters related to the dimensions of the seeds (length, width and weight) vary significantly depending on the fruit form and provenance.

The analysis of seed and almond parameters are reported in Tables 4 and 5. The results showed very highly significant differences ($p < 0.001$) among provenances and between shapes for all the characters involved. Indeed, the average values of SW ranged from 1.76 at Smimou to 3.65 at Amskroud. The SL was maximal at Amskroud (2.80) and the lowest value was noted at Admine (1.94). The average of the SW was between 1.07 at Smimou and 1.26 at Amskroud. The values of AN per seed ranged from 1.01 at Tamanar to 1.15 at Amskroud and Admine. Similarly, the SL/Swi ratio exhibited very highly significant differences between provenances. The highest values were observed at Tamanar (2.23) and the lowest in Admine samples (1.63). The values of the AW showed very highly significant differences between Amskroud, Argana and Aday, while the average was not significantly different between Aday, Admine, Smimou and Tamanar. The largest mean was observed in Amskroud (0.35) and the lowest in Aday (0.24). These results showed that the fruits collected in the region of Amskroud presented the highest average values and this for all the measured characters. In fact, the climate of this region is characterized by an annual average of greater precipitation (445 mm) and low average annual temperatures (13.4°C) compared to those of the other sources (Table 1).

Concerning the relationship between the weight of almonds and seeds (AW/SW), the analysis of variance showed that this character presented highly significant variations between provenances and shapes. It was highest at Smimou (0.147) followed by Argana (0.121), Admine (0.110) and Aday (0.104). The lowest values were noted for Amskroud samples. In this regard, studies conducted previously on the argan tree showed that the oil production yield increased depending on this ratio. Therefore, this ratio was considered as an important criterion in the evaluation of oil production [6, 11]. Thus, according to our study area, Smimou seeds would produce most oil compared to the other provenances. Similarly, Amskroud and Tamanar sites would be less productive even if the seeds from these regions had significantly higher weight. Increasingly, oval fruits presented the highest AW/SW, followed by those of round shape. These results constitute new data on argan tree and complete the observations reported in the literature on the interactions between the plant species genotype and the environment. The observed variations in morphometric characters of fruits between provenances were probably due to the differences in specific ecological and climatic factors in each region (soil type, altitude and humidity.). In this regard, the literature had extensively documented the many possible effects of the environment on the variability of the characteristics of the plant species fruits [12, 13, 14, 15]. For oilseeds such as canola, olive, sunflower and soya, variations in the quality of fruit (size and

weight) and overall productivity of crops (oil yields) were attributed mainly to the environment and its interaction with the genotypic factor [16,17,18].

In the case of the argan tree, previous studies reported that the variability in growth, fruiting and proportion of mature fruit, was related to the combined effects of the geographical location, climatic year and genotype of trees [10, 19]. Other works showed that production in fruits and oil from the argan tree was strongly influenced by climatic factors of the regions [20,21]. In fact, the productivity of the argan oil depends on both the relative AW/SW and fruit productivity in different individual argan trees. Our work showed that rainfall permitted fruits growth without improving argan oil productivity that may be more influenced by genotypic factors. This data could be a basis for the selection of candidates for domestication of the argan tree, or the reforestation of areas experiencing severe degradation of the species due to overexploitation.The biometric analysis also demonstrated the existence of high variability ($p < 0.001$) based on fruit shape (Table 5) and this for all measured characters (SW, SL, SWi, AN, CN and AW). The SL / Swi ratio also showed very significant differences depending on the shape of the fruit, thereby confirming the existence of three distinct forms: Fuiform, oval and round. The average of the highest ratio was noted for the fusiform shape (2.09) and the lowest for the rounded form (1.76). These results were in agreement with the literature data [8, 22], and were explained by a high polymorphism rate that support the different fruit morphotypes [19, 23, 24].

The correlation coefficients (table 6) showed that the weight was positively correlated with the characters related to the dimensions of the seed ($r = 0.744$ ***), followed by those in relation to the AN and the AW ($r = 0.638$ ***). This indicated that the largest seeds were those longer and wider. They were also the ones that contained the largest almonds. Furthermore, the Swi was positively correlated with the AN ($r = 0.319$ ***) and the SW ($r = 0.66$ ***). This showed that the large almonds are contained in the widest seeds. In contrast, lower correlations are recorded between the SL and the CN ($r = 0.006$), and between the SL and the AN ($r = 0.075$*), showing that the longest seeds were the least rich in almonds. These results would allow a combined improvement of different traits in order to select the most interesting morphotypes for agronomy and genetic variability. Therefore, they could be considered for domestication and conservation of the argan tree.

CONCLUSION

Our study showed that all the quantitative traits measured in seeds and almonds had very highly significant variations depending on the source and fruit shape. This reflects the existence of a great diversity of the *Argania spinosa* species in the southwest of Morocco. The characters that have recorded the greatest variability were the SW, the AW and AW / SW ratio. The results showed that the fruits from Amskroud contained seeds and almonds with the best average weight and the best dimensions. However, the AW / SW average ratio was relatively low in this region but high in fruits of Smimou. Since this parameter was positively correlated with oil yield, the seeds of the Smimou region will be most interesting for the production of argan oil, despite the small size of the fruits. The results also showed that the majority of the traits were positively correlated to each other. This would allow a combined improvement in order to select the most interesting morphotypes.

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Table 1. Climatic conditions of the different provenances of *Argania spinosa* fruits

Locality	Climate	Altitude	Temperature*	Rainfall *
Aday (1)	Upper aride with temperate winter	1055 m	18 °C	145 mm
Admine (2)	Medium Arid with warm winter	23 m	14.1 °C	393 mm
Argana (3)	Lower arid with cold winter	1132 m	15.8 °C	320 mm
Amskroud (4)	Upper arid with temperate winter	298 m	13.4 °C	445 mm
Smimou (5)	Upper arid with temperate winter	332 m	16.5 °C	302 mm
Tamanar 6)	Upper arid with temperate winter	332 m	16.6 °C	297 mm

*Annual average

Table 2. Descriptive statistics of seed and almond traits

	N	Mean	Min	Max	SD	CV (%)
SW	1020	2.52	0.70	6.88	0.98	38.99
SL	1020	2.28	1.05	3.90	0.47	20.83
Swi	1020	1.15	0.70	1.76	0.18	16
SL / Swi	1020	1.99	0.33	3.11	0.35	17.62
CN	1020	1.97	1	3	0.52	26.52
AN	1020	1.11	1	3	0.33	29.61
AW	1020	0.27	0.08	1.01	0.11	41.67

SD: Standard deviation, CV: Coefficient of vriability. SW: Seed weight, SL: Seed length, Swi: Seed width, CN: Carpel number, AN: Almond number, AW: Almond weight. Min: minimal value, Max: maximal value.

Table 3. Results of analysis of variance (GLM)

		SW	SL	Swi	SL/Swi	CN	AN	AW
Source	DF	F	F	F	F	F	F	F
Provenances	5	296.71 ***	4406.56 ***	177.57 ***	61.63 ***	5.46 ***	50.67 ***	263.45 ***
Shapes	2	710.90 ***	1178.13 ***	741.72 ***	45.73 ***	12.17 ***	153.69 ***	50.80 ***
Error	1012							
Total	1019							

SW: Seed weight, SL: Seed length, Swi: Seed width, CN: Carpel number per seed, AN: Almond number per seed, AW: almond weight. *** Significant difference at $p < 0.001$.

Table 4. Variation of seeds traits according to the provenance (X ± SD)

Provenances	SW	SL	Swi	SL/ Swi	CN	AN	AW	AW/SW
Aday	2.27 ± 0.36 b	2.53 ± 0.16 d	1.09 ± 0.09 ab	2.31 ± 0.28 e	1.68 ± 0.51 a	1.10 ± 0.30 ab	0.24 ± 0.06 a	0.104 ± 0.016 bc
Admine	2.54 ± 1.10c	1.94 ± 0.5 a	1.18 ± 0.24 c	1.63 ± 0.19 a	2.15 ± 0.41 b	1.15 ± 0.38 b	0.26 ± 0.14 a	0.110 ± 0.040 c
Amskroud	3.65 ± 0.93 e	2.80 ± 0.41 e	1.26 ± 0.12 d	2.22 ± 0.25 d	2.31 ± 0.46 c	1.15 ± 0.40 b	0.35 ± 0.10 c	0.121 ± 0.024 d
Argana	2.53± 1.20 c	2.29 ± 0.44 c	1.14 ± 0.27 b	2.24 ± 0.18 c	2.13 ± 0.37 b	1.09 ± 0.28 ab	0.30 ± 0.15 b	0.098 ±0.024 b
Smimou	1.76 ± 0.40 a	2.08 ± 0.19 b	1.07 ± 0.10 a	1.95 ± 0.23 b	1.62 ± 0.57 a	1.12 ± 0.32 b	0.26 ± 0.08 a	0.147 ± 0.029 e
Tamanar	2.82 ± 0.31 d	2.52 ± 0.19 d	1.13 ± 0.08 b	2.23 ± 0.19 d	2.06 ± 0.31 b	1.01± 0.10 a	0.25 ± 0.03 a	0.088 ± 0.009 a

SW: seed weight, SL: seed length, Swi: seed width, CN: carpels number per seed, AN: almonds number per seed, AW : almond weight. Different letters indicate significant differences between provenances at $p < 0.001$.

Table 5. Variation of seeds traits according to the fruit shape (X ± SD)

Shapes	SW	SL	Swi	SL/ Swi	CN	AN	AW	AW/SW
Fusiform	2.64 ± 0.65 b	2.30 ± 0.28 b	1.16 ± 0.14 b	2.09 ± 0.40 c	2.05 ± 0.63 b	1.16 ± 0.39 c	0.30 ± 0.13 c	0.063 ± 0.014 a
Oval	2.79 ± 1.04 c	2.44 ± 0.39 c	1.20 ± 0.17 c	1.98 ± 0.30 b	1.90 ± 0.48 a	1.10 ± 0.30 b	0.27 ± 0.10 b	0.141 ± 0.028 c
Round	1.25 ± 0.32 a	1.47 ± 0.29 a	0.89 ± 0.10 a	1.76 ± 0.22 a	2.03 ± 0.22 b	1.04 ± 0.20 a	0.17 ± 0.05 a	0.122 ± 0.029 b

SW: seed weight, SL: seed length, Swi: seed width, CN: number of carpels per seed, AN: number of almonds per seed, AW : almond weight. Different letters indicate significant differences between shapes at $p < 0.001$

Table 6. Correlations between seed traits

	SW	SL	Swi	SL/Swi	CN	AN
SL	0.744 (0.000)					
Swi	0.868 (0.000)	0.597 (0.000)				
SL/Swi	0.080 (0.010)	0.444 (0.000)	-0.081 (0.010)			
CN	0.349 (0.000)	0.05 (0.860)	0.241 (0.000)	-0.094 (0.003)		
AN	0.319 (0.000)	0.075 (0.016)	0.367 (0.000)	-0.028 (0.372)	0.210 (0.000)	
AW	0.662 (0.000)	0.363 (0.000)	0.603 (0.000)	0.088 (0.005)	0.337 (0.000)	0.638 (0.000)

The values correspond to correlation coefficient (Pearson Correlation) followed by (p-value).
SW: seed weight, SL: seed length, Swi: seed width, CN: number of carpels per seed, AN: number of almonds per seed, AW: almond weight.

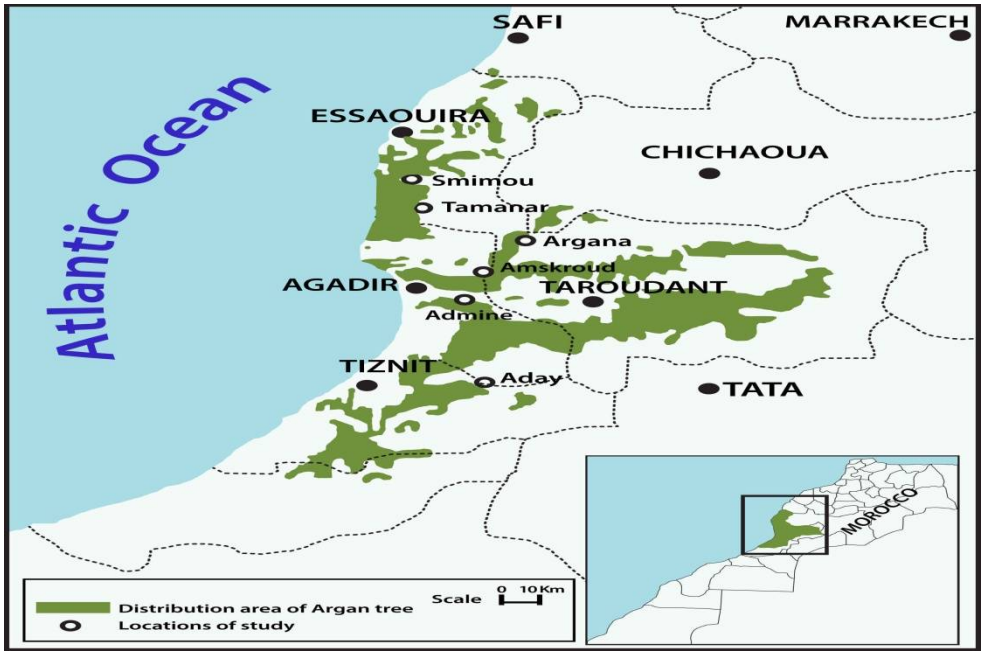

Figure 1. Geographical position of the studied areas of Argan forest

From north to south: Smimou, Tamanar, Argana, Amskroud, Admine, Aday.

Figure 2. Seeds of *Argania spinosa* from fruits of different shape.

1: Fusiform, 2: oval, 3: Round.

RESEARCH ARTICLE

The Rot-Ite Model-Emerging Green Techniques with Airborne Wind Energy

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ABSTRACT

The paper represents the various steps of Green Architecture with newer techniques to develop the concept of existing windmills with various elements which is to be used to implement the proposed idea in real life. Various new methods using the concept of Airborne and many techniques are introduced to improve the concept which is yet to be used and implemented for our benefits. With much of the non-renewable resources being used with the term “industrialization” the world today needs Green Techniques to be implemented, so as to reduce the risk of the environmental factors. In this paper, raw facts and data are collected to support each proposed system or material for the best cause, and proper detailed structures have also been proposed. Day by day due to urbanization, we need alternative ways to meet our demands otherwise there will be a time when there will be no growth and all the developments will stop due to shortage of resources. Detailed analysis of the proposed Rot-Ite Model has been and several components of the existing airborne model are researched to bring a new executive model for the best use.

Keywords: Green Energy; Green Architecture, Wind Energy, Energy Management, Airborne Technology, Sustainable Architecture**.**

INTRODUCTION

This paper is based on techniques of Green Architecture which is the need of the hour these days. The idea has put forward many promises in our world which would possibly make it better and provide a sustainable environment for us to live in. Lots of work has been done in the computing domain with little or fewer emphasis on the architectural field which is the matter of concern due to a number of environmental issues. Factors like population growth and high energy requirements are the key points in bringing up this idea into play.The study in the field of

Green Computing has been going on since the last decade while the Green Architecture concept is rather new. A good number of countries have taken steps to understand the need and formed organizations and committees to meet the requirements, some of them are LEED and IGBC.These organizations setup up some guidelines and standards for the Green Buildings which are to be followed by some lag specific detail.

The entire process starting from the manufacturing of the raw materials, planning of the building model, starting of the construction, execution of the model, processing of the construction, to the final maintenance of the structure the entire process if sustainable then only the building is called a Green Sustainable Structure. The benefits of such Green concepts are diverse and long turn, such as improved Bio-diversity, conservation of re-sources of future use and mainly causing less pollution to the environment. Uses of cheap, reusable materials are very much effective in this cause and can therefore make the environment safe and sustainable. Now due to the Global Warming the increase in CO2 in the atmosphere caused a major dis-balance in the entire eco-system so there is the need to reduce the use of carbon emitting installations and devices or else capture it by Carbon sequestration i.e. the method capture carbon emitted in the atmosphere by processes like Artificial Aquifers, Ocean Lifting Tanks or other Carbon sink tanks. The wind energy is very crucial for the development of any nation natural resource stock. Therefore, our research will provide a model which will harness wind at a mch higher level than earlier.

Specified Installation of Rooftop Turbine

Among all the resources which are renewable in nature wind energy is the least harnessed with Atomic Energy. The total wind energy, produced by the wind turbines which are rated between 1 – 2.9 Mega Watts, generally is estimated to be nearly 6 GW onshore and about 3.5 GW offshore in a country like the India (2011). The Energy Development Board of India (EDBI) estimated that the total wind energy harnessed is not also half-quarter of the total Energy required every year. The major problem with wind turbines is the efficiency which is greatly affected due to Turbulent Wind Flow (TWF). To manage this Rooftops can use Wind Tunnel based Turbines wherein the aerodynamic factors are much stable and suitable for wind Turbines. This would greatly reduce the effect of turbulent wind and will also help to make the wind speed constant. Now it is inefficient to place a wind pipe where the wind speed is less than 4.5-5 m/s as Wind Efficiency is Directly Proportional to the speed of the wind which is quite a concern for us as if the wind is unstable then the efficiency will decrease.

Boundary-Planet Layer Problem

The wind flow at the bottom level is very less and turbulent because of the presence of high towers, buildings and other installations which block the wind flow thus reducing the capacity of harnessing the wind energy. So, the wind flowing in the ground level is affected by the Boundary-Planet Layer Effect which decreases the efficiency of any wind mills present in that layer. The layer is about roughly hundreds to 2-3 K.M in height and depends upon several terrestrial factors and sub-tropical conditions and this region is known as “Critical Region of Efficiency” (CRE).

MATERIALS AND METHODS

The Introduction of Airborne Technology

In today’s technology, there are three dominating type of models available for Airborne Technology specifically the ball-rotor, the kite and the fly-balloon. Every approach is in innovative stage and much of research and development is required to fully harness such technologies. All of the technologies share some common advantages over the primitive ways of harnessing wind energy i.e. the Crust-Based Wind Mills but still we have to build effective designs so to fully utilize such valuable renewable resource.This kinds of devices are useful in the sense as they do not cause any harm to the flying birds which the traditional windmills do. Also, the noise factor is negligible in this case and thus this system will be acceptable for humans as they will not be interfered.A featuring advantage of Airborne architecture would be that mobility of the energy harnessing device i.e. As there is no need of bulk and tall tower

installments these airborne devices can be implemented in many temporary areas, having low impact in the surroundings.

Ball-Rotor Model

The Ball-rotor Model is almost similar to the primitive methods of Windmills like HAWts or VAWts. The technique that distinguishes this Model is that it has multiple high rotating devices which are made up in the high level and to the highest winds. The wind that flows in toe high altitudes gives a rotational Torque to the device and in turn produces the devices power. As for nature of the wind, it also provides and thrust to the device, which actually pushes the device into the de-sired level.The major disadvantage of this Model is that this de-vice needs the Generator to be connected with it so that the Model can transmit power through Cable. The acting tether which is used must be light-weight and at the same time provide support to overcome the disturbances made by the device at high altitudes and by the mass of themselves. Now these wires or cable must provide enough conductivity to bring the generated power to the ground station, and this can be tough job but many commercial organizations and NASA itself is researching upon how to improve the cables.The advantage of this kind of AWE system is that it can manage disturbance or turbulence perfectly. Massive disturbances are simply made resistive by small changes within the cable. The device additionally generates an incredible quantity of thrust that's directly proportional to the square of the wind speed, thus these characteristics of the model helps it to reach highest altitudes and levels.

Fly-Kite Model

This Fly-kite thought model usually uses a kite connected with cables to the ground level operator which are directly connected to the generators. This model uses sweep chopping motions to tackle across the wind and thus creating mechanical forces and these in turn generates power by the Turing the generator across.As the Fly-Kite model is very simple and easy to de-sign the materials can be easily made available to use. This system includes a safety factor as there is no heavy device falling off from the heights. Now, the action area of this model is large and therefore it gets enough sweeping space for the kite as it is not restricted to the dimensions of the Motor like in Ball-Rotor model.The main power in this model is generated by pulling the high-end cables by any mechanical device placed at the ground. The power generated can engulf a loss as the cable total weight add extra mass to the model design and can disturb the process of power generation if the device rises up at a level such as very high altitudes. Therefore, this System has a Critical Operating point. The COP varies from place to place and has several factors like Air density, air Stability, temperature etc.

Balloon Concept

A balloon like structure is used in the Balloon Model which is filled up with Helium gas and has flaps to draw the wind and hence generate power. On every sur-face of the model there is a generator, placed in between the device and the long cables. The winds blowing with high speed hit the device causing the upper part the flaps to hold the wind flowing and the lower end of the device to get flatten. This creates a large drag in the up-per surface and a low drag in the bottom surface which in turn engulfs a torque initiating a rotational motion in the device along the horizontal direction.Thus, the high drag on top, along with the low drag on the bottom, causes the rotational movement. This is a drag type machine does not use lift forces like a rotor and kite type systems, but uses only the drag forces of the wind for rotation.

The major drawback of this system is that it is only about half as efficient at obtaining power from the wind as a rotor-type system that can utilize both lift and drag. As compared to ground-based wind power generation reduction in efficiency, is expected to be more than offset by the increased wind speeds and consistency of the wind at the higher altitudes that it could reach. The overall cost for energy production per KPHT to be less than the cost of energy from the ground-based systems.One challenge faced is that the lift force available is dependent greatly on the size of the balloon. This means that, to reach great heights, jet streams with the highest wind speeds, the balloon would need to be quite large to overcome the weight of the tether.Another challenge is that a very large amount of

helium is required in the balloon system. This helium is a very important portion of the startup and operating costs. Over time helium tends to leak as well. But if the mem-brane of the balloon is designed well, the helium leakage rate can be kept down to about 6% per annum.

Proposed Rot-Ite Model

Introduction and Theory

The proposed Model comprises of the Rotor and the Kite Model combined together to give high stability and insurance.The model includes the fly-kite with the blades of the rotor or rather improved and Optimized Blades attached to the front end which generate electricity as the model floats the sky gaining rotational motion and thrust.Since this model is a clone type model it overcomes the disadvantages of both the fly-kite and the ball-rotor model and thus gives significant advantages over the other. Since this model is light-weight as compared to the ball-rotor, we do not have the fear of falling such heavy devices from high altitudes and damaging public property. Also, we will use a hybrid addition of insulated conductors of aluminum or silicon combined with fibers engulfed with Kevlar- type materials. Research has shown that these types of fibers can lift up to six cars as compared to the primitive steel cables which showed only three.

Components of the Model

Generators and Motors

The main components of the AWE rotor system need to be understood well so that it can be designed successfully. Generators and electric motors are essentially the same: the difference being, energy is put into the system and power is drawn from the device running it as a motor, while a mechanical power source drives the system to run it as a generator.There are two main kinds used in wind power applications—synchronous and induction generators. The key equations governing the performance of induction generators are:

$n=60f\div(p/2)$

$S = (Ns-n)\ Ns$

where,n is the rotational speed of the generator
s is the slip of the generator
Ns is the synchronous speed of the generator

If the speed of the generator is slower than the synchronous speed, then the generator operates as a motor.

$Ngen=Pgout/Pgin$

But, $Pgin=Pgout-Pmech\ loss$
where,f is the frequency of alternating current.

It is totally dependent upon the designer and the user the number of poles that the induction generator will have. The efficiency (ηgen) of the generator is:

$\eta gen= Pgout / Prout$

where,
Pgout is the electrical power output from the generator
Prout is the mechanical power supplied to the generator by the rotor.

The mechanical losses experienced in the shaft and in the gearbox between the rotor and the generator are represented by the mechanical loss (Pmech loss). Considering this information, will allow selection of a generator appropriate for an AWE design.

Mechanical Rotor

Another important component of an AWE rotor system is the Rotor. Rotors are similar in design to propellers, the only difference being, that the rotor is designed to convert the energy in the wind to mechanical, rotational energy, while the reverse is done by a propeller. Rotors are essentially airfoils that operate rotationally; thus, all of the same principles of lift and other aerodynamic forces that can be applied to wings are applicable to rotors.

Pwind=1/2pAU3

Pout=1/2pAU34a(1-a)

Nout=Pwind/Pout

Rotor power out (Pout) measures how much power the rotor has extracted from the wind. The axial induction factor is a measure of the ratio between the free stream velocity of the wind and the wind speed seen at the rotor plane. As with the power and wind equation given in Equation, the power output is proportional to the wind velocity (U) cubed. The difference between the two equations is that the power that one can extract from the wind by the rotor is limited by the laws of conversation of momentum, which is manifested in the axial induction factor (a). This knowledge is used to determine the efficiency (η) of a rotor in converting the power in wind to mechanical rotational power.

T(Thrust)=1/2pU24a(1-a)

Once power has been determined, thrust (T) is the important factor in determining the lift that the AWE device will have available to stay aloft. Notice that the equation for thrust is very similar to the equation for power, except that it is proportional to wind speed squared. This information allows calculation of the forces and loads required for the AWE design to man-age.

Effective Optimal Blade Design Technique

High-Altitude Wind Properties:For analyzing the performance of an AWE device, the first step is to determine the properties of the atmosphere at which the device would be operating.
Sea Level Std. Temperature (T0): 288.15 K
Sea Level Std. Pressure (P0): 101325 Pa
Reference Viscosity of Air (μ0): 1.827E-5kg/(m-s)
Ambient Lapse Rate (LapseR): 0.0065 K/m
The Universal Gas Constant (Ru): 8.31447 J/mol-K
Molar Mass of Dry Air (Mair):0.0289644 kg/mol
Gravity of Earth(g): 9.80665 m/s2
Specific Heat Ratio for Air (γ): 1.4
For generation of power from wind the most important characteristic of the atmosphere is the density of the air. This is because, the function for the power available in wind is directly proportional to the density of air.

T=T0LapseRh

Density= PMair/RuT

Density is depending on the pressure and temperature of the air. This equation shows that the temperature can be estimated as T0 (the sea level standard air temperature=288.15 K), and then decreases at a constant rate, Lapse (called the lapse rate), as altitude (h) increases.These estimates should be reasonable throughout the troposphere (the region of the atmosphere from 8,000 m to 16,000 m above sea level, depending on latitude). The troposphere is thinner at the poles and thicker at the equator. The air density estimates given by these equations will be sufficiently accurate for the altitudes, which is most important for analysis of AWE systems. This is because the strongest jet streams tend to

range from 8,000 m to 12,000 m, with the higher jet streams closer to the equator. The importance of the reduction of density with altitude can be understood by comparing the density at sea level with the density at 12,000 m of altitude. The density at 12,000 m is about ¼ the density at sea level. Hence, the power in the wind at 12,000 m of altitude would also be ¼ of the power of wind at sea level for the same wind speed.

Design Parameters

After understanding the atmosphere, the next step is the performance analysis of an AWE rotor by defining the design parameters for the device. Amongst all, one of the most important choices in design is the selection of the diameter (D) of the blade, because the generation of power is proportional to the area swept out by the rotor. This choice is totally dependent on the power output one desires—the larger the blade diameter, the more is the power produced, but the blade diameter will be constrained by the physical limits of the blade's structural strength.Firstly, the diameter (D), number of blades (B), and hub diameter (Dh) are selected. Initially, these numbers are estimated; however, they are then adjusted based on the results of the performance calculations. Once these are selected, we obtain the blade length. A blade element analysis will be used to analyze the blade/rotor performance, so the blade is divided into a certain number (N) of blade stations. Ten blade stations will be used for this project (ten to twenty is commonly used).

$L=(D-Dh)/2$

$Ls=L/n$

The next important parameter is the tip speed ratio (γ) as it affects the amount of power that can be pulled out of the wind and the amount of thrust on the wind-rotor. The tip speed ratio can be controlled in a number of different ways, such as adjusting the amount of power drawn from the wind or blade pitch angle or by using blade stall. For this performance analysis, focus is on the performance at the maximum power tip speed ratio.

$\gamma(maxpower)=4\pi 1.25/b$

Now we have the tip speed ratio set. The next thing to do is to calculate the speed of blade rotation (Ω). With (R) as the rotor radius (in meters) and the wind speed (U) (in meters per second), the result of this equation will be in radians per second (In order to get in rpm, we multiply by $60/2\pi$).

$\Omega= \gamma U/R$

Optimal Wind-Rotor Blade Design

Obtaining the ideal shape for a wind-rotor blade design can be done by using the combination of momentum theory and blade element theory.This method gives the ideal twist angle and chord lengths for each blade station, resulting in the maximum power. This maximum power is obtained when all the blade elements are at the angle of attack which produces the best lift-to-drag ratio for the airfoil used.

With the blade geometry defined, the properties at each blade station can then be computed. The following equation gives the local speed ratio (λR) for an intermediate radius (r). The blade element theory and momentum theory give the next two equations for the local angle of relative wind (ϕ) and ideal local chord length (c), respectively. The ideal chord length is one of the necessary blade design parameters,

$\lambda\_R=\lambda(r)/R$

$\phi= (2/3)$ 〖tan〗 ^ (-1) $(1/\lambda r)$

$c=8\pi r/BC\_l\ (1-\cos\phi)$

Once the angle of relative wind is found, the section pitch angle (θp) that will give the angle of attack for minimum drag is calculated. The section twist angle (θT) is then found by subtracting the blade pitch angle ($\theta p,0$), as shown in the equation. The pitch angle is an important operating parameter to the AWE system, and the twist angle is an important design parameter that defines the twist in the system blades.

$\theta\_P= \phi-\alpha$

θ_T=θ_P-θ_(P,0)

Once the blade chord and twist angles are defined, the rotor performance is estimated. The coefficient of power (Cp) is a measure of the fraction of power in the wind that is extracted by the wind-rotor.

Cp= (8/λ^2) ∫sin^2 ϕ (cosϕ-λ_R sinϕ) (sinϕ+λ_R cosϕ) [1-(C_D/C_L) cotϕ] λ_R^2 dλ_R

This integral could also be estimated by splitting the blade up into elements contributed by each blade station (ΔCp). The contribution of each blade station to the power coefficient is calculated with the equation.

ΔCp= (8/λ^2) sin□^2 ϕ (cosϕ-λ_R sinϕ) (sinϕ+λ_R cosϕ) [1-(C_D/C_L) cotϕ] λ_R^2 dΔλ_R

The term Δλ_R is the increment of tip speed ratio from one blade station to the next. All the blade stations have the same Δλ_R value of if the blade stations are each the same length.

Δλ_R=Δλ_ri-Δλ_r(i-1)

The coefficient of lift and drag (Cl and Cd) in Equations 25 and 26 are the lift and drag coefficients for the blade airfoil at the angle of attack of minimum drag. Next, sum the ΔCp's for all blade stations to get the total power coefficient (Cp), as in Equation 28. The power output of the optimized rotor is then calculated with Equation.

C_P=εΔC_P

P=C_P 1/2ϱAU^3

The coefficient of power essentially represents the efficiency of an AWE system. The power (P) represents the main goal of these calculations, finding the actual power output of the wind turbine's rotor. The power output will be used when designing the system for its applications. Thus far, these calculations do not include the effects of tip losses. Tip losses for optimal wind-rotor blade design can be accounted for and the equation for these losses will be given in the next section under-General Blade Design. Tip loss analysis is used to more closely reflect realistic flight for the AWE system.The calculation of the proposed model is done success-fully with a 50-m diameter, giving the altitude as 10,000 m and the speed of the wind to be maximum 25 meters/second. This Figure specifies the resulting functions and by the actions there is complete improvement of such optimal design technique over many primitive techniques and models'.

RESULTS AND DISCUSSION

The huge size of the high-altitude wind resource, together with its shut proximity to users and its consistency, create it a tempting, viable supply to faucet because the country searches for a lot of ways that to seek out clean, reliable, and index-pensive energy. The cheap element is that the most significant facet as a result of it makes AWE resources competitive with current energy resources. If AWE is actually competitive with current energy sources, the technology can rise on its own based mostly upon its profits, and this may enable the high-altitude technologies to be in-dependent of state subsidies.The Airborne Technology is still in R&D phase of many companies and is still in development phase.There are many drawbacks and several challenges that the AWE design face. The device needs specific and implemented area to work with. So, the People and the organization have to take the decision of finding the desired area. The device needs to be safe and dependable like if the model has high chances of falling of from heights and damaging property, so therefore it needs to be tested. Also, as days flow the technology will develop (for example, in such the way on cut back cable weight per unit length), then this can permit the AWE systems to systematically reach the highest altitudes. The capability issue reveals that if the systems are operated utterly, they'll

still not be as consistently obtainable as fuel sources. Even the most effective AWE sites can offer no power in situations, so we need alternative situations to take care of.

CONCLUSION

Yet lastly, following high-altitude energy is recommended as a result of this immense resource is additional reliable than ground alternative energy, and AWE are often additional cost-competitive than alternative resources. One reason it's important to drive the price of alternative energy down is that current ground alter-native energy is just cost-competitive once the worth of alternative energy (like the worth of oil or coal) is high. The impact of this is often that interest in wind-power and capital tends to dry up once the worth of energy drops. Therefore, if AWE costs are often competitive, even at low energy costs, it'll build interest and development within the AWE technology consistent regard-less of the worth of energy. To boot, the high-altitude systems can be useful as a result of they will be terribly near users, the systems are often transportable (they area unit solely connected to the bottom with a cable), and that they are often employed in varied locations and during a big selection of operating things. Realizing every of those things can facilitate the USAF to ac-accomplish the goals of the National Security Strategy. It appears that the objectives of AWE are often reached. The goal to get power at all-time low possible value per power unit created has been realistically value analyzed. Investigation has been done to use the mini-mum attainable land in these AWE systems in order that the utmost energy per space on the bottom (kW/km2) are often achieved. a number of the ad-vantages to AWE systems area unit financial (reduced land use and low environmental impact), however there's additionally a practical potential that the high-altitude approaches for additional effectively gathering alternative energy can put together give cheaper, cleaner, and inexhaustible energy technologies that the executive department will directly utilize.

With the demand for energy necessities increasing enormously, it is often met by energy re-sources like wind. notably, wind at high altitudes will generate additional power compared to the con-ventilation wind turbines. additionally, this energy supply offers ad-vantages like straightforward deploying, low installation value and maintenance systems, and fewer wind fluctuations. In terms of operational life, installation value and responsibility, mobile systems attract the interests of the many.Where as mobile wind turbines area unit thought-about as a promising alternate for ancient power sources, their blessings area unit questioned by several critics. Generating electricity at high altitudes has its own challenges, like unforeseen fucking, and so desires an automatic management piloting to style strong and reliable AWTs with extra safety. Like several alternative technologies, mobile wind turbines too have their own execs and cons, and nice efforts area unit created across the world to develop AWT as a good sup-porting technology for the eco-friendly wind energy market.

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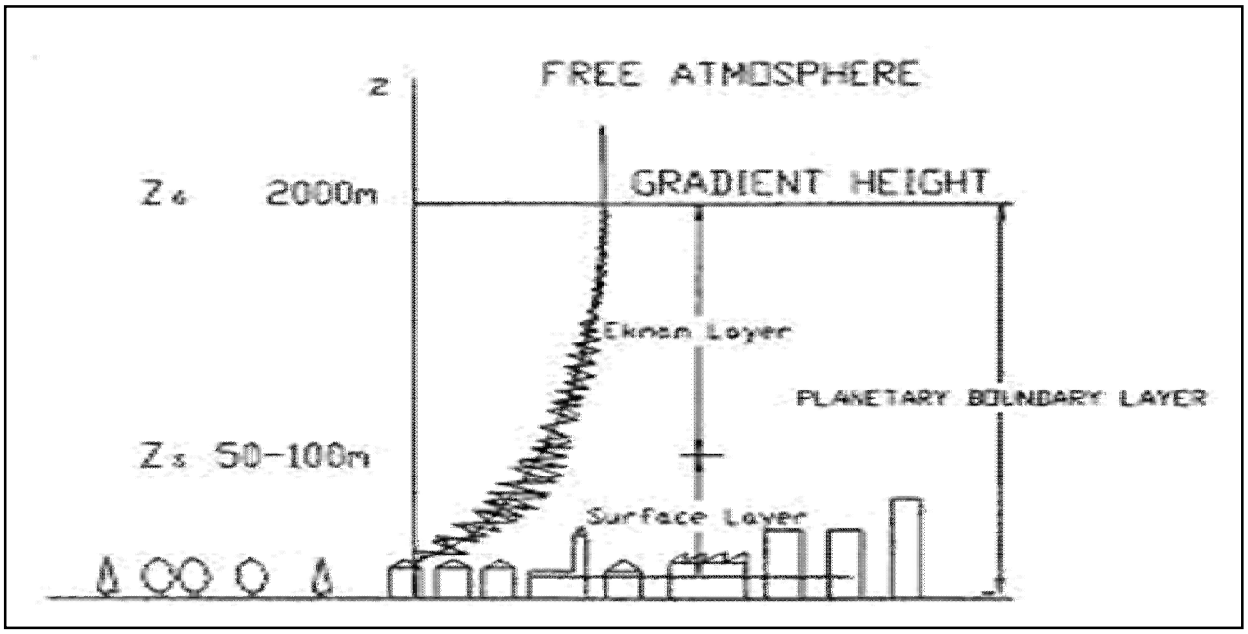

Fig.1 Critical Region of Efficiency (CRE)

Fig.2 The model includes the fly-kite with the blades of the rotor or rather improved and Optimized Blades at-ached to the front end which generate electricity as the model floats the sky gaining rotational motion and thrust

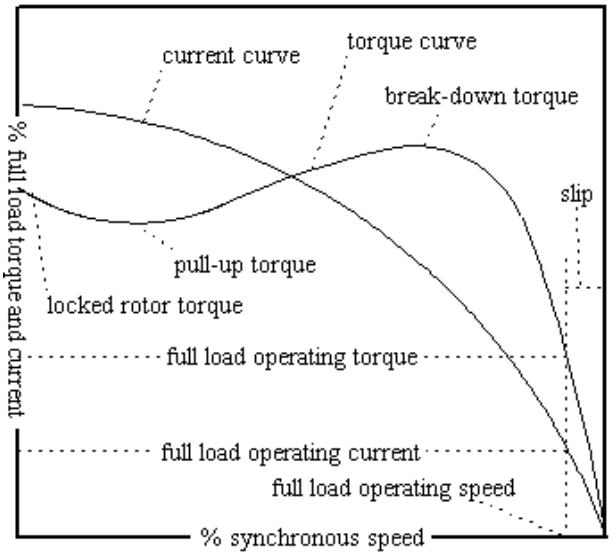

Fig.3 The Graph between the full load torque& current and the synchronus of speed

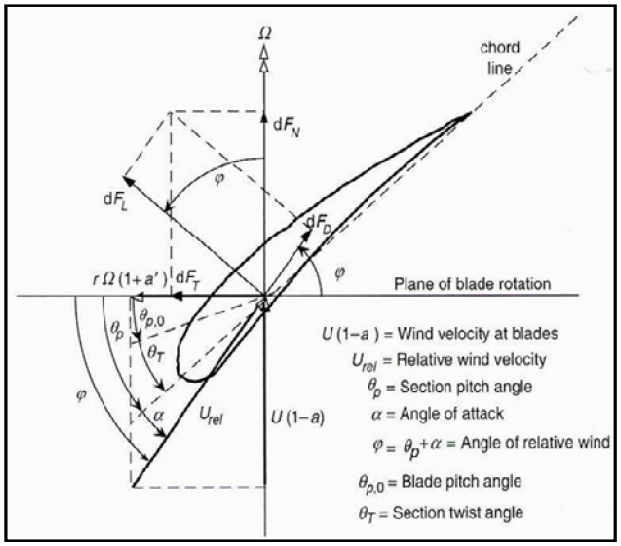

Fig.4 Effective Optimal Blade Design Technique

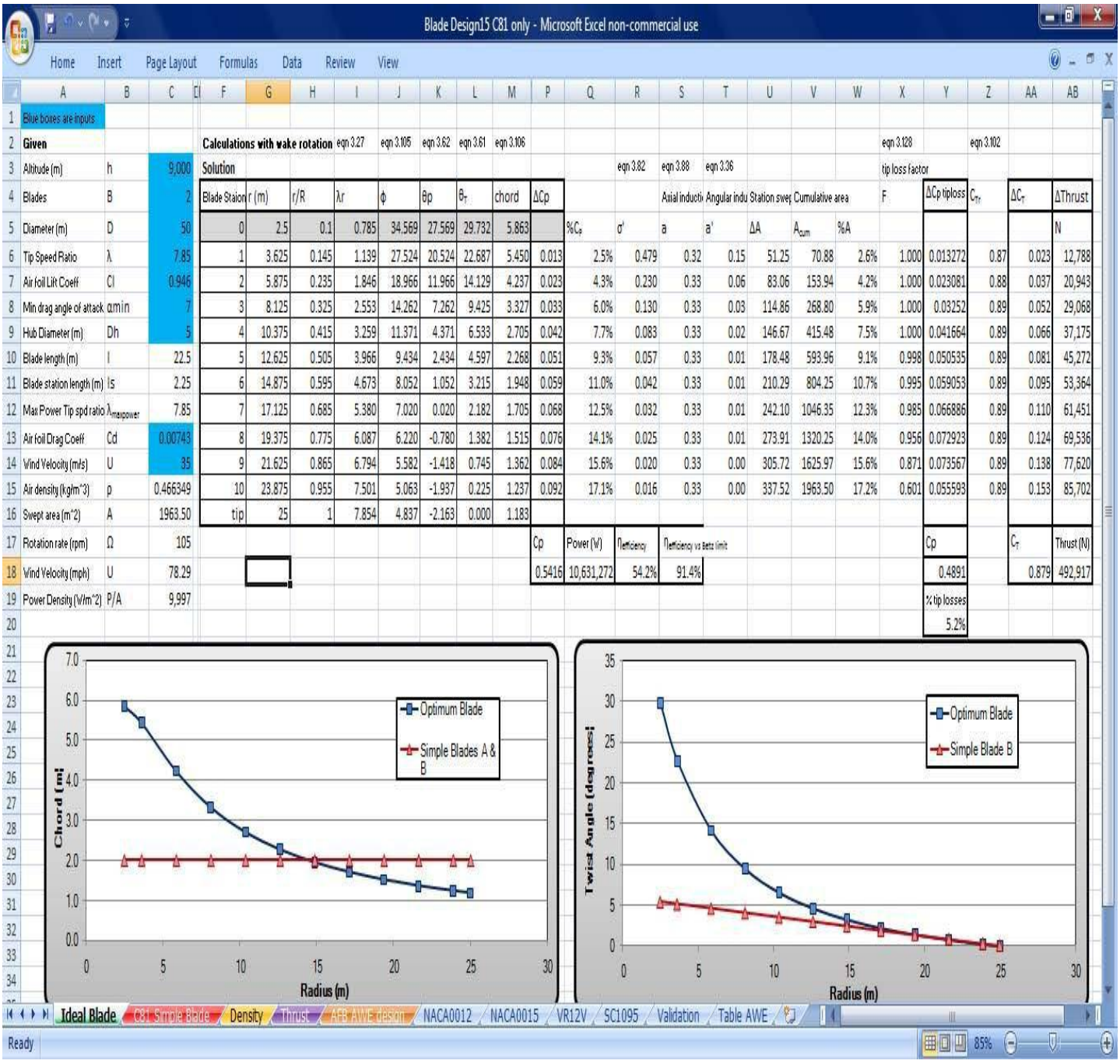

Fig.5 The resulting functions and by the actions there is complete improvement of such optimal design technique over many primitive techniques and models

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RESEARCH ARTICLE

Comparative Assessment of Monoherbal and Polyherbal Creams Incorporated with the Flower Extracts *Cassia auriculata* L., *Chrysanthemum indicum* L., *Tagetes erecta* L. for Acne

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ABSTRACT

The present investigation is envisaged on theformulation and evaluation of herbal cream incorporated with ethanolic extracts of flowers of *Cassia auriculata* L., *Chrysanthemum indicum* L. and *Tagetes erecta* L. intended for anti acne activity.The flower extracts were prepared by maceration method and its phytochemical studies were performed. Then the *in vitro* anti acne activity against *Staphylococcus epidermidis*and *Propionibacterium acnes* by disc diffusion methodwere evaluated.Further MIC was determined by Sahin *et al.* method.The extracts were formulated into 2% cream of individual flower extracts as well as in combination and then evaluated for its various standardization parameters. The macerate of flowers showed the presence of carbohydrates, flavonoids, cardiac glycosides, saponins, glycosides and tannins. According to results obtained the ethanolic extracts of flowers showed significant anti-acne activity ($p<0.001$) compared to standard drug clindamycin.The anti acne potency can be denoted as *C. indicum* L > *C. auriculata* L > *T. erecta* L. The quality control parameters performed for all the creams passed the tests and the values were within the standard limits. It was observed that the cream showed potent activity than the crude extract which depicts that the exiepients have given a synergistic effect. Among the four creams 2% *C.indicum* cream showed the highest activity.

Keywords: Anti-Acne, Polyherbal Creams, Flavonoids, Saponins.

INTRODUCTION

Acne or acne vulgaris is a general term used for eruptive disease of the human skin characterized by areas of skin with seborrhoea, comedones, papules, pustules, nodules and possibly scarring. Acne affects mostly skin with the densest population of sebaceous follicles and severe acne is inflammatory in nature. Acne occurs most commonly during adolescence, and often continues into adulthood. There are some major factors responsible for causing acne and them act together to cause the characteristic pimples, whiteheads and blackheads associated with acne. The factors include: overactive oil glands, blockage of the skin pores, activity of normal skin bacteria and inflammation. Although acne is not caused by a bacterial infection, bacteria do play a role in making the situation worse. The bacterium Propionibacterium acnes is a normal part of the skin surface. It keeps the skin from being invaded by harmful bacteria. When oil is trapped in the hair follicles, the normal skin bacteria P.acnes will grow in the blocked pore. The bacteria produce chemicals that alter the composition of the oil, which makes it more irritating to the skin and causes inflammation.

Propionibacterium acnes and Staphylococcus epidermidis are two of the major bacterial strains found in acne lesions and among these only P. acnes is implicated in acne inflammation [1-3].The present work is based on the study of anti acne property of three types of yellow coloured flowers namely *Cassia auriculata* L.(Fabaceae),*Chrysanthemum indicum* L.(Asteraceae), *Tagetes erecta* L.(Asteraceae), for their anti acne property as these are widely used by the people of Andhra Pradesh as a remedy for curing pimples.*Cassia auriculata* L. is a large shrub that grows up to 2 -3 meters in height. Its leaves are paripinnately compound with 10-12 pairs of leaflets. Flowers are yellowish, in axillary and terminal corymbs(Figure 1). Fruits are brown coloured pods, and when ripen contain 10-15 dark brown seeds[4]. Theplant is a rich source of terpenoids, tannins, flavonoids,saponins, bioflavonoids, cardiac glycosides and steroids. *Chrysanthemum indicum* L. is perennial flowering plant(Figure 1) which iserect andslender [4].It is reported to contain caffeic acid, luteolin and kaempferol.*Tagetes erecta* L is an erect, branching aromatic herb(Figure 1)[4]. It is a small shrub, which grows up to 1-2 m andis reported to contain lutein, lutein fatty acid ester, thiophenes, glycosides and flavonoids.

MATERIALS AND METHODS

Collection and Authentication

Cassia auriculata Lflowers were collected from surrounding area of Choutuppal village, Nalgonda district, Telangana, India. *Chrysanthemum indicum* L. and *Tagetes erecta* L. flowers were collected from local market of Choutuppal village, Nalgonda dist., Telangana, India. All plant materials were collected in the months of December to January 2012-13.The plant materials were identified and authenticated by Mr.Shankara Chary Professor of Botany, Government Degree College for women in Nalgonda. The specimens were prepared and submitted in the Department of Pharmacognosy, under the voucher no: *Cassia auriculata* L., family: Fabaceae.NCOP-NLG/Ph'cog/2011-2012/046, *Chrysanthemum indicum* L., family: Asteraceae.NCOP-NLG/ Ph'cog/ 2011-2012/ 047, *Tagetes erecta* L., family: Asteraceae.NCOP-NLG/Ph'cog/2011-2012/048.

Extraction by Maceration

The 500g of flower petals were subjected to maceration using ethanol.In this process, the drug was placed with the whole of the menstruum in a closed vessel for seven days with occasional shaking. After seven days, the liquid was strained by pressing the marc and the liquid thus obtained was filtered [5].

Preliminary Phytochemical Screening

The extracts were concentrated and made solvent free with the help of a rotary vacuum evaporator and concentrated extracts were subjected to preliminary chemical tests [6,7].

Thin layer chromatography

The TLC was performed for the phytoconstituents that gave positive response to the preliminary chemical tests and their R*f* values were calculated. The adsorbent used was pre-coated silica gel $60F_{254}$.The solvent system and detection system used for flavonoids was Ethylacetate: Formic acid: Glacial acetic acid: water (10:1.1:1.1:2.6) and UV light (short wavelength, long wavelength and day light), spray reagentused was2% $AlCl_3$ respectively. For glycosides the solvent system and detection system used was ethyl acetate: methanol: ethanol: water (8.1:1.1:0.4:0.8) and UV light (short wavelength, long wavelength and day light), spray reagentused wasAntimony-III-chloride reagent respectively. For tannins the solvent system and detection system used was chloroform: Ethyl acetate: acetic acid (6:4:4) and UV light (short wavelength, long wavelength and day light), spray reagentused was vanillin sulphuric acid reagent respectively. The solvent system and detection system used for saponins were ethyl acetate: ethanol: water: ammonia (6.5:2.5:0.9:0.1) and UV light (short wavelength, long wavelength and day light) respectively. Thin layer chromatography was performed as per standard procedure [8].

Procurement of Micro Organisms and Culture Conditions

The micro organisms *Propionibacterium acnes* MTCC 1951 and *Staphylococcus epidermidis* MTCC 435 were procured from Microbial type culture collection (MTCC), Chandigarhin freeze dried form. Each organism was sub cultured in different media. *Propionibacterium acnes* was sub cultured in sheep blood agar medium (Hi Media, Hyderabad, India) with sheep blood supplement then incubated at 37ºC for 48h where as *Staphylococcus epidermidis* was sub culture in nutrient agar medium (HiMedia, Hyderabad, India) at 37ºC for 24 h.

In-vitro Anti-Acne Activity by Hayes and Markovic (2002)

This experiment was performed by method of Hayes and Markovic (2002) with minor modifications. *Propionibacterium acnes* was incubated in sheep blood agar medium with sheep blood supplement and incubated it for 48h under anaerobic conditions at 37ºC and adjusted to yield approximately 1.0×108 CFU/ml. A prepared inoculum was added to molten agar, mixed and poured over the surface of the agar base and left to solidity. 25, 50, 75 and 100mg/ml concentration test solutions were prepared. Then solution was incorporated into a sterile disc. This sterile paper discs which was impregnated with test material were placed on the agar. Then plates were incubated at 37ºC for 48h under anaerobic conditions[9].*Staphylococcus epidermidis* was first inoculated in nutrient broth then transferred in to nutrient agar and incubated for 24h at 37^0C and adjusted to yield approximately1.0×10^8 CFU/ml. The procedures were the same as mentioned above except the plates were incubated at 37ºC for 24 h under aerobic conditions. All tests were performed in triplicates and the antibacterial activity was expressed as the mean of inhibition diameters (mm). Clindamycin at a concentration of 10μg/ml was used as a standard.

Determination of Minimum Inhibitory Concentration by Sahin *et al*., (2003)

The minimal inhibitory concentration (MIC) values were determined by micro dilution assay. The stock solution with 1mg/ml concentration was prepared. From the above stock solution serial dilutions were prepared and these were incorporated into the discs and then dried. These dried sterile discs were placed over the sheep blood agar medium inoculated with *Propionibacterium acnes* and then incubated at 37ºC for 48 h under anaerobic conditions. Same procedure was followed for the MIC determination of the plants extract in the case of *Staphylococcus epidermidis*. Here the dried sterile discs were placed over the nutrient agar medium andthe plates were incubated 37ºC for 24hrs. The plant activity was scored as per Table 1[9].

Preparation of 2% *Cassia auriculata* L. cream, 2% *Chrysanthemum indicum* L. cream, 2% *Tagetes erecta* L. cream and 2% Poly herbal cream

The ingredients for the oil phase for all the creams werestearic acid, mineral oil, cetyl alcohol, propyl paraben, sodium meta bisulphate, EDTA and perfume as rose oil. The aqueousphase consisted of water, respective flower extracts, triethanolamine, propylene glycol, glycerine andsorbital. The required ingredients of the oil phase and

aqueous phase were weighed and heated separately at 70°C Then the oil phase was mixed with aqueous phase and stirred well till the desired consistency was obtained and it was stored in well closed air tight containers[10].

Evaluation Parameters of Creams

Organoleptic Characters: By visual appearance, colour and odour was noted.

Presence of Foreign Particles/Grittiness: A small amount of cream was taken and spread on a glass slide free from grease and was observed against diffused light to check for presence of foreign particles.

Determination of pH: The pH of the formulations was determined at 27ºC using a pH meter.

Extrudability: The cream was filled in standard capped collapsible aluminium tubes and sealed by crimping to the end. The weight of the tube was recorded and was placed between two glass slides and clamped. 500gm was placed over the slides and then the cap was removed. The amount of the extruded gel was collected and weighed. The percent of the extruded gel was calculated (>90% extrudability: excellent, >80% extrudability,: good, >70%extrudability:fair).

Spreadability: The formulation was placed over the glass plate of 20cm×5cm. Another glass plate of the same dimension was placed on the top of the cream such that the formulation was sandwiched between the two slides by placing weight of 100g uniformly on the slides. The weight was removed and the excess of cream was scrapped off. Two slides in position were fixed to a stand at a 45º angle without the slightest disturbance so that only the lower slide was held firmly by the clamp, allowing the upper slide to slip off freely with the help of 20g weight tied to the upper slide. The time taken for the upper slide to separate away from the lower glass plate under the direction of the weight was noted as per ICH guidelines 10. Experiment was done in triplicate and spreadability was calculated as follows,

S = M × L/T Where S=spredabililty, L= length of glass plate

W= weight tied to the upper plate, T = time taken (sec).

Irritancy by Single Application Patch Test: In this single application patch test, 100mg of undiluted test material was moistened and applied to the lower arm using a small patch (smaller than test the pad of a standard plaster). The patch was allowed to stay in place for 4 hours, than the test site was observed for signs of irritation over the next 3 days if the test material was irritating, the patch site will be apparent as red, and may appear to be dry or rough. Typically, a minimum of 10 volunteer's panellists will participate in a single test.

Globule Size Determination: 1g of cream was diluted to 10ml with glycerine. A few drops of this are transferred on to a glass slide and is focused in a microscope. By using eyepiece micrometer, the diameters of 20 particles are determined randomly and averagevalue was noted.

Phase Separation: The formulation cream was kept intact in a closed container at 25-30ºC not exposed to light. Phase separation was observed carefully every 24 hrs for 30 days. Any change in phase separation was checked.

Sterility Test: As the herbal creams were more prone to microbial attack the formulation was subjected for microbial contamination test. Nutrient agar was prepared creams were added and incubated for 24hrs at 30ºC. The growth for microbes was observed [11].

Anti Acne Activity by Disc Diffusion Method: The formulated 2% of creams were evaluated for anti acne activity as per the method mentioned above and the zone of inhibition was calculated and compared with control and standards values.

Statistical Analysis: Averages ±S.E.M. of the means were calculated; statistical analysis of results was performed by Dunnet Multiple Comparison Test (One way analysis of variance, ANOVA), compared with control using Instat Graph pad #3. The Values of **P < 0.01 were considered significant.

RESULTS AND DISCUSSION

Nature of Extract

The three extracts were brown in colour with sticky consistency. The percentage yield was 1.3%w/w, 2.8%w/w and 2.78%w/w for *C.auriculata, C.indicum* and *T.erecta* respectively.

Preliminary Phytochemical Screening

The extracts of *C.auriculata* L exhibited the presence of carbohydrates, glycosides, and flavonoids, whereas the extracts of *C.indicum* L and *T.erecta* L showed the presence of carbohydrates, glycosides, flavonoids, saponins and tannins.

Thin Layer Chromatographic Analysis

Chromatographies are strongly recommended for the purpose of quality control of herbal medicines, since they might represent appropriately the chemical integrities of the herbal medicines and therefore be used for authentication and identification of the herbal products. The ethanolic extracts of three plants extracts were subjected to thin layer chromatography in order to detect and confirm phytoconstituents. The TLC analysis showed several spots for flavonoids, glycosides, saponins and tannins in varying colours under UV light in different wavelengths with R*f*values which are tabulated in Table 2, 3, 4.

Anti Acne Activity the Ethanolic Flower Extracts

Acne is a chronic inflammatory disease of the pilosebaceous units of skin, characterized by seborrhea, erythematous papules, nodules, deep pustules and pseudocysts[12].The microbial flora isolated from acne patients causing pathogenesis includes *P. acnes*, *S. epidermidis*, *S. aureus*, *Klebsiella pneumonia* etc. [13]. Plant secondary metabolites present in herbal drugs and food have shown to be very useful in the prevention and treatment of many diseases. Among these metabolites, flavonoids and saponins are a promising source of lipase inhibitors since they are present in plant extracts capable of inhibiting porcine pancreatic lipase activity [14]. These compounds are also present in several plant extracts that have been used for the treatment of lipase-related diseases such as acne. Recent studies have also demonstrated that flavonoids like quercetin and saponins are good lipase inhibitors.The three flower extracts exhibited potent anti acne activity and it was in dose dependent manner. Among the three plants *C.auriculata* and *C.indicum* have shown a better activity against *S.epidermidis* and *P.acnes* (Table 5 & 6) which was comparable to that of the standard drug clindamycin. *T.erecta* showed the least anti acne activity. The zone of inhibition obtained were found to be statistically significant where p<0.01(Table 5 & 6).The MIC of the plants extract was determined and is tabulated in Table 7& 8. From the preliminary phytochemical screening it was understood that the three extracts contained flavonoids and saponins, hence from this it can be concluded that these bioactive components were responsible for the potent anti microbial activity towards the *Staphylococcus epidermidis* and *Propionibacterium acnes.*

Evaluation of 2% Monoherbal and Polyherbal Creams

The colour and odour of topical preparations play a critical role in patient acceptance and compliance; hence it has to be taken into consideration [15].The 2% monoherbal and polyherbal creamshad a pleasant colour and fragrance. No foreign particles were observed; hence the creams were considered to be free from grittiness and had smooth texture. pH is an important evaluation factor of creams because if it alters there may be allergic reactions on the skin.pH of creams were found to be within the skin pH. The extrudability values obtained for all the creams were in the range of excellent (Figure 2-5).The spreadability was found to be with the standard limit. The skin irritation performed on human volunteers revealed that all the formulations were safe and free from creating any sort of irritations or allergic

reactions. These creams were prepared by o/w method hence it is easy to apply and wash from skin [15]. The skin irritation test showed no symptoms of irritancy and edema when applied on to skin. It was checked for 72 hours at regular intervals. No signs of itchiness, redness or rashes were observed (Figure 2-5). Globule size was determined and it was found to be as per the standard limits.None of the creams showed phase separation even after 30days and hence the cream appeared to be stable.It was found that there was no microbial growth and hence all the creams formulated were considered to be free of microbial contamination (Table 9).

Anti Acne Activity

The 2%creams of *Cassia auriculata* L., *Chrysanthemumindicum* L., *Tagetes erecta* L. and polyherbal creams were evaluated for the anti acne potency. It was observed that the 2% *Chrysanthemumindicum* L. cream (13.01±0.64**mm for *S.epidermidis*and 15.000±1.000**mm for *P.acnes*) and 2% polyherbal cream(12.0±1.0**mm for *S.epidermidis* and 13.67±1.53**mm for *P.acnes*) exhibited potent activity which was comparable with that of the standard clindamycin (Table 10). 2% *Tagetes erecta* L. cream showed the least activity. The anti acne potency can be attributed to the high flavonoid and saponin contents present in the flower extracts.Interestingly it was noted that the flower extracts after formulating into 1% cream exhibited potent anti acne activity than their individual extracts. Moreover the monoherbal formulation in which *C.indicum* flower extract was incorporated showed better activity than the polyherbal formulation. Hence from this study it can be concluded that 2% *Chrysanthemumindicum* L. cream proved to be a better anti acne agent when compared to the other monoherbal and polyherbal creams.

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Table 1: MIC Grading

S.No	Concentration	Grade
1.	≤20μg	more active
2.	20-100μg	Active
3.	100-250μg	Moderately active
4.	500μg	less active
5.	>500μg	Inactive

The table depicts the grading of minimum inhibitory concentration

Table 2: Thin layer chromatography of *Cassia auriculata* L.

Phyto constituents	Solvent system	Long wavelength (365nm)		Short wave length (256 nm)	
		Spot color	**R*f***	**Spot color**	**R*f***
Flavonoids	Ethyl acetate: Formic acid : Glacial acetic acid: water (10:1.1:1.1:2.6)	Green Blue fluorescence Blue Light blue Blue	0.93 0.81 0.71 0.60 0.52	Dark green Light green Green	0.96 0.93 0.83
Glycosides	Ethyl acetate: Methanol :Ethanol: water (8.1:1.1:0.4:0.8)	Blue fluorescence Blue Light blue Light blue	0.96 0.91 0.75 0.50	Green Light green	0.96 0.88

Table 3: Thin layer chromatography of *Chrysanthemum indicum* L.

Phyto constituents	Solvent system	Long wavelength (365nm)		Short wave length (256 nm)	
		Spot color	**R*f***	**Spot colour**	**R*f***
Flavonoids	Ethyl acetate: Formic acid : Glacial acetic acid: water (10:1.1:1.1:2.6)	Blue Blue fluorescence Light Blue fluorescence	0.88 0.83 0.80	Light green Light green	0.90 0.83

Glycosides	Ethyl acetate: Methanol :Ethanol: water (8.1:1.1:0.4:0.8)	Blue fluorescence Light Blue Light Blue fluorescence	0.96 0.90 0.83	Green Light green Green Green	0.95 0.83 0.75 0.66
Tannins	Chloroform:Ethyl acetate: Acetic acid (6:4:4)	Blue fluorescence	0.24	-	-
Saponins	Ethyl acetate : ethanol: water : ammonia (6.5:2.5:0.9:0.1)	-	-	Green Light green Light green Light green	0.94 0.84 0.75 0.71

Table 4: Thin layer chromatography of *Tagetes erecta* L.

Phyto constituents	**Solvent system**	**Long wavelength (365nm)**		**Short wave length (256 nm)**	
		Spot color	**R*f***	**Spot color**	**R*f***
Flavonoids	Ethyl acetate: Formic acid : Glacial acetic acid: water (10:1.1:1.1:2.6)	Blue Dark blue Light Blue Blue Fluorescence	0.91 0.83 0.80 0.66	Green Light green Green	0.96 0.83 0.53
Glycosides	Ethyl acetate: Methanol :Ethanol: water (8.1:1.1:0.4:0.8)	Dark Blue Blue fluorescence Blue	0.96 0.88 0.83	Green Green Brown Light blue	0.96 0.93 0.90 0.80
Saponins	Ethyl acetate : ethanol: water : ammonia (6.5:2.5:0.9:0.1)	-	-	Blue	0.84

Table 5: Antiacne activity of *Cassia auriculata* L., *Chrysanthemum indicum* L. and *Tagetes erecta* L. extracts against *Staphylococcus epidermidis*

Plant extracts	Control	25mg	50mg	75mg	100mg	Standard
Zone of Inhibition in mm						
Cassia auriculata L	5±0.000	7.667± 0.5774**	8.000± 1.000**	8.33± 0.578**	8.667± 0.5774**	20± 0.000**
Chrysanthemum indicum L	5±0.000	8.667± 1.528*	9.000± 1.000*	9.333± 2.082**	12.333± 1.528**	20.000 ± 0.000**
Tagetes erecta L	5±0.000	6.000± 0.5774 ns	7.000± 1.000 *	9.000± 2.309 **	9.67± 0.58**	20± 0.000**

Experimental data were expressed as mean ± SD. The difference between experimental group was compared by One way Analysis of variance (ANOVA) followed by Dunnet Multiple comparison test (control vs test) using the software Graph Pad Instat. The difference were considered to be statistically extremly significant when $p<0.001$.

Table 6: Antiacne activity of *Cassia auriculata* L., *Chrysanthemum indicum* L. and *Tagetes erecta* L. extracts against *Propionibacterium acnes*

Plant extracts	Control	25mg	50mg	75mg	100mg	Standard
Cassia auriculata L.	5±0.000	7.333± 0.5774**	10.333± 0.5774**	11.333± 0.5774**	12.000± 1.000**	20± 0.000**
Chrysanthemum indicum L.	5±0.000	6.667± 0.5774ns	9.667± 0.5774**	12.000± 1.000**	13.667± 1.528**	20± 0.000**
Tagetes erecta L.	5±0.000	7.667± 0.5774**	9.333± 0.5774**	11.667± 1.528**	12.67± 2.51**	20± 0.000**

Experimental data were expressed as mean ± SD. The difference between experimental group was compared by One way Analysis of variance (ANOVA) followed by Dunnet Multiple comparison test (control vs test) using the software Graph Pad Instat. The difference were considered to be statistically extremly significant when $p<0.001$.

Table 7: Minimum inhibitory concentration of flower extracts against *Staphylococcus epidermidis*

Micro organisms	MIC (μg/ml)	Grade
Cassia auriculata L.	25	Active
Chrysanthemum indicum L.	250	Active
Tagetes erecta L.	25	Moderately active

Table 8: Minimum inhibitory concentration of flower extracts against *Propionibacterium acnes*

Micro organisms	MIC (μg/ml)	Grade
Cassia auriculata L.	25	Active
Chrysanthemum indicum L.	250	Moderately Active
Tagetes erecta L.	250	Moderately active

Table 9: Evaluation of monoherbal and polyherbal creams

Parameters	2% *Cassia auriculata* L.cream	2% *Chrysanthemum indicum* L. cream	2% *Tagetes erecta* L. cream	2% *polyherbal* cream
Organoleptic characters	Colour Light pink Odour Pleasant	Colour Light yellow Odour Pleasant	Colour Light brown Odour Pleasant	Colour Brown Odour Pleasant
Presence of foreign particles	free from grittiness and had smooth texture.	free from grittiness and had smooth texture.	free from grittiness and had smooth texture.	free from grittiness and had smooth texture.
pH determination	6.4	6.6	6.5	6.3
Tube Extrudability	97.49%	95.64%	96.42%	95.92%
Spreadability	85.88gcm/sec	75.72gcm/sec	83.15gcm/sec	85.68gcm/sec
Irritancy	no symptoms of irritancy, irritancy and edema	no symptoms of irritancy, irritancy and edema	no symptoms of irritancy, irritancy and edema	no symptoms of irritancy, irritancy and edema
Globule size determination	72.8μ	81.90μ	76.54μ	83.21μ
Phase separation	No phase separation cream appeared to be stable.	No phase separation cream appeared to be stable.	No phase separation cream appeared to be stable.	No phase separation cream appeared to be stable.
Sterility test	free of microbial contamination	free of microbial contamination	free of microbial contamination	free of microbial contamination

Table 10: Anti acne activity of Monoherbal and polyherbal creams

Type of cream	Control (mm)	*S.epidermidis* (mm)	*P. acnes* (mm)	Standard (mm)
2% poly herbal cream	5±0.0	12.0±1.0**	13.67±1.53**	20±0.0**
2% *Cassia auriculata* L. cream	5±0.000	11.67±1.15**	13.333±0.5774**	20±0.00**
2% *Chrysanthemum indicum* L. cream	5±0.00	13.01±0.64**	15.000±1.000**	20±0.00**
2% *Tagetes erecta* L.cream	5±0.00	10.000±1.000 **	13.0±1.0**	20±0.00**

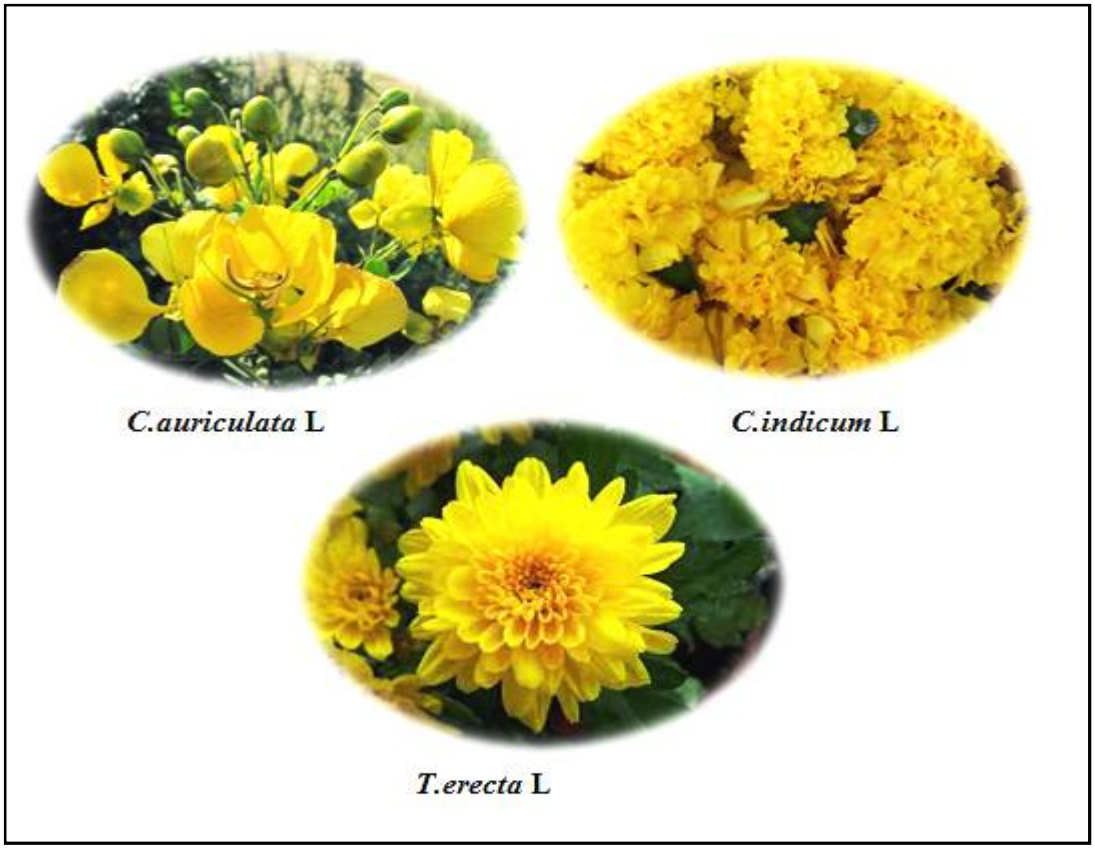

Figure 1: Flowers of the three plants

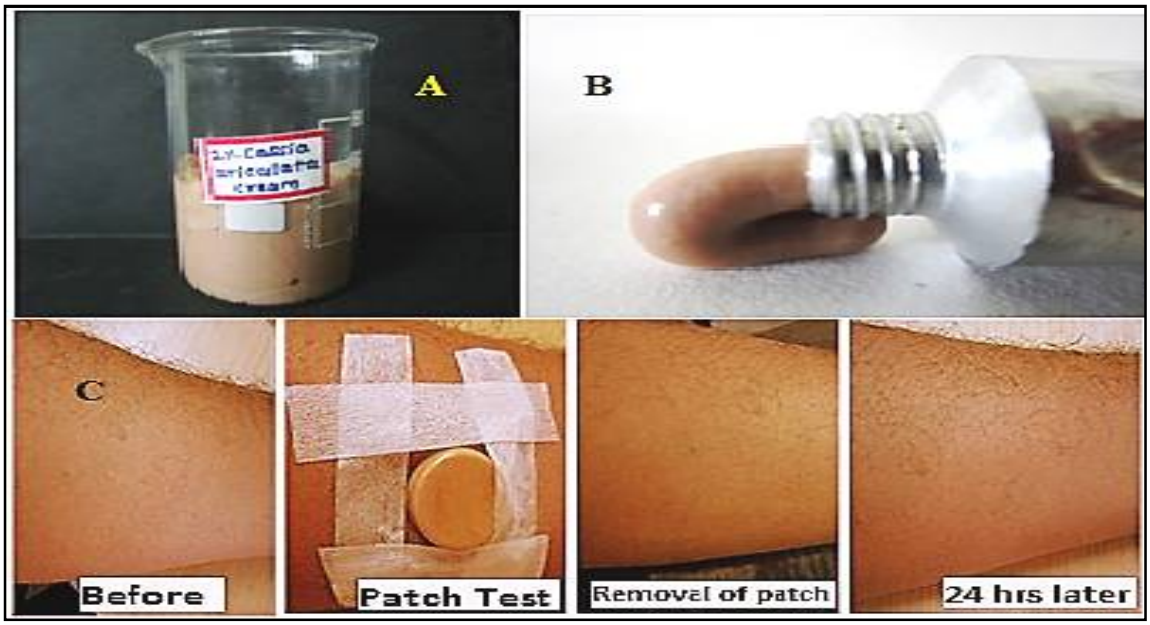

Figure 2: A- 2% cream of *C.auriculata*; B: Tube extrudability; C: Skin irritation test by single patch method

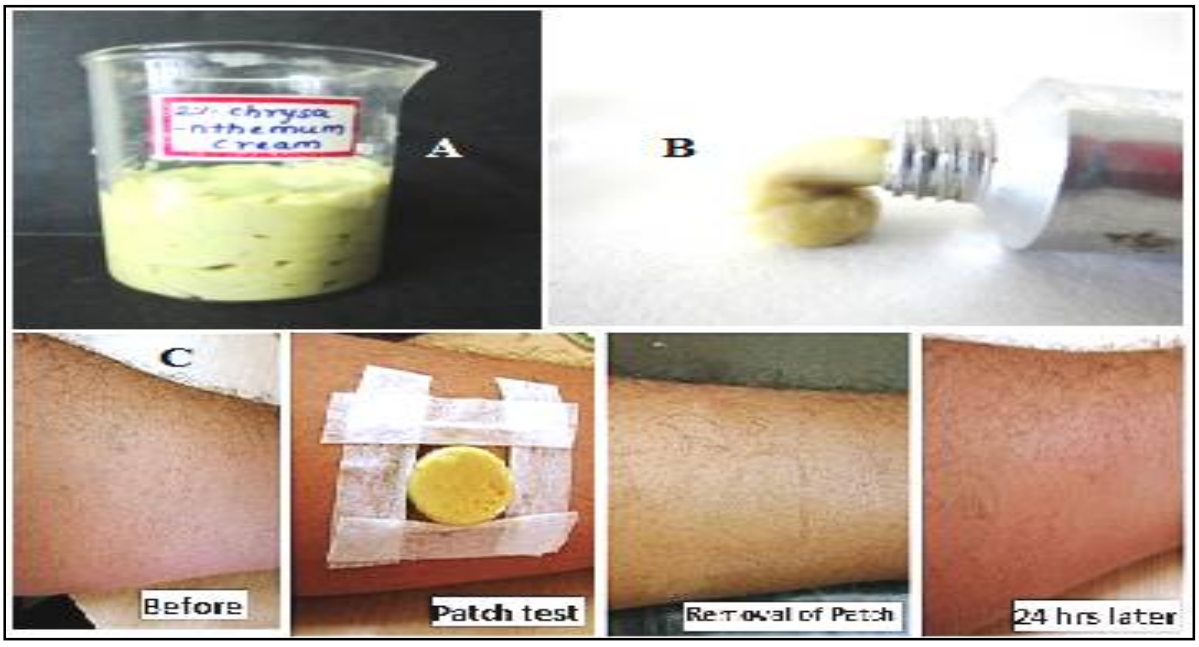

Figure 3: A- 2% cream of *C.cinerarifolium*; B: Tube extrudability; C: Skin irritation test by single patch method

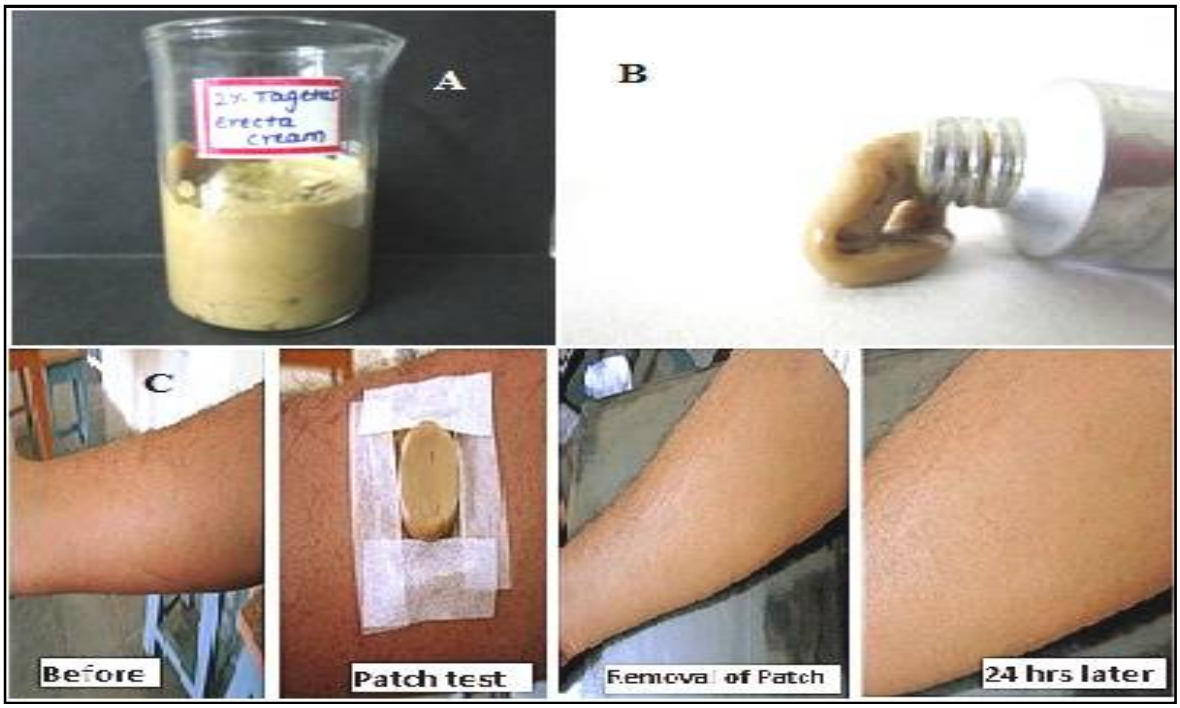

Figure 4: A- 2% cream of *T.erecta*; B: Tube extrudability; C: Skin irritation test by single patch method

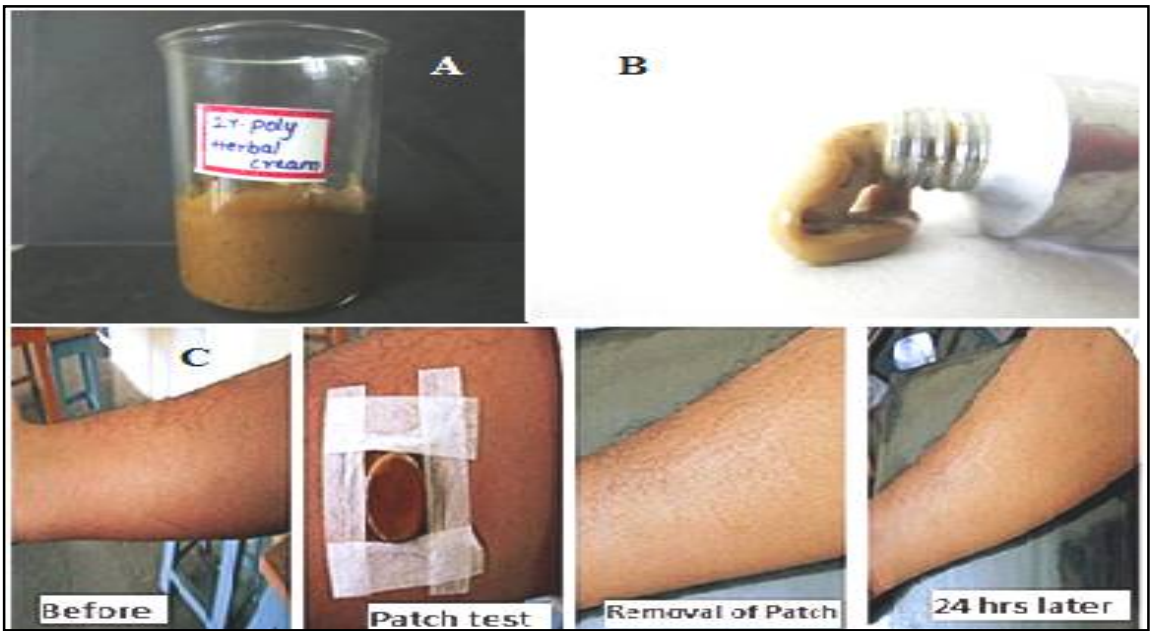

Figure 5: A- 2% polyherbal cream ; B: Tube extrudability; C: Skin irritation test by single patch method

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RESEARCH ARTICLE

Determine the Varietal Resistance in Indian Bean, *Lablab purpureus* (L.) Sweet against Pod Borer Complex

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ABSTRACT

Field experiment was conducted at Horticulture farm SKN college of agriculture, Jobner, Rajasthan, during *kharif* 2009. Out of 12 varieties/cultivars of Indian bean screened against pod borer complex based on pod damage both number and weight basis. None of them was found completely free from infestation of pest. Based on the statistically categorization, the varieties AnkurGoldy, JDL-79-1 and Pusa Early Profilic were found resistant had pod damage both number and weight basis below 16.29 and 16.21 per cent, respectively. Whereas, cultivars Local-2-1 and Local-2-2 were found highly susceptible had pod damage both number and weight basis above 27.39 and 27.29 per cent, respectively. The remaining varieties/cultivars SwarnaUtkrisht, IS-1, IS-2, Local-1-2, Local-1-1, Locan-3-2 and Local-3-1 were found moderately susceptible had 16.29 to 27.39 per cent pod damage on number basis and 16.21 to 27.29 per cent pod damage on weight basis.

Keywords: Pod Borer, Number and Weight Loss, Cultivars/Varietes and Resistance.

INTRODUCTION

The Indian bean, *Lablab purpureus* (L.) Sweet (Family: Fabaceae) originated from India and its wild form are found all over the sub-continent. It is a perennial twining or creeping rainy season herb that is generally cultivated as annual.Indian bean is a fast growing tropical and subtropical pulse-cum-vegetable suitable crop in India (Sprent*et al*. 2010). Considering it's potential, the high protein grain Indian bean (Khan *et al*. 2005). It is cultivated for its fresh tender, pods and seeds form an important source of protein diet of human beings also for fodder. The mature and

dark coloured seeds of Indian bean have trypsin inhibitor (Butani and Jotwani, 1984). Insect-pests are one of the major limiting factors for production of Indian bean. The crop is attacked by as many as 55 species of insects and one species of mite.Among them, the pod borers are considered to be the most devastating pests causing pod to the tune of nearly 54 per cent (20 q/ha) (Naik et al, 2009), where as a others were considered as minor pests.important group causing crop loss to the tune of 80 to 100 per cent (Katagihallimath and Siddappaji, 1962). The crop suffers from a variety of insect-pests Apporascaatkinsoni (Hewitson) and Helicoverpaarmigera (Hubner) which reduce yield (up to 70%) and productivity (BrachmapraKash*et al*. 2004) in field conditions (Rekha&Mallapur 2007).The pod borer complex include avare pod borer, *Adisuraatkinsoni*(Moore); gram pod borer, *Helicoverpaarmigera*(Hubner); spotted pod borer, *Marucatestulalis*(Geyer); bean pod borer moth / pea pod borer, *Etiellazinckenella*(Treitschke); soybean pod borer, *Lampiodesboeticus*(L.); and black cowpea seed moth, *Cydiaptychora*Meyrick. These borer causes substantial damage to flowers by webbing and boring into the pods.

MATERIALS AND METHODS

The experiment was laid out in a simple randomized block design (RBD). Twelve varieties as treatments including local cultivar each replicated four times. The plot size was 1.8 x 1.5 m^2 with row to row and plant to plant distance of 60 and 30 cm, respectively. The crop was sown on 21th July, 2009. The varieties screened and their source of supply is shown in Table 1.

Observation

To assess the incidence of pod borers, observations on pod borer damage was taken at the time of peakings at weekly intervals on randomly selected five tagged plants and per cent damage was estimated by counting and weighting both damaged and total number of pods. Besides, observations on flowering and pod initiation time were also taken.

RESULTS AND DISCUSSION

In the present investigations 12 varieties/cultivars of Indian bean viz., Pusa Early Prolific, SwarnaUtkrisht, AnkurGoldy, IS-1, IS-2, JDL- 79-1, Local-1-1, Local-2-2, Local-3-1 and Local-3-2 were screened for their relative resistance against pod borer complex infestation. The results revealed that none of the variety/cultivars of Indian bean were found completely free from the infestation of pod borer complex. The data revealed that significant difference existed among the varieties/cultivars in their resistance/susceptibility. During peak minimum infestation on pod of Indian bean was observed on AnkurGoldy followed by JDL-79-1 and Pusa Early Profilic and maximum was on Local-2-1 followed by Local-2-2. The peak per cent infestation on pod of different varieties/cultivars of Indian bean both number and weight basis were categorized on the basis of formula $x \pm \sigma$. The varieties AnkurGoldy, JDL-79-1 and Pusa Early Profilic were categorized as resistant had below 16.29 and 16.21 per cent infestation on mummer and weight basis respective, whereas, cultivars Local-2-1 and Local-2-2 were categorized as high susceptible had more than 27.39 and 27.29 per cent infestation on number and weight basis, respectively. The remaining varieties/cultivars *viz*., SwarnaUtkrisht, IS-1, IS-2, Local-1-2, Local-1-1, Local-3-2 and Local-3-1 were categorized as moderately susceptible had 16.29 to 27.39 per cent infestation on number basis and 16.21 to 27.29 per cent infestation on weight basis.

These finding showed wide range of diversity among tested varieties/cultivars of Indian bean with respect to pod borer damage. These observations are in conformity with the findings of Chakravarthy and Lingappa (1986), Rashid (1999), Kundari*et al*. (2000), Sultana (2001), Mass *et al*. (2003) and Rudranaik*et al*. (2009) in field bean. The partially support revealed that the accession, EC-92956 recorded the least pod damage (9.58%) and highest yield of 1.55 kg(Parvathy*et al*. 2011).Similarly, Shivashankar*et al.* (1989) found cultivar PLS-24 and PLS-16-1 as resistant, PLS-93 on as highly tolerant and M-39579 and IC-661-1 moderately tolerant. However, they observed a high degree of resistance in *L. purpureus* var. *ignosus*. Likewise, Ghuguskar (2001) observed least infestation of pod borer on Kokanbhushan

followed by AKW-9303, AKW-9304 and AKW-9301. However, AKW-9306, AKW-9611 and AKW-9312 had significantly high infestation which indicates the presence of diversity with respect to reaction pattern against pod borer complex in Indian bean.

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Table 1. The varieties screened and their source of supply

Name of varieties / cultivars	Source of supply
Pusa Early Profilic, SwarnaUtkrisht, AnkurGoldy, IS-1, IS-2, JDL-79-1	Department of Horticulture, Agricultural Research Station, Durgapura, Jaipur (Rajasthan)
Local-1-1 (Laxmi seeds), Local-1-2 (Laxmi seeds), Local-2-1 (Ekta seeds), Local-2-2 (Ekta seeds), Local-3-1 (Pooja seeds), Local-3-2 (Pooja seeds)	Local market, Jaipur (Rajasthan)

Table 1. Varietal resistance of Indian bean varieties/cultivars against pod borer complex in 2009 (Number basis)

S. No.	Varieties	Per cent pod infestation* (at weekly picking)															Mean
		15/09	22/09	29/09	06/10	13/10	20/10	27/10	03/11	10/11	17/11	24/11	01/12	08/12	15/12	22/12	
1.	Pusa Early Profilic	3.33 (10.51) **	5.00 (12.93)	8.19 (16.63)	8.91 (17.37)	11.32 (19.66)	12.56 (20.76)	13.68 (21.70)	13.70 (21.72)	14.53 (22.41)	15.38 (23.09)	14.02 (21.99)	13.69 (21.71)	13.56 (21.61)	0.00 (0.00)	0.00 (0.00)	11.37 (19.71)
2.	Swarna Utkrisht	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	19.18 (25.97)	17.34 (24.61)	16.50 (23.97)	16.12 (23.67)	15.45 (23.15)	14.75 (22.58)	16.56 (24.01)
3.	Ankur Goldy	3.03 (10.02)	4.70 (12.52)	7.98 (16.41)	8.56 (17.01)	11.15 (19.51)	12.31 (20.54)	13.39 (21.46)	13.48 (21.54)	14.35 (22.26)	15.22 (22.96)	13.82 (21.82)	13.43 (21.50)	13.29 (21.38)	0.00 (0.00)	0.00 (0.00)	11.13 (19.49)
4.	IS-1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.81 (24.20)	18.05 (25.14)	19.27 (26.04)	17.46 (24.70)	16.59 (24.03)	16.21 (23.74)	15.58 (23.24)	14.93 (22.73)	16.86 (24.24)
5.	IS-2	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.90 (24.26)	18.11 (25.19)	19.34 (26.09)	17.59 (24.79)	16.71 (24.13)	16.37 (23.86)	15.69 (23.33)	15.04 (22.82)	16.96 (24.33)
6.	JDL-79-1	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.56 (21.61)	13.61 (21.65)	14.41 (22.31)	15.29 (23.02)	13.91 (21.90)	13.58 (21.62)	13.42 (21.49)	13.24 (21.33)	12.37 (20.59)	13.71 (21.73)
7.	Local-1-1(Laxmi seeds)	5.74 (13.86)	8.01 (16.44)	11.51 (19.83)	12.90 (21.05)	15.65 (23.30)	17.86 (24.99)	18.96 (25.81)	20.48 (26.91)	21.88 (27.89)	23.48 (28.98)	21.18 (27.40)	19.78 (26.41)	19.20 (25.99)	0.00 (0.00)	0.00 (0.00)	16.66 (24.09)
8.	Local-1-2(Laxmi seeds)	5.61 (13.70)	7.88 (16.30)	11.39 (19.72)	12.76 (20.93)	15.59 (23.26)	17.79 (24.95)	18.92 (25.78)	20.42 (26.86)	21.84 (27.86)	23.43 (28.95)	21.13 (27.37)	19.83 (26.44)	19.11 (25.92)	0.00 (0.00)	0.00 (0.00)	16.59 (24.04)
9.	Local-2-1 (Ekta seeds)	6.77 (15.08)	9.34 (17.79)	14.55 (22.42)	16.61 (24.05)	18.02 (25.12)	20.51 (26.93)	21.96 (27.94)	23.95 (29.30)	26.14 (30.75)	32.02 (34.46)	25.25 (30.16)	23.73 (29.15)	22.29 (28.17)	0.00 (0.00)	0.00 (0.00)	20.09 (26.63)
10.	Local-2-2 (Ekta seeds)	6.86 (15.18)	9.44 (17.89)	14.65 (22.50)	16.73 (24.13)	18.10 (25.18)	20.57 (26.97)	20.05 (28.01)	24.07 (29.38)	26.32 (30.08)	32.16 (34.55)	25.42 (30.28)	23.89 (29.26)	22.44 (28.27)	0.00 (0.00)	0.00 (0.00)	20.21 (26.71)
11.	Local-3-1 (Puja seeds)	5.88 (14.03)	8.15 (16.59)	12.81 (20.97)	14.51 (22.39)	15.73 (23.37)	17.97 (25.08)	19.15 (25.95)	20.68 (27.05)	22.09 (28.03)	23.69 (29.12)	21.38 (27.54)	20.38 (26.84)	19.38 (26.12)	0.00 (0.00)	0.00 (0.00)	17.06 (24.40)
12.	Local-3-2 (Pua seeds)	5.78 (13.91)	8.07 (16.50)	12.73 (20.90)	14.63 (22.49)	15.68 (23.33)	17.92 (25.04)	19.07 (25.89)	20.57 (26.97)	21.98 (27.96)	23.58 (29.05)	21.27 (27.46)	20.27 (26.76)	19.27 (26.04)	0.00 (0.00)	0.00 (0.00)	16.99 (24.34)
	S.Em.±	0.29	0.35	0.39	0.43	0.45	0.48	0.52	0.73	0.87	0.98	0.89	0.78	0.68	0.51	0.51	
	CD at 5%	0.84	1.00	1.14	1.24	1.30	1.38	1.48	2.10	2.49	2.83	2.55	2.23	1.96	1.46	1.47	

* Per cent pod infestation of four replications

** Figures in parentheses are angular transformed values

Table 2. Varietal resistance of Indian bean varieties/cultivars against pod borer complex in 2009 (Weight basis)

S. No.	Varieties	Per cent pod infestation* (at weekly picking)															Mean
		15/09	22/09	29/09	06/10	13/10	20/10	27/10	03/11	10/11	17/11	24/11	01/12	08/12	15/12	22/12	
1	**Pusa Early Profilic**	3.29 (10.45)**	4.91 (12.80)	8.09 (16.52)	8.81 (17.27)	11.22 (19.57)	12.47 (20.68)	13.58 (21.62)	13.64 (21.67)	14.47 (23.36)	15.32 (23.05)	13.96 (21.94)	13.63 (21.66)	13.50 (21.56)	0.00 (0.00)	0.00 (0.00)	11.30 (19.64)
2	**Swarna Utkrisht**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	19.08 (25.90)	17.24 (24.53)	16.40 (23.89)	16.01 (23.58)	15.33 (23.04)	14.61 (22.47)	16.44 (23.92)
3	**Ankur Goldy**	2.98 (9.94)	4.65 (12.45)	7.89 (16.31)	8.47 (16.92)	11.05 (19.41)	12.22 (20.46)	13.29 (21.38)	13.39 (21.46)	14.26 (22.19)	15.13 (22.89)	13.73 (21.75)	13.34 (21.42)	13.20 (21.30)	0.00 (0.00)	0.00 (0.00)	11.05 (19.41)
4	**IS-1**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.72 (24.13)	17.95 (25.06)	19.17 (25.96)	17.36 (24.62)	16.48 (23.95)	16.10 (23.66)	15.49 (23.17)	14.81 (22.64)	16.76 (24.17)
5	**IS-2**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	16.81 (24.20)	18.05 (25.14)	19.28 (26.04)	17.53 (24.75)	16.65 (24.08)	16.29 (23.80)	15.59 (23.25)	14.91 (22.71)	16.89 (24.27)
6	**JDL-79-1**	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	13.47 (21.53)	13.50 (21.56)	14.32 (22.23)	15.19 (22.94)	13.81 (21.81)	13.48 (21.54)	13.32 (21.40)	13.15 (21.26)	12.24 (20.48)	13.61 (21.65)
7	**Local-1-1(Laxmi seeds)**	5.64 (13.74)	7.90 (16.33)	11.41 (19.74)	12,80 (20.96)	15.55 (23.22)	17.78 (24.94)	18.86 (25.74)	20.38 (26.84)	21.78 (27.82)	23.38 (28.92)	21.08 (27.33)	19.68 (26.33)	19.10 (25.91)	0.00 (0.00)	0.00 (0.00)	16.56 (24.02)
	Local-1-2(Laxmi seeds)	5.50 (13.56)	7.78 (16.20)	11.28 (19.62)	12.67 (20.85)	15.50 (23.18)	17.70 (24.88)	18.82 (25.71)	20.32 (26.97)	21.74 (27.79)	23.33 (28.88)	21.03 (27.29)	19.73 (26.37)	19.01 (25.85)	0.00 (0.00)	0.00 (0.00)	16.49 (23.96)
9	**Local-2-1 (Ekta seeds)**	6.67 (14.97)	9.24 (17.70)	14.46 (22.35)	16.52 (23.98)	17.92 (25.04)	20.41 (26.86)	21.86 (27.87)	23.84 (29.23)	26.04 (30.68)	31.91 (34.39)	25.14 (30.09)	23.62 (29.08)	22.18 (28.10)	0.00 (0.00)	0.00 (0.00)	19.58 (26.55)
10	**Local-2-2 (Ekta seeds)**	6.76 (15.07)	9.34 (17.79)	14.55 (22.42)	16.63 (24.07)	18.01 (25.11)	20.47 (26.90)	21.96 (27.94)	23.99 (29.33)	26.22 (30.80)	32.07 (34.49)	25.32 (30.21)	23.80 (29.20)	22.35 (28.21)	0.00 (0.00)	0.00 (0.00)	20.11 (26.64)
11	**Local-3-1 (Puja seeds)**	5.82 (13.96)	8.09 (16.52)	12.75 (20.92)	14.40 (22.30)	15.64 (23.29)	17.87 (25.01)	19.05 (25.88)	20.60 (26.99)	22.03 (27.99)	23.63 (29.08)	21.32 (27.50)	20.31 (26.79)	19.32 (26.07)	0.00 (0.00)	0.00 (0.00)	16.96 (24.32)
12	**Local-3-2 (Pua seeds)**	5.69 (13.80)	7.97 (16.40)	12.63 (20.82)	14.52 (22.40)	15.58 (23.25)	17.83 (24.99)	18.98 (25.83)	20.48 (26.90)	21.88 (27.89)	23.48 (28.98)	21.17 (27.39)	20.18 (26.69)	19.17 (25.96)	0.00 (0.00)	0.00 (0.00)	16.89 (24.26)
	S.Em.±	0.28	0.34	0.38	0.41	0.46	0.48	0.51	0.73	0.86	0.96	0.87	0.76	0.67	0.49	0.46	
	CD at 5%	0.81	0.99	1.08	1.17	1.32	1.38	1.48	2.09	2.49	2.76	2.51	2.20	1.93	1.42	1.38	

* Per cent pod infestation of four replications

** Figures in parentheses are angular transformed values

Table 3. Categorization of Indian bean varieties/cultivars against pod borer complex in 2009

S.No.	Per cent pod damage at peak period		Varieties/cultivars	Category
	Number basis	Weight basis		
1.	Below 16.29 ($< \bar{x} - \sigma$)	16.21	Ankur Goldy, JDL-79-1 and Pusa Early Profilic	Resistant
2.	16.29 to 27.39 ($\bar{x} - \sigma$ to $\bar{x}$)	16.21 to 27.29	Swarna Utkrisht, IS-1, IS-2, Local-1-2 (Laxmi seeds), Local-1-1 (Laxmi seeds), Local-3-2 (Puja Seeds) and Local-3-1 (Puja Seeds)	Moderately susceptible
3.	Above 27.39 ($> \bar{x} + \sigma$)	27.29	Local-2-1 (Ekta seeds) and Local-2-2 (Ekta seeds),	Highly susceptible

X x = 21.84 and 21.75;X σ = 5.55 and 5.54

Where,$\bar{x}$ = mean of peak per cent pod infestation ;σ = standard deviation ;on number basis $\bar{x} \pm \sigma$;on weight basis $\bar{x} \pm \sigma$

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RESEARCH ARTICLE

Effect of Duration of Dystocia on Maternal and Fetal Survival Following Caesarean in Crossbred Cows

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ABSTRACT

The present study was conducted on forty crossbred cows with a history of calving difficulty presented to the clinic during April 2015 to May 2016. The influence of duration of dystocia on the outcome of caesarean section of the animal was evaluated. Results revealed that the duration of dystocia is one of the important factors which dictate the outcome and subsequent recovery rate of the dams. The survival rate following caesarean showed a significant inverse relation with duration of dystocia.

Keywords: Caesarean, Crossbred Cow, Dam Survival Rate, Dystocia.

INTRODUCTION

Dystocia has been a long-standing problem in dairy cows, occurring in 3 to 25% of cattle pregnancies. It is associated with numerous factors such as pelvic area of the cow, birth weight of the calf, age of dam, twin pregnancy, presentable disposition, gestation length, sex of the calf, and body condition of the cow at calving, hormonal status and nutrition of dam (Noakes *et al*., 2001). Dystocia can lead to increased incidence of retained fetal membranes, uterine infections, reduction of milk production, failure to conceive, long calving intervals, and reduced health of cows and survival of calves (Bellows and Lammoglia, 2000).Under Indian conditions most cases of dystocia often are first handled by owners and Para-veterinary personnel using the wrong obstetrical techniques and are referred to a hospital at a very late stage. The treatment of choice in these cases is largely dictated by the duration of dystocia, the state of the birth canal and the general body condition of the animal. Analysis of such cases may provide an insight into the various factors which dictate the outcome of dystocia under Indian conditions. The present study was

envisaged to study the factors influencing maternal and fetal survivability in crossbred cows undergoing caesarean section.

MATERIALS AND METHODS

The present study was conducted on forty crossbred cows maintained by the farmers in and around Bangalore city with a history of calving difficulty presented to the clinic of the Department of Veterinary Gynecology and obstetrics, Veterinary College, Bengaluru during April 2015 to May 2016.To analyse the influence of duration of dystocia on the outcome of caesarean section of the animal, the information regarding the approximate duration of dystocia immediately after presentation, complete history regarding the obstetrical clinical status of the cows was obtained and were classified into following four groups:

a. Animals referred within 12 h from the onset of labour.
b. Animals referred between 12 to 24 h after the onset of labour.
c. Animals referred between 24 to 48 h after the onset of labour.
d. Animals referred 48 h after the onset of labour.

The condition of the birth canal was classified as normal, edematous, torn and lacerated, which in turn reflected the degree of handling of the cases prior to its reference to this clinic and also the duration of dystocia. The number of cases where live, dead, dead and emphysematous fetuses were encountered in relation to the approximate duration of dystocia was obtained for analysis of cases.To establish the temporal relationship between the duration of dystocia, condition of the birth canal, and the maternal/fetal recovery rate were compared by Univariate Chi-square test as per the methods of Steel *et al*. (2006). P value of $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

The prevalence of dystocia was 22.50, 27.50, 17.50 and 32.50 per cent in cows presented to referral center within 12 h, 12-24 h, 24-48 h and more than 48 h, respectively, after the onset of labor (Table 1).Irrespective of the duration or nature of dystocia, the birth canal was normal in 2.50 per cent, edematous in 92.50 per cent, while it was lacerated or torn in 2.50 per cent of cases. Significantly higher percentage of cows showed edema of birth canal (Table 2). The studies on the effect of duration of dystocia and its effect on condition of birth canal revealed, birth canal was normal when the cases were presented at less than 12 hours, while the incidence of edematous condition was 17.50%, 27.50%, 17.50% and 30.00%, respectively in cases presented to the referral centers at less than 12 h, 12-24, 24-48 and more than 48 h, respectively. In 2.50 % cases each the birth canal was torn or lacerated when the cases of dystocia presented within 12 h and more than 48 hours after the onset of labour (Table 2).In a previous study in cows with dystocia, higher percentages of cows with edema of birth canal were found (Kanakapur, 1992).

The higher rate of edematous condition noticed indicated previous handling of the cases prior to presentation of cases to referral center.In the present study, higher incidence of foetal dystocia (57.50) was observed than maternal dystocia (42.50 percent) (Table 3). These findings are well supported by the previous study in buffaloes (Purohit *et al*., 2012) and cattle (Singla *et al*., 1990; Phogat *et al*., 1992).Survival rates of the dams following caesarian operation among 40 cases of dystocia are presented in Table 4. Irrespective of duration of dystocia, the overall survival rate after caesarian operated cows was 62.50 per cent. This finding consistent with previous study who reported a dam survival rate of 62.50 per cent following caesarian in dystocia cows (Kumar, 2012).However, a much lower survival rate of 25.00 per cent (Prabhakar, 1995), 34.90 per cent (Singh *et al*., 2013), 46.30 per cent (Dhindsa *et al*; 2010) and higher incidence of 66. 67 per cent (Dhahake *et al*; 2005) and 76.90 per cent (Purohit and Mehta, 2006) were reported. The difference between the present study and previous reports may have been due to condition of dam at presentation, duration of dystocia, previous handling may have contributed to the observed discrepancies (Amer *et al*., 2008; Amin *et al*., 2011).The perusal of Table 4, reveals the survival rate of the cows which were in labour for less than 12 h, 12-24 h, 24-48 h and more than 48 h was 30.00 %, 20.00 %, 0.00 % and 12 .50 %, respectively.These

observations suggested that the survival rate following caesarean showed a significant inverse relation with duration of dystocia and these observations are in consonance with several studies who have also reported that the survival rate of the dams showed a significant inverse relationship with the duration of dystocia, in cows and in buffaloes (Srinivas *et al*., 2007; Dhindsa *et al*., 2010; Mane and Bhangre, 2015). Out of twenty eight cows which were in labour for less than 24 hours, the survival rate of dam was 50.00 per cent as against 12.50 per cent survivability in cows experiencing dystocia for more than 24 h (Table 4). The time elapse between onset of dystocia and the caesarean operation determines the outcome. Previous studies have also shown that the dam survival rates was high (64.7% to 100.0%) when caesarean was performed within 24-36 h onset of dystocia (Singh and Dhaliwal, 1998; Murty *et al*., 1999) and the survivability decreased to 25%-33.0% when the operation was performed after 72 h (Murty *et al*.,1999; Srinivas *et al*., 2007).Based on the observations made in the present study that duration of dystocia is one of the important factors which dictate the outcome and subsequent recovery rate of the dams.

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Table 1. Effect of duration of dystocia on incidence of dystocia in crossbred cows

Duration of dystocia	Number of cases	Per cent
< 12 hours	09	22.50
12---24	11	27.50
24---48	07	17.50
>48	13	32.50
Total	40	100
$\chi^2 = 2.00$		

Table 2. Effect of duration of dystocia and condition of birth canal

Duration of dystocia	Condition of birth canal			Total
	Normal	Edematous	Torn/lacerated	
< 12 hours	01(2.5)	07(17.5)	01(2.5)	09(22.5)
12---24	00	11(27.5)	00	11(27.5)
24---48	00	07(17.5)	00	07(17.5)
>48	00	12(30.0)	01(2.5)	13(32.5)
Total	01(2.5)	37(92.5)	02(5.0)	40(100.0)
$\chi^2 = 38.500^*$ (3df)				

Note: Values in parenthesis refers percentage

Table 3. Incidence of fetal and maternal dystocia

Causes of dystocia	Number of cases	Percentage
Maternal	17	42.50
Fetal	23	57.50
Total	40	100

Table 4. Dams survival rate

Duration of dystocia	Maternal dystocia		Fetal dystocia		Total	
	Survived	Dead	Survived	Dead	Survived	Dead
< 12 hours	3 (7.50)	1 (2.50)	9 (22.50)	1 (2.50)	12 (30.00)	2 (5.00)
12-24 hours	4 (10.00)	2 (5.00)	4 (10.00)	4 10.00)	8 (20.00)	6 (15.00)
24-48 hours	00	2 (5.00)	00	2 (5.00)	00	4 (10.00)
> 48 hours	3 (7.50)	2 (5.00)	1 (2.50)	1 (2.50)	5 (12.50)	3 (7.50)
Total	10 (25.00)	7 (17.50)	15 (37.50)	8 (20.00)	25 (62.50)	15 37.50)

Note: Values in parenthesis refers percentage