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RESEARCH ARTICLE

Impact of Textile Dyeing and Bleaching Effluents on Surface Water Quality of River Noyyal at Tirupur, Tirupur Dist, TamilNadu, India.

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ABSTRACT

River Noyyal is a seasonal river and it flows through the two urbanised and well known industrial cities namely Coimbatore and Tirupur before it reaches River Cauvery as a tributary. More than 700 textile dyeing and bleaching units are situated in Tirupur region across the river basin release partially or untreated effluents into R.Noyyal.To understand the extent of pollution on surface water quality, physicochemical characteristics of surface water were analyzed monthly at selected sites of River Noyyal during January 2008 to December 2010. Maximum level of total dissolved solids, total hardness, chemical oxygen demand, biological oxygen demand, sodium and nitrate were recorded as 9100, 3100, 611, 155, 4992 and 48 mg/l, respectively.

Key words: physico-chemical characteristics, dyeing and bleaching effluents, River Noyyal, anthropogenic pollution

INTRODUCTION

Freshwater ecosystem offers numerous ecosystem services to humanity and it act as a prime inland water resources for domestic, industrial and irrigation purpose. Rapid urbanization, mushrooming of industries and its associated economic activities creates tremendous pressure on water resources. Evidently, rivers and their tributaries passing through the cities are receiving huge amount of contaminants released from point and non-point sources of pollution [1]. Further, seasonal variations in rainfall, surface run-off, interflow, groundwater flow and pumped in and outflows have a strong effect on river discharge and subsequently on the concentration of pollutants in river water [2].

In India, industry and agriculture coexist in the same geographical area and share the same water resources of a river basin as like other developing countries. Industries and cities withdraw large quantities of water from rivers for cater their needs and discharge more or less an equal amount of wastewater into the river. Disposal of untreated/ partially treated or diluted industrial effluents on surface water bodies could transfer a large cost to society in terms of environmental pollution and related human health hazards. Broadly, industrial pollutants often alter the physico-chemical characteristics, such as temperature, acidity, salinity, or turbidity of receiving water bodies, leading to ecosystem alterations and also lead to higher incidence of water-borne diseases. It is estimated that industries are responsible for dumping 300-400 million tons of heavy metals, solvents, toxic sludge, and other waste into water bodies each year in worldwide [3]. According to recent estimates, more than 70 per cent of industrial wastes in developing countries are dumped untreated into adjacent waters resources [4]. Such kind of industrial pollution was reported in most of river basins in India [5-11].

The River Noyyal a tributary of the Cauvery, originates from Velliyangiri hillocks on the Western Ghats and flows from this mountain valley region towards fairly level areas in the lower catchments and it reaches the river Cauvery about 170 km downstream. The total catchment area is 3510 km^2 and is located between 10°56′ N, 76°41′E and 11°19′ N, 77°56′E. Noyyal is a seasonal river, fed by the monsoons. The water flow is moderate for a short period during the monsoon season. The basin annually receives more than 3000 mm and 600 mm of rainfall during the southwest monsoon and northeast monsoon, respectively [12].

River Noyyal receives massive quantity of industrial effluents and municipal waste water while flowing through urban areas. A well known 'industrial city' Tiruppur is located on the bank of the Noyyal River. In this city, about 9000 knitting, processing and manufacturing units are functioning at present [13]. The bleaching and dyeing units consume large quantities of water, but most of the water used by these units is discharged as effluents containing a variety of dyes and chemical (acids, salts, wetting agents, soaps, oil etc.). These units discharge nearly 90 MLD of effluents into the Noyyal River, leading to contamination of surface and groundwater and soil in and around Tirupur and downstream. Most of published reports mainly concentrated on groundwater contamination in this region. There is no detailed study on surface water quality in River Noyyal was undertaken. In this context, the present study is focused to assess the impact of industrial effluents on surface water quality of River Noyyal in downstream region.

MATERIALS AND METHODS

Surface water samples were collected in acid washed polythene bottles from River Noyyal in three sites (Kasipalayam, Orathupalayam reservoir and Muthur barrage) during January 2008 to December 2010 at monthly intervals. Sampling site characteristics are as below,

1. Kasipalayam – The sampling site receives industrial effluents and municipal sewages from Tirupur city.
2. Orathupalayam reservoir – The reservoir is located about 20 km downstream of Tirupur on the border between Kangayam and Perundurai Taluks. It receives huge amount of industrial effluents and municipal sewage from upstream industries and urban settlements.
3. Muthur barrage – The sampling site is located about 15 km downstream of Orathupalayam reservoir and ahead of mixing point of River Noyyal and River Cauvery.

The parameter such as pH, electrical conductivity and total dissolved solids were measured in the field using portable water monitoring kit (make: Deep vision). Total alkalinity, total hardness, calcium, magnesium, chloride were estimated by titration method. Sodium and potassium level was measured using flame photometer (make: Systronics). Sulphate, nitrate and phosphate were estimated spectrophotometrically. All the analysis was carried out as per standard methods of APHA [14]. The results obtained were evaluated in accordance with the norms prescribed under Bureau of Indian Standards (BIS) and World Health Organization (WHO). Correlation analysis was performed using Statistical Package for Social Sciences (SPSS) software.

RESULTS AND DISCUSSION

The descriptive statistics of physico-chemical characteristics of surface water are given in Table 1. Irrespective of sampling locations, pH value of the surface waters samples ranged between 7.06 and 8.82. The minimum pH value was recorded as 7.06 during the month of March (2010) at Muthur barrage. The maximum pH level (8.82) was recorded at Kasipalayam during the month of February. In all the three sample sites, alkaline condition was observed throughout the study period and falls within the maximum permissible limit of World Health Organization (6.5 to 9.2). Electrical conductivity (EC) is an indicative of the total concentration of total dissolved ions in water [15]. EC value of the water samples ranged between 0.82 and 8.85 mS/cm in the River Noyyal. The maximum (8.85 mS/cm) and minimum level (0.82 mS/cm) of EC was recorded during the month of May (2009) and October at Orathupalayam reservoir. The value of total dissolved solids (TDS) and total suspended solids (TSS) was figured from 590 to 9100 mg/l and 215 to 1392 mg/l, respectively. Enhanced level of EC and TDS attributed to discharge of ionic content rich bleaching and dyeing effluents into river system. Significant reduction in the TSS and TDS during the period of September to December was noted in all three sampling sites implies monsoonal influenced dilution.
The turbidity value was ranged between 5 and 22 NTU. Maximum level of turbidity recorded at Orathupalayam during the month of June (2008 and 2010). The lowest value was observed at Kasipalayam during monsoon months (September and October). In general, higher level of turbidity was recorded during summer season (February to June). High level of turbidity observed all three sites might be attributed to dyeing and bleaching effluents which are released by industries located on either sides of the river basin.

DO may be a potential indicator of river quality in assessing urban impacts on river ecosystem [16]. DO level was found between 0.20 and 5.40 mg/l. Very low level of DO recorded at Orathupalayam (0.8 to 4.2 mg/l) throughout the study period. The level of DO at Kasipalayam and Muthur barrage was observed in the range from 0.2 to 4.8 and 2.15 to 5.40, respectively. While comparing with Kasipalayam and Orathupalayam site, Muthur barrage recorded moderately high level of DO throughout the study period. The low DO levels observed in Kasipalayam and Orathupalayam may be an indication of oxidative decay of organic debris by utilization of DO, it indicates the unhealthy condition of the River, a scenario similar to Hooghly estuary on the east coast of India [17].

Chemical oxygen demand (COD) is a measure of organic matter in the sample including biological as well as chemically degradable fractions which survive bacterial attack. Level of COD in water samples found between 39 and 611 mg/l. Maximum level of COD was observed at Muthur barrage during the month of May (2008) and minimum level was recorded at Orathupalayam in the month of October (2010). The mean COD level at Kasipalayam, Orathupalayam and Muthur barrage were recorded as 330.6, 356.2 and 299.3 mg/l, respectively.

Biochemical oxygen demand (BOD) is a measure of the amount of food for bacteria that is found in water. BOD content of the water samples figured between 3.4 and 155 mg/l with mean level of 50.7 mg/l. Maximum level of BOD (155 mg/l) was observed at Muthur barrage during the month of June (2008) and low level (3.4 mg/l) was recorded at Orathupalayam in the month of October (2010). According to Mukherjee et al. [18], about 30% of BOD and 47% of COD come from industrial sources in Indian River system. More than half number of samples not falls within the permissible limit of Bureau of Indian standards (6 mg/l). High level of COD and BOD indicates high organic load in surface water which depletes oxygen for its oxidation process.

The alkalinity of the water samples ranged between 114.4 and 682 mg/l with a mean of 349.7 mg/l. The alkalinity level at Kasipalayam, Orathupalayam and Muthur barrage were observed in the range from 115 to 682, 185 to 628 and 114.4 to 402 mg/l, respectively. The increasing trend of alkalinity was noted in all sampling sites during the summer season (February to June) in the study period. Alkalinity level fluctuation indicates in accordance with dilution of pollution load [19]. In the present study, the bi-carbonate content of all samples falls within the permissible limit (500 ppm) of World Health Organization.

Total hardness level of water samples ranged between 118.8 and 3100 mg/l with a mean of 1110.6 mg/l. Maximum level of total hardness was observed at Orathupalayam (3100 mg/l) during the month of June (2008). Minimum level was recorded at Muthur barrage (118.8 mg/l) in the month of April (2008). A slight decline in the total hardness value was observed at Muthur barrage (118.81 to 712 mg/l) while comparing sampling sites. Desirable limit of total hardness is 300 mg/l however in the absence source it is permissible up to 600 mg/l [20]. Moderately high concentration recorded during summer months probably attributed to evaporation induced concentration. Principal cations imparting hardness are calcium and magnesium. Mean concentration of calcium (Ca) and magnesium (Mg) were observed as 428, 293 & 209 mg/l and 630, 509 & 333 mg/l at Kasipalayam, Orathupalayam and Muthur barrage, respectively. Maximum level of Ca (580 mg/l) and Mg (840 mg/l) was recorded at Kasipalayam in the month of May (2010). Comparatively, low level of Ca (146 to 288 mg/l) and Mg (261 to 411 mg/l) was recorded in Muthur barrage than other sampling sites. The desirable limit of Ca and Mg in drinking water is 75 and 30 mg/l, respectively [21]. Sodium (Na) and potassium (K) concentration was ranged between 268.3 and 4992 mg/l, 16.5 and 82 mg/l, respectively. Maximum level of Na was recorded at Orathupalayam in the month of May (2009). Lowest level was recorded at Muthur barrage in the month of August (2008). In all three years of sampling, very high level of Na was observed in the Orathupalayam site (1074 to 4992 mg/l) followed by Kasipalayam (415 to 1608 mg/l). Maximum level of K was recorded at Kasipalayam (82 mg/l) in the month of May (2010). Elevated level of sodium may adversely affect the cardiac, renal and circulatory functions [22]. The level of chloride (Cl) was ranged from 163.4 to 4291 mg/l with a mean value of 1969.6 mg/l. Maximum level of Cl (4291 mg/l) was recorded at Orathupalayam site in the month of May (2008). Concentration of Cl at Orathupalayam was observed in the range between 274 and 4291 mg/l. Kasipalayam and Orathupalayam had almost same level of Cl in the study period. Higher level of chloride in water samples probably contributed by discharge of chloride rich effluents. Maximum permissible limit of chloride is 1000 mg/l [21]. The concentration of TDS, Cl, Na and total hardness of the present level is much higher than the earlier investigations reported by Senthilnathan [23].

Nitrate (NO_3) concentration in surface water samples ranged between 6.2 and 48 mg/l. The amount of NO_3 found to be high at Orathupalayam (8.7 to 48 mg/l) compared to Kasipalayam (6.8 to 31 mg/l) and Muthur barrage (6.2 to 31 mg/l). Nitrate concentration above 10 mg/l in most of samples indicates surface water contamination in all three sampling sites [24]. High concentration of nitrate can cause health problems in infants and animals, as well as eutrophication of water bodies. There were no notable changes in the nitrate levels throughout the study period. Enhanced concentration of nitrate in drinking water is toxic and causes methemoglobinemia and gastric carcinomas [25]. Mean maximum level of phosphate was recorded at 0.56 mg/l at Orathupalayam followed by Muthur barrage at 0.93 mg/l. Kasipalayam and Orathupalayam had almost same values in the study period. The phosphate values exceeded the permissible limit (0.1 mg/l) of US Public Health Standards [20]. The high concentration of phosphate is due to the domestic sewage discharge from Tirupur city.

The level of Sulphate (SO_4) falls within the range from 148 to 736 mg/l with a mean of 405.5 mg/l. Maximum level of SO_4 (736 mg/l) was recorded at Orathupalayam site. SO_4 content in most of the water samples exceeded the prescribed standards limit of Bureau of Indian standard (400 mg/l) for SO_4. The oil and grece level was figured between 0.38 and 3.84 mg/l with a mean concentration of 1.58 mg/l. The maximum level of oil and grece was recorded at Muthur barrage in the month of October (2010). Fluoride content in all samples falls within the optimum concentration of 1.5 mg/l, as recommended by WHO.

Correlation between the physico-chemical characteristics is given in the Table 2. Total hardness showing positive correlation with Mg and Ca suggest that hardness of water samples is mainly due to the presence of the Mg and Ca ions. The strong positive relationship between TDS with Cl and total hardness implies notable contribution of these parameters in TDS content in surface water. The negative correlation between phosphate and dissolved oxygen revealed that enrichment of nutrients lead to the eutrophication in the River system and inversely affects the dissolved oxygen content of water.

CONCLUSION

The physico-chemical characteristic of surface water samples reveals that most of the parameters are exceeds the prescribed standard limit for drinking water. The analysis of variance among all physicochemical parameters showed significant variation between sampling months and sampling locations at 95% confidential level. Higher level of electrical conductivity and total dissolved solids in water samples attributed to discharge of salt content rich bleaching and dyeing effluents into river system. Elevated concentration of COD and BOD indicates organic load enrichment in River Noyyal. In conclusion, surface water of River Noyyal is not suitable for domestic and agricultural purposes as per drinking water standards of Bureau of Indian Standards and WHO guidelines. Rapid industrialization and urbanization in Tirupur city may further aggravate the pollution level in future. Implementation of zero discharge facilities and integrated waste water resource management practices is paramount necessary in order to prevent the further deterioration.

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Table 1. Descriptive statistics for surface water characteristics of River Noyyal at Tirupur, Tamil Nadu, India during January (2008) to December (2010).

Parameters	Unit	Kasipalayam				Orathupalayam				Muthur barrage			
		Minimum	Maximum	Mean	Std.dev	Minimum	Maximum	Mean	Std.dev	Minimum	Maximum	Mean	Std.dev
pH	-	7.24	8.82	7.77	0.30	7.29	8.40	7.70	0.27	7.06	8.54	7.69	0.42
EC	µS/cm	4.30	8.37	5.90	0.87	1.53	10.22	5.49	2.71	0.82	5.02	3.10	1.10
TDS	mg/l	3600.00	7125.00	5032.06	785.20	1428.00	9100.00	4767.75	2343.32	590.00	4368.00	2609.44	1009.45
TSS	mg/l	510.00	1032.00	762.97	141.66	255.00	1392.00	748.36	297.43	215.00	960.00	525.11	181.91
TS	mg/l	4215.00	7997.00	5795.03	864.06	1913.00	10162.00	5516.11	2611.05	805.00	4894.00	3134.56	1129.07
Turbidity	NTU	5.00	21.00	14.42	4.16	5.00	22.00	13.02	4.71	6.00	22.00	12.63	3.80
Cl	mg/l	1550.00	3550.00	2546.64	468.13	163.42	4291.00	2067.55	1141.83	266.00	3180.00	1294.64	770.40
Alkalinity	mg/l	115.00	682.00	416.88	155.03	160.00	628.00	355.72	136.62	114.37	541.00	276.62	98.27
Hardness	mg/l	840.00	2515.00	1685.75	444.88	118.81	3100.00	1184.40	815.50	154.61	1260.00	461.61	260.36
Ca	mg/l	232.00	580.00	428.25	96.87	151.23	492.00	296.89	94.56	112.00	288.00	204.59	43.14
Mg	mg/l	344.00	840.00	630.08	123.04	288.00	751.00	465.92	132.71	260.56	552.00	375.92	76.12
Na	mg/l	415.00	1608.00	919.00	246.71	314.00	4992.00	2794.14	1642.06	268.34	4100.00	1386.73	1304.69
K	mg/l	16.50	82.00	51.88	15.54	18.80	62.00	39.43	11.39	21.35	62.00	38.89	12.04
DO	mg/l	0.20	4.80	2.25	1.26	0.80	4.80	2.27	1.04	0.90	5.40	3.26	0.96
COD	mg/l	135.00	518.00	330.58	115.59	115.00	608.00	332.47	156.49	39.00	611.00	283.08	143.43
BOD	mg/l	5.50	75.00	41.48	18.30	14.85	110.00	61.72	28.09	3.40	155.00	48.91	32.74
SO_4	mg/l	390.00	649.00	504.78	53.85	196.00	736.00	408.56	154.37	147.96	571.00	303.23	125.89
NO_3	mg/l	6.80	31.00	18.31	7.19	12.00	48.00	23.59	8.85	6.22	21.91	12.46	3.56
PO_4	mg/l	0.10	2.85	1.39	0.67	0.40	1.60	0.92	0.30	0.12	1.20	0.57	0.28
Oil and grease	mg/l	0.50	3.25	1.69	0.79	0.38	3.40	1.78	0.85	0.59	3.84	1.27	0.67

Table 2. Correlation between physico-chemical characteristics of surface water.

	pH	EC	TDS	Turbidity	Cl	Alkalinity	Hardness	Ca	Mg	Na	K	DO	COD	BOD	SO_4	NO_3	PO_4	Oil and grease
pH	1																	
EC	.363**	1																
TDS	.377**	**.993****	1															
Turbidity	.337**	.400**	.413**	1														
Cl	.362**	**.881****	**.891****	.532**	1													
Alkalinity	.451**	.590**	.595**	.533**	.689**	1												
Hardness	.380**	.865**	**.864****	.505**	.878**	.686**	1											
Ca	.128	.577**	.566**	.274**	.678**	.643**	.705**	1										
Mg	.238*	.642**	.638**	.404**	.770**	.730**	.811**	.876**	1									
Na	.223*	.484**	.494**	.130	.523**	.375**	.324**	.115	.248**	1								
K	.336**	.502**	.497**	.414**	.541**	.389**	.495**	.492**	.418**	-.032	1							
DO	-.341**	-.596**	-.616**	-.388**	-.739**	-.671**	-.578**	-.661**	-.624**	-.499**	-.476**	1						
COD	.271**	.506**	.498**	.372**	.564**	.571**	.394**	.48**	.434**	.438**	.392**	-.686**	1					
BOD	.305**	.305**	.308**	.430**	.198*	.227*	.129	-.044	-.067	.11	.340**	-.250*	.440**	1				
SO_4	.342**	.738**	.750**	.409**	.851**	.657**	.809**	.632**	.744**	.457**	.457**	-.648**	.379**	-.010	1			
NO_3	.258**	.549**	.550**	.498**	.474**	.476**	.620**	.273**	.396**	.299**	.189	-.313**	.245*	.46**	.36**	1		
PO_4	.295**	.563**	.569**	.470**	.647**	.543**	.658**	.584**	.636**	.123	.563**	**-.531****	.427**	.161	.61**	.30**	1	
Oil and grease	.115	.370**	.370**	.182	.293**	.367**	.223*	.353**	.199*	.21*	.360**	-.366**	.449**	.35**	.174	.06	.48**	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

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RESEARCH ARTICLE

Preliminary Investigation and Computational Identification of New Genes from Leguminaceae

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ABSTRACT

The Fabaceae, the third largest family of plants and the source of many crops, has been the target of many genomic studies. Currently, only the grasses surpass the Chena legumes for the number of publicly available expressed sequence tags (ESTs). The quantity of sequences from diverse plants enables the use of computational approaches to identify novel genes in specific taxa. We used BLAST algorithms to compare unigene sets from Chickpea to nonlegume unigene sets, to GenBank's nonredundant and EST databases, and to the genomic sequences of rice and Arabidopsis. As a working definition, putatively legume-specific genes had no sequence homology, below a specified threshold, to publicly available sequences of nonlegumes. As a first step toward predicting function, related sequences were clustered to build motifs that could be searched against protein databases. Evolutionary analyses of the genomic sequences of several CCPs in M. truncatula suggest that this family has evolved by local duplications and divergent selection.

Key words: Genomic, Chena legumes, BLAST, Arabidopsis.

INTRODUCTION

Legumes constitute a large plant family that presents humans with a treasure trove of resources for a variety of uses. Throughout the world, legumes provide important sources of protein, oil, mineral nutrients, and nutritionally important natural products. An important feature of legumes is their ability to obtain nutrients via symbioses with soil microbes. The formation of nitrogen fixing nodules via interaction with bacteria collectively known as rhizobia is virtually unique to legumes, although some species in eight families of the eurosid I clade of dicots can form nodules in association with nitrogen fixing actinomycetes [22]. An exchange of specific signal molecules between host and

Microbe triggers many developmental events in the host, including extensive modulation of gene expression [5]. Although there has been recent rapid progress in identification of genes regulating early stages of nod-ule formation [16], much is still to be learned about nodule organogenesis.

Legumes can also form symbiotic associations with arbuscular mycorrhizal fungi, which aid the acquisition of minerals from the soil. Unlike the formation of nitrogen fixing nodules, symbioses with mycorrhizal fungi are found in the majority of higher plants. However, some of the same plant genes are required for both symbioses, so that legumes have emerged as major study systems for mycorrhizal interactions [14]. While these symbioses are beneficial to the plant, many fungal and bacterial pathogens have a large negative impact on crop yields of legumes.

The many uses of legumes and the variety of symbiotic and pathogenic interactions found provide numerous targets for functional genomics research. Currently, there are nearly 7,05,000 nucleotide sequences representing the Fabaceae available from the National Center for Biotechnology Information (NCBI taxonomy browser, http://www.ncbi.nlm.nih.gov/ Taxonomy, November, 2003). In particular, significant strides have been made in the functional genomics of the model legumes M. truncatula and Lotus japonicus and the crop legume soybean [21]. There are over 1,85,000; 36,000 and 3,40,000 Expressed Sequence Tags (ESTs) available for these species, respectively (http://www.ncbi.nlm.nih.gov/ dbEST/ dbEST_ summary.html). Key outputs from EST projects are public database of sequence that allow widely distributed investigators to access information on gene structure, expression, and putative function [13,19].

The wide availability of ESTs and other gene containing sequences from plants, including the near complete rice (*Oryza sativa)* and Arabidopsis genomes, provides an opportunity for comparative sequence analysis. For example, the well characterized Arabidopsis genome is an asset to identify orthologous genes in other, less well characterized species. However, comparative approaches may also be used to identify genes that are taxon specific.

In the case of legumes, it is clear that many of the genes involved in the hallmark legume functions of nodulation or isoflavonoid biosynthesis likely evolved from pathways shared among many plant species. For example, several classes of broadly conserved receptor kinases have been found to be required for nodule formation or regulation of nodule numbers [16], including a homolog of CLAVATA1, which controls shoot meristem size in Arabidopsis [4]. Using Boolean searches of TIGR M. truncatula Gene Index 4, identified 340 TCs with nodule specific expression patterns. Many of the genes identified shared sequence homology to sequences from nonlegumes.

In addition to the recruitment of broadly conserved genes for novel legume functions, we hypothesize that legumes may have novel genes involved in legume specific functions. These genes may be truly unique to legumes, or may have diverged so much from their progenitors that they appear to be unique. If so, a comparative analysis of expressed sequences would bring to light novel genes whose function would be difficult to study in other taxa.

However, this would have removed those sequences that have diverged significantly from nonlegume ancestral genes. Further, it would have provided few biological insights into the function of the remaining genes. By using motif analysis, we have identified novel gene families including F-box related proteins. The results of this bioinformatic study will enable laboratory assessment of gene function and analysis of evolutionary patterns of these and other unique gene families.

MATERIALS AND METHODS

Upon request, all novel materials described in this publication will be made available in a timely manner for noncommercial research purposes, subject to the requisite permission from any third-party owners of all or parts of the material. Obtaining any permission will be the responsibility of the requestor. Perl scripts will be made available upon request.

RESULTS

Identification of Legume Specific Genes

A series of BLAST analyses was used to identify and remove broadly conserved sequences from the set of expressed sequences from legumes. A summary of the approach and the results are presented in Table I. Initially, the TIGR M. truncatula, L. japonicus, and soybean (G. max and G. soja) TCs were compared to the TIGR maize (Zea mays, ZmGI), tomato (Lycopersicon esculentum, LeGI), rice (OsGI), and Arabidopsis (AtGI) gene indices [17] ; http:// www.tigr.org/tdb/tgi) using BLASTN and TBLASTX. While the gene indices included both TC and singleton EST sequences, only legume TCs were analyzed be-cause they were more likely to represent full length transcripts. By using a limited dataset of four non-legume species, many legume TCs with sequence homology to nonlegume sequences could be identified with minimal computer processing time. Perl scripts were used to parse the BLASTN and TBLASTX reports and to identify legume TCs with homology to nonlegume sequences. If a legume TC had BLAST homology to any nonlegume sequence with an E-value more significant than 10^{24}, it was not considered legume specific. Only TCs without significant hits or with hits only to other legumes species were considered as putatively legume specific. Roughly 90% of legumes TCs were eliminated using this approach.

One area of concern was that some of the putative legume specific TCs might be too short to yield in-formative BLAST hits with nonlegume sequences. One example would be if the TC corresponded to the untranslated portions of a transcript. Since untranslated regions do not code for protein, they are less likely to be conserved among species. In order to identify sequences falling into this category, we used TBLASTX to compare the remaining legume-specific genes to the original set of legume TCs. If a legume-specific TC showed homology to a lengthier legume TC that was not legume specific, it was unlikely to be legume specific. However, if a legume-specific TC had homology only to itself or other remaining legume-specific TCs, it was retained in the legume-specific category. After this analysis, 2,525 total legume-specific TCs remained from all three species (Table I and Supplemental Table I, which can be viewed at www.plantphysiol.org).

To confirm that the identified C.arietinum legume specific TCs were of plant origin and not that of the nitrogen-fixing symbiont, the legume specific TCs were compared to the complete genome of Sinorhizobium meliloti [9] and a portion of the M. truncatula genome. No sequences matches were found to the S. meliloti genome. However, 63 M. truncatula TCs had exact matches to the available M. truncatula genome (Table I).

Characterization of Legume-Specific TCs

Following the analyses above, 92; 861; and 1,572 legume-specific TCs remained in L. japonicus, M. truncatula, and G. max/soja, respectively. A comprehensive list of all TCs identified is presented in Supplemental Table I, while a summary of TC length and the number of ESTs per TC in the legume specific TCs can be found in Table II. BLASTX analysis to the GenBank NR database revealed that less than three percent of the identified legume specific TCs had homology to other legume sequences with an E value less than 10^{24} (Supplemental Table II). Of the five most highly expressed TCs in M. truncatula, two (MtTC59272 and MtTC60015) had sequence homology (E-value # 10^{215}) to seed albumins (C.aritinum, GenBank accession no. Q9ZQX0), while the other three (MtTC68206, MtTC59435, and

MtTC60038) had sequence homology (E-value # 10^{24}) to a hypothetical protein from Galega orientalis (GenBank accession no. CAB51773). Twelve percent of legume specific TCs had sequence homol-ogy to sequences present in dbEST from other legume species. Previous data suggests the lack of homology among legume species may be due to tissue bias in sequencing projects rather than true species differ-ences [10]. A complete description and analysis of all the legume-specific TCs is provided in Supplemental Table I.

Single Linkage Clustering and Motif Analysis

One approach for hypothesizing function for legume-specific TCs lacking significant BLAST hits was to scan the TCs for conserved motifs identified from other proteins. Of these motifs, 41 were rich in specific amino acids. The remaining 14 included hits to a cyclin-like F-box (IPR001810), pectinesterase inhibitors (IPR006501 and TIGR01614), a zinc finger (IPR001878 and PS50158), and a nodulin (IPR003387 and PF02451). These are listed in Supplemental Table I.

A second approach was to mine groups of related legume-specific genes for common, uncharacterized motifs. Motif searching can be more sensitive than BLAST analysis for several reasons. First, highly con-served residues carry more weight in motif analysis. Second, a minimum exact word match is not required. Finally, the information from many homologs can be combined in a single motif. Once a novel motif de-scription is generated, it can be used to scan the public protein databases for matches. Common motifs among target proteins suggest possible functions.

In order to identify families of legume-specific TCs for motif analysis, single linkage clustering analysis of legume-specific TCs was performed. Including as many diverse sequences as possible within a cluster increases the likelihood of identifying conserved mo-tifs. Therefore, the 2,525 legume-specific genes were combined with 50, 672, and 688 homologous single-tons from G. soja/max respectively. Clustering identified 665 groups that corresponded to potential gene families or cross-species homologs. The groups are identified in Supplemental Table I, with their size distribution. The majority of TCs did not cluster with other sequences and are denoted as Group 0 in Supplemental Table I. Sixty-seven groups were chosen for motif analysis based on their size and/or tissue-specific expression patterns. Nine additional groups were almost certainly not legume specific. In these cases, TCs clustered with singletons that had strong hits to nonlegumes. Motif analyses in many of the remaining groups were quite fruitful. Thirteen groups had Motif Alignment Search Tool (MAST) or hidden Markov model (HMM) hits (E-value, 10^{24}) to nonlegume Swiss-Prot/TrEMBL sequences or to the Arabidopsis genome. The member sequences, motifs, and sequence alignments for each group are provided in additional supplemental data available at www. medicago.org/documents/Publications/Graham04_ supplement. Three classes of families are particularly noteworthy and are as novel F-box proteins.

F-Box Proteins

Two groups (640 and 630) of sequences without significant BLAST similarity with one another clearly shared motifs with discrete families of F-box proteins. Group 640 had five sequences from M. truncatula, from a variety of root and shoot libraries (Table III). Multiple EM for Motif Elicitation (MEME; [1,2] identified two ordered motifs (widths 35 and 21 amino acids) shared by all five sequences. MAST [2] was unable to find any significant hits (E-value, 10^{24}) to other proteins using these motifs. However, an HMM trained on the trimmed alignments containing these motifs found two hits to the Arabidopsis genome (At4g12560 and At4g22390)

DISCUSSION

Using increasingly stringent BLAST searches, we have identified over 2,500 legume-specific genes from C.arietinum, L. japonicus, and G. max/soja. The analyses included comparisons to the GenBank NR and EST_others databases, as well as comparisons to the rice and Arabidopsis genomes. By the very nature of the analysis, only a small subset of

the legume-specific genes identified have homology to previously charac-terized genes or gene products. The observed results are consistent with the representation of legume sequences in the GenBank NR database relative to those of better characterized groups. In contrast, for all of the legume species within the Fabaceae, there are only 11,300 protein sequences. While Arabidopsis has proven to be a useful model for many aspects of plant biology, it is not a good model for studying nodule development. In contrast, only 22% of the ESTs correspond-ing to all Medicago TCs comes from nodules.

Given the low representation of legume sequences in the GenBank databases, several approaches were taken in order to assign putative functions to the legume-specific genes. All of the legume-specific genes and homologous singletons were grouped together into families of related sequences. The sequences within a group were then mined for conserved motifs that could be used to scan the protein databases at Swiss-Prot/TrEMBL. Proteins of known function that shared these motifs could provide a hint of function. Using this technique, we identified several groups of interest. However, the most interesting were families of F-box related proteins.

A Diverse Family of F-Box-Related Genes from Legumes

F-box-related proteins have been identified with a wide array of cellular functions. F-box proteins are involved in transcriptional regulation and signal transduction through Skp1, Cdc53/Cullin1, F-box protein ubiquitinligase complexes, transcript elongation, cell cycle transition, and self-incompatibility in plants [12,20]. The range in F-box protein functions is due in part to the diversity of secondary motifs. Comprehensive analyses of the Arabidopsis genome identified between 560 and 690 predicted F-box proteins [7,8]. Leurich and kelch repeats were the most abundant secondary motifs identified. Other motifs such as WD-40, Armadillo, and tetratricopeptide repeats were also found [15]. A large number of F-box proteins had associated regions with no similarity to previously characterized motifs.

In our analysis, we identified two groups with dis-tant homology to F-box proteins and related domains. In Arabidopsis, roughly 35% of genes encoding F-box-related proteins exist in clusters of two to seven F-box genes [15]. This arrangement would allow shuffling of domains among F-box genes. The presence of F-box proteins with similar N-terminal and dissimilar C-terminal domains support the idea that motif shuffling has lead to further F-box protein diversification [15]. Of the 22 M. truncatula genome hits from group 640, 10 came from BACs containing two or more F-box proteins (data not shown). Motif shuffling between clusters of F-box related proteins would allow sequence diversification to occur, possibly leading to the evolution of novel functions in legumes.

One gram of soil can contain between 6,800 and 34,000 different taxa of bacteria [15]. Also present in the soil are fungal and insect pathogens. We believe that CCPs are induced as a secondary defense to protect the nodule from pathogenic organisms, while allow-ing the symbiosis to continue. Given the broad spec-trum of pathogens present in the soil, the secondary defense response must be able to target specific pathogens without harming beneficial rhizobia. This requires an arsenal of different defense compounds, like the CCPs. As pathogen populations evolve, so must plant defense responses.

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BLAST Analyses to Identify Legume-Specific Genes

Unless otherwise stated, all computer analyses were performed on a single Macintosh PowerG4 computer running Mac OS 10.22 with dual 800 MHz processors. Locally installed versions of the NCBI BLASTN, BLASTX, and TBLASTX (Altschul et al., 1997) programs were used to find regions of sequence homology between legume and nonlegume sequences (Table I). Filtering of repetitive sequences was turned off for all BLAST searches to increase the stringency for finding legume-specific genes, unless noted. Perl scripts were used to parse the results of each BLASTanalysis described below. Legume TCs with BLAST homology to any nonlegume sequence from a variety of databases with an E-value more significant than 10^{24} were not considered legume specific and were removed from the list of putative legume-specific genes. Legume TCs with BLAST homology to nonlegume sequences with an E-value between 10^{24} and 10^{28} were analyzed by hand, to ensure that homology was not due to the polyA tail or highly repetitive sequences. A summary table of the identified legume-specific TCs is provided in Supplemental Table I.

In the first BLAST iteration, (Table I) the TIGR Medicago truncatula, Lotus japonicus, and soybean (Glycine max and Glycine soja) TCs were compared to the TIGR maize (ZmGI), tomato (LeGI), rice (OsGI), and Arabidopsis (AtGI) gene indices using BLASTN and TBLASTX (Table I; Michelle et al., 2004; http://www.tigr.org/tdb/tgi). In the second BLAST iteration, the remaining legume-specific genes were compared to the NR using BLASTX. This was followed by the third BLAST iteration in which TBLASTX comparisons were made to the remaining TIGR plant gene indices from barley (*Hordeum vulgare*), *Chlamydomonas reinhardtii*, cotton (Gossypium spp.), grape (*Vitis vinifera*), ice plant (*Mesembryanthemum crystallinum*), lettuce (*Lactuca sativa),* Pinus spp., potato (*Solanum tuberosum*), rye (*Secale cereale*), sorghum (*Sorghum bicolor*), sunflower (*Helianthus annuus*), and wheat (*Triticum aestivum*). BLAST iteration five used TBLASTX to compare legume-specific TCs to the Arabidopsis and rice genomes. Unlike previous searches, the sequences were filtered prior to BLAST to remove low complexity sequences. In the final BLAST iteration, the remaining legume-specific TCs were then compared to EST_others using the DeCypher Bioinformatics Accelerator running the Tera-BLAST hardware accelerated version of BLAST (TimeLogic, Crystal Bay, NV), which was housed in the Center for Computational Genomics and Bioinformatics at the University of Minnesota.

Cys-Rich Proteins

A large fraction of the legume-specific genes encoded Cys-cluster proteins (CCPs). These share several common features: (1) an N-terminal signal sequence, (2) a small, highly charged or polar-mature protein sequence, (3) a

characteristic arrangement of 4, 6, 8, or 10 Cys residues likely involved in disulfide bridges, (4) an apparent tissue-specific expression profile, and (5) low similarity to other expressed CCPs (Table III). The largest group of CCPs, group 31, is made up almost entirely of M. truncatula sequences. The group contains 197 M. truncatula singleton sequences and 136 TCs, all of which are expressed almost exclusively in nodules. A small fraction (less than 1%) of EST sequences from this group was also found in other root tissues. While the majority of sequences in this group had no BLAST homology to sequences within NR, approximately 20% had low homology (approximately equal to 1024) hits to the same group of sequences: a hypothetical protein from G. orientalis (CAB51773; [11], late nodulins from V. faba (CAB96471, CAB96474, CAB96476; Fruhling et al., 2000), and a nodule-specific protein and ENOD3 [18] from pea (BAB40944 and AAB23537, respectively).

The MEME program, when applied to the nodulespecific CCPs (group 31), generated three motifs of 21, 15, and 11 amino acids, respectively. The new hits included several more genes from legumes annotated as early and late nodulins, plus a set of five potassium channel-blocking neurotoxins from Manchurian scorpion (Mesobuthus martensii.) Further, the dozen hits with E-value between 0.1 and 1024 were almost all K1 channel-blocking neurotoxins from a variety of scorpion species. All scorpion toxins hit only motifs 2 and 3, having their own distinctive signal peptide.

The extensive divergence among the nodule-specific CCPs made the creation of an accurate multiplesequence alignment impossible for the whole group. However, an accurate alignment is a prerequisite for building an effectiveHMM. Hence, 261 sequences from group 31 were distributed among 11 distinct subgroups. A final set of HMMs was created to describe each of the 11 subgroups of nodule-specific CCPs. Alignments of the largest two subgroups, 31.01 and 31.02, are displayed in Figure 1A. ForHMMgeneration, the subgroups were deliberately modeled without the signal peptide, since only the mature peptide itself proved to be similar to the scorpion toxins in the MEME/MASTanalysis. SinceHMMs were specifically designed for each subgroup, none of the 11 HMMs picked up as many significant hits as had the MEME motifs when scanning Swiss-Prot/TrEMBL. However, they proved to be useful in subdividing the large diverse family. For example, only subfamily two picked up any hits to scorpion toxins.

The 11 HMMs were then used to scan the Arabidopsis genome, which was translated into all six reading frames. These searches yielded eight hits with E-values more significant than 1024, but seven of these hits were accounted for by subgroups two (subgroup 31.02; Fig. 1B) and nine (subgroup 31.09). Seven of the eight hits to the Arabidopsis genome lie in regions with no predicted genes on chromosomes one, two, and four of TIGR Arabidopsis sequence 4.0 (TAIR). The remaining hit, At1g43720, was a predicted hypothetical gene. In contrast to Arabidopsis, the rice genome had no significant hits to any of the 11 subgroups.

In addition to the group of nodule-specific CCPs, we also identified several groups of predominantly seedspecific CCPs (Table III). Group 645 was composed of 10 M. truncatula TCs and two singletons corresponding to the pods with seeds and immature seed libraries. Groups 38 (5 TCs, 6 singletons), 40 (2 TCs and a singleton), and 41 (2 TCs and a singleton) were soybean specific and were composed of ESTs from immature seed coats, seed coats, and very young seeds. Group 36 contains one M. truncatula singleton, one soybean singleton, and two soybean TCs composed of ESTs from mature and immature seed coats, mature pods and immature cotyledons. Group 655 (2 TCs and a singleton) corresponded to developing flowers and pods with seeds. Unlike the nodule-specific CCPs, none of these groups had significant BLAST hits in the NR database. However, if a lower E-value cutoff of 1024 were used, many would cluster with the nodulespecific CCPs.

The first round of MEME motif building did not yield any hits more significant than 1024 for any of the seed-specific Cys-rich protein groups. Adding this single sequence to the second round of MEME analysis resulted in a set of motifs that had significant hits to nearly 100 proteins in Swiss-Prot/TrEMBL. Collectively, these included sequences annotated as gamma thionins, protease inhibitors, insect and plant defensins, and sodium channel-blocking scorpion toxins. A few examples of these can be seen in Figure 1C.

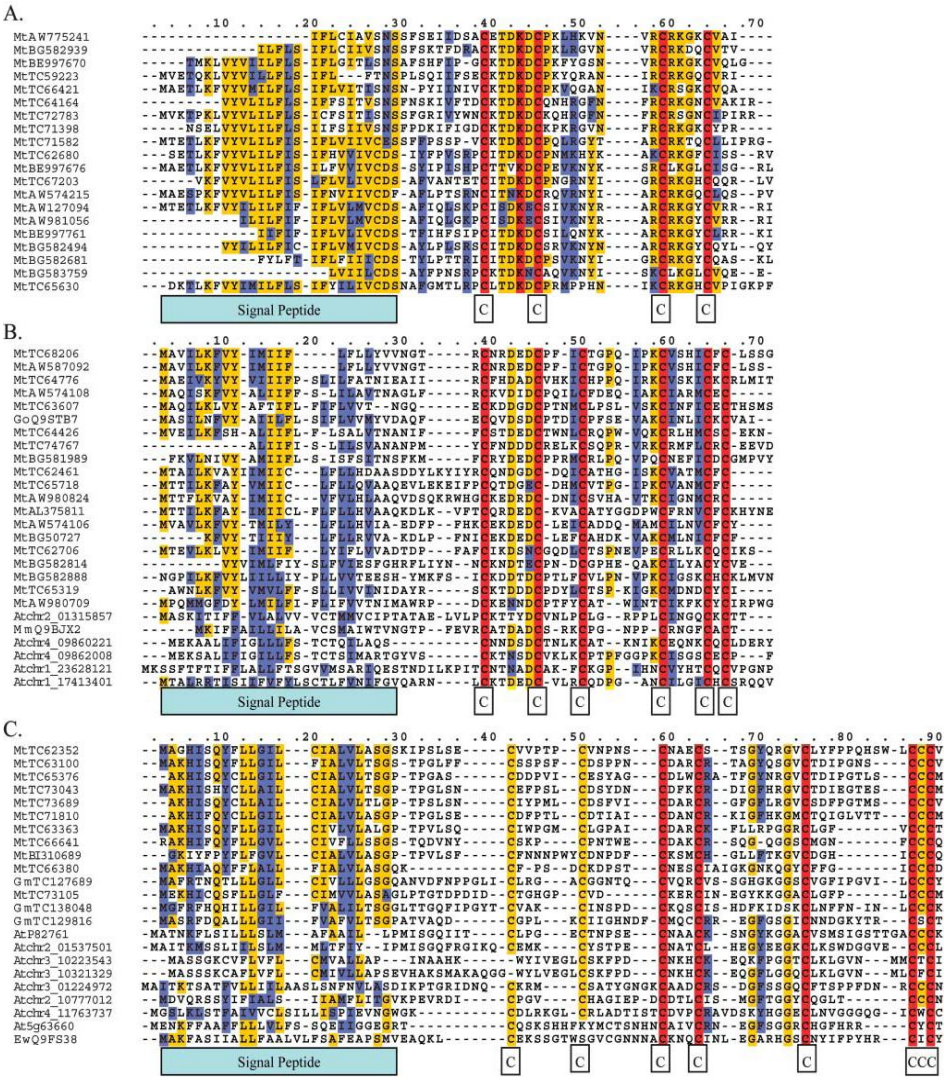

Figure 1. Motif analysis of different groups of Cys cluster proteins. Small figures below each of the alignments depict the pattern of conserved residues. Gaps (-) were introduced to optimize the alignments. Given the large number of sequences in the alignments, not all sequences are shown. A, Motif analysis of nodule-specific CCP group 31.01 identified no homologous sequences from other species. B, Motif analysis of nodule-specific CCP group 31.02 identified sequences from unannotated regions of the Arabidopsis genome and from Swiss-Prot/TrEMBL. MmQ9BJX2 is a neurotoxin protein identified from scorpion; GoQ9STB7 is a hypothetical protein from G. orientalis. C, Prior to motif analysis, seed-specific CCP groups 645 and 40 were merged into a single alignment. The resulting motif identified sequences from the Arabidopsis genome and from Swiss-Prot/TrEMBL. Hits to Arabidopsis came from both annotated (At5g63660) and unannotated regions of the genome. Hits from Swiss-Prot/TrEMBL included a putative gammathionin from Eutrema wasabi (EwQ9FS38) and putative self-incompatibility factor LCR46 from Arabidopsis (AtP82761).

A single HMM was created from an alignment of all 12 original members from group 645, the largest seedspecific CCP group. Like the first iteration of the MEME motif, the HMM also did not have any significant hits to Swiss-Prot/TrEMBL. However, it had eight hits to the Arabidopsis genome and one hit to the existing M. truncatula genomic bacterial artificial chromosome (BAC) sequence (E-value , 1024). An alignment of these sequences is shown in Figure 1C. At5g63660 was predicted to be a plant defensin by TAIR. P82761 (Swiss-Prot/TrEMBL) is the putative self-incompatibility protein LCR46. The remaining six hits lie in regions not predicted to encode genes on chromosomes two, three, and four (Fig. 1C; TAIR).

Group 666 also appeared to be Cys-rich and had strong sequence homology to the soybean albumin 1 precursor (GenBank accession BAA04219). This group was composed of one TC and three singletons from soybean and three TCs and singletons from M. truncatula. Two of the TCs from M. truncatula had high levels of expression. MtTC59272 and MtTC60015 were composed of 61 and 36 ESTs, respectively, mainly from roots and mycorrhizal roots. Unlike the CCPs in group 31, members of group 666 are longer, contain more conserved Cys residues, and show diminished tissue specificity.

Construction and Searching of Novel Legume-Specific Motifs

The following procedure was used to identify and refine motifs shared within a group of related legume-specific sequences. Each TC or singleton within a clustered group was first translated in all six frames. The translated sequences were then modeled as a set of ungapped position-specific scoring matrices using the MEME program (Bioinformatics Department, Jamal Mohamed College, Tamilnadu; [15]. An expectation maximum algorithm is used by the program to identify optimal-width motifs conserved among the protein sequences. Only a few reading frames had conserved motifs free of stop codons. All other reading frames were removed from the set, and the MEME motifs were regenerated.

The protein sequences in the proper frame identified above were aligned using ClustalW (Jamala Mohamed college) and manually edited with JALVIEW [3]. Note that the HMMs generated by HMMER statistically model the entire alignment including the likelihood of finding insertions and deletions, whereas the profiles generated by MEME model small, ungapped segments within an alignment. Each of these methods has a different level of sensitivity that is dependent on the protein family studied. Hence using both complementary methods increases the likelihood of finding significant hits.

The family models generated by MEME and HMMBUILD were used by the MAST (Computer Science and Bioinfomatics Department, JMC;[2] and HMMSEARCH [6] programs, respectively, to scan all the sequences in Swiss-Prot/ TrEMBL [15] , a comprehensive nonredundant collection of known protein sequences. The available sequence from the Arabidopsis and M. truncatula genomes (February, 2009) were also scanned in this step. Sequences with significant scores less than 10^{24} (unless noted) were added to the original group of legume-specific genes. The motif building and searching procedures were repeated until no additional significant hits were found or until new sequences identified began to dominate the model. In this case, further iterations would be uninformative.

Only groups with 10 or more TCs or singletons were analyzed. Smaller groups were also analyzed if they showed a tissue-specific pattern of expression, based on the library of EST origin. In order to remove redundancy caused by overlapping singletons, the singletons were contigged using Sequencer software (Gene Code, Ann Arbor, MI). Motifs, sequences and sequence alignments for each of the groups analyzed are provided in a downloadable directory structure in the supplemental data.

One problem encountered during motif analysis was the presence of chimeric clones. A single chimeric clone could bring two unrelated groups of legume-specific genes together into one. Sequence alignments and BLAST analyses were used to identify the chimeric clone. A clone was considered chimeric if: (1) in a multiple sequence alignment, this single sequence was the only link joining two distinct alignments, and (2) alignments of the clone to members of one group do not overlap the sequence boundaries of alignments to members in the other group. Chimerism was confirmed in cases where Medicago genome sequence was available. The chimeric clone was removed from the alignment, and the two groups of sequences were assigned new group numbers.

Motif Analysis of Subgroups of Nodule-Specific CCPs

To create the subgroups, an initial automated alignment of all the CCPs in group 31 was performed using ClustalW. Sequences that did not fit the alignment were removed. Subgroups within the family were identified based on the automated dendrogram from ClustalW and realigned separately. HMMs were then generated for each subgroup and were used to scan MtGI 6.0. Several sequences were identified from MtGI 6.0 that had significant scores within a subgroup, but were not legume specific. In some cases, these sequences had been omitted by our original BLAST filtering procedure because of obvious chimerism with nonlegume-specific sequences. The CCP portion of these TCs was added to the alignment. Sequences within the alignments were shuffled between subgroups until the members in each subgroup scored better in the associated HMM than any sequence outside the group, and the greatest apparent separation among the subgroups was achieved. For CCP-like sequences identified from the Arabidopsis genome, intron sequences were predicted using the NetPlantGene prediction server (http://www.cbs.dtu.dk/services/NetPGene).

CONCLUSION

One of our hypotheses was that some of the identified legume specific genes were derived from non legume origins, but have diverged so much they appear unique to legumes. Using single-linkage clustering and motif analysis, we were able to identify gene families with conserved motifs. In some cases, such as the defensin-like CCPs and F-box related proteins, the motifs identified were clearly represented across diverse taxa. Thus, as hypothesized, these genes may be examples of fast-evolving genes that are so divergent that similarity to their progenitors is not readily detectable by BLAST algorithms. Sequences that are truly novel in legumes may be present among the families that were too small for motif analysis, families where motifs could not be detected, or fam-ilies whose motifs failed to detect similarity to any known proteins. Experimental analyses and sequence information from a wider diversity of organisms will aid in determining if these genes are indeed novel.

While the function of many legume specific genes could not be predicted by computational approaches, their expression patterns suggest they are worth investigating experimentally in the future. All of the legume-specific genes we have identified have been made publicly available in the supplemental data, representing a rich resource for legume biologists (see supplemental data for this article and at www.medicago.org/documents/Graham04_ supplement). Among these legume-specific genes, we identified many gene families with nonspecific ex-pression patterns. Additionally, we have identified 10 gene families specifically expressed in roots and nod-ules, eight in seeds, four expressed only in leaves and flowers, and seven from stressed or pathogen-inoculated tissues. The tissue specificity of these genes suggests they would make excellent candidates for transformation or gene silencing in future analyses of gene function.

Table I. Legume TCs from the *C. arietinum* gene indices were used as query sequences for sequential BLAST analyses against various sequence data sets from nonlegumes. The TCs retained as legume specific from each analysis served as the query for the subsequent BLAST analysis.

BLAST Algorithm, E-Value Cutoff	**Data Set**	**Total Sequences in Data Set**	**Legume-Specific *C. arietinum L.* TCs Retained**
BLASTN, 10^{24}	TIGR AtGI, LeGI, OsGI, ZmGI	142,492	7,938
TBLASTX, 10^{24}	TIGR AtGI, LeGI, OsGI, ZmGI	142,492	2,412
BLASTX, 10^{24}	GenBank nonredundant database	1,335,905	2,230
TBLASTX, 10^{24}	Remaining TIGR Plant GIs	334,347	2,101
TBLASTX, 10^{24}	Arabidopsis and rice genomes	–	2,081
Tera-BLASTN, 10^{24}	EST_others	6,985,891	2,020

Table II . Summary statistics of *C. arietinum* specific TCs

Types	**specific TCs**
No. of legume	1,572
Size range of TCs (bp)	103-2,962
Average size of all TCs (bp)	476
Average size of all TCs $ 300 bp	527
Maximum number of ESTs per TC	76
Average number of ESTs per TC	2.4

Table III. Characteristics of predicted F-box, Pro-rich, and Cys-rich proteins

Protein Description	Group	Lengths of predicted Proteins (aa)	Species Distribution of sequences	Predominant Expression pattern	Total number of ESTs in Group
F-box associated	640	150–400	Mt(5)	No specificity	6
F-box	630	400	Mt(4)	No specificity	17
Pro-rich	5	200–500	Mt(57) Gm(7)	No specificity	65
Pro-rich	485	95	Gm(2) Mt(1)	Above-ground	22
Pro-rich	669	100	Mt(3)	No specificity	5

[a]Further descriptions of predicted proteins are provided in the text
[b]Group numbers are arbitrary identifiers assigned during single-linkage
[c]Amino acid (aa) lengths of predicted proteins were determined using ClustalW alignments with At4g22390)

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Supplemental Table 1. Summary of Analyses of Legume-specific TCs from *M. truncatula*, *G. max*/*soja* and *L. japonicus*

Group Number	TC or Singleton Name	Length of TC (bp)	Number of ESTs in TC	Library distribution of TCs and Singletons (cDNA library number of ESTs from cDNA library)	Strongest NR BLASTX HIT Description line, E-Value, Score	Other Legume BLASTX NR hit (Yes or No)	Other Legume TBLASTX EST_others hit (Yes or No)	100% Nucleotide Identity to *M. truncatula* BAC with BLASTN, Score, E-value	InterProScan Hit
0	GmTC119017	604	3	Gm-c1016 1 Gm-c1068 1 Gm-c1073 1	No NR BLASTX Hits	No	No	-	No Inter ProScan Hit
0	GmTC119039	298	2	Gm-c1027 2	No NR BLASTX Hits	No	No	-	No InterProScan Hit
0	GmTC119077	436	2	Gm-c1016 1 Gm-r1070 1	No NR BLASTX Hits	No	No	-	No InterProScan Hit
0	LjTC1169	418	2	young_plants_2weeks 2	No NR BLASTX Hits	No	No	-	No InterProScan Hit
0	LjTC1232	422	2	young_plants_2weeks 2	No NR BLASTX Hits	No	No	-	No InterProScan Hit
0	MtTC60187	1022	2	Developing_flower 1 MTGIM 1	No NR BLASTX Hits	No	No	No BLASTN 100% identity match	No InterProScan Hit
0	MtTC60458	690	2	KV2 2	No NR BLASTX Hits	No	No	No BLASTN 100% identity match	No InterProScan Hit
1	GmAW757366	-	-	Gm-c1027	-	-	-	-	-
1	GmTC131460	500	2	Gm-c1018 1 Gm-c1066 1	No NR BLASTX Hits	No	Yes	-	No InterProScan Hit
2	GmAI416565	-	-	Gm-c1003	-	-	-	-	-

Supplemental Table II. BLASTX Analysis of Legume-specific TCs to GenBank's Nonredundant Database Using an E-value Cutoff of $10E^{-04}$

TC Number	Group	ESTs per TC	Strongest BLASTX Hit	E-value
GmTC120738	432	53	Phloem-specific protein Vein1 (*V. faba*, S66340)	7.00E-12
GmTC126059	78	5	Hypothetical protein (*C. arietinum*, CAB71133	2.00E-16
MtTC64434	5	3	Leginsulin (*G. max*, CAA11040)	3.00E-15
MtTC73113	563	3	MtN17 (*M. truncatula*, CAA75587)	7.00E-04
MtTC75027	31	2	Late nodulin (*V. faba*, CAB96475)	8.00E-06

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RESEARCH ARTICLE

Exploring the Floristic Diversity and Economic Value of Plant Species in Three Selected Sacred Groves of Pudukkottai District, Tamilnadu, India.

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ABSTRACT

Sacred groves are small patches of native vegetation traditionally protected and managed by local communities which act as a nursery and storehouse of many medicinal plants. Groves are rich heritage of India, and play an important role in religious and socio-cultural life of the local people. These ecosystems harbour many threatened, endangered and rare plant and animal species. Even the smallest groves harbor some old and magnificent specimens of trees and climbers. The present study has been carried out in three selected sacred groves namely Pulvayal, Mylappatti and Kallampatti of Pudukkottai District, TamilNadu, India, to asses the current floristic composition and their status of availability in the area. About 66 species were collected from Pulvayal grove (Shivan kovil), Marayappatti grove (Ayyanar kovil) and Kathavampatti grove (Muneeswarar kovil) of IlluppurTaluk, Pudukkottai district, Tamil Nadu, South India. One endangered species, viz. *Cayratia pedata was* recorded in the sacred grove of Pulvayal. Rare plant species like *Alangium salvifolium*, *Barleria cuspidata, Enicostemma axillare*, *Gymnema sylvestre* and a very rare plant species like *Phyllanthus reticulatus* was recorded from the study.

Key words: ecosystems, conservation, endangered, floristic diversity, sacred groves, heritage.

INTRODUCTION

Sacred groves are a group of trees or a patch of vegetation is a mythological landscape and cult practice of great religious importance to a particular culture that are left untouched by the local inhabitants, and protected in the name of the local village folk deities evolved to minimize destruction [1-3]. Moreover, these forests are the true indicators of the type of vegetation that once existed here before the dawn of modern civilization. Their existence is mostly due to certain taboos, strong beliefs, and supplemented mystic folklores. Generally sacred groves are believed to be a treasure house of medicinal, rare and endemic plants, as refugee for relic flora of a region and as centers of seed dispersal [4]. Their plant wealth and conservation potential were impressive enough to acknowledge them as 'mini biosphere reserves [5]. Since the tropical forests have impressive species diversity contained in diverse formation types, attention has been diverted to the sacred groves of the tropical tracts in the recent past. A systematic survey of the sacred groves of India in 1997 has recorded the existence of thousands of such groves along the plains and hills of the Indian subcontinent and confirmed their floristic richness confined within islets of diverse habitats [6].

Sacred groves are not restricted to India alone. They are also found in Afro-Asian countries. These sacred groves may range in size from a group of few trees to a forest of trees. In India, as elsewhere in many parts of the world, a number of communities practice different forms of nature worship [7,8]. One such significant tradition is that of providing protection to patches of forests dedicated to deities and/or ancestral spirits. Groves are rich heritage of India, and play an important role in religious and socio-cultural life of the local people. These ecosystems harbour many threatened, endangered and rare plant and animal species [9]. Sacred groves are dedicated to local deities and raised in honour of heroes and warriors and maintained by the local community with religious fervor. The importance of sacred groves in conserving the local biodiversity has been acknowledged only recently, though this practice has been long back hailed by the British Forester Dietrich Brandis as an example of 'vernacular conservation' [10].

Sacred groves of India

Several groves are reported in Assam, Bihar, Meghalaya, Manipur, Madhya Pradesh, Himachal Pradesh, Uttar Pradesh, Orissa, Rajasthan and in other parts of India and Western Ghats. The tracts of Sacred groves have been guarded from human interference on the grounds of religious beliefs. Gadgil and Vartak [5], state that these traditional practices owe their origin to the hunting-gathering stage of the society. Sacred groves harbour vegetation in its climatic formation, and probably constitute the only representation of forest in near-virgin condition in many parts of India [11]. The sacred groves are the repositories of unique and rare plants. They are the home for myriad's of insects, birds, reptiles, animals and store houses of the country's diverse natural wealth. The estimated number of sacred groves in India in about two lakhs [12]. Despite the vast and varied flora in the Southern Peninsular of India, information on the biodiversity of the sacred grove is still limited and only a few studies have been made to understand the phytodiversity of the region [13-18].

Sacred groves of TamilNadu

Scrub jungles, which have disappeared in most regions of the Tamil Nadu are protected in these sacred groves due to religious and traditional beliefs. Sacred groves help to retain the subsoil water of the area, providing life sustenance for the villagers. These groves are left untouched by the local inhabitants and protected by the local deity. They may be preserved out of belief, fear or reverence, but the practice of conserving them is deep-rooted and cuts across caste and communal barriers. Sacred groves probably represent the single most important ecological heritage of the ancient culture of the India. They are a kind of conservation area as well as a spiritual retreat. These small thickets of wooded area which remain undisturbed amidst development are the last remnants of rural biodiversity. From ancient times to till today, the village people believed that anyone damaging these groves would be punished by the gods. Women folk, in particularly, were afraid even to go near to these groves.

In Tamil Nadu, these groves are found in Dharmapuri, Erode, Perambalur, Pudukkottai, Salem, Sivaganga, Namakkal, Nilgiri, Tiruchirappalli and Tiruvannamalai districts. *Kovil Kaadus* (temple forests) are found in every village settlement in Tamil Nadu and are regarded as the abode of the Mother Goddess and the guardian spirits of the village such as *Ayyanar, Muneeswarar, Karuppuswami, Veeran,* and so on, who are powerful and can fulfil wishes. These deities are generally of an extremely primitive nature. The deities, are often in the form of an anthropomorphic slab of stone, a hero stone, *sati* stone or a trident even, irregular lumps of stone serve as the deity in some places. Mostly, they lie under a tree/ shrub or open to the sky, smeared with vermilion and turmeric powder. Often a thread is tied around a tree or miniature cradles are hung from the branches. The first is a form of prayer, while the second is a prayer for a child, particulary male. The cults are often associated with ancestor worship. A hero stone, *sati* stone, or small round stone representing ancestors are generally placed by the side of deities. The worshippers of these deities fear that even breaking a dead wood in a grove may result in a serious illness or in violent death. Such strict taboos indirectly preserved these sacred groves in their virgin form, relics of the forest that must have once covered much of the Peninsular India. These sacred groves are the only remnants of the original forest maintained in many parts of Tamil Nadu. As such, these groves now play a vital role in the conservation and preservation of species diversity.

The sacred groves represent a variety of vegetation types from semi-evergreen to dry deciduous. In Tamil Nadu, they range from a clump of few trees to 20 hectares, though the majority are fairly small, being only about 1.5 hectares. Most of them are distributed over the plains of the districts as well as in the hill regions of the eastern and western ghats. Folklore plays an important role in the conservation of sacred groves. Not only the tribal people, the rural people also preserved the sacred groves by their traditional customs, rituals, ceremonies and folk-beliefs. Folklore gives rewards and blessings for good behaviour, and punishes the non-believers or atheist. Several stories depict various facets of life and culture of the people. The annual festival is celebrated in all the groves of all districts accompanied by community offerings of *pongal* and animal sacrifice. The most favoured deity of TamilNadu is *Ayyanar*. Sacrifice of fowl, goat, sheep is offered to all the deities except *Ayyanar*. Next to *Ayyanar*, is *Karuppuswami*. It is believed that if cut coins are offered to deity, he will punish one's enemy. People of Puthupet near Pondicherry believe that a string tied below the knee of the horse has the power to do good or to cause harm to an adversary. Next to *Karuppuswami* is Muneeswarar who is worshiped either as a fierce God or a peaceful God in the form of a Soolam (Trident) and stone (usually brick or Lingam-shaped stones). The other names are Muniappan, Aandiappan, Munisamy. Since his weapon is the trident, Muneeswarar temples will contain a trident placed in the ground, and limes are placed upon the prongs of the trident [19]. The worship of Shiva is a pan-Hindu tradition, practiced widely across all of India, Nepal and Sri Lanka. Shiva is often depicted as the destroyer, and will appear as a naked ascetic accompanied by demons, encircled with serpents and necklaces of skulls [20,21]. However, he is also associated with reincarnation, since in Hinduism death is believed to be a necessary step for rebirth.

Even the smallest groves often harbor some old and magnificent specimens of trees and climbers. As an ecosystem sacred groves help in soil and water conservation besides preserving biological wealth. Preservation of these groves is crucial need of the hour. Assessment of diversity proves extremely practical for determining decreasing natural diversity, effect of exotic species, migration and threat to the species. In this context, conservation of biodiversity calls for reorientation of strategies where cultural traditions are also incorporated. Pudukkottai district consists of 27 sacred groves covering 15.2% of the total area. Out of 27 sacred groves, only three are studied ecologically in detail [22-24]. In light of this the present study was undertaken to study the floristic richness and botanical significance of three selected sacred groves of Pudukkottai district namely Pulvayal, Mylappatti and Kallampatti.

MATERIALS AND METHODS

Study area

Pudukkottai District was carved out of Tiruchirappalli and Thanjavur districts in January 1974. Pudukkottai district covers an area of 4663 Sq. Km. which has a coast line of 39 Kms. The district is located between 78.25' and 79.15' of the East of Longitude and between 9.50' and 10.40' of the North of Latitude (Map 1 and 2). It is bounded by Tiruchirappalli district in the North and West, Sivaganga district in the South, Bay of Bengal in the East and Thanjavur district in the North East.

The study area Pulvayal, Marayappatti and Kathavampatti are situated in Illuppur Taluk of Pudukkottai district in Tamilnadu. The land is not fertile, so agriculture is not very common in these areas. Mostly people are engaged in the mining of granite and laterite. The average rainfall is 513.1 mm rainfall from January to September as against the normal rainfall of 528.7 mm. The lowest average temperature is 21.6 ^{o}C and the highest measuring as 38.7^{o}C.

Pulvayal, Marayappatti and Kathavampatti are lying west of the road from Pudukkottai to Ponnammaravathy. The details of the sacred groves are given in Table 1. The present study is focused on Sivan of Pulvayal, Ayyanar of Marayapatti and Muneeswarar of Kathayampatti (Plate 1).

Field Study

An extensive floristic survey was carried out in the sacred grove at Pudukkottai district of Tamil Nadu, southern peninsular India at monthly intervals for 2 years between November 2009 to November 2011. The entire sacred grove was surveyed thoroughly at every visit. All the plants growing over the study area were recorded in all the seasons of the study period.

Identification of Plant species

Specimens of flowering plants were collected and identified taxonomically with the help of different floras [25-29] and by using field keys devised by Pascal and Ramesh [30]. The Rapinant Herbaria, St. Josephs College, Tiruchirappalli were consulted for correct identification of plant specimens. The nomenclature of species follows the regional flora. Lists of endangered, rare and endemic plants found in the sacred grove were prepared with the help of the published work of Nayar and Sastry [31].

RESULTS AND DISCUSSION

Taxonomically, a total of 65 species belonging to 51 genera and 29 families was identified from the three sacred grove (Table 2). Among these twenty were trees, ten shrubs, four under shrubs, one thorny shrub, four climbers, one straggler and twenty five herbs. The secondary invasive species were confined to the periphery and in disturbed patches of the grove. Some interesting herbaceous plants were also found inside the sacred grove. In Pulvayal sacred grove, 34 species were recorded. Out of 34 species, 10 are trees, 15 are herbs, 7 are shrubs and 2 are climbers. Herbs are dominated in this sacred grove followed by trees, shrubs and climbers. One endangered species, viz. *Cayratia pedata was* recorded in the sacred grove. In Marayappatti sacred grove, 33 species were recorded. Out of 33 species, 14 are trees, 13 are herbs, 4 are shrubs and 2 are climbers. Trees as well as herbs are dominated in this sacred grove followed by trees, shrubs and climbers. In Kathavampatti sacred grove, 30 species were recorded. Out of 30 species, 13 are trees, 10 are herbs, 4 are shrubs and 3 are climbers. Herbs are dominated in this sacred grove followed by trees, shrubs and climbers.

The floristic composition of the sacred grove is similar to the low elevation tropical dry evergreen forest as described by earlier studies [13]. This is evident by the presence of evergreen species such as *Azadiracta indica, Albizia lebbeck, Ficus benghalensis, F. religiosa* and *Syzygium jambolanum* in the sacred grove. The population is largely concentrated in five species, viz. *Albizia amara, Strychnos nux vomica, Zizyphus oenoplia, Acacia arabica and Acacia nilotica.* The over storey consisted of trees like *Albizia amara , Albizia lebbeck, Phyllanthus emblica* and *Syzygium jambolanum*, and the canopy layer was dense and continuous. *Atlantia monophylla, A. racemosa, Cassia occidentalis* and *Pongamia glabra* belonged to the understorey vegetation in the sacred grove. Leguminosae with 13 species was by far the largest and dominant family in these groves. Euphorbiaceae with 9 species occupies the second position followed by Amaranthaceae, Lamiaceae and Arecaceae (4 species each), Asclepiadaceae and Rubiaceae (3 species each) were well represented in the sacred grove. The three families, Compositae, Gramineae and Moraceae were represented by two species each; Acanthaceae and Vitaceae were represented by two species each, whereas 14 families namely Cactaceae, Cleomaceae, Cordiaceae, Cucurbitaceae, Gentianaceae, Loganiaceae, Liliaceae, Malvaceae, Meliaceae, Moringaceae, Myrtaceae, Nymphaceae, Rutaceae and Rhamnaceae were monospecific. Multi-species genera have been reported in many tropical forests [32,33]. Coexistence of congeneric species in the sacred grove indicates differences in flowering phenologies, pollination and dispersal agents of the species [32]. Data on vegetation analysis indicates the presence of mostly age-old specimens in this sacred grove is a worrisome factor. A few rare and endangered taxa were also found in this sacred grove. About 2% of the total species recorded in the groves were rare in nature. An analysis of the distribution pattern of rare, endangered and endemic species in the sacred grove reveals that the majority of them were either small trees or shrubs (Table 3). Interestingly, all plant species inventoried in this sacred grove are economically important. Among these species 52 plants were used as medicine by the indigenous people, 6 plants are with high timber value and 10 plant species are minor forest produce used by local people.

CONCLUSION

As degradation of sacred groves and fragmentation of habitats have been rampant worldwide, reservation of natural habitats, however small they might be, has become imperative, along with the reorientation of the strategies for the conservation of biodiversity towards the sacred groves, and the cultural traditions associated with them. Thus, the enumeration of the sacred grove at Illupur taluk and the assessment of the floristic wealth, medicinal importance, rarity and endemism would provide a strong basis for evolving measures for their protection.

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Table 1: Details of the miniature sacred grove selected for the study

S.NO	Name of the groove	Area (Acres)	Deity	Distance from Pudukkottai District
1	Pulvayal	0.5	Sivan	**12**
2	Marayappatti	0.3	Ayyannar	10
3	Kathavampatti	0.5	Muneeswarar	13

MAP OF INDIA SHOWING TAMILNADU WHICH IN TURN SHOWING PUDUKKOTTAI

Map 1 (Inside view) and 2 (Marking Area)

Table 2. List of plant species recorded in the three sacred groves (Pulvayal, Marayappatti and Kathavampatti) of Pudukkottai District

S. No	Botanical name	Family	Occurrence of plant species		
			Pulvayal	Marayappatti	Kathavampatti
1	*Abrus precatorius .*	Fabaceae	-	✓	✓
2	*Acacia nilotica*	Mimosaceae	✓	✓	-
3	*Acacia arabica*	Mimosaceae	✓	-	-
4	*Acalypha indica*	Euphorbiaceae	✓	✓	-
5	*Achyranthes aspera .*	Amaranthaceae	✓	✓	✓
6	*Alangium salvifolium*	Alangiaceae	-	-	✓
7	*Albizia amara*	Mimosaceae	✓	✓	✓
8	*Albizia lebbeck*	Mimosaceae	-	✓	-
9	*Amaranthus spinosus*	Amaranthaceae	-	✓	-
10	*Amaranthus viridis*	Amaranthaceae	-	✓	-
11	*Asteracantha longifolia*	Acanthaceae	-	-	✓
12	*Aloe vera*	Liliaceae	-	✓	-
13	*Atalantia monophylla*	Rutaceae	-	✓	-
14	*Azadirachta indica*	Meliaceae	-	✓	✓
15	*Barleria cuspidata*	Acanthaceae	-	✓	✓
16	*Borassus flabellifer*	Arecaceae	-	-	✓
15	*Barleria cuspidata*	Acanthaceae	-	✓	✓
16	*Borassus flabellifer*	Arecaceae	-	-	✓
17	*Bothriochloa odorata*	Poaceae	✓	-	-
18	*Calotropis gigantea*	Asclepiadaceae	-	✓	-
19	*Canthium parviflorum*	Rubiaceae	✓	-	✓
20	*Cissus quadrangularis*	Vitaceae	✓	-	-
21	*Cayratia pedata*	Vitaceae	✓	-	-
22	*Cleome viscosa*	Cleomaceae	-	✓	-
23	*Cassia fistula*	Caesalpiniaceae	✓	-	✓
24	*Coccinia indica*	Cucurbitaceae	✓	✓	-

25	*Cassia auriculata*	Caesalpiniaceae	-	✓	✓
26	*Cassia occidentalis*	Caesalpiniaceae	✓	✓	-
27	*Cassia roxburghii*	Caesalpiniaceae	✓	-	✓
28	*Cassia tora*	Caesalpiniaceae	✓	-	✓
29	*Cassia elongata*	Caesalpiniaceae	-	✓	✓
30	*Cynodon dactylon*	Poaceae	✓	-	-
31	*Enicostemma axillare*	Gentianaceae	-	-	✓
32	*Cocos nucifera*	Arecaceae	-	✓	✓
33	*Cordia obliqua*	Cordiaceae	✓	-	-
34	*Croton sparsiflorus*	Euphorbiaceae	-	✓	-
35	*Euphorbia antiquorum*	Euphorbiaceae	✓	-	-
36	*Euphorbia hirta*	Euphorbiaceae	✓	✓	-
37	*Ficus bengalensis*	Moraceae	✓	-	✓
38	*Ficus religiosa*	Moraceae	✓	-	-
39	*Gomphrena globosa*	Amaranthaceae	-	✓	
40	*Gymnema sylvestre*	Asclepiadaceae	-	-	✓
41	*Jatropha gossypifolia*	Euphorbiaceae	✓	-	-
42	*Jatropha glandulifera*	Euphorbiaceae	✓	-	-
43	*Lawsonia inermis* .	Lythraceae	-	✓	-
44	*Leucas aspera*	Lamiaceae	✓	✓	-
45	*Morinda tinctoria*	Rubiaceae	✓	✓	-
46	*Moringa pterygosperma*	Moringaceae	-	✓	-
47	*Ocimum sanctum*	Lamiaceae	✓	✓	-
48	*Ocimum basilicum*	Lamiaceae	-	-	✓
49	*Ocimum tenuiflorum*	Lamiaceae	-	✓	-
50	*Opuntia dillenii*	Cactaceae	✓	-	-
51	*Phoenix dactylifera*	Arecaceae	-	-	✓
52	*Phoenix loureirii*	Arecaceae		✓	✓
53	*Phyllanthus amarus*	Euphorbiaceae	✓	✓	
54	*Pergularia daemia*	Asclepiadaceae	-	-	✓
55	*Pongamia glabra*	Fabaceae	-	✓	✓
56	*Phyllanthus maderaspatensis* L.	Euphorbiaceae	✓	✓	-
57	*Phyllanthus reticulatus*	Euphorbiaceae	-	-	✓
58	*Randia longispina*	Rubiaceae	✓	-	-
59	*Sphaeranthus indicus*	Asteraceae	✓	-	✓

60	*Strychnos nux-vomica .*	Loganiaceae	-	-	✓
61	*Syzygium jambolanum*	Myrtaceae	-	-	✓
62	*Thespesia populnea*	Malvaceae	✓	✓	✓
63	*Tamarindus indica*	Caesalpiniaceae	-	✓	-
64	*Tridax procumbens*	Asteraceae	✓	-	-
65	*Zizyphus oenoplia*	Rhamnaceae	✓	✓	✓

Table 3: Vernacular name, life form, economic value and conservation status of different plant species of three sacred groves in Pudukkottai District.

S. No	Botanical name	Vernacular name	Life form	Econ	Conservation
1	*Abrus precatorius .*	Gundumani	Climber	M	Occasional
2	*Acacia nilotica*	Karuvelam	Tree	FT	Occasional
3	*Acacia arabica*	Karuvai	Tree	FT	Occasional
4	*Acalypha indica*	Kuppai-meni	Herb	M	Common
5	*Achyranthes aspera .*	Nayuruvi	Herb	M	Occasional
6	*Alangium salvifolium*	Alangi	Tree	M	Rare
7	*Albizia amara*	Usila maram	Tree	MFT	Common
8	*Albizia lebbeck*	Vaagai	Tree	MFT	Occasional
9	*Aloe vera*	Katrazhai	Herb	M	Common
10	*Amaranthus spinosus*	Mullukkirai,	Herb	M	Occasional
11	*Amaranthus viridis*	Kuppaikkirai,	Herb	M	Occasional
12	*Asteracantha longifolia*	Neermulli	Herb	M	Common
13	*Atalantia monophylla*	Kattu-elumichai,	Tree	MT	Common
14	*Azadirachta indica*	Veppamaram	Tree	MT	Common
15	*Barleria cuspidata*	Not Recorded	Un-shrub	NR	Rare
16	*Borassus flabellifer*	Panaimaram	Tree	MT	Common
17	*Bothriochloa odorata*	Pillu	Herb	M	Common
18	*Calotropis gigantea*	Eruku	Shrub	M	Common
19	*Canthium parviflorum*	Marukkarai	Shrub	M	Common
20	*Cassia auriculata*	Avarai	Shrub	M	Common
21	*Cassia occidentalis*	Pon-avarai	Un-shrub	M	Occasional
22	*Cassia roxburghii*	Seemaraela	Tree	M	Occasional
23	*Cassia elongata*	Nilavirai	Herb	M	Occasional
24	*Cassia fistula*	Sarakkonrai kai	Tree	M	Occasional
25	*Cassia tora*	Oosithagarai	Un-shrub	M	Occasional
26	*Cayratia pedata*	Kattu Pirandai	Climber	M	Endangered
27	*Cissus quadrangularis*	Pirandai	Straggler	M	Common
28	*Cleome viscosa*	Naikkaduku	Herb	M	Occasional
29	*Coccinia indica*	Kovai	Climber	M	Common
30	*Cocos nucifera*	Thennai	Tree	M	Common

31	*Cordia obliqua*	Naruvili	Tree	M	Occasional
32	*Croton sparsiflorus*	Siru-kattamanakku	Herb	M	Common
33	*Cynodon dactylon*	Arugampillu	Herb	M	Common
34	*Enicostemma axillare*	Vellaruku	Herb	M	Rare
35	*Euphorbia antiquorum*	Chadura-kalli	Shrub	M	Common
36	*Euphorbia hirta*	Ammam pachcharisi	Herb	M	Occasional
37	*Ficus bengalensis*	Alamaram	Herb	M	Common
38	*Ficus religiosa*	Arasha-maram	Herb	M	Common
39	*Gomphrena globosa*	Civappu vatamallikai	Tree	M	Occasional
40	*Gymnema sylvestre*	Shirukurinja	Climber	M	Rare
41	*Jatropha glandulifera*	Erikkaraikattamanakau	Shrub	M	Occasional
42	*Jatropha gossypifolia*	Kattamanakku	Herb	M	Occasional
43	*Lawsonia inermis* .	Marutani	Herb	M	Common
44	*Leucas aspera*	Thumbai	Tree	M	Occasional
45	*Morinda tinctoria*	Nunaa	Tree	M T	Occasional
46	*Moringa pterygosperma*	Murungai	NR	M	Common
47	*Nelumbo nucifera*	Thamarai	Aquatic	M	Occasional
48	*Ocimum basilicum*	Tiruneetruppachchilai	Shrub	M	Occasional
49	*Ocimum sanctum*	Nallathulasi	Climber	M	Common
50	*Ocimum tenuiflorum*	Karuttulasi	Shrub	M	Occasional
51	*Opuntia dillenii*	Cappatti-k-kalli	Herb	M	Common
52	*Pergularia daemia*	Velipparutti	Climber	M	Common
53	*Phoenix dactylifera*	Pereecham	Tree	MFT	Occasional
54	*Phoenix loureirii*	Sittreechu	shrub	MFT	Occasional
55	*Phyllanthus amarus*	Kilanelli,	Herb	M	Occasional
56	*Phyllanthus maderaspatensi*	Mela nelli	Herb	M	Common
57	*Phyllanthus reticulatus*	Karunelli	Shrub	M	Very rare
58	*Pongamia glabra*	Pungam-maram	Tree	M T	Common
59	*Randia longispina*	Marukarai chedi	Shrub	MFT	Common
60	*Sphaeranthus indicus*	Kottakkarandai	Herb	M	Occasional
61	*Strychnos nux-vomica* .	Kanjirai	Tree	M	Occasional
62	*Syzygium jambolanum*	Naval	Tree	MT	Occasional
63	*Tamarindus indica*	Puliyamaram	Tree	MT	Common
64	*Thespesia populnea*	Puvarasu	Tree	MT	Occasional
65	*Tridax procumbens*	Vettukkaya puntu	Herb	M	Common
66	*Zizyphus oenoplia*	Soorai	Thorny	M	Occasional

AYYANAR TEMPLE OF MARAYAPPATTI

MUNEESWARAR TEMPLE OF KATHAVAMPATTI

SIVAN TEMPLE OF PULVAYAL

PLATE 1

ALANGIUM SALVIFOLIUM

PHYLLANTHUS RETICULATUS

CAYRATIA PEDATA

BARLERIA CUSPIDATA

GYMNEMA SYLVESTRE

ENICOSTEMMA AXILLARE

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RESEARCH ARTICLE

An Effective Biochemical Preservation Method for *Daucus carota* L. Roots

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ABSTRACT

Lactic acid, a natural chemical compound plays an important role in several biochemical processes. In the present study, the fleshy roots of *Daucus carota L.* (Carrot) are taken as a subject to measure the preservation capacity of lactic acid of various concentration for 0-12 days of time using 4% acetic acid as a control. The presence of microorganisms and their growth rate are monitored by nutrient agar plate method. Chemical parameters such as total titratable acidity and pH are determined. Also the taste and texture of the samples are tested. The results of the study demonstrate that the growth rate of the microorganisms is minimized and the preservative capacity of lactic acid is increased at higher concentrations with time.

Keywords: *Daucus carota L.* – Carrot ,Biochemical parameters,Lactic acid,Preservation.

INTRODUCTION

Plants and plant products are part and parcel of human society to combat diseases from the dawn of civilization. Natural food has unlimited power to sustain and restore when health is lost. Fresh fruits and vegetables have possessed the property of curing almost all kind of diseases. There has been an increase in the variety and the frequency of diseases in proportion to the process of degeneration of the food stuffs through refining and cooking. Food such as fruits and vegetables have short growing season. For the human body vitamins and carotenoids are essential nutrient for healthy eyesight, skin and age related diseases. Antioxidant characters of carotenoids protect human health from diseases. *Daucus carota L.* (Carrot) is well known throughout the world including India as one of

the most versatile medicinal plants having a wide spectrum of biological activity. Carrot is enriched with full of biomolecules such as amino acids, proteins, sugars, soluble phenols, vitamin-A, β-carotene and anthocyanin. Fruits and vegetables have a short keeping quality and preservation makes them available to use throughout the year and avoids wastage of surplus of them. So, it has been proposed to study the simple short term method of preservation of carrot to enhance and extend its shelflife period.

Food preservation

Common objective of preservation is decreasing the number of living organisms in since stuffs or at least holding them in check against multiplication. Fresh vegetables are highly perishable and are easily spoiled by a number of factors including the growth of spoilage microorganisms. Acidity and temperature are the commonly involved factors in the spoilage of foods. Naturally occurring enzymes present in the food produce chemical reactions and structural changes. Food preservation is the process of treating and handling food in such a way as to stop or greatly slow down spoilage due to food borne microorganisms while maintaining nutritional value, density, texture and flavour. Food preservation involves the action taken to maintain food with the desired properties or nature for as long as possible. Preservation method starts with complete analysis and understanding of the whole food chain including growing, harvesting, processing, packaging and distribution. Common methods of applying these processes include drying, spray drying, freeze drying, vacuum-packing, canning, preserving in syrup, sugar crystallization, food irradiation and adding preservatives. The use of preservatives must be strictly limited to those substances which are recognized as being less harmful to human beings health and are accepted by international standards.

Lactic acid bacteria perform an essential role in the preservation and production of whole some foods. Lactic acid fermentation, preferably at low cost is expected to become even more important in preserving fresh vegetables, fruits, cereals and legumes for feeding humanity. Lactic acid is naturally occurring organic acid present in food stuffs and is constituent of animal blood and muscle tissues. Lactic acid also known as a milk acid is a carboxylic acid with a chemical formula of $C_3H_6O_3$. It has a hydroxyl group adjacent to the carboxyl group, making it as a α-hydroxy acid. Lactic acid is chiral and has two optical isomers. One is known as L-(+)-lactic acid or (S)-lactic acid and the other, its mirror image, is D-(+)- lactic acid or L-(+)-lactic acid. L-(+)-lactic acid is the biologically important isomer.

Biopreservation refers to extended storage life and enhanced safety of foods using the natural micro flora and or their antibacterial products. Lactic acid bacteria (Figure 1) have a major role and potential for use in biopreservation because they are safe to consume and during storage they do not produce any harmful effects. Lactic acid preservation is one of the common technologies with relatively low operating costs. Hence the present study is focused to measure the preservation capacity of lactic acid on carrot using acetic acid as a control.

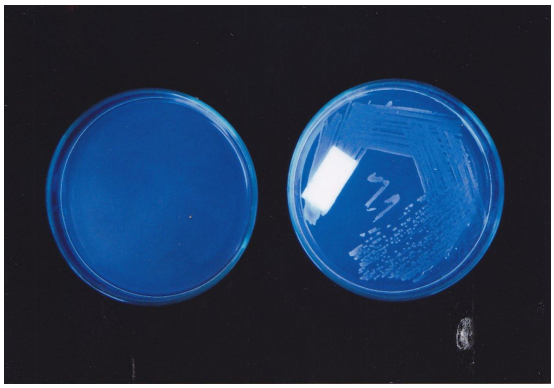

Figure -1.Lactic acid bacteria

MATERIALS AND METHODS

Collection of the sample

Approximately 90 days old growth of carrots (Figure2) from a healthy plant was collected from Nilgiri district and the surface of the root was sterilized.

Figure -2
45 Days and 90 days old carrots

About the plant *Daucus carota* L.

There are two major groups of carrot, Asiatic and Temperate. Asiatic types are more popular in northern India whereas Temperate types are sown for late supply in the season. Three important varieties of carrot are grown in different parts of the world.

a. Asiatic varieties- Pusakesar, Pusa meghali, Pusa yamdagni and India gold etc.
b. European varieties- Nantes, Coreless, Chantaney, Oxheart, Scarlet horn, Danvers half long, Orange prince and Orange Geno etc.
c. Improved varieties- Sel-5a, Sel-5B, Weibulls Briallent and Sel-233 etc.

The Taxonomic position of *Daucus carota* L.

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Apiales
Family : Apiaceae (or) Umbelliferae.
Genus : Daucus
Species : carota

Biological activity of carrot

Biological activities and uses of the king of the root vegetable carrot are listed below:

a. Cleanses the blood.
b. Increases the RBC count in the blood.
c. To promote flow of urine.

e. Used for the treatment of eye diseases, beneficial for the kidneys and the urinary tract, skin problems, eczema and psoriasis which is a chronic skin disease characterized by scaly red patches on the skin and duodenal ulcers, expelling worms, stomach ache, amenorrhea or absence of menstruation, feminine hysteria and chronic bronchial inflammation, painful abscess near finger or toe nails, other inflammations of the fingers and open wounds.

Botanical Description

Daucus carota L. belongs to the family Apiaceae (or) Umbelliferae, biennial but is ready for harvesting in one year. The root has typical conical appearance. The plant has characteristic pinnately compound leaves. It bears an erect umbel bearing stem in the second year of cultivation to produce flowers and seeds. Although most carrots are dark yellow or orange, some have other colours normally varies from purple to white. Carrots vary in shape, size, colour and quality depending upon the soil conditions. The swollen tap root possesses wide cortex and phloem layers which store the reserve food. Carrot is a cool season crop which grows well in the well drained, deep, loose and loamy soil. Looseness of soil help in the production of good round shaped roots. The growth and the colour development of roots are affected by temperature. Carrots grown at 10 -15 ^{o}C develop a poor colour and those grown at 15 -20 ^{o}C develop a dark colour.

Storage and Nutritive values

Carrot may remain fairly fresh for 3-4 days at ordinary temperature. They could be stored only at 12 ^{o}C for a long period without loss of quality. The nutritive value of carrot in 100g of edible portion is as given Table -1

Table -1 :Nutritive value of carrot in 100g of edible portion

Contents	Quantity	Contents	Quantity
Moisture	86.00g	Iron	2.20mg
Protein	0.90g	Sodium	0.80mg
Fat	0.20g	Phosphorous	3.00mg
Minerals	1.10g.	Copper	0.13mg
Fibre	1.20g	Riboflavin	0.02mg
Carbohydrate	10.60g	Vitamin C	3.00mg
Calcium	80.00mg	Oxalic acid	5.00mg
Magnesium	14.00mg	Nicotinic acid	0.60mg
Potassium	0.80mg	Vitamin A	3,151.U
Sulphur	27.00mg	Energy	47Cals.

Enumeration of total bacterial and fungal load

Total bacterial and fungal load were enumerated by using nutrient agar plates and Rose Bengal chloramphenicol agar plates. Bacterial and fungal growth was performed in a selective media and isolated. The nutrient agar plates inoculated with bacterial organism under test were incubated at 35-37 ^{o}C for about 24 hours. But the plates streaked with fungal organism under test were incubated at the same temperature for about 48 hours. The isolated pathogens were identified based on colony morphology. The colonies from the nutrient plates were plated for the identification of cultural characteristics in a selected culture media. Gram's staining procedure was followed for the cultures obtained from nutrient agar plate.

Preservation Method

Fresh matured and cleaned carrots were washed in plain water followed by 200ppm chlorinated water and then rinsed with plain water. The rinsed carrots were washed smoothly with luke warm water (about 68 °C) for one minute and cooled with running tap water of potable quality. The blanched carrots were rinsed in 5ppm chlorine water and sliced into ¾ inch pieces. Lactic acid solution was prepared at four different concentrations such as 100ppm, 150ppm, 200ppm, and 250ppm using required volume of sterilized water. The carrot pieces were soaked in the prepared lactic acid solutions for 15 minutes. And the soaked carrots were sealed in polythene pouches using hand sealer. 4% acetic acid solution was prepared and the sliced carrots were soaked in the solution for 15minutes and used as a control. The soaked carrots were sealed in polythene pouches using hand sealer. The filled pouches were stored in normal temperature (Figures 3 and 4) the stored carrots were tested for taste, flavour, texture and microbial load on 3rd, 6th, 9th and 12th days.

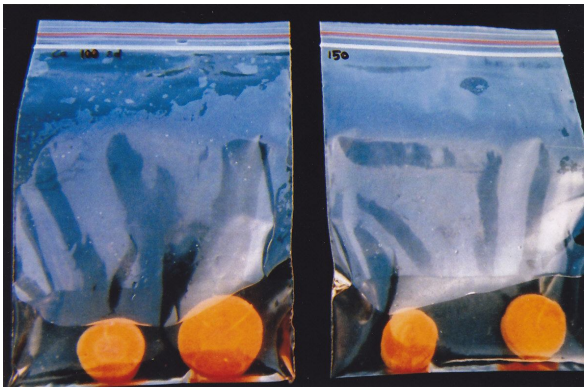

Figure-3
Sealed pouches with 100 and 150ppm
Lactic acid solutions

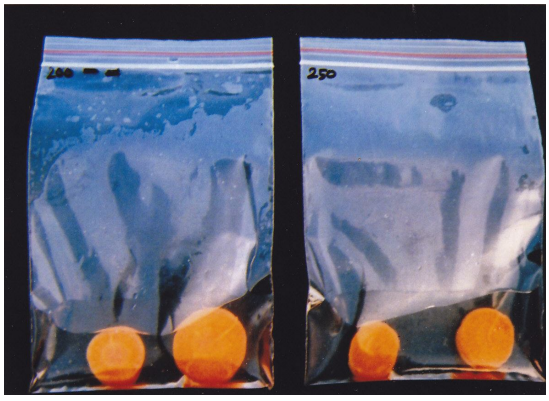

Figure -4
Sealed pouches with 200 and 250pp
Lactic acid solutions

Determination total titratable acidity

25 g of carrot was taken and minced using pestle and mortar. 10g of minced sample was dissolved in 50ml of distilled water in a 250ml standard flask and made up to a mark 10ml of the made up solution was taken in a Erlenmeyer flask and diluted with 25ml of distilled water and titrated against 0.05N NaOH till the appearance of permanent pale pink colour using phenolphthalein indicator. Using the titre value the total acidity was calculated. Total titrable acidity was calculated on 3rd, 6th, 9th and 12th days.

Determination of pH

The pH meter was standardized with pH buffer and the electrode was checked against additional buffer near the pH of the sample. The electrode was immersed in the sample solution and pH was read directly on 3rd, 6th, 9th and 12th days.

RESULTS AND DISCUSSION

Vegetables supply many nutrients besides make the food attractive by their colour, texture and flavour. Plants like tapioca, radish, carrot, beet root, potato, sweet potato and turnip etc. acts as staple food crop in which the underground part being the principal storage organ. Fermentation and preservation of food stuff using lactic acid is

the one of the biochemical processes practiced by mankind. One of the important contributions of lactic acid bacteria is their ability to produce antimicrobial compound known as bacterocin. These antimicrobial compounds have high potential as natural substitute for the chemical preservatives in the production of foods with enhanced shelf life period and safety.

In the present study preservation of carrot by using lactic acid medium and acetic acid as control were investigated. The preservation is carried out from 0-12 days. The presence of microorganisms, the total acidity, pH and texture are monitored during the preservation period. The existence of microorganisms and their biochemical characters in carrot is confirmed by biochemical tests and the results observed are given in Table-2. Bacterial growth and fungal growth are shown in Figure 5 and 6.

Table -2 . Biochemical characters

Organisms Tested	Bacillus	Pseudomonas	Lactobacillus
Gram's Staining	Rod, +	Rod, +	Rod, +
Motility	Motile	Motile	Motile
Indole	◎	◎	◎
MR	◎	◎	◎
VP	+	◎	+
Citrate	◎	+	◎
Catalase	+	+	◎
Oxidase	◎	+	+
TSI Slant	Acid	◎	Acid
Butt	◎	Acid	Acid
H_2S	◎	◎	◎

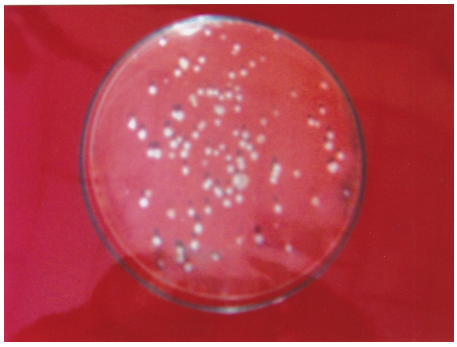

Figure -5.Bacterial growth on carrot

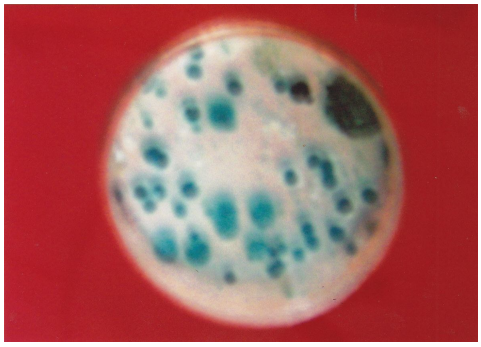

Figure -6. Fungal growth on carrot

The results of bacterial and fungal count detected by plate count method during the preservation period of carrots in various concentration of lactic acid and 4% acetic acid as a control are given in Table-3 and 4. Preservation of carrot by lactic acid showed reduction in number of organism with increasing concentration and no change in original and flavour even at 250ppm. It is obvious that lactic acid can reduce and controls the growth of bacteria and fungi day by day. This may be due to the strong antagonistic activity of lactic acid against several foods - spoilage and pathogenic organisms. But acetic acid causes change in taste and flavour even though it reduces bacterial and fungal load appreciably.

Table – 3. Effect of Lactic acid in bacterial count

Storage days	Control 4% acetic acid (cfu/ml)	Bacterial count (cfu/ml)			
		Concentration of Lactic acid (ppm)			
		100	150	200	250
0	TNTC	TNTC	TNTC	TNTC	TNTC
3	135×10^8	250×10^8	235×10^8	215×10^8	138×10^8
6	86×10^8	178×10^8	162×10^8	165×10^8	83×10^8
9	75×10^8	150×10^8	136×10^8	122×10^8	68×10^8
12	58×10^8	87×10^8	73×10^8	64×10^8	37×10^8

Table – 4. Effect of Lactic acid in fungal count

Storage days	Control 4% acetic acid (cfu/ml)	Fungal Bacterial count (cfu/ml)			
		Concentration of Lactic acid(ppm)			
		100	150	200	250
0	TNTC	TNTC	TNTC	TNTC	TNTC
3	165×10^3	187×10^3	175×10^3	158×10^3	95×10^3
6	110×10^3	173×10^3	153×10^3	109×10^3	79×10^3
9	83×10^3	159×10^3	108×10^3	78×10^3	58×10^3
12	58×10^3	81×10^3	93×10^3	54×10^3	43×10^3

The physical and chemical parameters such as acidity, pH, taste and texture for the test period are observed both in lactic acid and the control acetic acid medium and the results are given in Tables - 5 and 6.The compliment of organic acid and phenolic acids in carrots is responsible for the titratable acidity and is commonly measured as over all index of the quality of the vegetable. From the table values it has been observed that there is a small increase in the acidity for the samples preserved in lactic acid as compared to values the fresh carrots and remains constant up to day 9. But in 12^{th} day a marked difference is observed, correspondingly there is a decrease in the pH values from 5.73 to 2.25. However, the taste of the carrot is not varied significantly but the texture of the preserved carrots became very soft than the original sample taken before. This may be due to the chemical changes such as enzymatic activities and oxidative reactions.

Table – 5. Physico chemical parameters determined for carrot in Lactic acid

Parameters	Storage days					
	Fresh carrot	**0th**	**3rd**	**6th**	**9th**	**12th**
Acidity	0.85	2.34	2.35	2.37	2.39	3.01
pH	5.73	3.56	3.47	3.23	3.11	2.25
Taste	Better	Better	Better	Good	Good	Good
Texture	Firm	Firm	Firm	Soft	Soft	Soft

Table – 6.Physico chemical parameters determined for carrot in Acetic acid

Parameters	Storage days					
	Fresh carrot	**0th**	**3rd**	**6th**	**9th**	**12th**
Acidity	0.85	3.01	3.01	3.01	3.02	3.02
pH	5.79	2.45	2.50	3.23	2.49	2.41
Taste	Good	Acidic	Acidic	Acidic	Acidic	Acidic
Texture	Firm	Firm	Firm	Firm	Firm	Firm

The results of the control experiment with carrot in acetic acid evidences that there is a remarkable change observed in the acidity (0.85-3.02) and the pH (5.79-2.41) values of the samples studied. Consequently the taste of the samples became very acidic and the texture changed to very firm. This may be due to the difference in pka values between lactic acid (pKa = 3.86 at 25 ^{o}C in water) acetic acid (pKa = 4.76 at 25 ^{o}C in water). The results showed that the potential for using bacteriocin producing lactic acid as a preservative and the improvement of safety and quality of the final preserved product varied with the acid strength of the acids used.

CONCLUSION

Carrots are used by people throughout the world and universally recognized as good natural food for its healthy properties and sweet taste. Carrot is also an important crop because its root mobilizes lot of minerals and phenolics and alkaloids which are beneficial to mankind. The perishable food like carrot can be preserved for short term period using chemical preservatives. Fermentation of food using lactic acid bacteria ensure not only increased the shelf-life and microbiological safety of food, but also may make some food more digestible. Biochemical study results and other physicochemical parameters studied showed a considerable reduction in the level of microorganisms and no remarkable change in the taste and texture of the samples. Form the overall results of the study it is concluded that the lactic acid bacteria method of preservation could be suitable for the perishable vegetable carrot to save important phytochemicals and thus reaping the fruits of healthy nutrients.

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RESEARCH ARTICLE

Studies on Standardization of Citric acid Production by using *Aspergillus niger* Strains

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ABSTRACT

Many microorganisms such as fungi and bacteria are capable of producing citric acid. The production of citric acid by *Aspergillus niger* is one of the most commercially utilized examples of fungal overflows metabolisms. In this study *Aspergillus niger* was isolated from the soil by using Czapekdox agar medium and it was used as inoculum. Sucrose is the traditional commercial substrate for citric acid production. Glucose, sucrose and maltose have also been used as substrates for citric acid production. The effect of different sugar concentrations (120-180 g/l) on citric acid production by *Aspergillus niger* was experimentally carried out. The maximum amount of citric acid (92.50 g/l) production was obtained in the medium containing 150g/l sugar. In the present study, sucrose was used as substrate for citric acid production and the initial sucrose level condition was 140 g/l. UV-mutagenesis (physical) process was also employed to obtain the maximum yield of citric acid, . The fungal organism was exposed to UV-radiation at two different times of intervals (15 and 30 minutes). Doelger and Prescott fermentation medium (1934) was prepared and inoculated with the strains of *Aspergillus niger* (approximately $2x10^5$ spores/ml) and incubated at 25°C for 8-days in a mechanical shaker.

Key words: Citric acid, *Aspergillus niger,* Czapekdox agar medium.

INTRODUCTION

Citric acid i.e., 2-hydroxy 1,2,3 propane tricarboxylic ($CH_2COOH.COH.CH_2COOH$) is ubiquitous in nature and exists as an intermediate in the citric acid cycle when carbohydrates are oxidized to carbon dioxide. Citric acid is solid at room temperature, melts at 153°C and decomposes at higher temperatures into other products [7]. *Aspergillus niger* is the principal mold used in citric acid production although various other molds are known to be able to make the acid and may have seen tried in experiments molds such as *Aspergillus clavatus*, *A. wentii*, *Penicillium leuteum*, *P. citrinum*, *Mucor pyriformis* and others. Apparently different strains of *Aspergillus niger* are preferred for surface methods of citric acid production than the submerged method of growth. Citric acid is responsible for the tart taste of various fruits in which it occurs, such us lemons, limes, figs, oranges, pineapples, pears and gooseberries. Citric acid can be recovered from its calcium salt by adding sulfuric acid [2]. It is non toxic and easily oxidized in the human body. Because of its high solubility, palatability and low toxicity, it can be used in food, biochemical and pharmaceutical industries. These uses have placed greater stress on increased citric acid production and search for more efficient fermentation process. The worldwide demand of citric acid is about 6.0x105 tons per year and it is bound to increase day by day [1]. At present time citric acid is produced commercially by using mutant strains of *Aspergillus niger*, and with a significant amount by *Saccharomycopsis lipolytica* [5].

MATERIALS AND METHODS

Collection of soil samples

The soil samples from fertile land at 4 to 6 inches depth were collected from Sembanarkoil, Nagai district, Tamil nadu, India. The collected soil samples were brought to the laboratory in sterilized polythene bags, handpicked, air dried and stored in sterile containers for future use.

Serial Dilution

The serial dilution of soil (10^{-1} to 10^{-7}) were made in a sterile water blanks. The samples were taken from the dilution of 10^{-3}, 10^{-4}, and 10^{-5}.

Isolation of *Aspergillus niger*

The above dilutions were spreaded in Czapekdox agar plates and incubated at 25°C for 7 days.

Pure Culture

Identified colonies of *Aspergillus niger* were pure cultured in the same medium stained with lactophenol cotton blue.

Fermentation Process

Doelger and Prescott fermentation medium (1934) [3] was prepared and inoculated with the strains of *Aspergillus niger* (approximately $2x10^5$ spores/ml) and incubated at 25°C for 8-days in a mechanical shaker.

UV-Mutagenesis

To obtain the maximum yield of citric acid, UV-mutagenesis (physical) process was employed. The organism was exposed to UV-radiation at different time of intervals i.e.15 and 30 minutes.

Biomass

The whole fungal culture growth was filtered with Whatman No.1 filter paper, washed in distilled water (250 ml) and dried at 105^0C to get a constant weight. Results were expressed in terms of g/l.

Citric acid determination

Citric acid (A) was determined titrimetrically (AOAC, 1995) by using 0.1N NaoH and phenolphthalein as indicator and calculated as % according to the following formula.

$$\% \text{ CA} = \frac{\text{Normality x volume x Equivalent wt. of. CA}}{\text{Weight of Sample (g) x 10}}$$

CA → Citric acid

RESULTS AND DISCUSSION

Isolation

Fungal species were isolated from the soil by using czapekdox agar medium. Among the fungal species, *Aspergillus niger* was isolated, identified with the help of standard Manual of soil fungi [4] based on macroscopic and microscopic observations.

Aspergillus niger was maintained in czapekdox as purified form (Plate-1, Fig-A and B).

Cultural characters of *Aspergillus niger*

Conidia are brown coloured. Conidiophores arise directly form prostate hyphae, smooth septate or non-septate, varying greatly in length and 200-400x7-10 diameter respectively. Some times even above this range. Conidial heads are fucous, blackish brown or purple colour. Phalides are typically arranged in two series and thickly covering the vesicle.

Biomass

Biomass of *Aspergillus niger* was estimated on dry weight basis from three treatments. Among the three treatments, wild strain showed minimum growth and maximum biomass production was observed in U-V treated organisms at 30 minutes.

Estimation of Citric Acid

After completion of 8 days of incubation, citric acid quantity was estimated by using titration method.
Wild strain showed maximum quantity of citric acid production than the mutated. U-V treated strain did not develop spore and citric acid production (Plate-1 and Fig-C, D and E).

CONCLUSION

In this study, *Aspergillus niger*, was isolated from soil using Czapexdok agar medium and the culture was maintained in the same medium. The culture was U-V treated for 15 and 30 minutes. Three strains were inoculated in the Doelger and Prescott fermentation medium [3] (i.e) wild strain, 15 and 30 minutes U-V treated. After incubation period the fermented broth was taken, filtered and titrated. Wild strain were produced more amount of citric acid than U-V treated strains. U-V treated strains, showed more mycelial formation and less spore formation than wild. In this study due to mycelial formation in mutated strains did not enhance citric acid production. Mattey and Allan (1990)[6] described that due to the increase of mycelial formation in the medium, there was reduction in the yield of citric acid. In the present study, 30 minutes of U-V treated strain of *Aspergillus niger* enhanced the mycelial formation and do not form fungal spores. Hence there was reduction in the yield of citric acid.

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Table 1 . Titration of filtered broth for estimation of the amount of Citric acid

Volume of filtrate (ml)	Volume of 0.1 N NaOH (Burette reading) concurrent value			
	Initial (ml)	Wild strain (ml)	15 minutes U-V treated strain (ml)	30 minutes U-V treated strain (ml)
10	0	28.4	22.7	17.1

Table 2 . Biomass of *Aspergillus niger* in fermented broth

Organisms	Biomass (g)
Wild strain	1.0573
15-min mutated strain	1.1473
30-minutes mutated strain	3.831

Table 3 .Production of Citric acid from various strains of *Aspergillus niger*

Organisms	Production of Citric acid (g)
Wild strain	56.45
15-min mutated strain	41.58
30-minutes mutated strain	9.38

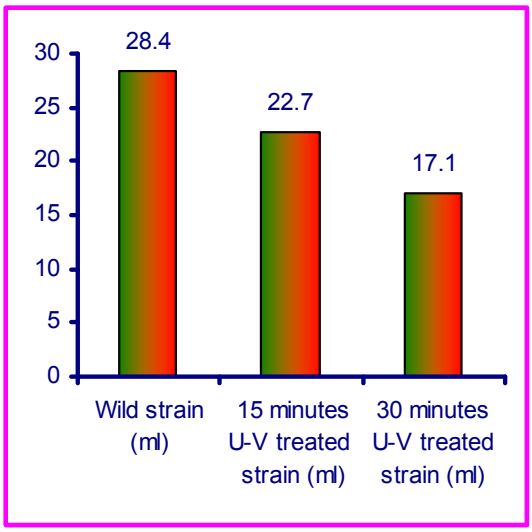


Fig-1

Estimation of the amount of Citric acid from filtered broth

X axis - Fungal strains

Y axis - Milliliters (ml)

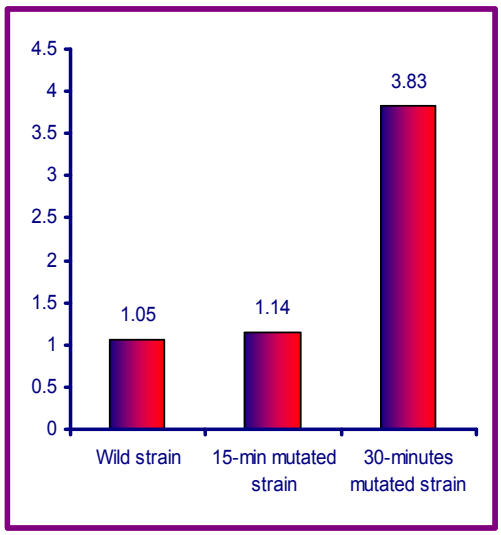


Fig-2

Biomass production by *Aspergillus niger* strains

X axis - Fungal strains

Y axis - Grams

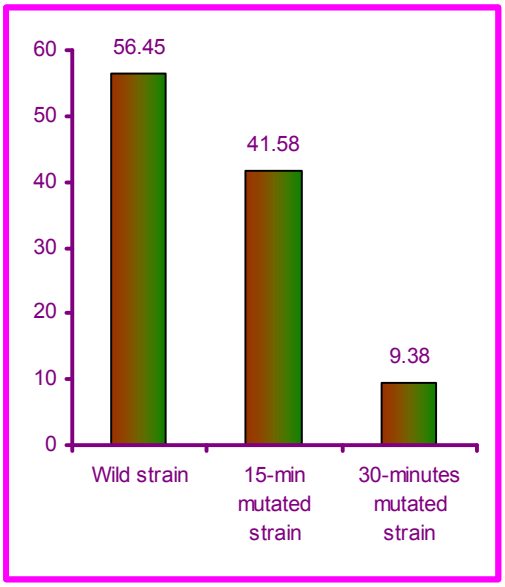


Fig-3

Citric acid production from *Aspergillus niger* strains

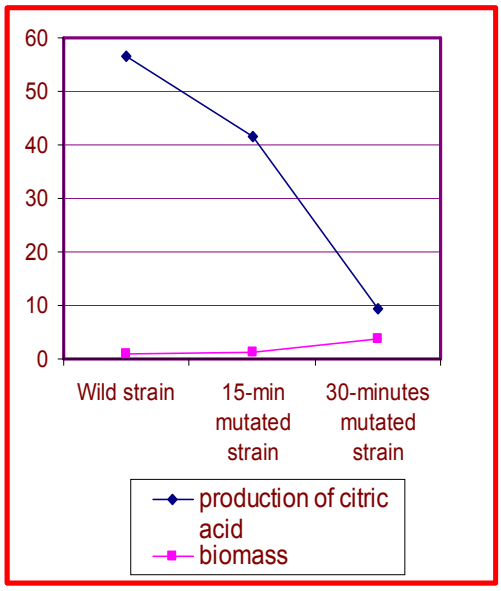
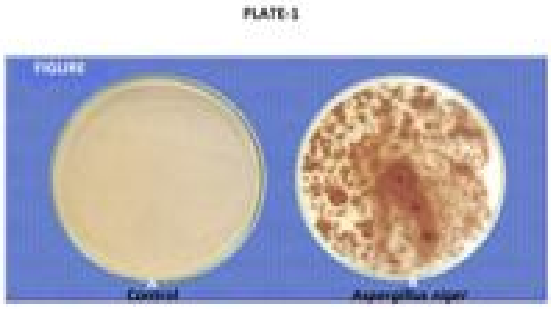


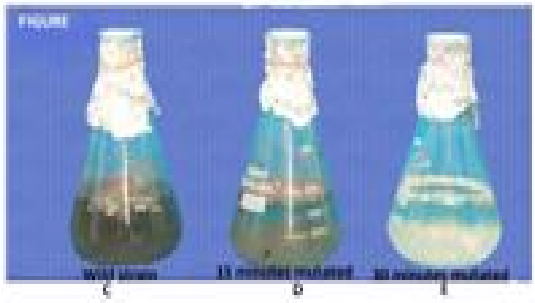
Fig-4

Comparative analysis of the production of Citric acid and biomass

Fig – 3&4: X axis - Fungal strains

Y axis - Grams





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RESEARCH ARTICLE

Effect of Weather Parameters on Growth and Yield Parameters of Tomato under Natural Poly House

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ABSTRACT

The experiment was conducted to study the effect of changes in microclimate produced by poly house conditions on plant growth characteristics and fruit yield of tomato was studied during 2009-2010 at TNAU campus, Coimbatore in the PFDC farm field located behind AEC & RI, workshop complex, which is situated at 11^0 N latitude and 77^0 E longitudes. Daily climatic parameter fluctuations of temperature, relative humidity and light intensity were measured at various stages of crop growth. Temperature (39.88 ^{0}C) was high in poly house during afternoon time. The relative humidity (91.06 %) was higher during morning time, and in afternoon (38.48 %) it was lower under polyhouse. The light intensity inside the poly house was higher at afternoon (58865, lux) and it was also observed that during morning and evening hours there was low light intensity. The changes in microclimates are positively influenced on growth and yield parameters of tomato under poly house condition.

Keywords: Temperature, Relative humidity, Tomato, Poly house, Soilless culture.

INTRODUCTION

Greenhouse is a framed structure, creating barrier between plant microclimate and ambient climate. Generally, greenhouse is covered with transparent or translucent materials in which crops may be grown under the conditions of partially or fully controlled environment. With a greenhouse, it is possible to create a microclimatic environment, which is better suited for the development of crop than the outside environment, thus giving better production and uniform quality produce. It was estimated that yield of greenhouse cultivation was nearly 15 times higher than those of open field.

The greenhouse environment has a profound effect on crop productivity and profitability. Climate decides crop selection while weather decides crop production and productivity. Greenhouse structures have distinct effect on several environmental parameters particularly temperature, light, carbon dioxide and humidity. The plant response to specific environmental parameter is related to the physiological processesand to yield and quality. Since the microclimate components inside the structures influences the functional aspects of plant, the emphasis is normally given to the maintenance of the optimal level of the factors for the successful and better productivity in the protective cultivation. The maintenance of crop photosynthesis is essential under the protective structure as it is responsible for 90% dry matter accumulation and plant productivity. Hence an experiment was carried out to study the influence of weather parameters on yield of tomato under different growing conditions.

MATERIALS AND METHODS

The study was conducted at TNAU campus, Coimbatore in the PFDC farm field located behind AEC & RI, workshop complex, which is situated at 11^0 N latitude and 77^0 E longitude. The mean altitude is 437 m above the mean sea level. The tomato hybrid Vaishali from Indo-American Company was chosen for the study since it has a vibrant market potential in domestic market. The duration of the crop is 126 days. The experiment was carried out in a natural ventilated poly house of 14 m length, 4 m breadth and the center height of the poly house was 4 m and the experiment was conducted in 3 replication. For keeping the soil less media intact and growing crop, a M.S. frame work of channel spacing of size 300 x 30 x 45 cm was designed. The media was supported by a trough made out of 750 micron thick HDPE black poly film; firmly attached to the frame work by means of M. S. bolts and screws. The depth of media can also be adjusted. With this set up it is possible to have a media depth 17 to 20 cm at the center of the trough. Twenty four numbers of such troughs were fabricated, painted with rust resistant proof paint and installed inside the house according to the treatment schedule.

Treatments: There were 8 treatments was taken for experiment. The treatments contain coir pith, perlite, vermiculite and peat and combination with vermicompost (1:1) volume basis. Treatments are T_1 - Coir pith, T_2 - Perlite, T_3 - Vermiculite, T_4 - Peat, T_5- Coir pith: Vermicompost (1:1), T_6 - Perlite: Vermicompost (1:1), T_7 -Vermiculite: Vermicompost (1:1) and T_8 - Peat: Vermicompost (1:1).

The micro climate data such as minimum and maximum temperature, relative humidity and light intensity were periodically (8.22AM, 10.22AM, 12.22PM, 2.22PM and 4.22PM) recorded in poly house during crop growing season and mean weekly values are represented in Table 1, 2 and 3. Observations on vegetative, growth, and yield parameters were recorded. Reproductive characters like days to first flower, first harvest, single fruit weight, number of fruits and harvests per plant and yield per plant were recorded.

RESULTS AND DISCUSSION

Temperature

In poly house during the crop period between November 2009 and March 2010, the morning temperature was maximum (25.10°C) in the month of March (12-18th days) and minimum (20.62°C) in December (11-17th days). The afternoon temperature was maximum (39.88°C) in the month of March (5-11th days) and minimum (35.13°C) in December (18-24th days) (Table 4.1 and Fig. 4.1). In every evening hour (4.22 pm) the temperature has been decreased.

Higher temperature during daytime was due to trapping of short wave radiation in the greenhouse under partially closed conditions.Nimje and Shyam [8] also obtained similar results.

Ganesan (1999) [4] reported that the air temperature in the open field condition was lower than in the poly-greenhouse treatments throughout the growth period. Poly-greenhouse with ventilation gaps in the triangular roof and four sidewalls was found more suitable for better plant growth and yield of tomato than the open field condition.

Air temperature is the main environmental component influencing vegetative growth, cluster development, fruit setting, fruit development, fruit ripening, and fruit quality The average 24-h temperature is believed to be responsible for the growth rate of the crop-the higher the average air temperature the faster the growth. It is also believed that the larger the variation in day-night air temperature, the taller the plant and the smaller the leaf size [9].

Relative humidity

The representative variations of relative humidity for poly house are presented in Table 2 and the data indicated that minimum relative humidity was observed in poly house during afternoon (2.22 pm) for the entire crop period.In poly house conditions, the morning relative humidity was maximum (91.06 %) in the month of December (11-17th days) and minimum (74.82 %) in March (5-11th days). The afternoon relative humidity was maximum (38.48 %) in the month of December (11-17th days) and minimum (25.13 %) in March (12-18th days) during the crop period between November 2009 and March 2010.

The variation in the relative humidity with time in poly house may be due to the increase in temperature. Higher humidity was observed inside the poly house during morning hours and gradually decreased in the afternoon because of increase in temperature.

Moreover, relative humidity inside the poly house was found to be high at early morning hours. The possible reason for this might be that the poly house was filled with the vegetation and plants were well watered, the ground surface of the greenhouse was always wet. During night, certain quantum of water from soil gets evaporated. Since poly house was covered with ultra violet stabilized sheet and also due to absence of solar radiation, the escape of water vapour from the poly house to outside was comparatively less during night. Besides, at early morning, when sun starts shining, there will be more transpiration from the leaves. Both these factors together caused higher relative humidity inside the poly house. Since in this study, poly houses was naturally ventilated, this effect does not prolong for longer period, but it occurred hardly for an hour after sunrise.

Light intensity

Light is a prerequisite of plant growth. Plant matter is produced by the process of photosynthesis, which takes place only when light is absorbed by the chlorophyll (green pigment) in the green parts of the plant, mostly in the leaves.
In the poly house, the morning light intensity was maximum (33845 lux) in the month of March (12-18th days) and minimum (23687 lux) in December (4-10th days). The afternoon light intensity was maximum (58865 lux) in the month of March (12-18 th days) and minimum (47950 lux) in December (19-25th days) during the crop period between November 2009 and March 2010. The representative variations of light intensity for poly house are shown in Table 3.

The results revealed that the light intensity inside the poly house was found to be much lower than in open field. Further, it was also observed that during morning and evening hours, there was low light intensity. These results are in accordance with those of Albright (1990) [2] who observed that the light intensity was less in poly house. A fully grown tomato crop benefits from any increase in natural light intensity, provided the plants are well supplied with water, nutrients, and carbon dioxide, and the air temperature is prevented from becoming too high.

Effect of weather parameters on days to first flowering, 50 per cent flowering and days to first harvest

In poly house the earliest flowering was recorded in T2F1 after 15.66 days of transplanting and T5F2 was late by three days. Remaining treatments have no significant difference in first flowering. Whereas, the treatment T5F2 (clay

loam soil) took higher number of days (39.33 days after transplanting) for 50 percent flowering. T3F1 attained 50 percent flowering in 33.66 days whereas, T4F1took (35.66) days after transplanting.

The results revealed that the optimum levels of nutrient status in the media and favorable growing conditions (temperature, light and relative humidity) in the poly house might be the reasons for early flowering. The crop response could be altered by achieving specific climatic conditions in the poly-house. In the case of tomato, 96% increase in shoot length and 27% increase in yield were observed inside the poly-house as compared to open field. For brinjal, the shoot length increased by 55 % and the yield increased by 85 % [6].

Growth in general is favoured by high relative humidity; high relative humidity during the day can also improve fruit setting. However, high relative humidity, when not managed properly, can easily lead to water condensation on the plants and the development of serious diseases.

From the Table 4, it was observed that early maturity was in poly house with T1F1, T2F2, and T3F2 in (61 days), followed by T3F1 and T4F1 (62 days) and the highest number of days were recorded in T_5F_2 (70days).

The maximum growth of any crop is known to occur at a day and night temperature of approximately 25°C, maximum fruit production is achieved with a night temperature of 18°C and a day temperature of 20° C [9] this temperatures can be easily maintained in poly house for better crop production.

Effect of weather parameters on fruit weight, number of fruit per plant and fruit size

The highest fruit weight (135.00 g) was noticed in T4F2 up to last harvest and followed by T2F2 (128.00 g) and least values was recorded in T2F1 (60.00 g). The highest number of fruits per plant (20.00) was obtained in T1F1 and there is no significant difference in T1F1, T2F1, T3F2 and T5F1 and least values was recorded in T2F2 (15.00), followed by T4F2 (16.00). In all the stages fruit size (65.00 mm) was highest in T4F2 and followed by T2F2 (59.20 mm) and lowest values was noticed in T2F1 (46.11 mm).

Marcelis (1998) [7] reported that an increase in temperature of 1°C (within the range 19-23°C) promoted yield and fruit size on an average by 4 % and 3 % respectively and it simulated increase in fruit fresh weight. Gul and Savgican (1994) [5] reported that green house grown tomato the growing media had significant effect on fruit size.

Higher yield in the poly house grown cucumber could be attributed to optimum light intensity and equal distribution of radiation over the crop canopy resulting in high photosynthetic activity than at a high light intensity [1]. Also, optimum light intensity might have led to optimum stomatal functioning as already reported by Bakker [3].

CONCLUSION

Temperature (39.88 ^{0}C) was high in poly house during afternoon time. The relative humidity (91.06 %) was higher during morning time, and in afternoon (38.48 %) it was lower under polyhouse. The light intensity inside the poly house was higher at afternoon (58865, lux) and it was also observed that during morning and evening hours there was low light intensity. The changes in microclimates are positively influenced on growth and yield parameters of tomato under poly house condition.

Table 1. Temperature (˚C) recorded in the poly house during crop period

Date from Transplanting	Temperature (˚C)				
	8.22 A.M	**10.22 A.M**	**12.22 P.M**	**2.22 P.M**	**4.22 P.M**
(13 Nov - 19 Nov)	21.25	26.49	30.61	36.71	27.51
(20 Nov - 26 Nov)	21.65	26.99	32.33	36.41	27.44
(27 Nov - 3 Dec)	22.74	26.91	34.88	37.37	28.61
(4 Dec - 10 Dec)	23.82	26.83	34.43	37.95	29.74
(11 Dec – 17 Dec)	20.62	30.17	33.85	37.34	29.00
(18 Dec - 24 Dec)	21.75	29.97	31.35	35.13	29.32
(25 Dec - 31 Dec)	21.24	29.90	35.10	37.78	28.97
(1 Jan - 7 Jan)	23.71	31.05	35.53	38.18	28.29
(8 Jan - 14 Jan)	23.59	32.26	35.66	38.03	28.55
(15 Jan - 21 Jan)	23.11	32.14	35.78	38.34	27.88
(22 Jan - 28 Jan)	23.68	32.56	35.81	38.76	29.04
(29 Jan - 4 Feb)	23.86	32.79	35.75	37.99	29.45
(5 Feb - 11 Feb)	24.13	33.01	35.83	38.45	30.33
(12 Feb -18 Feb)	24.45	33.44	36.02	38.89	30.89
(19 Feb - 25 Feb)	24.78	33.21	36.14	39.03	30.65
(26 Feb - 4 Mar)	24.65	33.87	36.65	39.34	31.33
(5 Mar - 11Mar)	25.02	34.12	36.77	39.88	32.32
(12 Mar - 18Mar)	25.10	34.43	37.05	39.65	32.76

Table 2. Weekly mean Relative Humidity (%) recorded in poly house during crop period

Date from Transplanting	Relative Humidity (%)				
	8.22 A.M	**10.22 A.M**	**12.22 P.M**	**2.22 P.M**	**4.22 P.M**
(13 Nov - 19 Nov)	88.07	58.32	48.94	37.35	48.41
(20 Nov – 26 Nov)	87.25	57.51	47.90	35.53	48.96
(27 Nov - 3 Dec)	84.95	59.18	48.97	35.53	48.47
(4 Dec - 10 Dec)	85.24	58.87	37.51	34.93	45.88
(11 Dec – 17 Dec)	91.06	50.82	33.82	38.48	43.92
(18 Dec - 24 Dec)	81.00	45.48	36.11	32.30	43.84
(25 Dec - 31 Dec)	81.32	43.56	38.08	31.23	41.58
(1 Jan - 7 Jan)	79.45	42.44	35.98	29.35	40.60
(8 Jan - 14 Jan)	78.47	41.76	35.15	27.90	40.88
(15 Jan – 21 Jan)	78.13	41.53	35.11	27.88	40.56
(22 Jan- 28 Jan)	77.11	41.65	34.89	27.23	39.78
(29 Jan- 4 Feb)	76.05	40.89	34.56	26.89	39.23
(5 Feb- 11 Feb)	76.44	40.67	34.23	26.90	38.88
(12 Feb-18 Feb)	75.21	40.23	33.78	26.74	38.43
(19Feb- 25 Feb)	75.23	39.98	33.34	25.67	37.67
(26 Feb- 4Mar)	75.01	39.45	33.43	25.34	37.34
(5Mar-11Mar)	74.82	38.77	32.56	25.22	36.49
(12Mar-18Mar)	74.89	38.43	32.41	25.13	36.11

Table 3. Weekly mean light intensity (lux) recorded in poly house during crop period

Date from Transplanting	Light Intensity (Lux)				
	8.22 A.M	**10.22.A.M**	**12.22 P.M**	**2.22 P.M**	**4.22 P.M**
(13 Nov - 19 Nov)	27032	31174	37322	48931	6207
(20 Nov – 26 Nov)	27171	31505	38512	47950	6622
(27 Nov - 3 Dec)	26572	31242	37764	49107	6433
(4 Dec - 10 Dec)	24237	33740	39880	49450	11234
(11 Dec – 17 Dec)	26604	32670	40314	50535	15165
(18 Dec - 24 Dec)	25794	31595	40716	51612	12771
(25 Dec - 31 Dec)	24687	31600	42465	52462	16375
(1 Jan - 7 Jan)	28409	34009	42783	52498	16021
(8 Jan - 14 Jan)	29429	34110	43032	53844	17329
(15 Jan – 21 Jan)	29354	35612	43412	53121	17943
(22 Jan- 28 Jan)	30213	35896	43171	54109	18320
(29 Jan- 4 Feb)	30975	36421	44123	54461	18721
(5 Feb- 11 Feb)	31673	36124	44621	54920	19201
(12 Feb-18 Feb)	31483	36823	45316	55391	19393
(19Feb- 25 Feb)	31935	37271	45931	55923	20702
(26 Feb- 4Mar)	32812	37423	46310	56120	21021
(5Mar-11Mar)	33611	37845	46921	57341	21481
(12Mar-18Mar)	33845	38142	47215	58865	22031

Table 4. Effect of weather parameters on days to first flowering, 50 per cent flowering and days to first harvest

Treatments	Days to first flowering	Days to 50 percent flowering	Days to first harvest
T1F1	16.33	34.66	61.33
T1F2	17	37	63
T2F1	15.66	35.33	64.66
T2F2	17.33	36	61.33
T3F1	16.66	33.66	62.66
T3F2	16	36.33	61
T4F1	16.66	35.66	62.66
T4F2	16.66	37.33	63.66
T5F1	17.66	38	69.66
T5F2	18.33	39.33	70.33
Mean	16.82	36.33	64.02

T	SEd	0.314	0.432	0.778
	CD(0.05)	0.661	0.907	1.635
F	SEd	0.199	0.273	0.492
	CD(0.05)	0.418	0.574	NS
T ×F	SEd	0.445	0.611	1.100
	CD(0.05)	0.935	1.283	2.312

Table 5. Effect of weather parameters on fruit weight, number of fruit per plant and fruit size

Treatments	Yield(kg/plant)	Yield(t/ha)
T1F1	1.48	76.74
T1F2	1.37	71.03
T2F1	1.07	55.48
T2F2	1.93	100.07
T3F1	1.87	96.96
T3F2	2.08	107.85
T4F1	1.89	98
T4F2	2.16	112
T5F1	1.35	70
T5F2	1.22	63.25
Mean	1.64	85.13

T	SEd	0.029	1.572
	CD(0.05)	0.061	3.304
F	SEd	0.018	0.994
	CD(0.05)	0.039	2.089
T ×F	SEd	0.041	2.224
	CD(0.05)	0.087	4.672

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RESEARCH ARTICLE

Treatment of Dairy Wastewater using Low Cost Adsorbents such as Powdered Coconut Shell and Powdered Bagasse Activated Carbons

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ABSTRACT

The main aim of this study was the assessment of reduction of chemical oxygen demand (COD) and biological oxygen demand (BOD) of wastewater from dairy processing plant using low cost adsorbents such as powdered coconut shell activated carbon (PCSAC) and powdered bagasse activated carbon (PBAC). The complete study was done in batch mode to investigate the effect of operating parameters. The result of PCSAC and PBAC were compared and optimum operating conditions were determined for maximum reduction. The parameters were analyzed in laboratory according to the methods prescribed by APHA (American Public Health Association) before and after treatment with adsorbents twice and average values are considered. Adsorption isotherms were also studied besides the calculation of optimum treatment parameters for maximum reduction of COD and BOD concentration from effluent of dairy processing plant. The maximum percentage reduction of COD and BOD concentration under optimum operating conditions using PCSAC was 76.47% and using PBAC was 65.54% resp. As the adsorption capacity of PCSAC is comparable with PBAC for removal of COD and BOD concentration it could be lucrative technique for treatment of dairy waste water generated in different sectors.

Keywords: Adsorption, Coconut shell, Bagasse, COD , BOD, dairy wastewater.

INTRODUCTION

India has a rich tradition in dairying since the time of Lord Krishna. Dairying has been inherent in Indian culture, for centuries. Milk and milk products have always been an integral part of our consumption habits [1].Today, India is 'The Oyster' of the global dairy industry and emerging as sunrise industry [2].It is an important economic sector [3] offers opportunities galore to entrepreneurs worldwide, who wish to capitalize on one of the world's largest and fastest growing markets for milk and milk products. Indian dairy sector contributes the large share in agricultural gross domestic products. Presently there are around 70,000 village dairy cooperatives across the country. The co-operative societies are federated into 170 district milk producers unions, which is turn has 22-state cooperative dairy federation. Milk production gives employment to more than 72 million dairy farmers. In terms of total production, India is the leading producer of milk in the world followed by USA. The milk production in India accounts for more than 13% of the total world output and 57% of total Asia's production. The top five milk producing nations in the world are India, USA, Russia, Germany and France [4].

Dairy industry was chosen as it requires high volume of water [5]. Large quantity of wastewater originates due to their different operations [6]. The organic substances in the wastes comes either in the form in which they were present in milk, or in a degraded form due to their processing. As such, the dairy wastewaters, though biodegradable, are very strong in nature [7]. Wastes from dairy processing industry contain high concentration of organic materials like proteins, carbohydrates and lipids, high concentration of suspended solids, high biological demand (BOD) and chemical oxygen demand (COD), high nitrogen concentration, high suspended oil/grease contents and large variations in pH. Therefore special treatment is needed to minimize these problems [8].

Among the unit operations in treatment processes adsorption occupies an important position [9]. Adsorption is a physical treatment process where the pollutants (the adsorbate) physically adsorb onto the surface of the adsorbent via weak electrostatic forces of attraction. Due to the some drawbacks, the potential exists for replacing granular activated carbon (GAC) with Innovative, yet cost effective natural adsorbents. The candidate adsorbents tested in this research were Powdered Coconut Shell Activated Carbon (PCSAC) and Powdered Bagasse Activated Carbon (PBAC) from sugar industry to treat the dairy waste water.

Agricultural products and by- products are an abundant waste materials and needs proper disposal. When disposed by burning it generates CO2 and other forms of pollutants. This creates a need for the conversion of agricultural products and by- products to useful and hopeful, value added products .One possible avenue could be as in expensive sorbent material in the treatment of dairy industry waste [10].The present study is focused on Powdered Coconut Shell Activated Carbon (PCSAC) and Powdered Bagasse activated carbon (PBAC) for treating dairy effluent [11].

MATERIALS AND METHODS

Materials

The wastewater generated from dairy processing plant is highly diversified nature. Various product processing, handling and packaging operations create waste of different quality and quantity which is not treated, could lead to increased disposal and severe pollution problems [12].For the study samples of dairy wastewater were collected from Dharwad Milk Union Ltd., Dharwad for analysis. Samples were collected in clean air tight plastic containers of five liter capacity, and the samples were collected twice in a week and as for as possible fresh samples were used for analysis and some times samples were preserved in the refrigerator at 4^0C and while using preserved samples

first it was brought back to the ambient room temperature and then used for analysis. The wastewater was filtered to remove suspended solid particles; the filtered wastewater was used for batch studies and laboratory investigation.

In the study the following two materials were used as adsorbents i.e. Powdered Coconut shell activated carbon (PCSAC) and Powdered Bagasse activated carbon (PBAC).As their relative cheapness, availability, high stability, high adsorptive capacity compares to other adsorbents made to use these[13,14].Coconut trees are plentiful and the green empty coconut finds their way in the cities refuse. Coconut shells are made up of stone cells and are hard, porous, impregnated with lignin, tannin and little oil. It is felt that shells can be used for producing an adsorbent for treating dairy waste water. Coconut shells were gathered from vendors and dried in the sun for a week. All the outer fibers were removed and the inside of the shells are cleaned and dried. Coconut shell carbon was prepared by treating 1 part of coconut shell pieces with 1.5 parts by weight of Concentrated Sulphuric acid and keeping it in an air –oven maintained in the temperature range of 140-160 ^{0}C for a period of 24 hrs. The carbonized matter was washed with distilled water to remove the free acid and dried at 105±5^{0}C.The dried material was ground and sieved to separate particles of 20-50-mesh (ASTM).Thus activated carbon from coconut shell is prepared and kept in air tight container [15,16].

India produces more than 400 million tons (MT) of agricultural waste annually which include a very large % of the total world production of bagasse, coconut fiber, jute, rice husk.etc. India became largest producer of sugar cane/sugar in the world. Thus bagasse production in India has reached a little above 100MT. Bagasse is the waste product obtained from the near by sugar mills. It is composed largely of cellulose, pentose and lignin [17,18]. The bagasse obtained from country side was dried under the sunlight until all of the bagasse evaporated and was ground to a fine powder. The ground bagasse was sieved, so that the size of fiber used was between £0.33 mm. One part of bagasse was mixed with one part of sulphuric acid and heated in a muffle furnace for 24 hr at 150^{0}C.The heated bagasse was washed with distilled water and soaked in 1% sodium bicarbonate solution overnight to remove residue acid. The material was dried in an oven at 150^{0}C for 24 hrs. Then, the material was kept in air tight container for further use.

Methods

The pH and temp of the wastewater samples were measured on collection site.Colour, Odour, pH , Total Solids, Total Suspended solids, Total Dissolved Solids, BOD at 20°C ,COD ,Chlorides, Sulphate, Residual Sodium Carbonate, Oil and Grease, TKN (As Nitrogen),Conductivity/Conductance, Alkalinity as $CaCO_3$ were analyzed in laboratory according to the methods prescribed by APHA(American Public Health Association).In this paper we have studied the removal of COD and BOD concentration only. The COD and BOD conc. of wastewater samples were measured in lab before and after its treatment with adsorbents.

Batch mode treatment of wastewater samples

All the experiments were carried out at ambient temperature in batch mode. The batch experiments were conducted in different flasks of 250 ml capacity using an average speed magnetic stirrer (shaker). Adsorption experiments were conducted in different batches for all the experimental conditions like pH of the solution, adsorbent dose, adsorbent treatment (contact) time and agitation speed. The influence of various operating parameters was studied by varying one parameter and keeping the others constant.

The extent of adsorption is strongly influenced by the pH at which adsorption is carried out. The effect of pH on COD and BOD concentration was studied by performing equilibrium adsorption tests at different initial pH using both adsorbents. The pH solution was adjusted by using HCL (0.1 N) or 0.1 N NaOH, the pH at which maximum COD and BOD concentration removal gives optimum pH.

For the adsorption capacity studies different dosages accurately weighed and added to batch reactor containing 100 ml of waste water and half inch Teflon coated magnetic bar is placed in the reactor and stirred for optimum contact time. After equilibrium period the samples were withdrawn and filtered waste water samples were analyzed to determine

COD and BOD concentration by standard methods. The dosage which gives minimum residual concentration is chosen as the optimum dosage.

The adsorption process is strongly influenced by the contact time. For study of effect of contact time a known weight of sorbent (2.5 gms) is brought into the contact with a 100 ml of solution of known concentration of COD (1180 mg / L) in the batch reactor and BOD (465 mg / L). Sample was stirred in a magnetic stirrer for varying length of time (30, 60, 90, 120, 150 minutes). At the end of desired time the samples were withdrawn and filtered through Whatmann-No.1 filter paper and filtrates were analyzed to determine COD and BOD concentration by standard methods. The time which gives minimum residual forms optimum contact time.

The extent of adsorption is strongly influenced be the agitation speed at which adsorption is carried out. The effect of agitation speed on COD and BOD adsorption was studied by equilibrium adsorption tests at different agitation speed (30, 60, 90, 120 and 150 rpm). The speed at which maximum COD and BOD concentration removal forms optimum agitation Speed.

RESULTS AND DISCUSSION

Initially the optimum operating parameters were obtained by conducting separate experiments with PCSAC and PBAC for pH, dosage, contact time, and agitation speed. After obtaining these optimum parameters, detailed treatment study on dairy wastewater i.e. physical and chemical characteristics, like colour, pH, total dissolved solids, COD, BOD, chlorides, sulphate and oil and grease were analyzed but much emphasis was given to remove COD and BOD concentration was carried out using PCSAC and PBAC separately and results were compared. Further these results were analyzed for adsorption isotherm models namely i) Freundlich isotherm ii) Langmuir isotherm and iii) BET isotherms. Throughout the study 100 mL of wastewater sample was used.

The physico-chemical properties of the wastewater sample collected from dairy processing plant is shown in Table 1. The important operating parameters were taken under consideration for the present study where pH of wastewater, adsorbent dosage, contact time, and agitation speed.

Effect of pH

Figure 1 shows the effect of pH of wastewater on the adsorption properties of PCSAC and PBAC. Maximum COD and BOD reduction using PCSAC were74.57% and 80.00% and using PBAC were 61.86 % and 65.30% are observed at pH – 6 for both the adsorbents. The COD and BOD reduction is found maximum using PCSAC. This may be due to the generation of positive charge on the surface of particles in the acidic pH which attracts negatively charged organic molecules abundantly present in the wastewater and removed by charge neutralization.

Effect of Adsorbent dose

Figure 2 shows the effect of adsorbent dosage on removal of COD and BOD. Maximum COD and BOD reduction using PCSAC were75.40% and79.60% and using PBAC were 70.33 % and 73.79% are observed for both the adsorbents at 2.5 gm of adsorbent dosages. The COD and BOD concentration reduction is found maximum using PCSAC. It is observed that the percentage COD and BOD removal increases with increase in the adsorbent dosage. From figure it is noted that the rate of decrease of percentage removal of COD and BOD has been found to be rapid in the beginning, which remains constant due to equilibrium condition as the dose increases. The rate of adsorption increase because of increase in surface area of the adsorbent.

The adsorption isotherm studies conducted at fixed initial COD and BOD concentration and varying adsorbent dose were fitted to Freundlich isotherm of the form

$$x / m = k * C^{1/n}$$

Where x/m = amount of COD and BOD removed(x) per unit mass of adsorbent (m),C(mg/Lt) was residual COD and BOD concentration of aqueous solution, *k* and *1/n* are Freundlich constants and measure of adsorption capacity and adsorption intensity respectively.The values of Freundlich isotherm corresponding to the experimental measurements for PCSAC and PBAC were plotted for COD removal using on log scales as shown in the figure 3 and for BOD removal is as shown in the figure 4 From the following figures, it can be observed that the data is well fitted to Freundlich isotherm.

Values of regression coefficient (r^2) had been calculated from the linear fit and based on the fit, the respective values of the slope *1/n* and intercept on y-axis taken as *k* were also calculated. The values of *1/n, k* and regression coefficient $r^2$for PCSAC were 0.92, $1.38X10^{-4}$ and 0.667 corresponding to COD reduction and corresponding to BOD reduction. The values of *1/n, k* and regression coefficient $r^2$for PBAC were 0.235, $2.8X10^{-4}$ and 0.0639 corresponding to COD reduction and corresponding to BOD reduction resp.

The constant 1/*n* and *k* are of definite importance in determining the adsorption capacity of organic pollutants from wastewater and reduction of COD and BOD concentration by adsorbents. The slope *1/n* is dependent on the order of the change of reduction in COD and BOD concentration with adsorbent dose, while *k* is dependent on the extent of removal of COD by adsorbents.

Effect of contact time

The optimum contact time for the adsorption is up to 120 minutes for both the adsorbents i.e. PCSAC & PBAC. Maximum COD and BOD reduction using PCSAC were77.11% and81.55% and using PBAC were 66.10 % and 70.07% respectively. Using PCSAC maximum reduction in the organic matter is found. Figure 5 shows that in the batch studies it is concluded that the optimum contact time for the adsorption is up to 120 minutes for both the adsorbents i.e. PCSAC &PBAC, after that the removal rate is very low, the rate of adsorption increase with time and after some time it remains constant due to equilibrium condition. As the contact time progressed, the adsorbents had tendency towards saturation.

Agitation speed

For both the adsorbents i.e. for PCSAC and for PBAC the higher COD and BOD removal is at 120 rpm. Maximum COD and BOD reduction using PCSAC were78.81% and82.01% and using PBAC were 63.90 % and 66.66% respectively. Maximum reduction is found using PCSAC. Figure 6 shows that there is an increase in the rate of COD and BOD removal with respective increase in agitation speed, this is because the resistance to the mass transfer which mainly lies around the surface of adsorbent breaks down with increasing agitation speed, as a result more amount of organic matter penetrates into the adsorbents with ease.

Optimum operating conditions for maximum COD and BOD reduction

We tried to obtain conditions for the maximum reduction of COD and BOD concentration for both the adsorbents. The optimum operating conditions for getting maximum COD and BOD concentration reduction from dairy processing plant with PCSAC and PBAC;
For coconut shell activated carbon (PCSAC) Optimum conditions are:

pH =6 , Dosage=2.5 grams/100mL , Agitation speed=120 rpm, Contact (Stirring) time=120 minutes.

For bagasse activated carbon (PBAC) Optimum conditions are:

pH =6 , Dosage=2.5 grams/100mL , Agitation speed=120 rpm, Contact (Stirring) time=120 minutes**.**

The other parameters like Colour, Odour, pH ,Total Solids, Total Suspended solids, Total Dissolved Solids, BOD at 20°C , COD, Chlorides, Sulphate , Residual Sodium Carbonate, Oil and Grease , TKN (As Nitrogen), Conductivity/Conductance, Alkalinity as $CaCO_3$ were also reduced effectively using both the adsorbents.

CONCLUSION

The experimental investigation reveals that PCSAC is effective for reduction of COD and BOD concentration from dairy processing plant. Adsorption of COD and BOD was found to be dependent on pH of wastewater, adsorbent dosage, contact time, and agitation speed. The studied adsorption data fitted well to Freundlich isotherm adsorption model. Both the low cost adsorbents i.e. PCSAC and PBAC have good adsorbent capacity and hence can be recommended in the dairy wastewater treatment.

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Table – 1 Characteristics of raw dairy waste water along with KSPCB tolerance limits.

SI. No.	Particulars	Value	KSPCB tolerance limits
1.	Colour and Odour	Light Milky& Bad smell	See Note
1.	pH	10.46	5.5-9.0
2.	Total Solids (mg/L)	2076	2300
3.	Total Suspended solids (mg/L)	375	200
4.	Total Dissolved Solids (mg/ L)	1701	2100
5.	BOD_5 at 20°C (mg/ L)	645	100
6.	COD (mg/ L)	1180	250
7.	Chlorides (mg/ L)	96	350
8.	Sulphates (mg/ L)	117	1000
11	Residual Sodium Carbonate (mg/L)	Nil	60
12	Oil and Grease (mg/ L)	260	10
13	TKN (As Nitrogen) (mg/L)	340	50
14	Conductivity/Conductance MHO/cm	3160	2250
15	Alkalinity as $CaCO_3$(mg/L)	510	--------

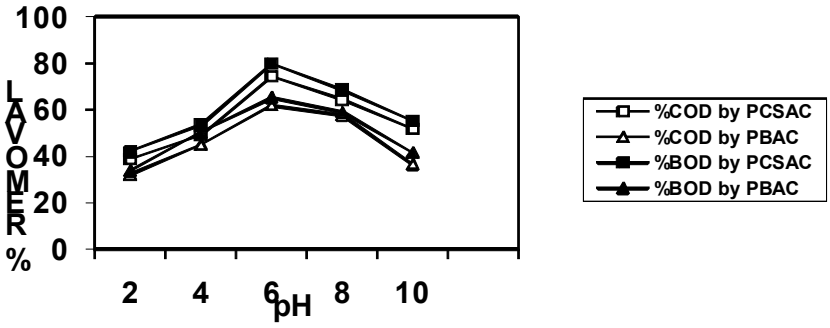

Fig 1.Effect of pH on COD and BOD of Dairy Wastewater

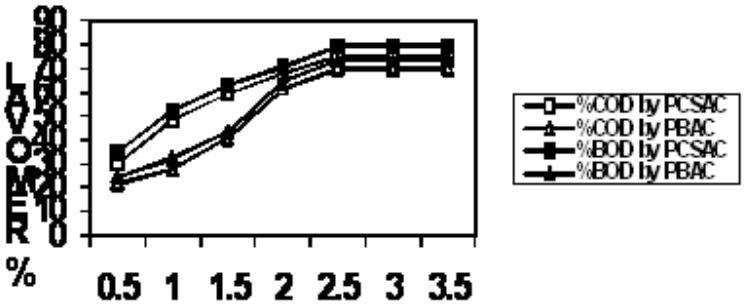

Fig 2.Effect of Adsorbent closage on Dairy Waste Water

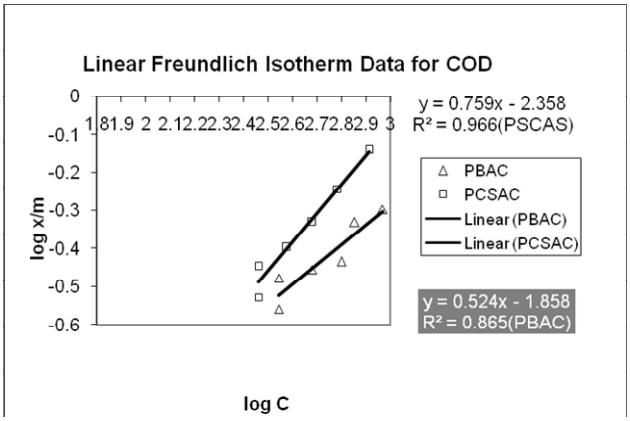

Fig. 3. Linear Freundlich Isotherm Data for COD removal using PCSAC and PBAC

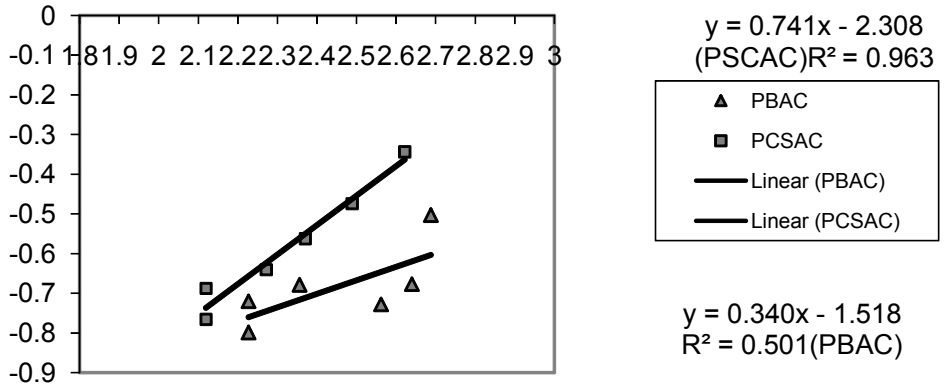

Fig. 4. Linear Freundlich Isotherm Data for BOD removal using PCSAC and PBAC

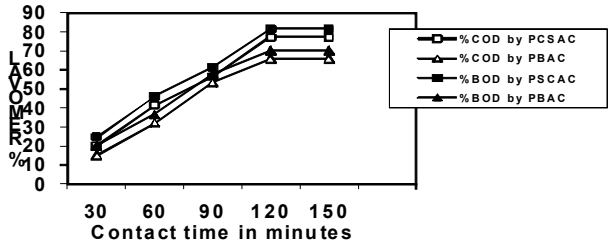

Fig.5 Effect of contact time on removal of COD and BOD of dairy wastewater

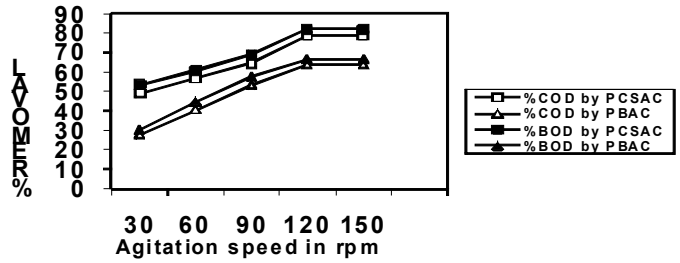

Fig. 6. Effect of contact time on removal of COD and BOD of dairy wastewater

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RESEARCH ARTICLE

Phytochemical Evaluation of Crude Methanol Extract of Some Lamiaceae

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ABSTRACT

Plants have been a source of bioactive agents for 1000's of years, and their discovery and use are increasing in pharmaceutical, herbal, cosmetic, fragrance, food and drink industries. Detailed analyses of active natural product skeletons have led to the identification of of a number of precursor molecules. It has also enabled studies of structure activity relationships and has led to the generation of various analogues for synthetic production [1].Therefore a phytochemical analysis of crude foliar extracts of some lamiaceae was taken, to provide a scientific basis for further study.

Keywords: Phytochemical analysis, Lamiaceae, Pharmaceutical, Precursor molecules, Foliar extracts.

INTRODUCTION

Green plants support all life on earth. Starting with water , carbon dioxide and minerals and utilizing sunlight they have the remarkable capacity of synthesing carbohydrates , proteins , fats , vitamins and also produce myriads of other products ,many of which are used by man. These have been in use as drugs, as stimulants, as dyes, and for many other purposes since ancient times. They have been called as secondary metabolites these compounds occur in great numbers throughout the plant kingdom. The chemistry of plants is as varied as the great variety of forms in which plants occur. The number of species of plants, based on morphological differences, is estimated to be in excess of 335,000[2].

These special compounds occur in a great variety of structures, they are often found in remarkably high concentrations, sometimes in great purity, and in some instances, a large number are found in a single species are on the rise. About 3000 alkaloids and 3000 glycosides have been isolated from plants and new compounds being reported daily. In addition to amino acids necessary for proteins, over 200 special amino acids are known. Over 100 nonessential sugars have been isolated.In general; the individual essential oils (about 9000) are known to contain very many ingredients [3].

Comparitive studies have shown extreme differences in some groups of secondary compounds among species of similar general morphology. In other instances, plants very different in appearance have a nearly identical array of these compounds. I n the latter situation, one is not able to find any common feature that is peculiar to the two taxa except their chemistry [4].The so-called secondary plant constituents indeed represents a major contribution to the therapeutic armamentarium of the physician.

Members of lamiaceae are known for their aromatic essential oils. The phytoconstituents of these are of therapeutic value and it has been scientifically proved that many of them have potential as antioxidants, anti bacterial, anti fungal, cytotoxic, hepato-protective, and so on.The present study is a part of research focusing on finding out the chemical constituents, in order to explore the possibility of use of these as adaptogens.

MATERIALS AND METHODS

The following plants were collected.*Anisochilus carnosus* .Wall, *Ocimum canum. Sims, Hyptis suaveolens* L, Poit, *Lentoid nepetaefolia* Br., *Pogostemon auricularis, Leucas pubescens , Teucrium tomentosum* Heyne.The plants were washed in running water to remove dust particles . Healthy leaves were separated and they were dried in shade and stored in air tight plastic containers.The leaves were ground and the methanol extract was analysed by GCMS.

The powdered leaf samples were dissolved in methanol and subjected to GCMS at IIT, Chennai.The details regarding GCMS. The make of the machine JEOL GC MATE-II. The column used was HPS and the column temperature was maintained between 80°c -250°c. the rate of temperature 20°c and the injection temperature 220°c.Helium was used as the gas. The software used to identify the compounds was NIST library mass EI.

RESULTS AND DISCUSSION

The identified compounds are listed below the table.

S.No	Plant Name	Identified compounds
1	*Anisochilus carnosus*	Hexadecanoic acid , Methyl ester.NIST MS 1 of 50 (112-39-0)
		10- Octadecanoic acid , Methyl ester. NIST MS I of 50 (13481-95-3
		9-Octadecanoic acid (Z)-,2- hydroxyl-1-(hydroxymethyl)ethyl ester.NIST MS 6 of 50 (3443-84-3)
		Ricinoleic acid. NIST MS 2of 50(141-22-0)
2	*Teucrium tomentosum*	Pentadecanoic acid,14- methyl-, methyl ester. NIST MS 1of 50(5129-60-
		Tridecanedioic acid, diethyl ester NIST MS 32of 50(15423-05-
		10-Octadecenoic acid , metyl ester NIST MS 1of 50(13481-95-3.
		Linoleic acid ethyl ester NIST MS 25of 50(544-35-4)
3	*Leucas pubescens*	Pentadecanoic acid , 13- methyl-, methyl ester. NIST MS 1of 50 (5487-50-3)

		1,2- Benzenedicarboxylic acid , butyl cyclohexyl ester NIST MS 1of 50 (84-64-0)
		Cyclopropanebutanoic acid, 2-{ {2-{ {2-{(2 pentylcyclopropyl)methyl}cyclopropyl}methyl}- cyclopropy]-methyl]- methyl ester. NIST MS 1of 50 (56051-53-7.
		9- Octadecenoic acid (Z)-, 2- hydroxyl-1-(hydroxymethyl)ehylester. NIST MS 1of 50 (3443-84-3)
		Aspidofractinine-3-methanol,17-methoxy-,(2a`,3a`,5a`)- NIST MS 7of 50(55103-44-1)
4	*Leonotis nepetaefolia*	Pentadecanoic acid, 14-methyl-,methyl ester. NIST MS 1of 50(5129-60-2)
		1,2- Benzenedicarboxylic acid, butyldecyl ester. NIST MS 1of 50 (89-19-0)
		Dasycarpidan-1-methanol, acetate(ester) NIST MS 5of 50(55724-48-6
		Oleic acid NIST MS 1of 50(112-80-1)
5	*Hyptis suaveolens*	Cyclopropanebutanoic acid,2-[{2-[{2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]mehyl],-methyl ester. NIST MS 1of 50 (56051-53-7
		5-(p-Aminophenyl)-4-(o-tolyl)-2-thiazolamine NIST MS 23of 50 (DB#13285)
		Gibb-3-ene-1, 10-dicarboxylic acid, 2,4a-dihydroxy-1-methyl-8-methylene-,1,4a-lactone,10-methyl ester, (1a`,2a`,4aa`,4ba`,10a`)- NIST MS 11of 50(5508-47-4
		1-Monolinoleoylglycerol trimethylsilyl ether NIST MS 1of 50(54284-45-6.
6	*Pogostemon auricularis*	Hexadecanoic acid , methyl ester NIST MS 1of 50 (112-39-0)
		10- Octadecanoic acid, methyl ester. NIST MS 1of 50 (13481-95-3)
		1H-Pyrrolo[2,3-c] pyridine -3-propanoic acid,5(4H)-oxo-6,7-dihydro-,methylester. NIST MS 12of 50 (32682-61-
7	*Ocimum canum*	G ibb-3-ene-1,10-dicarboxylicacid,2,4a-dihydroxy-1-methyl-8-methylene-,1,4a-lactone,10-methylester,(1a`,2a`,4aa`,4ba',10a`)- NIST MS 11of 50(5508-47-4
		Prednisolone Hemisuccinate NIST MS 6of 50 (2920-86-7)
		1,2-Benzenedicarboxylic acid, butyl decyl ester. NIST MS 1of 50(89-19-0)
		Dasycarpidan-1-methanol, acetate(ester) NIST MS 1of 50(55724-48-6
		4-Thiazoleethanol,2-(p-chlorophenyl)_, acetate (ester) NIST MS 22of 50(27551-11-

Primary healthcare is still a major problem in most of the developing countries. The cost of drugs is on the increase as are the number of ailments. Better health, with minimum expense can be within the reach of common man if steps are taken to provide locally available herbal products for common ailments. The problem of side effects also could be ruled out. Moreover some of these plants products could be used as supplements to ensure better health and development of immunity. Based on the findings of this study further work has been undertaken to study the efficacy of some of these plants on fungi.

Much of the work centres around analysis of essential oils and their efficacy on microbes and animals. The study on efficacy of aqueous extract is considerably fewer in number [5]. The constituents of Lamiaceae, particularly di and triterpenoids have been found to be antiseptic, antibacterial anti-inflammatory, cytotoxic, cardioactive, etc [6,7]The leaves of the studied plants are rich in fatty acids.The prescence of Ricinoleic acid in Anisochilus is probably the first of the kind.

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REVIEW ARTICLE

Cuscuta reflexa ROXB. – A Wonderful Miracle Plant in Ethnomedicine

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ABSTRACT

Cuscuta reflexa Roxb. is a twining parasite and makes a tangled mass covering the host plants. It is called in *Akasvalli* in Tamil. This plant stem and seeds have highly important medicinal values. Some research studies say those Indian tribes and other traditional communities are used this plant as purgative, carminatives and external application for skin diseases. Stem decoction is used for constipation and liver complaints. In vitro studies showed that the Cuscuta stem extraction had antiviral and anti cancerous activities. Further research work is necessary to isolate, characterize the phytochemical constituents with effective pharmacological study.

Keywords: *Cuscuta reflexa* Roxb., parasite, carminatives, phytochemical, pharmacological, constipation.

INTRODUCTION

Cuscuta reflexa Roxb. (Cuscutaceae a division of Convolvulaceae) is an extensive climber parasite. It occurs throughout the plains of India. It is more often called dodder in English. Traditional healers called in Hindi Akash bel in Tamil Akashavalli. Other names include hell weed, devil's gut, and beggar weed, strangle tare, scald weed, dodder of thyme, greater dodder, and lesser dodder. In Chinese, Cuscuta seeds are called *tu si zi*. It has no chlorophyll and cannot make its own food by photosynthesis. Some research studies say that the plant has very low levels of chlorophyll and can slightly photosynthesis. But other species of Cuscuta are entirely dependent on the host plants for nutrition. The stem is thread like filaments it is begin to grow and attach themselves to nearby host plants. The nature plants lives its entire life without attachment to the ground. It has long history of ethnomedicinal use. Cuscuta is a genus of about 100 – 170 species.

Plant description

Kingdom ..... Plantae
Subkingdom .....tracheobionta
Superdivision ..... spermatophyta
Division ..... Angiospermes
Class ..... Eudicots
Subclass ..... Asterids
Order ..... Solanales
Family ..... Cuscutaceae alternate Covolvulaceae - Dodder
Genus ..... *Cuscuta*
Species ..... *reflexa* Roxb. 100-170 Species Available.

Geographical Distribution

India, China, E.Asia and Afghanisthan

Vernacular names

Tamil	:	Verillakothan
English	:	Dodder Plant
Hindi	:	Amarabela
Sanscrit	:	Akasavalli,Amaravalli,Khavalli
Punjabi	:	Zarbut
Malayalam	:	Moodillathali
Urdu	:	Akashbel
Bengali	:	Akashbel

Review Study

For our recent investigation, we referred many research Articles. Among the variety of references, 11 research papers can be retrieved from Indian journal of traditional knowledge. From these retrieved references, we can gather many research informations regarding *cuscuta reflexa* and the collected informations are included in this research article. Apart from these 11 references we can also collected variety of sources for this article. From these articles, we observed that a minimum amount of research activities carried out in this miracle plant. The scientific information gathered from these research articles is that traditional usage of the plant and pharmacological activity. From these references we concluded that *cuscuta reflexa* has some scientific effects to prevent the diseased factors. If we can utilize this emerging plant in India we can able to provide disease free environment. Table 1 show that the information is observed from the study. The plant part is used for headache, body ache and itches. IUCN 2004 (International Union for Conservation of Nature) report is say that the plant also used for asthma, bronchitis, rheumatism and skin diseases. Whole plant extract is considered as an antiviral activity and analgesic. This plant extract has diaphoretic, demulcent, laxative and tonic properties. Cuscuta is an antifertility agent.

Contemporary Chinese herbalists use Cuscuta in formulas to treat a range of conditions, including: impotence premature ejaculation ,sperm leakage ,frequent urination ,ringing in the ears ,lower back pain ,sore knees ,white discharge from the vagina (leucorrhea) ,dry eyes ,blurred vision ,tired eyes. Cuscuta is one of nine herbs included in the manufacture of Equiguard, a Chinese herbal medicine recommended for kidney and prostate disorders. Research performed at New York Medical College indicates that the combination of ingredients in Equiguard may well be

effective in the treatment of prostate cancer. The preparation inhibited the growth of cancer cells, increased the rate of self-destruction (apoptosis) of cancer cells, and prevented the surviving cells from forming colonies.

SCIENTIFIC RESEARCH

In Ayurvedic medicine, the plant is said to be useful in diseases of eye and heart (Chopra, Chopra Handa anD Kapoor, 1958). The chemical examination of the plant has been done by Aggarval and Dutt (1935). Some Pharmacological studies on this plant were conducted by G.S Singh and K.W. Garg (1973). Their research studies are found to have anti histamines action in this plant. Some recent studies show that the following chemical constituents are identified i.e. Quercetin, Cuscutine, and Cuscutamide etc. Cuscuta's seed and stem are highly medicinal values. The seeds are used for carminative and anodyne. The stem is purgative. Dixit et al reported that hair growth activity of this plant stem through the periodic transformation of hair follicle from telogen to anagen phases. Some in vitro studies are indicated antioxidant activity of the plant stems. This plant extracts are very close and identical in magnitude and comparable to that of standard antioxidant compounds used. Another one article reported that in vitro studies of free radicals scavenging activity may be to Phenolic compounds in *Cuscuta reflexa* extract. A cold infusion of the seeds is given as a depuration and carmination is pains and aches of the stomach. Seed poultice can also apply locally for pains. The stems in decoction are useful in constipation, flatulence, liver complaints and bilious affection.

List of chemical constituents Isolated from species of Cuscuta

Kaempferol ,Kaempferol – 3- O-glucoside (Astragalin),Myricetin,Myricetin glucoside, Quercetin ,Quercetin -3-O-glucoside, Kaempferol -3- O-galactoside , Quercetin -3-O- galactoside, Isorhamnetol, Azaleatin Cuscutalin, Cuscutin, Linolenic acid, Linoleic acid, Oleic acid , Stearic acid, Palmitic acid , Amarbelin , Beta sitosterol , Bergenine ,Dulcitol, Myricetin, Myricetin glucoside , Luteolin,Coumarin, Maragenin, n-Pentacosane , n-Heptacosane, Cusctutamine , n-Octacosane, n-Nonacosane ,n-Triacontane ,n-Hentriacontane ,1- Triacontane, Cuscutoside-A, Cuscutoside-B, Arbutin Chlorogenic acid, Caffiec acid, p-Coumaric acid , Stigmasterol , Avenasterol, Campesterol, Matrine , Saphoronal , Methylcytisine ,Cus-1,Cus – 2 ,3,5 Dicaffeoyl quinic acid, 4,5 Dicaffeoyl quinic acid, Laceeroic acid Australiside A, Cuscutic acid A, Cuscutic acid B, Cuscutic acid C, Cuscutic acid D, Hydroxyoleanane , 6,7,8- Trimethoxy -2H-1-benzopyran-2-one Lupeol, Alpha – Amyrin, Beta – Amyrin, Alpha Amyrin Acetate, Beta Amyrin Acetate , Oleanolic acetate , Oleanolic acid ,Sesamin ,Trihydroxy auran, Daucosterol, Propenamide ,7-Propenamide, 6,7-Dimethoxy-2H-1-benzopyran-2-one ,Ethyl 3-(3,4-dihydroxyphenyl)-2-propeonate 3-2-Propenol,2-3-5-dihydroxy-7-0-beta-D-glucopyranoside -4H-1-benzopyran-4-one.

Cuscuta reflexa Roxb. is prevalent in various regions of Bangladesh. *Cuscuta reflexa* is known to contain a number of alpha -glucosidase inhibitory compounds. A new flavanone- reflexin, tetrahydrofuran derivatives and a coumarin have been isolated from stems of the plant. Methanol extracts of the stem reportedly demonstrated anti-steroidogenic and antibacterial activities.

The hypoglycemic effects of methanol and chloroform extracts of whole plants of *Cuscuta reflexa*, investigated in oral glucose tolerance tests in Long Evans rats and Swiss albino mice, respectively. Both methanol and chloroform extracts of *Cuscuta reflexa* whole plant demonstrated significant oral hypoglycemic activity in glucose-loaded rats at doses of 50, 100 and 200 mg/kg body weight. When tested at doses of 100 and 250 mg/kg body weight did not demonstrate any oral hypoglycemic effect when tested in glucose-loaded mice.*Cuscuta reflexa* contains a number of compounds like flavonoids (kaempferol, quercitin), coumarins, and flavonoid glycosides .Earlier studies have shown that both kaempferol and quercetin could significantly improve insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes. It was further reported that these two compounds act at multiple targets to ameliorate hyperglycemia.

It has been investigated for antispasmodic, heamodynamic, bradycardia, antisteroidogenic,antihypertensive, antiviral and anticonvulsant activities.Many chemical constituents have been isolated from Cuscuta *reflexa* such as, cu scutin, amarbelin, betasterol, stigmasterol, kaempferol, dulcitol, myricetin, qurecetin, coumarin and oleanolic acid.

This plant reported to have *in vitro* antioxidant activity (non-enzymatic hemoglobin glycol sylation), antibacterial activity, onset of puberty and ovarian steroid genesis .The study to evaluate the free radicals scavenging activity by using DPPH radical scavenging assay and reducing power assay of methanolic extract of *Cuscuta Reflexa* (MECR). The DPPH assay results were expressed as IC50 value. Ascorbic acid which was used as a standard showed an IC50 9.22µg/ml, whereas, the methanolic extract of *Cuscuta Reflexa* (MECR) showed antioxidant activity with IC50 value 359.48µg/ml. The reducing power of MECR was found to be increase with increasing amount of extract concentration. All concentrations of MECR showed significant antioxidant activities when compared to control and these differences were statistically significant ($p < 0.001$).

In Ayurvedic medicine, the Cuscuta plant is said to be useful in diseases of eye and heart. The chemical examination of the plant has been done by Aggarval and Dutt (1935). Some Pharmacological studies on this plant were conducted by G.S Singh and K.W. Garg (1973). Their research studies are found to have anti histamines action in this plant. Some recent studies show that the following chemical constituents are identified i.e. Quercetin, Cuscutine, and Cuscutamide etc. Cuscuta's seed and stem are highly medicinal values. The seeds are used for carminative and anodyne. The stem is purgative. Dixit et al reported that hair growth activity of this plant stem through the periodic transformation of hair follicle from telogen to anagen phases. Some in vitro studies are indicated antioxidant activity of the plant stems. This plant extracts are very close and identical in magnitude and comparable to that of standard antioxidant compounds used. Another one article reported that in vitro studies of free radicals scavenging activity may be to Phenolic compounds in *Cuscuta reflexa* extract. A cold infusion of the seeds is given as a depuration and carmination is pains and aches of the stomach. Seed poultice can also apply locally for pains. The stems in decoction are useful in constipation, flatulence, liver complaints and bilious affection.

Reinvestigation of the chemical constituents of the stem of c.reflexa was under taken by M.K.Jain and R.K.Miahra in 1963 who successively extracted the dried stem with petroleum ether and alcohol. The petroleum ether extract on careful chromatography afforded a white solid identified as beta sitosterol reported earlier by gopinath et al, while kaempferol and bergenin were obtained from the alcoholic extract of the plant. These studies were in complete agreement with the report of Patil Amol,2009.

Maragenin a triterpenoid from crude petroleum ether of *C.reflexa* has been isolated and its chemical nature eas determined by US.Srinivastava and co workers. In the year of 1992 AGR.Nair and G.Thiripurasundari isolated 6,7-dimethoxycoumarin(scoparone),6-hydroxy -7-methoxy -4- hydroxyl phenyl –coumarin(melanettin),quercetin and hyperoside from C.reflexa collected over Bougainvillea spectabilis.The carotenoid pigments of *Cuscuta* were characterized with the help of HPLC and chemical properties.Major carotenoids characterized were,Beta carotene,lycopene,rubixanthin, lutein, violaxanthin along the esters of Beta –cryptoxanthin lutein, ,rubixanthin and violaxanthin.

The invitro antioxidant activity of *Cuscuta reflexa* stem extract has been investigated by S.B Yadav *et al*.,2000,by estimating degree of non enzymatic heamoglobin glycosylation measured colorimetrically at 440nm.The ethyl acetate fraction of ethanol extract showed higher activity tan the other fractions. The antioxidants activity of extracts is very close and identical in magnitude and comparable to that standard antioxidant compounds used.

Another one important research report says that the possibility of introduction of trehalose as a unit into cellulose and thus causing abrupt termination of chain growth was investigated by studying the incorporation of trehalose in cuscuta shoot tips. However the heterogeneous distribution of radioactivity showed that trehalose was, at least partially, hydrolyzed in the tissue resulting in the labeling of different cellular fractions. The reported earlier that extracts of Cuscuta have trehalose-hydrolysing activity though low.Threfore from trehalose feeding experiments it could not be ascertained whether trehalose as a unit was introduced into cellulosic material or not.

According to Ramya et al (2010) the whole plant Cuscuta were collected, cleaned with tap water and dried under shade.The dried stem parts of medicinal plants were ground well to find powder. The ground sample was made alkaline with 39% ammonia and extracted with chloroform at room temperature for a total period of 24 hrs and then the extract was partitioned between 5% HCl and chloroform. The aqueous phase was made alkaline again with ammonia and partitioned between water and chloroform. Finally chloroform was totally evaporated from the organic phase to form the phenolics powder.From the above mentioned results this study reveals the presence of various phenolics compounds including flavonoids, flavones, flavonones etc. in *Cuscuta reflexa L.* In this study, UV-Vis and FT-IR procedure was applied for the identification of secondary metabolites. In the effort to study plant of the *Cuscuta reflexa* from the identified localities subjected to phenolics screening. From this total samples, 78% gave positive result for phenolics, from this 33% gave a positive reaction for flavones. So the final result is indicating the identified plant may be used for antimicrobial, anti helminthetic and anti-inflammatory agent in phyto-pharmaceutical applications.

In recent research (2011) the antitumor activity of the chloroform and ethanol extracts of *Cuscuta reflexa* was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice at doses of 200 and 400 mg/kg body weight orally, respectively, while acute oral toxicity studies were performed to determine the safety of the extracts. Briefly, the EAC cells were injected (i.p.) into ninty six mice (divided into 6 numerically equal groups), and after a one-day incubation period, the extracts were administered to the mice daily for 16 days. On day 21, six animals in each group were sacrificed for observation of antitumor activity and the remaining animals were observed to determine host the life span. Antitumor effect was determined by evaluating tumor volume, viable and nonviable tumor cell count and hematological parameters of the host. The standard antitumor used was 5-fluorouracil. Administration of the extracts resulted in a significant ($p < 0.05$) decrease in tumor volume and viable cell count, but increased non-viable cell count and mean survival time, thereby increasing the life span of the tumor-bearing mice. Restoration of hematological parameters - red blood cells (RBC), hemoglobin, white blood cells (WBC) and lymphocyte count - to normal levels in extract-treated mice was also observed.The results suggest that the chloroform and ethanol extracts of *C. reflexa* exhibit significant antitumor activity in EAC-bearing mice that is comparable to that of the reference standard, 5-fluorouracil.

CONCLUSION

From this study, we suggested that Cuscuta is unpopular with community but the traditional and tribal people aware in some states of India. It has been used for their health care (particularly epilepsy and jaundice) and ethnovertinary practices. The research activities carried out in this plant was very less in India and abroad. Advanced scientific research will urgently need for make healthy India through this miracle ethno medicinal plant.

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Table :1 .Traditional Usage of *Cuscuta reflexa* Roxb. in different states of India.

S.No	Place / State	Usage	Reference
1	Uttaranchal	Bone fracture, lock of jaw	PC Pande et al (2007)
2	Kandhamal Dist. Orissa	Stem decoction with honey is taken every morning for 7 days to cure Epilepsy.	Mk misra et al (2006)
3	Tripura	Plant juice mixed with coconut water is taken early morning for 2 weeks to cure jaundice. It is also used in cough and Diabetes.	BK Datta et al (2006)
4	West Rarrh region of West Bengal.	Stem juice is used for cow's diarrhea	Ashish Ghosh (2008)
5	Hill Tracts district Bangladesh	To treat eczema, plant part is applied to affected areas until recovery	M. Atiqur Rahman et al (2007)
6	Upper Assam	Decoction of aerial plants of Cuscuta is used for Jaundice	J. Mahanta et al (2006)
7	West Bengal	Abortifacient activity	K.R. Mukherjec (2009)
8	Kunihar forest Division Solan district Himachal Pradesh	Plant juice to remove the works in intestine. It also control the heart beat in weakness plant juice is also applied on hair to increase the length	Saroj verma (2007)
9	Bijagarah west NimarDist Madhya Pradesh	Antihelminthetic	S.K. Mahajan (2007)
10	Bibdod, Madhya Pradesh	Stem extract is used to cure epilepsy	Dinesh Jadhav (2006)
11	Southern Aravalli Hills, Rajasthan	Plant juice causes depression with nausea, Vomiting and abortion.	S.S Katewa (2008)
12	-	Antibacterial activity of Cuscuta reflexa stem	D.K Pal et al (2006)

Fig 1. *Cuscuta reflexa* Roxb

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RESEARCH ARTICLE

Nature's Gift- Organic Chemical Factories (Lamiaceae) to Provide Safe Solutions for Most Human Needs

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ABSTRACT

The Lamiaceae is one of the most diverse and widespread plant families in terms of Ethnomedicine. In today's world where modernization and urbanization are rapidly changing our environment, in the name of progress, we are also producing the beginnings of a crisis. The crisis is of the increasing loss of endemic/ native plant species as a result of declining natural areas. This is in turn adding numerous species to the "Threatened and Endangered list all over the world. The numbers are increasing alarmingly every year.The study forms a part of the research work.

Key words: Lamiaceae, ethnomedicine, endemic, environment, urbanization, modernization.

INTRODUCTION

The original family name is Labiatae, is because the flowers have their petals fused into an upper lip and lower lip. Botanists now use the name Lamiaceae .It is also known as the mint family. It had traditionally been considered closely related to the Verbenaceae,[1].But 1990's phylogenetic studies that many genera classified in verbenaceae belong instead in lamiaceae [2].It is one of the climax groups of dicots with about 200 genera and 3200 species[3].

The family has a cosmopolitan distribution, but the centre is chiefly in the Mediterranean region, where they form a dominant part of vegetation.Some of the subfamilies are localized in distribution, as the prostantheroideae, in Australia and Tasmania , the Prasioideae in Malaya, India, China and the Catopherioideae Central America. Most of the North American genera belong to the more cosmopolitan subfamilies , Stachydoideae and Ajugoideae.The

family is widespread throughout this country where it is represented by 48 genera, of which the larger include Salvia, Pycnostachys,Scutellaria,Stachys, Monarda, Monardella. The enlarged lamiacae has about 236 genera and 6900 to 7200 species. The largest genera are Salvia (900), Scutellaria (360), Stachys (300), Plectranthus (300), Hyptis (280), Teucrium (250), Vitex (250), Thymus (220) and Nepeta (200). Clerodendrum was once a genus of over 400 species but by 2010, it had been narrowed down to 150.

In India the family represented by about 69 genera and 420 species. Approximately 261 species are endemic.The plants are generally aromatic herbs or shrubs (Hyptis- trees and shrubs. They have quadrangular and simple leaves which are e ither opposite or whorled (Icomum leaves alternate). Inflorescence generally verticillasters. Flowers bisexual, zygomorphic and hypogynous (Mentha : flowers regular, corolla 4- lobed, actinomorphic). The calyx is irregular (bilabiate) 5- lobed. Corolla of five petal lobes, bilabiate, upper lip smaller or absent, lower lip larger,3 lobed, stamens 2 or 4. Disc present at the ovary. Ovary 4 celled 4 lobed with te gynobasic. Ovules 4 in basal axile placentation. Fruit of four nutlets enclosed in the persistent calyx (carcerule). In Prasium the fruit is a drupe. Nutlets often contain hardened mucilage which helps in dispersal. The other genera with exceptional characters are, Coleus: stamens monadelphous and Salvia: connective much elongated and anterior cell absent.

Herbs are staging a comeback. Herbal products today symbolize safety in contrast to synthetic ones that are regarded as unsafe to humans and the environment. From available trade data, it is clear that there is huge demand in the global market for medicinal plants. A good example of the trade volumes in 1980 is one report commissioned by the World Wide Fund for Nature, the total import of "vegetable materials used in pharmacy" by the European Economic Community was 80,738 tons [4]. India was the largest supplier with 10.055 tons of plants and 14 tons of vegetable alkaloid and their derivatives. The rising International demand has resulted in a number of plant species becoming scarce and quite a number of plant species are facing the prospect of becoming extinct. Therefore, it is very important to conserve the extensively traded medicinal plants in its natural environment/ cultivating in favourable environment.

Of the 2,50,000 higher plant species on earth , more than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agro- climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes. Of these about 15000-20,000 plants have good medicinal value. However only 7000-7,500 sps are used for their medicinal values by traditional communities. The family is represented in India by ca. 64 genera and 350 species, of which (60 species and 5 varieties) 18% is strictly endemic to peninsular India. These endemic taxa are spread over genera, of which Anisochilus, Eusteralis, Leucas, Plectranthus and Pogostemon have a large representation of endemic taxa. As many as 18 of these endemic plants are rare in their habitat.

MATERIALS AND METHODS

The plants belonging to the following were collected from Narthamalai, in Puddukkotai dist, Tamil Nadu, and Kolli hills. The members of the following genera were collected. Anisochilus, Anisomeles, Leonotis, Hyptis, Geniosporum, Leucas, Teucrium, Pogostemon , Orthosiphon, and Ocimum.They were identified and samples were prepared for herbarium. These plants were reviewed for the medicinal properties .Morphological studies were carried out, and the leaf samples of a few species were selected for further studies.

Ocimum

Strongly scented herbs , undershrubs , or shrubs. Whorls 6-10 fid, spiked or racemed tips of pedicels recurved; bracts minute , caduceus; flowers small. Calyx ovoid or campanulate, deflexed in fruit; upper tooth, broadest, decurrent, 2lower acuminate. Corolla- tube short , not annulate within; upper lip subequally 4-fid, lower hardly

longer declinate entire. Stamens declinate, exerted , filaments free or the lower connate below, entire or 3-4 lobed. Style- lobes subulate or flattened. Nutlets smooth or subrugose, mucilaginous when moistened-. Species about 40, tropical and chiefly Asiatic.

O. canum,Sims.

Herbaceous, erect, pubescent, leaves petioled narrowly ovate toothed or entire,bracts petioled, two lower calyx- teeth ovate- lanceolate awned longer than the rounded upper , lateral smaller than the lower, corolla ,1/6 in long . Plants branched from the base , 1-2 ft high. Leaves 1- 1 ½ in; petiole very slender, usually ciiate. Spikes 3-8 in; whorls rather close; flowers subsessile; bracts ovate awned, not so large as the nearly glabrous calyx, ciliate. Filaments twice as long as the white corolla, hairy at the knee. Nutlets pitchy- black, narrowly ellipsoid, punctulate.

Teucrium

Herbs or shrubs. Whorls in 2-6fld, axillary or terminal spikes racemes or heads,. Calyx 10 nerved, teeth 5, equal or the upper largesr. Corolla –tube not annulate, limb 1- lipped, the 2 upper and lateral lobes cuneate and very short or obsolete, lower lobe very large. Stamens4, ex, exerted;anther reniform, cells short, at length confluent. Disc symmetrical. Style lobes subequal. Nutlets minute, reticulate, smooth or rugulose; hilum large, oblique or lateral.

2a) T. tomentosum, Heyne. ; Pubescent, leaves ovate serrate, toothed or crenate, base cuneate, racemes panicled, calyx1/4 in., villous, lower teeth triangular acuminate, petiole ½- 1.5in . slender.

Orthosiphon,Benth.

Under-shrus or shrubs. Whorls 6- or fewer fid, racemose. Calyx ovoid, campanulate or tubular, fruiting deflexed ,upper tooth broad ,membranous, margins decurrent on the tube, lateral and lower distinct or shortly connate, usually subulate. Corolla –tube often slender, straight or incurved; upper lip 3-4 fid; lower entire,concave. Stamens-4, declinate, filaments free, toothless; anther cells confluent. Disc usually gibbous. Style with a minute capitate or clavate entire or notched stigma. Nutlets ovoid or orbicular,smooth or nearly so- species 16, tropics of the old world.

O. diffuses, Benth- Woody, branched, viscidly tomentose, leaves ovate or elliptic obtuse crenate, corolla tube shortly exerted, fruiting calyx1/8-1/4 in. Branches many and straggling from the woody stock, stout or slender; whorls rather distant,2-4 fid floral leaves minute ,subulate calyxpubecent, throat with long hairs,. Corolla about ¼ in long. Nutlets, oblong, obscurely reticulate, brown, nearly smooth.

Anisochilus

Herbs or under shrubs. Flowers, small, in dense oblong 4- gonal or cyclindric spikes. Calyx suberect, inflated below the middle, 2- lipped or 5- toothed, rarely 1- lipped, upper lip entire and deflexed or short and 3- crenate. Corola 2-lipped; tube slender, decurved; throat inflated; upper lip short, entire or 3-4 fid; lower elongate, concave. Stamens 4 ,filaments free. Style 2 –fid. Disc lobed

A..carnosus,Wall Annual, erect,glabrous or tomentose, leaves petolate, ovate or rounded obtuse, crenate fleshy, floral ovate obtuse, fruiting spikes 4- gonous then cyclindric obtuse, calyx glabrous pubescent or ciliate ,lip deflexed ovate acute ciliate.

Hyptis

Herbs or undershrubs. Inflorescence various, capitate in the following species. Corolla 5- lobed. Lower lobe or lip abruptly deflexed,saccate,contracted at the base. Stamens 4, declinate; anther cells confluent. Style subentire or 2- fid. Nutlets various-species 250, all American.

H .suaveoens, Piot- stem hairy, leaves petioled broadly ovate ,sinuate and aerrulate pubescent villous or tomentose lower cordate, peduncles racemose equaling the globose heads, bracts minute setaceous, calyx striate, mouth villous, teeth erect subulate.

Pogostemon. Desf.

Herbs or undershrubs. Leaves opposite, very rarely 3-nately whorled. Flowers small, in solitary or panicled spikes or contracted racemes formed of many and dense fid. Subcapitate cymes (whorls). Calyx subequally 4-5 toothed. Corolla tube exerted or included; limb spreading, sub 2- lipped; lobes 4, lower usually longest. Stamens 4, exerted, straight or declinate, filaments usually bearded; anther cells confluent. Didc subentire, equal. Styles2- fid. Nutlets smooth, ovoid or oblong-species about 30, Indian and Asiatic.

Anisomeles , Br.

Tall,erect, branching, coarse herbs. Flowers in axillary whorls or lax- fid, branched panicled cymes, purplish. Calyx ovoid or tubular, straight, equally 5 –toothed. Corolla-tube short, annulate within; upper lip erect entire; lower broad, midlobe notched. Stamens exerted; anthers conniving, of th longer pair dimidiate, of the shorter 2- celled, cells transverse parallel. Style subequally 2- fid. Nutlets smooth – species about 8, warmer Asia and Australia.

A. malabarica,R.Br.

It is a shrubby herb 0.5-1.5 m tall. The stem is tetragonous, and densely woolly. The leaves are ovate to oblong 3-8 cm x 1.5-3.0 cm. The leaves are sparsely hirsute above and densely woolly beneath., leaves short – petioled oblong linear- oblong or oblong- lanceolate, obtuse acute or acuminate crenate, calyx1/4-1/3 in. villous or wooly, teeth narrow lanceolate.,

A. **indica** (Nepeta indica L, A. ovata R. Br.)

It is a woody shrub, growing wild along borders of settled areas at low and medium altitudes in South East Asia, including India, China, Vietnam, Phiippines, Taiwan, Thailand and Indonesia as well as Australia. It is erect , grows upto 1-2 m in height, stems 4- angled, pubescent. The leaves are thin , ovate 3-12 cm long stalked and pointed at the tip with round- toothed margins. Inflorescence is a spike like raceme almost 5-25 cm long and 2-3 cm in diameter. The flowers are numerous , crowded and almost stalkless. The calyx is about 6mm long, hairy and pointed toothed, the tube is long and bell- shaped. The corolla is purplish, 10-12cm long, strongly zygomorphic, the upper lip being oblong ovate and lower lip has 2 middle lobes.The leaves emit a camphor like smell when crushed.

Leonotis, Br.

Herbs or shrubs. Whorls axillary, densely many- fid; bracteoles many, slender, flowers scarlet or yellow. Calyx 10-nerved, often incurved, mouth oblique; teeth8-10, rigid, upper largest. Corolla tube exerted;upper lip longg, concave, crown villous; lower very small, spreading, concave, midlobe largest. Stamens 4, ascending; anthers conniving cells divaricate. Disc equal. Style subulate, upper lobe very short. Nutlets oblong .

L. nepetaefolia, Br.

Atall annual, 4-6ft, stem of the thickness of the finger, 4- angled with concave faces, puberulous. Leaves 4-8 by 2-5 in ;floral lanceolate, deflexed; petiole1-3 in, winged above slender. Whorls distant, globose,2-3in. diam, squarrose; bracts slender, linear, deflexed. Calyx3/4in long, ribbed and reticulate, pubescent or villous, tubular, incurved, teeth spinescent, upper ¼ in long; throat glabrous. Corolla orange –red, 1in long; tube slender, exerted, villous like the upper lip, lower ip minute, nutlets linear oblong.

Leucas, Br.

Wolly or villous, rarely glabrate herbs or undershrubs. Whorls axillary, usually distant. Calyx 10 nerved, striate; mouth equal or obique, eqully or unequally 6-10 toothed. Corolla tube included, annulate or not within; upper lip erect, concave, crown vilous; lower spreading,3 –fid, midlobe very large. Stamens 4, ascending; anthers conniving,cells divaricate at length confluent. Style subuate, posterior lobe obsolete, Nutlets ovoid, triquetrou, obtuse- species 50,Asiatic and African..

L.pubescens, Benth.

Pubescent or tomentose, rather stout, erect, leaves petioled rounded or ovate, coarsely crenate-serrate, whorls densely many fid, bracts linear nearly as long as the calyx or less, calyx ½ in. hispid, teeth. Subulate, ciliate half as long as the tube.

L. aspera ,Spreng.

Annual , erect or diffuse, stem stot hispid or scabrid, leaves 1-3 in. linear or oblong obtuse entire or crenate, whorls large terminal and axillary, bracts long , linear and filiform, calyx 1/3-2/3 in. tubular curved smooth below green and ribbed and scabrid above contracted above the nutlets, mouth small glabrous very oblique shortly irregularly toothed.

Geniosporum, Wall.

They are mostly herbs, whorls many fid, in long lax racemes or spikes; bracts, often coloured, flowers small. Calyx ovoid in flower, in fruit tubular, suberect,or declinate, 5 toothed, upper tooth broadest, not decurrent; lateral free, or connate with the upper ; lower short, free or connate. Corolla- tube short, upper lip 4- fid, lower declinate entire. Filaments free, toothless. Disk tumid, gibbous. Style-arms short, flattened. Nutlets ovoid or oblong, smooth or punctulate. – species 6or 7 , Indian or African.

G. tenuiflorum, Heyne.

Stems many from a woody stock. Leaves in distant pairs rather thick, base narrowed. Spikes elongate, slender; whorls close , bracts ovate, acute reflexed; flowers minute, pedicilled. Calyx hairy, upper lip very variable in size,, throat hairy. Corolla hairy , filaments areexerted. Nutlets are extremely small, ellipsoid, smooth, naked[5].

DISCUSSION

Many papers on the biological activity of essential oils have been published and the data show discordance between the same essences. The reasons for this variability can be understood only if all the factors influencing the chemical

composition of the oils, like climatic, seasonal geographic conditions, harvest period ,distillation technique, plant maturity at the time of oil production, chemotypic differences are taken into account. Moreover essential oils are complex mixtures comprising many single compounds. Each of these constituents contributes to the beneficial or adverse effects of these oils. Therefore, the intimate knowledge of essential oil composition allows for a better and specially directed application [6].

According to the WHO 80% of the world's population still depend on traditional medicine for their primary health care needs. There is also considerable economic benefits in the development of indigenous medicines and in the use of medicinal plants for the treatment various diseases [7]. Therefore the crude methanol extract of the plants were studied to validate the claims of their use in traditional medicine.

Medicinal plants obtained from wild habitats are found in different natural ecosystems of the forests, grasslands, woodlands, gardens etc, as weeds are harvested when the need arises. These are freely accessible to those who use them for the family or the traditional healers.The traditional culture worldwide are more or less endangered as a result of increasing legislative and moral supports accorded orthodox practice over native medicine[8]. There are very few reports regarding the extent to which water extracts of various herbs contain valuable bioactive ingredients
.
Natural antioxidants are important for their physiological functions after absorption into the body [9,10,11]. Aromatic plant have been studied in great detail for the presence of natural antioxidants with emphasis on the constituents of essential oils and / or to the hexane , acetone, ethanol or methanol extracts less polar than water. . This is out of reach for the common man in underdeveloped and developing countries .Some flavonoids present in tea infusions may have protective effects against coronary heart disease, cancer or allergy [12,13,14,15,16].

In a study by [17], it was reported that the aqueous extracts had higher content of phenolics than 12% ethanol extracts samples. It has also been reported that the aqueous extract of dittany is more effective than the methanol, ethanol and acetone extract in scavenging hydroxyl radicals as generated by the Fenton reaction and in reducing oxygen consumption when initiated by metamyoglobin [18].Therefore one can conclude that the ample distribution of these plants provide a means of use as nutraceuticals , adaptogens, insecticide, etc , in an economic way with minimum side effects. A few of the above mentioned plants were selected for further studies

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Table :1. Biodiversity of Lamiaceae (s.l) in India (V. Sampath Kumar and G.V.S. Murthy).

Sl.No	Name of the Taxon	Distribution
1	Acrocephalus palniensis Mukerjee	TN
2	A.axillaris Benth	AS
3	Anisochilus argenteus Gamble.	KL, TN.
4	A.dysophylloides Benth. Var.dysophylloides, Var.purpureus(Wight)G	TN
5	A.plantagineus Hook.f.	KN
6	A.robusta Hook.f.	TN
7	A.scaber Benth.	TN,KL
8	A.sericeus Benth	TN,

9	A.suffruticosus Wight	TN
10	A.verticillatus Hook.f.	AP, KN, MH.
11	A.wightii. Hook.	TN
12	Callicarpa psilocalyx C,B.Clarke	MG
13	C,moldenkiana Rajendran&Daniel.	MG
14	Clerodendrum cecil-fisheri Rajendran& Daniel.	AR.
15	C. lakawiense King& gamble Var amanense Moldenke	AN
16	C.nicolsonii Rajendran&Daniel.	AS
17	Elsholtzia major(Hook. F,)V.S.Kumar&B.D.Sharma	UP,SK
18	Geniosporum tenuiflorum(L) Merr.var longiracemosum(Raman. Et.al)	TN
19	Gomphostemma eriocarpa Benth.	TN
20	G.G.heyneanum Wall var heyneanum&var. rottleri Prain	KL,TN.
21	G .keralensis Vivek., Gopalan&Ansari.	KL
22	G.nayarii Chauhan	MG
23	G.thompsonii Benth,ex Hook.f.	MG
24	Isodon assamicus(Mukerjee)H.Hara.	AR.
25	I .coetsa Kudo var Hookeri(Hook.f.) V.S.Kumar &G.V..S. Murthy	MG
26	I. kurzii(Prain) H.Hara	SK
27	I. Nilghericus (Benth) H. Hara.	TN, KL.
28	I. rivularis(Wight ex Hook.f.) H.Hara	TN, KL.
29	I. wightii(Benth) H.Hara.	TN
30	Lavandula gibsoni Grah.	KN,MH,TN.
31	Leucas angustisima Sedgwick.	KN.
32	L. clarkei Hook.f.	BR
33	L. deodikarii Billore&Hemadri.	MH
34	L. diffusa BENTH.	AP,TN.
35	L. eriostoma Hook.f.var eriostoma, var.latifolia, var. lantana	MH,KL,KN,TN.
36	L. heliathemifolia Desf.	TN
37	L. helicterifolia Haines.	BR.
38	L.hirta Spreng var. hirta ,var. beddomei Hook.f.	AP, KL, KN,TN,.
39	L. indica(l) Br. ex Vatke var.nagalapuramiana.	AP
40	L. lanceafolia Desf.	TN
41	L.lamifolia Desf	TN.
42	L. macrantha Blatter& Hallb.	RJ
43	L. mollisma Wall. Var. sebastiana Subba Rao &Kumari	N
44	L. mukerjiana Subba Rao& Kumari.	AP
45	L. nepetaefolia Benth.	AP
46	L . prostata Gamble.	TN
47	L. pubescens B enth.	TN
48	L. rosmarinifolia B enth.	TN
49	L. stelligera Wall	KN,MH,TN.
50	L. suffrucicosa Benth	TN
51	L. ternifolia Desf.	KL,TN.

52	L. vestita Benth. Var. vestita, var.devicolamensis Shetty&Vivek.	KL,KN,TN.
53	L. wightiana B enth.	KL,TN,KN.
54	Meriandra strobilifera Benth.	HP, UP.
55	Micromeria capitellata. Benth.	BR,OR,MP,RJ,,MH,TN
56	Microtoena griffithii. Prain.	AS,AR
57	M. wardii.Stearn.	AR
58	Nepeta bombaiensis Dalz.	MH
59	N.campestris Benth.	JK,HP,,UP.
60	N. duthiei Prain ex Mkerjee	UP
61	N. eriostachya Benth.	JK,UP.
62	N. gelesii Mukerjee	JK.
63	Orthosiphon cosmosus Wight& Benth.	KL,TN.
64	O. diffusus Benth.	AP,TN.
65	O. glandulosum C.E.C.Fischer	MZ.
66	O.robustus Hook.f.	AS.
67	O.rubicundus Benth var. hohenackerii Hook.f.	TN.
68	O. wattii. Prain.	AS, MR, MZ, NG.
69	Plectranthus bishopianus .Gamble	TN.
70	P. bourneae. Gamble.	TN.
71	P. fruticosus Wight ex Hook.f.	TN.
72	P.urticifolius Hook.f.	TN.
73	P. vettiveroides (Jacob) Singh& Sharma.	TN.
74	Pogostemon artopurpureus Benth.	TN.
75	P.dasianus De & Mukerjee.	MG.
76	P. erectus (Dalz) Kuntze var.erectus, var. gracilis var.tomentosa	MH,KN,KL.
77	P.gardneri Hook.f.	KL,KN,TN.
78	P. hedgei. V.S.Kumar& B.D.Sharma.	TN.
79	P.mollis	KL,KN,TN.
80	P. myosuroides(Roth) Kuntze.	AP,KN,TN
81	P.paludosus Benth.	TN.
82	P. pressii Panigrahi	OR
83	P. pubescens B enth.	GA,KN,KL.
84	P. rotundatus Benth.	AP.
85	P. salicifolius(Dalz. Ex Hook.f.) Kuntze	KN,MH.
86	P.speciosus Benth. Var. speciosus, var .filiformis	KN,TN
87	P. stocksii(Hook.f.)Press	KN,MH.
88	P. travancoricus var. travancoricus,var. devicolamensis	KL.
89	P. vestitus Benth	AP,KL.
90	P.wightii Benth	KN,KL,TN.
91	Premna coriacea C.B. Clarke	AN,KL,KN,MH,TN.
92	P. glaberrima Wight	TN.
93	P. khasiana C.B.Clarke.	AS,MG
94	P.milleflora C.B.Clarke	AR,AS.

95	P.mundathuraiensis Rajendran& Daniel	TN.
96	P. paucinervis Gamble	KL,TN.
97	P.villosa C.B. Clarke	KL,KN.
98	Salvia ampicalyx Peter-Stibal	SK.
99	Scutellaria andamanica Prain	AN,KL,KN,MH,TN.
100	S. assamicaMukerjee	AS,MR,MZ,NG.
101	S. colebrookiana Benth	TN.
102	S.khasiana Clarke ex Hook.f.	AS,MG.
103	S.wightiana Benth	TN.
104	Stachys scaberula Vatke	MG.
105	Teucrium plectranthoides Gamble	TN.
106	T. wattii Prain.	AS,MR,KN,TN.
107	Vitex. abta Heyne	KN,TN
108	V. diversifolia Kurz	AN
109	V. negundo var. purpurescens Sivaranjan& Moldenke	KL
110	V. witherleyi Kurz	AN

Abbreviation: AN-Andaman and Nicobar Islands; AP-Andhra Pradesh; AR-Arunachal Pradesh; AS-Assam; BR-Bihar; GA-Goa; HP-Himachal Pradesh; JK-Jammu and Kashmir; KL-Kerala; KN-Karnataka; MG-Mrghalaya; MH-Maharashtra;MR-Manipur; MZ-Mizoram; NG-Nagaland, OR-Orissa; RJ-Rajasthan; SK-Sikkim; TN-Tamil Nadu; UP-Uttar Pradesh.

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RESEARCH ARTICLE

Assessment of Water Quality in the Karaivetti Lake, Ariyalur district, Tamil Nadu,India.

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ABSTRACT

Understanding the water quality is important as it is the main factor determining its suitability for drinking, domestic, agricultural, and industrial purposes. In order to assess the water quality, 10 water samples have been collected in year 2010. The water samples collected in the field were analyzed for electrical conductivity, pH, total dissolved solids (TDS), and major cations like calcium, magnesium, sodium, potassium, and anions like chloride, nitrate, and sulfate, in the laboratory using the standard methods given by the American Public Health Association. The water locations were selected to cover the entire study area and attention was been given to the area where contamination is expected. The expected water contaminants were chloride, nitrate, TDS, etc. The results were evaluated in accordance with the drinking water quality standards given by the World Health Organization (WHO 1993). To know the distribution pattern of the concentration of different elements and to demarcate the higher concentration zones, the linear maps for various elements were also generated, discussed, and presented.

Key words: Water ,Water quality,Permissible limit ,Irrigation,Karaivetti Lake, Contaminants.

INTRODUCTION

Water is one of the most important and basic natural resources. Water is not only one of the most essential commodities of our day-to-day life, but the development of this natural resource also plays a crucial role in economic and social development processes. While the total amount of water available in the world is constant and is generally said to be adequate to meet all the demands of mankind, its quality and distribution over different regions of the world is uneven and causes problems of scarcity and suitability. It is therefore imperative that man develops, uses and manages this scarce commodity as rationally and efficiently as possible. In order to execute this task, accurate

and adequate information must be available about the quality of this natural resource under constantly changing human pressures and natural forces.

The knowledge of hydrochemistry is essential to determine the origin of chemical composition of water (Zaporozec 1972). The hydrology and geochemistry of waters have been further discussed in the classic works of Stumm and Morgan (1981), Hem (1991), Drever (1988), Domenico and Schwartz (1990a, b), Adverse conditions increase investment in irrigations and health and decrease agricultural production, which, in turn, reduce agrarian economy and retard improvement in living conditions of rural people. Poor quality of water adversely affects the plant growth and human health (Wilcox 1948; Thorne and Peterson 1954; US Salinity Laboratory Staff 1954; Holden 1971; Todd 1980; ISI 1983; WHO 1984; Hem 1991; Karanth 1997).

The geographical area of India is 3,287,590 sq km. The length of its Coastline is about 7500 km. The climate of India varies from tropical monsoon in south to temperate in north. Its terrain have upland plain (Deccan Plateau) in south, flat to rolling plain along the Ganges, deserts in west, Himalayas in north. India is enviably endowed in respect of water resources. The country is literally criss-crossed with rivers and blessed with high precipitation mainly due to the southwest monsoon, which accounts for 75% of the an-nual rainfall. There are thirteen major river basins (area more than 20,000 square kilome-tre) in the country, which occupy 82.4% of total drainage basins, contribute eighty five percent of total surface flow and house eighty percent of the country's population. Major river basins are Brahmaputra, Ganga (including Yamuna Sub Basin), Indus (including Satluj and Beas Sub Basin), Godavari, Krishna, Mahanadi, Narmada, Cauvery, Brahmini (including Baitarni Sub Basin), Tapi, Mahi, Pennar and Sabarmati. The classification of river basin based on catchment area is given in Table 1. There are few desert rivers, which flow for some distance and get lost in deserts. There are complete arid areas where evaporation equals rainfall and hence no surface-flow. The medium and minor river ba-sins are mainly in coastal area. On the east coast and part of Kerala State, the width of land between mountain and sea is about 100 km, and hence the riverine length is also about 100 km. whereas, the rivers in the west coast are much shorter as the width of the land between sea and mountains is less than 10 to 40 km. Yet, in spite of the nature's bounty, paucity of water is an issue of national concern resulting in deterioration of water quality in aquatic resources.

Water quality is influenced by natural and anthropogenic effects including local climate and irrigation practices. The chemical character of any water determines its quality and utilization. The quality is a function of the physical, chemical, and biological parameters and could be subjective, since it depends on a particular intended use. Various workers in our country have carried out extensive studies on water quality. Laluraj et al. (2005) have studied water chemistry of shallow aquifers in the coastal zones of Cochin and concluded that waters present in the shallow aquifers of some of the stations were poor in quality and beyond potable limit as per the standard set by WHO and ISI. Rapid increase in Urbanization and Industrialization leads in to deterioration in water quality. Srinivas et al. (2000) and Jha and Verma (2000) have reported the degradation of water quality in Hyderabad and Bihar, respectively. Untreated industrial waste effluents when discharged in unlined drains can percolate under directly affecting the quality of water. Patnaik et al. (2002) have studied water pollution generated from major industries. Similarly, waste effluents discharged in to streams may enter the aquifer body downstream, which also affects the water quality. Abbasi et al. (2002) have studied the impacts of wastewater inputs on the water quality. Jagdap et al. (2002) and Sunitha et al. (2005) classify the water in order to assess the water quality for various purposes

Study area

The Karaivetti Lake extends over approximately 454 hectors and lies between 10◦45_N and 10◦58_N latitudes and 79◦11_ and 79◦22_ E longitudes in the southern part of Tamilnadu, India (Fig. 1). The western, northwestern, and southwestern parts are characterized by the presence of residual hills. The basin is generally hot and dry except during winter season. The mean maximum monthly temperature varies from 14◦C in August to 33◦C in February while as mean minimum monthly temperature ranges from35◦C in June and 20◦C in January. The area receives an average annual rainfall of about 464 mm. The surface runoff goes to stream as instant flow. Rainfall is the direct

recharge source and the irrigation return flow is the indirect source of water in the Karaivetti Lake. Most of the farmers depend on the water for their irrigational needs.

MATERIALS AND METHODS

In order to assess the water pollution, 10 water samples have been collected. The water samples collected in the field were analyzed for electrical conductivity (EC), pH, total dissolved solids (TDS), major cations like calcium, magnesium, sodium, potassium, and anions like chloride, nitrate, and sulfate, in the laboratory using the standard methods given by the American Public Health Association (APHA 1995). The water locations were selected to cover the entire study area, and attention was been given to the area where contamination is expected. The expected water contaminants were chloride, nitrate, TDS, etc. Sampling was carried out using precleaned polyethylene containers. The results were evaluated in accordance with the drinking water quality standards given by the World Health Organization (WHO 1993).

RESULTS AND DISCUSSION

Water chemistry Results of the various physicochemical parameters is shown in Table 1 and their statistical measures such as minimum, maximum, average, median, and mode are given in Table 2. The number and percentage of samples exceeding the allowable limits set by WHO (1993) is given in Table 3.

The EC values ranges from 1.01 to 361 μS/cm with an average value of 284.7 μS/cm. The occurrence of high EC values in the study area might also be due to addition of some salts through the prevailing agricultural activities. The pH value of surface water ranges from 4.84 to 6.5 with an average value of 5.18. This shows that the surface water of the study area is mainly of acidic in nature during the summer season. (Fig. 2).

TDS values ranges from 113 to 256 mg/l with an average value of 159 mg/l. To know the distribution pattern of the concentration of different elements and to demarcate the higher concentration zones, the linear maps for various elements were also generated, discussed, and presented. (Fig 10).

Ionic chemistry

From the Fig. 13, it is obvious that Na+ ion (average concentration of 10.55 mg/l) dominates the cation chemistry of the study area. Sample no. 5 showed the highest concentration of sodium ion (14.69 mg/l). While as the K+ ions is found in a least concentration (average value of 4.27 mg/l). While as Cl− dominates the anionic chemistry of the study area (Fig. 14).

Total dissolved solids

To ascertain the suitability of water of any purposes, it is essential to classify the surface water depending upon their hydrochemical properties based on their TDS values (Davis and DeWiest 1966; Freeze and Cherry 1979). The study shows that only 90% of the sample is below 500 mg/l of TDS which can be used for drinking without any risk. Higher content of TDS can be attributed to the contribution of salts from the thick mantle of soil and the weathered media of the rock and further due to higher residence time of water in contact with the aquifer body. As the host rocks belongs to charnockites and granitic suits, there can be some oxidation and reduction processes in water and surface water, thereby also causing enrichment in the total dissolved solids.

Total hardness

The hardness values range from 45 to 305 mg/l with an average value of 105 mg/l (Table 2). The maximum allowable limit of TH for drinking purpose is 500 mg/l and the most desirable limit is 100 mg/l as per the WHO international standard. (Fig. 12).

Chloride

Chloride concentrations ranging from 28.36–216.21 mg/l have been found in shallow surface water, and its possible source is tanneries where sodium chloride is used as a raw material. The spatial distribution of chloride concentration in water of the study area is illustrated in (Fig. 8).

Nitrate

The nitrate ion concentration varies from 0 to 52 mg/l with an average value of 7.41 mg/l. The concentration of nitrogen in water is derived from the biosphere (Saleh et al. 1999). Nitrogen is originally fixed from the atmosphere and then mineralized by soil bacteria into ammonium. One sample exceeds the desirable limit of 45 mg/l as per WHO standard. The high concentration of nitrate in drinking water is toxic and causes blue baby disease/methaemoglobinemia in children and gastric carcinomas (Comly 1945). The high Nitrate concentration is due to the in-tensive urbanization and industrialization. The spatial variation of nitrate in water of the study area is illustrated in (Fig. 10).

Sulphide

The concentration of sulphide is likely to react with human organs if the value exceeds the maximum allowable limit of 400 mg/l and causes a laxative effect on human system with the excess magnesium in water. However, the sulphide concentration in water of the study area is within the maximum allowable limit in all the sample locations. (Fig. 9).

Potassium

As per WHO (1993), the maximum allowable limit for potassium is 12 mg/l. From the analysis of water samples of the study area, 20% of the collected samples (i.e., two sampling stations) exceed this permissible limit. The spatial distribution map for potassium is shown in (Fig. 7).

Irrigation water quality

Excessive amount of dissolved ion such as sodium, bicarbonate, and carbonate in irrigation water affects plants and agricultural soil physically and chemically, thus reducing the productivity. The physical effects of these ions are to lower the osmotic pressure in the plant structural cells, thus preventing water from reaching the branches and leaves. The chemical effects disrupt plant metabolism. It is the quantity of certain ions, such as sodium and boron, rather than the total salt concentration that affects plant development (Sahinci 1991). Excess salinity reduces the osmotic activity of plants and thus interferes with the absorption of water and nutrients from the soil (Saleh et al. 1999).

Sodium concentration plays an important role in evaluating the water quality for irrigation because sodium causes an increase in the hardness of soil as well as a reduction in its permeability (Tijani 1994). Na% in eight water samples (viz. 1, 16, 20, 22, 24, 25, 26, 30) are high and are not suitable for irrigation (Fig. 6).

More than 16 (16.7%) percentages of the water samples are permissible for irrigation in almost all types of soil with little danger of exchangeable sodium. While as sample numbers 2, 4, 6, 7, 8, 9, 12, 13, 14, 15, 17, 19, 21, 23, 27, 28, 29 (comprising 56.7%) are categorized under doubt full for irrigation.

CONCLUSION

The hydrochemical analysis of the study reveals that the water in the study area is hard to very hard, fresh to brackish, and alkaline in nature. Na+ ion (with average concentration of 164.83 mg/l) dominates the cation chemistry of the study area, while as Cl− dominates the anionic chemistry of the study area. The occurrence of high EC values in the study area reflected the addition of some salts through the prevailing agricultural activities. The water of the area is fresh water for 56.7% of the sample locations and the rest of the samples represent brackish water based on Freeze and Cherry (1979). The study showed that only 40% of the sample is below 500 mg/l of TDS which can be used for drinking without any risk. Higher content of TDS can be attributed to the contribution of salts from the thick mantle of soil and the weathered media of the rock and further due to higher residence time of water in contact with the aquifer body. As the host rocks belong to charnockites and granitic suits, there can be some oxidation and reduction processes in water and surface water, thereby also causing enrichment in the total dissolved solids. Na% in eight water samples (viz. 1, 16, 20, 22, 24, 25, 26, and 30) are high and are not suitable for irrigation (Table 8). More than sixteen (16.7%) percentages of the water Samples are permissible for irrigation in almost all types of soil with little danger of exchangeable sodium.

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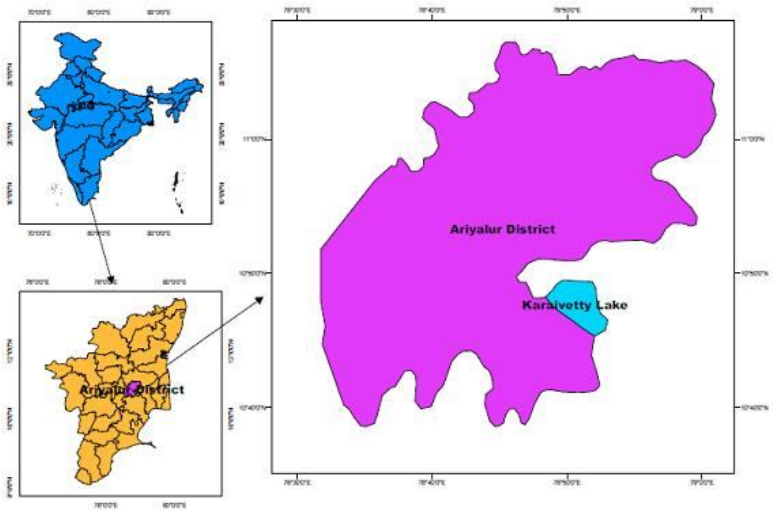

Fig. 1 Location of the Karaivetti Lake

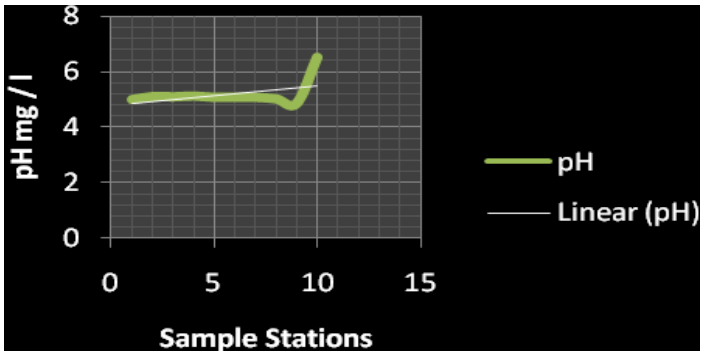

Fig2. pH concentration in Karaivetti Lake

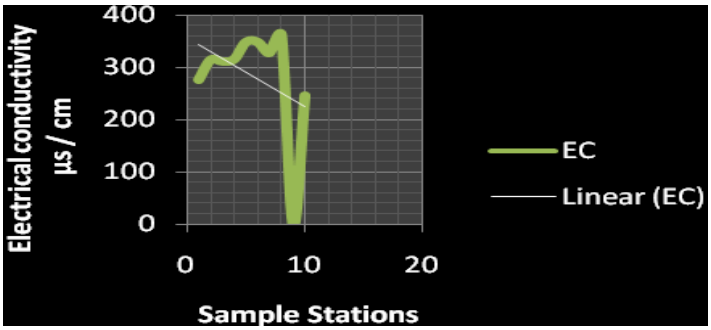

Fig 3. Electrical conductivity in Karaivetti Lake

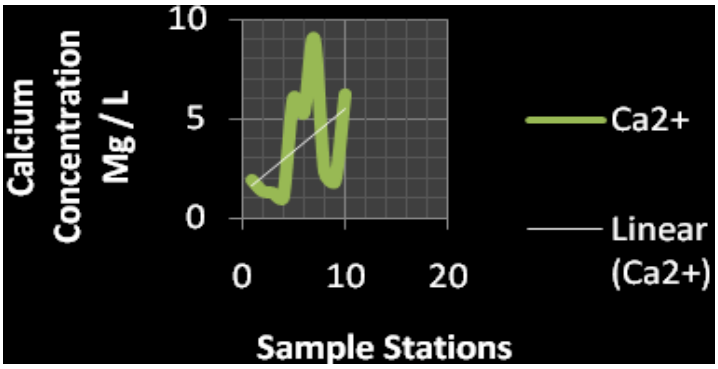

Fig 4. Calcium concentration in Karaivetti Lake

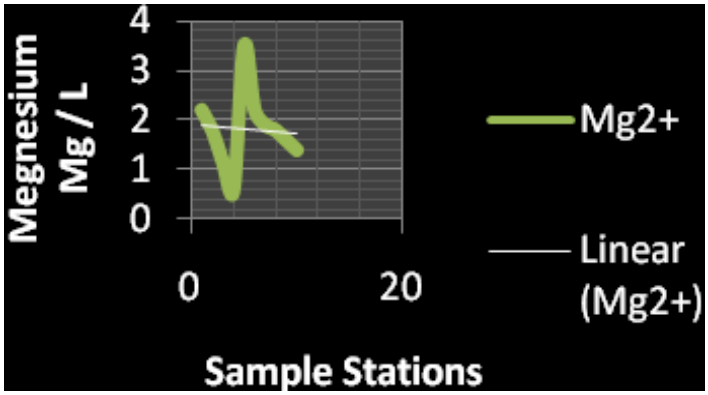

Fig 5. Magnesium Concentration of the Karaivetti Lake

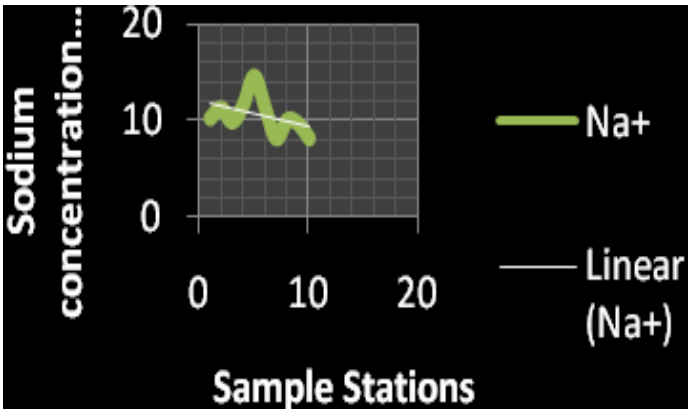

Fig 6. Sodium concentration in Karaivetti Lake

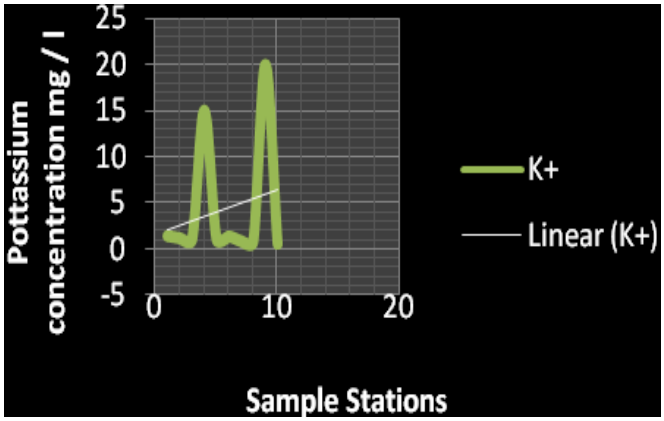

Fig 7. Potassium Concentration of the Karaivetti Lake

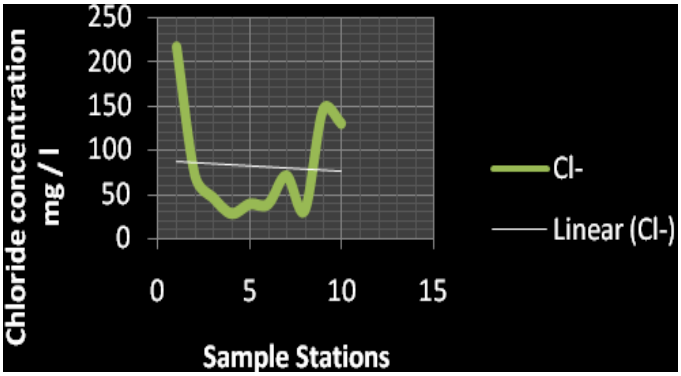

Fig 8. Chloride Concentration of the Karaivetti Lake

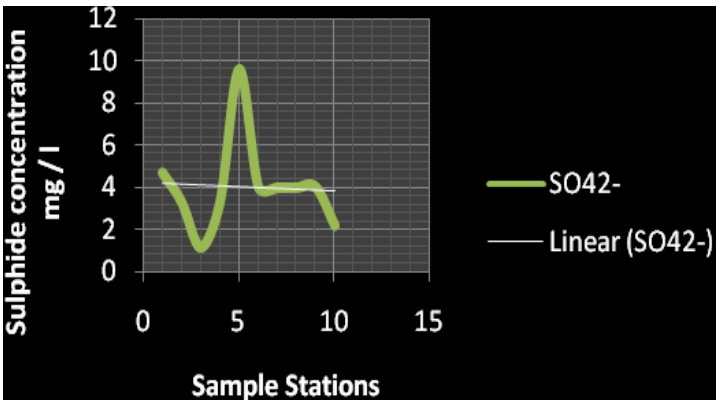

Fig 9. Sulphide concentration in Karaivetti Lake

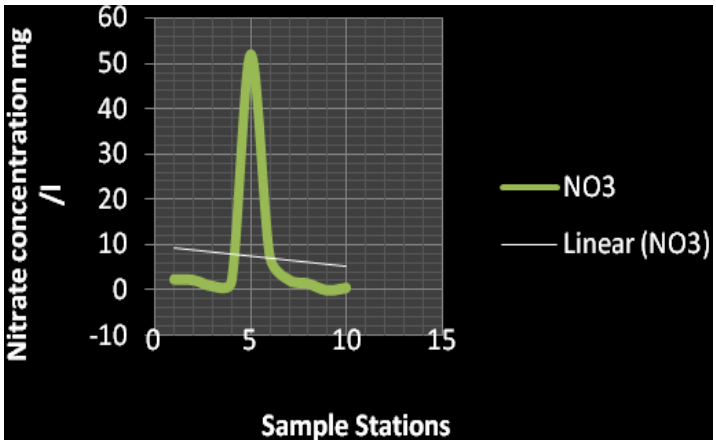

Fig 10. Nitrate Concentration of the Karaivetti Lake

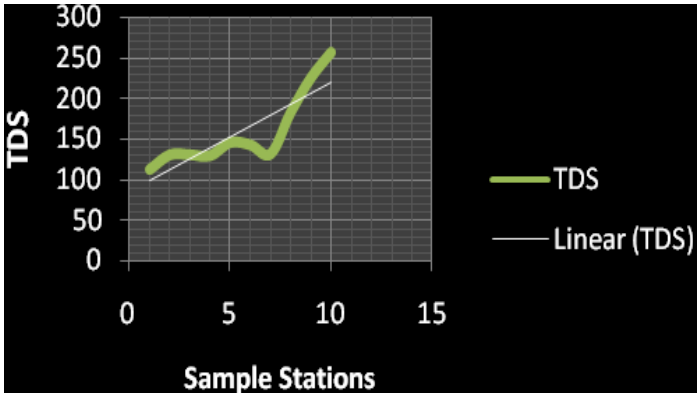

Fig 11. TDS concentration in Karaivetti Lake

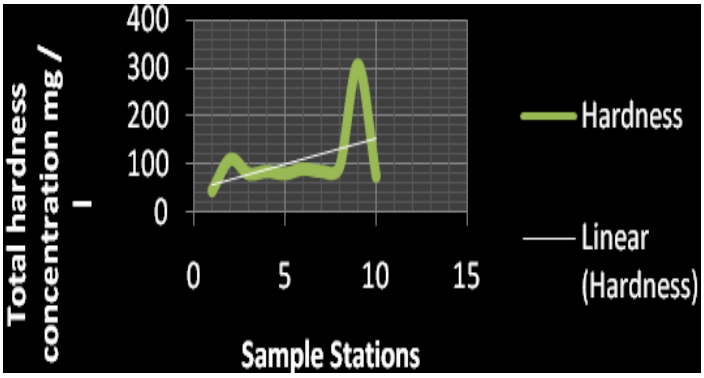

Fig 12. Total hardness of the Karaivetti Lake

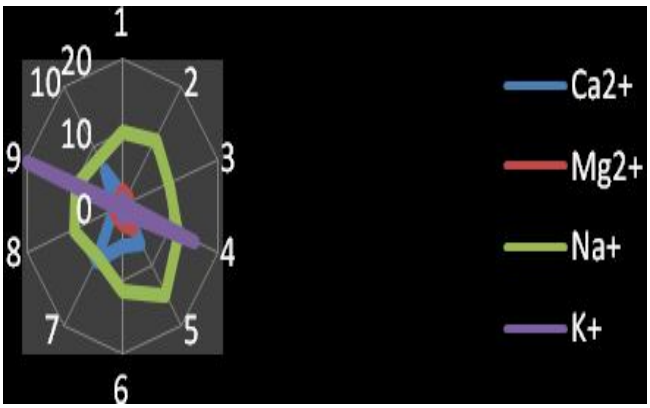

Fig 13. Cation Chemistry

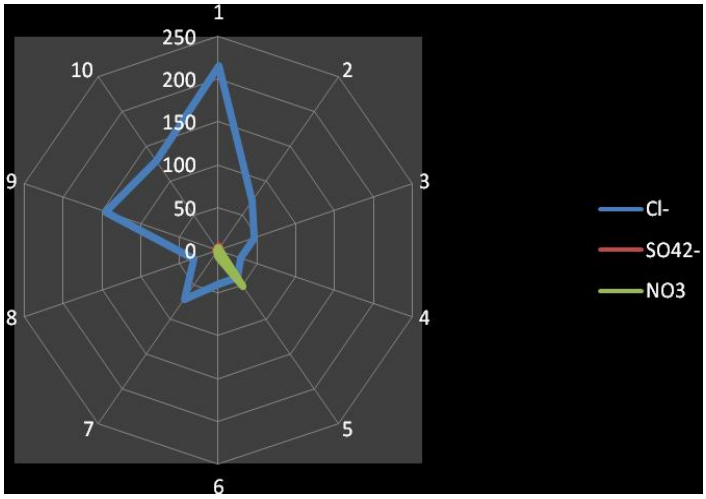

Fig 14. Anion Chemistry

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RESEARCH ARTICLE

Stress Ameliorating Effect of *Abutilon indicum* G. Don Dried Powder on the Growth and Biochemical Characteristics of Metal Treated V*igna radiata,* (L.)Wilczek

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ABSTRACT

The effect of lead acetate on the growth and biochemical characteristics of *Vigna radiata,* (L.)Wilczek. at different concentrations. The metal treatment has caused a steep decline in its growth, pigment content, other biochemical characteristics and enzyme activities. On the other hand, a bioadsorption study carried out with the biomass of Abutilon indicum G.Don in different concentration on lead acetate treated Vigna radiata.All the above characteristics was found to be improved significantly than in plants subjected to untreated metal solution. The pigment content and biochemical parameters were increased after the application of bioadsorbed metal solution. But in contrast, anthocyanin, aminoacid, proline, leaf nitrate and enzyme activities such as catalase and peroxidase were found to be reduced in the bioadsorbed metal treated seedlings. For the present study, it is concluded that Abutilon indicum G.Don is reducing the toxic effect from the metal and relieved the *Vigna radiata* plants from metal stress.

Key words: Heavy metals, Lead, Bioadsorbent, *Abutilon indicum*

INTRODUCTION

The world's ever increasing population and her progressive adoption of industrial based life style and urbanization have yielded large quantities of sewage. Environmental pollution, especially by chemicals has received a great deal of attention in recent years. A major environmental concern due to dispersal of industrial and urban waste generated by human activities is the contamination of soil.

Heavy metal toxicity causes multiple direct and indirect effects on plant growth and alters many physiological functions [1]. The toxicity of heavy metals is mainly attributed to their ability of binding enzyme resulting in the alteration of their catalytic functions and inactivation [2]. Heavy metals are metallic elements which have a high atomic weight and density much greater than water. Heavy metals influence and interface with a variety of process in higher plants such as protection and enzyme synthesis, disturbances in cytokinesis, lowering DNA synthesis and stability. Lead does not evaporate, but it can be present in air as particles. A wide range of organic and inorganic and organic compounds cause contamination these include heavy metals, combustible and putricible substances hazardous wastes, explosives and petroleum product.

Lead as an environmental Contaminant

Metal toxicity in plants has been reported by many workers [3]. The effect of lead on growth of an invasive weed *Lythrum salicaria*,L. and explained that application of lead caused complete withering and death of the above ground parts of all plants. The effect of lead on seed germination and early seedling growth of *Vigna ambacensis* [4]. He observed that the apical and shoot length of *Vigna ambacensis* L. seedlings were significantly inhibited by lead. The lead treated sunflower (*Helianthus annuus)* plants at highter concentration showed stunted growth and reduced leaf expansion[5]. Hence, an investigation has been undertaken to study the effect of lead acetate on the growth and biochemical characteristics of bioadsorbed *Vigna radiata.* Bioadsorption is one of the new approaches that offer more ecological benefits and a cost efficient alternative. Although it is cheaper method, but requires technical strategy, expert project designers with field experience that choose the proper species and cultivars for particular metals and regions.The plant used in the bioadsorption technique must have a considerable capacity of metal absorption, its accumulation and strength to decrease the treatment time.

Bioadsorption of Lead

Helianthus annus accumulate lead in the leaf and stem. So it could be used in restoration of abandoned mines and factories sites contaminated with elevated lead levels in the soil[6].*Hemidesmus indicus* has also been shown to be a lead hyper accumulating plant species, but the heavy metal was mainly accumulated in roots and shoots [7].Free floating plants such as *Spirodela, Lemna minor, Saloinia natum* and *Pista* had a greater significance in the treatment of sewage water [8]. The use of *Albizzia lebback* for the removal of alhyl benzene sulphonates from aquous solution was worked out [9].In *Typha*, roots can hold heavy metal in the cell walls reducing the heavy metal combination with large weight molecules in plant cell plasma [10]. *Typha angustifolia* is able to tolerate heavy metals such as N_1, Cu and Zn. The cell walls of the plant have the capacity to bind metalions in negatively charged sites [11]. *Brassica Juncea* is more effective in the re Bioadsorption removal Zinc from soil [12].It was suggested that bioadsorption would be a suitable alternative to conventional remediation. This was proved experimentally with the help of *Pleris vittata* and *Brassica Juncea* plants to remediate the arsenic and lead contaminated soil [13]. Planting Indian mallow (*Abutilon avicennae*) in (TNT) 2,4,6 Trinitrotoluence contaminated soil enhanced TNT reduction both by stimulating microbical activity that enhances microbial TNT transformation, and by direct uptake and bioadsorption of TNT [14]. The present investigation attempts to study the *Abutilon indium* D.Gon for the remediation of lead on the growth and biochemical profile of *Vigna radiata* (L) wilczek.

MATERIALS AND METHODS

Healthy and viable seeds of uniform size *Vigna radiata* (L). Wilczek were germinated in plastic troughs of uniform size containing acid washed riverbed sand. Seeds of equal number were sown in each pot. The three day old seedlings were irrigated everyday with 50ml of different concentration (5mM, 10mM, 15mM, 20mM, 25mM) of lead acetate containing half- strength of Hoagland's Nutrient Solution[15]. Plants irrigated with half- strength of Hoagland's Nutrient Solution served as control. Various concentration of dried powdered biomass of *Abutilon*

indicum G.Don (2g/L, 4g/L and 6g/L) was mixed with 15mM lead acetate and used for further study. 15Mm lead acetate was found to be optimum by LSD analysis [16].

After ten days of the treatment the seedlings of *Vigna radiata* were used for measuring the growth parameters such as shoot length, root length, leaf area, fresh weight, dry weight, pigment content such us chlorophyll *a, b*, total chlorophyll and carotenoid[17]. Anthocyanin content[18]. total soluble sugar [19] protein content [20], starch , aminoacid content [20] proline content [21], *invivo* nitrate reductase activity [22] catalase [23] and peroxidase activity [24].

RESULTS AND DISCUSSION

Effects of five different concentrations (5mM, 10mM, 15mM, 20mM, and 25mM) of lead acetate on the growth, biochemical and enzyme activities are represented in table 1 to 4. The result shows that the growth parameters such as root length, shoot length, leaf area, fresh weight and dry weight decreased with the increase in the concentration of lead acetate. Similarly chlorophylls, carotenoid, total soluble sugar, protein, starch content and NR activity also follow a declining trend. In contrary the pigment anthocyanin, total free amino acid, proline and the antioxidant enzyme such as peroxidase and catalase increased with the increase in the metal concentration. Bioadsorption studies shows that the growth parameters such as root length, shoot length, leaf area, fresh and dry weight of the plant were increased by increasing the amount of dried powdered biomass of *Abutilon indicum* G.Don with15mM lead acetate solution treated *Vigna radiata* plants (Table5) The chlorophyll and carotenoid contents had been significantly increased after the application of in *Vigna radiata* seedlings. The anthocyanin content decreased with the application of biomass treated metal solution (Table 6).

Total soluble sugar and soluble protein contents were significantly increased in the seedlings after the application of *Abutilon indicum* G.Don treated heavy metal solution. In contrary, total free amino acid and proline contents got reduced after the application of treated lead acetate(Table 7). The dried powdered biomass of *Abutilon indicum* G.Don mixed with 15mM lead acetate caused increase in the pigment content than the untreated lead acetate treated plants and also. An increase in protein content and decrease in free aminoacid and proline after the application of bioadsorbed lead acetate observed in the present study indicates the use of plants natural ability to degrade and remove toxic effect from soil. In present investigation the *in vivo* nitrate reductase activity increases with the increase in the application of dried powder plant biomass. This may be due to the increase in the uptake of nitrate by the plants.

The activities of enzymes such as catalase and peroxidase in the *Vigna radiata* seedlings had been reduced after the application of *Abutilon indicum* G.Don treated lead acetate solution, where as the nitrate reductase activity was increased by the application bioadsorbed metal solution.(Table 8). Peroxidase and catalase are the enzymes responsible for scavenging the plant materials from the stressed impact. Upon the addition of driedpowdered biomass of *Abutilon indicum* G.Don in 15mM lead acetate treated seedlings of *Vigna radiata*, these enzyme activities decrease considerably than in plants treated only with the said metal. The present study shows that, the toxic effects of lead on plants can be almost removed by the addition of dried powdered biomass of *Abutilon indicum* G.Don. AAS study also revealed that the accumulation of lead got reduced after the bioadsorption treatment (Table9&10) and was in accordance with findings [25]. The result of present investigation clearly showed the weed plant *Abutilon indicum* could used to remove the toxicity of lead in the pollution environment for sustainable agriculture. It is inferred from the study that *Abutilon indicum* has brought that normalcy in plants treated with lead acetate proving its capacity as a good bioadsorbant and also a viable alternative to the conventional chemical absorbents.

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Table 1: Effect of Various Table 1: Effect of Various concentration of Lead acetate on the growth of *vigna radiata* (L.) Wilczek

Parameters	Control	5mM	10mM	15mM	20Mm	25mM
Shoot Length (cm)	32.50 ± .057** (100)	24.1 ± 0.057** (74)	21.0 ± 0.577** (64)	19.0 ± 0.577** (58)	18.2 ± 0.233** (56)	16.6 ± 0.333** (51)
Root Length (cm)	16.2 ± 0.057** (100)	13.4 ± 0.088** (82)	9.6 ± 0.305** (59)	9.5 ± 0.251** (58)	8.2 ± 0.088** (50)	7.2 ± 0.145** (44)

Fresh Weight (mg)	0.653 ± .008** (100)	0.490 ± 0.006** (75)	0.380 ± 0.005** (58)	0.283 ± 0.003** (43)	0.260 ± 0.005** (39)	0.186 ± 0.008** (28)
Dry Weight (mg)	0.146 ± .026** (100)	0.076± 0.0005* (51)	0.063± 0.0008** (43)	0.046± 0.0003** (31)	0.040 ± 0.0008** (27)	0.038 ± 0.0003** (25)
Leaf area (cm^2)	3.33 ± 0.166** (100)	2.86 ± 0.033** (86)	2.63 ± 0.033** (79)	2.23 ± 0.016** (67)	2.11 ± 0.016** (63)	1.95 ± 0.028** (58)

Values are an average of five observations values in parenthesis are percentage.
activity with respective control Mean ± SE** Significance at P <0.05 level

Table 2: Effect of Various concentration of Lead acetate on the Photosynthetic pigment of *Vigna radiata (L.)* Wilczek

Parameters	Control	5mM	10mM	15mM	20mM	25mM
Chlorophyll a (mg/gLFW)	0.123± 0.0005** (100)	0.106± .0008** (86)	0.098± 0.0005** (79)	0.095± 0.0005** (77)	0.085± 0.0005** (69)	0.082± 0.001** (66)
Chlorophyll b (mg/gLFW)	0.056 ± 0.001** (100)	0.043 ± .001** (77)	0.034 ± 0.0008** (61)	0.026± 0.0005** (46)	0.021± 0.0008** (37)	0.013± 0.001** (24)
TotalChlorohyll (mg/gLFW)	0.179± 0.0006** (100)	0.154± 0.001** (85)	0.135± 0.0005** (75)	0.116 ± 0.001 ** (65)	0.105 ± 0.001** (58)	0.095± 0.001** (52)
Carotenoids (mg/gLFW)	1.124 ± 0.001**	0.894± 0.0008**	0.765 ± 0.002**	0.615 ± 0.001**	0.576 ± .001**	0.525± 0.001**

	(100)	(79)	(68)	(54)	(51)	(46)
Anthocyanin (mg/gLFW)	4.230 ± 0.011** (100)	5.546 ± 0.017** (131)	5.750 ± .011** (135)	6.673 ± 0.012** (157)	6.753 ± 0.017** (159)	7.346± 0.017** (173)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table 3 : Effect of Various concentration of Lead acetate on the Biochemical Characteristics of *Vigna radiata(L.)Wilczek*

Parameters	Control	5mM	10mM	15mM	20mM	25mM
Glucose (mg/gLFW)	64.23± 0.145** (100)	29.60± 0.115** (46)	25.43± 0.145** (39)	22.70± 0.115** (35)	17.60± 0.115** (27)	14.70± 0.152** (22)
Protein (mg/gLFW)	4.63 ± 0.088** (100)	3.25 ± 0.011** (70)	2.64 ± 0.012** (57)	2.50 ± 0.023** 53)	2.06 ± 0.017** (44)	1.46 ± 0.011** (31)
Starch (mg/gLFW)	2.05 ± 0.006** (100)	0.86 ± 0.014** (42)	0.64 ± 0.011** (31)	0.54 ± 0.014** (26)	0.44 ± 0.015** (21)	0.34 ± 0.012** (16)
Amino acid mole/gLFW)□(	26.66 ± 0.088** (100)	43.43 ± 0.233** (162)	47.39 ± 0.049** (177)	53.24± 0.023** (199)	56.06± 0.021** (210)	60.30 ± 0.050** (226)
Proline (mg/gLFW)	1.246 ± 0.008** (100)	1.763 ± 0.014** (141)	1.84 ± 0.011** (147)	1.953± 0.020** (156)	2.066± 0.017** (165)	2.150 ± 0.023** (172)
Leaf nitrate	15.16 ±	20.64 ±	25.63 ±	28.45±	33.46 ±	36.69 ±

(mg/gLFW)	0.088** (100)	0.023** (136)	0.027** (169)	0.017** (187)	0.015** (220)	0.015** (241)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table4: Effect of Various concentration of Lead acetate on Enzyme activities of *Vigna radiata(L.)Wilczek*

Parameters	Control	5mM	10mM	15mM	20mM	25mM
Nitrate reductase activity mole/gLFW)□(	7.65 ± 0.005** (100)	4.15± 0.017** (54)	3.16± 0.021** (41)	2.14± 0.008** (27)	1.63± 0.027** (21)	0.94± 0.023** (12)
Catalase activity mole/gLFW)□(	2.1± 0.057** (100)	3.36 ± 0.015** (160)	3.73± 0.020** (177)	4.65± 0.018** (221)	6.71± 0.142** (319)	8.27± 0.011** (393)
Peroxidase activity mole/gLFW)□(	0.0077± 0.0003** (100)	0.016 ± 0.001** (212)	0.017± 0.0003** (216)	0.019± 0.0003** (242)	0.026 ± 0.0008** (341)	0.035± 0.00** (458)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table 5: Effect of Lead acetate (15 mM) and *Abutilon indicum*, G. Don. on the growth parameter of *Vigna radiata* (L.) Wilczek

Parameter	Control	15mM	2g/L(w/v)	4g/L(w/v)	6g/L(w/v)
Shoot Length (cm)	32.50± 0.057** (100)	19.00 ± 0.577** (58)	21.73 ± 0.371** (66)	24.86 ± 0.133** (76)	28.63 ± 0.317** (88)
Root Length (cm)	16.20 ± 0.057** (100)	9.50 ± 0.251** (58)	12.43 ± 0.233** (76)	13.83 ± 0.166** (85)	15.53 ± 0.290** (95)
Fresh Weight (mg)	0.653 ± 0.008** (100)	0.283 ± 0.003** (43)	0.436 ± 0.008** (66)	0.566 ± 0.008** (86)	0.610 ± 0.005** (93)
Dry Weight (mg)	0.146 ± 0.026** (99)	0.046 ± 0.0003** (31)	0.097± 0.001** (66)	0.109 ± 0.010** (74)	0.130 ± 0.005** (88)
Leaf Area (cm^2)	3.33 ± 0.166** (100)	2.23 ± 0.016** (67)	2.30 ± 0.003** (69)	2.66 ± 0.0251** (79)	3.06 ± 0.033** (92)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table 6: Effect of Lead acetate (15 mM) and *Abutilon indicum*, G. Don. on the pigment content of *Vigna radiata* (L.) Wilczek

Parameter	Control	15mM	2g/L(w/v)	4g/L(w/v)	6g/L(w/v)
Chlorophyll *a* (mg/gLFW)	0.123 ± 0.0005** (100)	0.095 ± 0.0006** (77)	0.099 ± 0.0003** (80)	0.107 ± 0.001** (87)	0.113 ± 0.0008** (92)
Chlorophyll *b* (mg/gLFW)	0.056 ± 0.001** (100)	0.026 ± 0.0005** (46)	0.029 ± 0.0006** (51)	0.042 ± 0.001** (75)	0.047 ± 0.001** (84)

Total chlorophyll (mg/gLFW)	0.179 ± 0.0006** (100)	0.116 ± 0.001** (65)	0.136 ± 0.002** (75)	0.142 ± 0.001** (79)	0.158 ± 0.001** (88)
Carotenoids (mg/gLFW)	1.124 ± 0.0012** (100)	0.615 ± 0.002** (54)	0.811 ± 0.0008** (72)	0.911 ± 0.004** (80)	1.024 ± 0.003** (91)
Anthocyanin (mg/gLFW)	4.23 ± 0.011** (100)	6.67 ± 0.012** (157)	6.33 ± 0.0088** (149)	5.84 ± 0.0933** (138)	5.18 ± 0.041** (122)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table 7: Effect of Lead acetate (15 mM) and *Abutilon indicum*, G. Don. on the Biochemical parameter of *Vigna radiata* (L.) Wilczek

Parameter	Control	15mM	2g/L(w/v)	4g/L(w/v)	6g/L(w/v)
Glucose (mg/gLFW)	64.23 ± 0.145** (100)	22.70 ± 0.115** (35)	28.60 ± 0.057** (44)	35.10 ± 0.321** (54)	41.13 ± 0.272** (64)
Protein (mg/gLFW)	4.63 ± 0.088** (100)	2.50 ± 0.023** (53)	3.22 ± 0.066** (69)	3.75 ± 0.018** (81)	4.26± 0.027** (92)
Starch (mg/gLFW)	2.05 ± 0.006** (100)	0.54 ± 0.014** (26)	1.27 ± 0.061** (62)	1.45 ± 0.020** (70)	1.74 ± 0.052** (84)
Amino Acid mole/gLFW)□(	26.66 ± 0.088** (100)	53.24 ± 0.023** (199)	48.51 ± 0.364** (181)	32.91 ± 0.303** (123)	30.33 ± 0.158** (113)

Proline (mg/gLFW)	1.24 ± 0.008** (100)	1.95 ± 0.020** (156)	1.63 ± 0.015** (130)	1.44± 0.012** (115)	1.29 ± 0.005** (103)
Leaf Nitrate (mg/gLFW)	15.16 ± 0.088** (100)	28.45 ± 0.017** (187)	23.33 ± 0.283** (153)	19.25 ± 0.159** (126)	17.25 ± 0.038** (113)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table 8: Effect of Lead acetate (15 mM) and *Abutilon indicum*, G. Don. on the enzyme content of *Vigna radiata* (L.) Wilczek

Parameter	Control	15mM	2g/L(w/v)	4g/L(w/v)	6g/L(w/v)
NRA mole/gLFW)□(	7.65± 0.005** (100)	2.14 ± .008** (28)	3.85± 0.070** (50)	4.87 ± .090** (63)	6.46 ± 0.211** (84)
Catalase mole/gLFW)□(	2.10 ± 0.057** (100)	4.65 ± 0.018** (221)	4.10 ± 0.057** (195)	3.40 ± 0.058** (161)	2.66 ± 0.088** (126)
Peroxidase mole/gLFW)□(	0.0077± 0.0003** (100)	0.0187± 0.0003** (242)	0.0163± 0.0002** (212)	0.0143 ± .0001** (186)	0.0097± 0.0003** (125)

Values are an average of five observations values in parenthesis are percentage activity with respective control Mean ± SE** Significance at P <0.05 level

Table 9: AAS study on the concentration of lead in heavy metal treated *Vigna radiata* (L.)Wilczek

S.NO	Treatment	Lead in PPM
1	Control	0.0782
2	5mM	0.4313
3	10mM	0.8136
4	15mM	1.3273
5	20mM	1.9042
6	25mM	2.7410

Table 10: AAS study on the concentration of lead in *Abutilon indicum* ,G.Don treate *Vigna radiata* (L.)Wilczek

S.NO	Treatment	Lead in PPM
1	Control	0.0782
2	15mM	1.3273
3	2g/L	0.6841
4	4g/L	0.3021
5	6g/L	0.1764

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2. Volume with supplement: Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.
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4. Personal author(s): Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.
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