National Seminar on

"Recent Trends in Plant Sciences"

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Department of Botany

Satavahana University

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Dedicated

to our beloved

Prof. B. Bhadraiah

Former Registrar
Satavahana University, Karimnagar, Telangana, India
(Pioneer & Founder father of the Department of Botany, Satavahana University)

With love & affection

Dr. N. Venu Madhav
Dr. E. Narasimha Murthy
(Founders of the Department of Botany, Satavahana University)

About the Satavahana University, Karimnagar

The University College of Satavahana is located in Rekurthi of Karimnagar District & Univ. of Science College in the left flank of Manair Dam of Karimnagar District. Satavahana University has its roots in the erstwhile Post Graduate Centre of Osmania & Kakatiya Universities. In the month of June 2008, the Post Graduate Centre of Kakatiya University has been upgraded as Satavahana University Prof. Mohd. Iqbal Ali, a well known Academician & Economist has been appointed as the 1st Vice Chancellor of this University. The University is named after the Satavahana dynasty that ruled this region. Now the present VC Prof. Veera Reddy, a well-known academician & eminent Professor in Chemistry as the 2nd Vice Chancellor of this University. At present totally there are 145 Colleges (including PG & UG) affiliated Satavahana University which are offered all major courses of Science & Arts groups.

About the Karimnagar District

Karimnagar District is a heart of Northern region of Telangana state. The District derived its name from Karimnagar, its head quarters town, it is situated at a distance of 160 Kms. from Hyderabad, the state capital. The component part of the District were ruled at different times by various dynasties namely, the Satavahana's, Eastern Chalukyas, Kakatiyas, Mughals and Asaf Jahis. The Godavari and Manair are the two important rivers that flow in this District. Across Karimnagar, there are several famous Hindu Temples (Kaleshwaram, Vemulawada and Dharmapuri), Mosques, and Gurdwaras. People belonging to every religion respect each other and co-exist with peace and happiness. Moreover, it is the birthplace of intellectuals like Late P.V.Narasimha Rao, former Prime Minister of India. Since the geographical distribution of this district is enriched with several natural mineral resources, several public and private industries including Singareni Mines, NTPC-Ramagundam, Birla- Kesoram and Orient Cement companies are operating from this place.

Brief history of the Department of Botany

The Department of Botany was come into existence in the academic year 2013-2014 with the introduction of M.Sc- Botany course as a regular course. The course is taught only in English Medium. The initial intake of students was only 30. **Dr. N. Venu Madhav**, as a In-charge Head, along with another faculty member **Dr. E. Narasimha Murthy** founded the Department of Botany at left flank of LMD in University College of Sciences, Karimnagar. A classroom was made into the Laboratory, which was having only skeletal structure to start with equipment, which is sufficient to conduct all the practical experiments of the subject.

The Department of Botany was came into existence in the academic year 2013-2014 with approval of **Monitoring & Development Committee held on 1st October 2012.** M.Sc. Botany course was introduced as a regular course from the academic year 2013-14. The initial intake of students was only 30. Dr. N. Venu Madhav, as an In-charge Head in the Department Botany, initially it is established the department with another faculty member Dr. E. Narasimha Murthy. The faculty members have good academic potentials and studied prestigious institutions like Meerut University and University of Hyderabad. Dr EN Murthy visited Portugal and Germany. It was started at left flank of LMD, University College of Science, SU,, Karimnagar. A classroom was made into the Laboratory, which was having only skeletal structure to start with equipment, which is sufficient to conduct all the practical experiments of the subject. We are conducting regular field demonstrations to develop curiosity and basic understanding of plants in and around Satavahana University campus.

Acknowledgements

The papers presented in this volume are both extempore speeches that are; Transformed into paper form, given significance of the ideas expressed therein. Some others are presentations that are read out in the UGC sponsored National Seminar on "Recent trends in Plant Sciences" held during 22nd and 23rd August, 2014 in Satavahana University College for Science, Karimnagar. We extend our gratitude to the APSCHE, Hyderabad, for extending financial support both for the conduct of seminar as well as for the publication of proceedings. We are also grateful to the commission of collegiate education AP, Hyderabad, for according permission to conduct the seminar. We are deeply indebted to the Honorable Vice Chancellor Prof.K.Veera Reddy of our University for his valuable guidance and encouragement in all aspects. We are thankful to Our beloved Registrar, Prof.M.Komal Reddy for his unforgettable role in grand success of this department first National Seminar, we also acknowledgements to the paper presenters and all the students and staff of the local Govt and Private P.G Colleges, of satavahana university, and other participants,Prof.C.Mnohara Chary, Prof.S.M.Reddy and Prof. Bir Bahadur whose active participation and co-operation Prof.T.Pullaiah, Prof.N.Rama Swamy, and DR.C.Sudhakar Reddy definitely added the needed touch of seriousness to the proceedings of the seminar that is expected in a National Seminar. Finally, our deep sense of gratitude goes to Principal, Dr.V.Namrath, and all other Teaching and Non-Teaching Staff Members of Our University College, Karimnagar, all the other sponsorers of the seminar.

The spirit, which with they have come forward to help in their own way, is very encouraging and makes one feel optimistic to take up the activities of this sort. The help extended by the other Faculty of the department is invaluable. To conclude, we are hopeful, the lessons that we learnt, the fresh insights that we have about human nature, may go a long way in tempering me.

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- **Editor(s)**

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1.

A STUDY ON MILK ADULTERATION IN KARIMNAGAR CITY, TELANGANA STATE, INDIA.

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ABSTRACT

Milk is a whitish liquid containing proteins, fats, lactose and various vitamins and **is** an important nutritive food for vast population on earth. Any change in milk composition is considered as milk adulteration. The study was carried out keeping in view the recently emerging concern of adulteration of natural milk with various illegal substances to increase its marketability. This study explains in detail the hygienic status of milk supplied to various cafes, small hotels and other public and educational institutions. A total of 10 samples were collected from different localities in Karimnagar city, Telangana state, India and tested for determination and extent of adulteration. Qualitative analyses were carried our on 10 milk samples; a standard milk adulteration kit manufactured by Himedia laboratories, Mumbai, India was used. Following are the significant observations of the study: - Sucrose, Skim milk powder was present in 10% and 80% of the milk samples respectively. Neutralizers, Sodium chloride and Urea were present in 30%, 70% and 10% of the milk samples respectively. Hydrogen peroxide and Detergents were present 40% and 60% of the milk samples obtained. All percentage values are indicative of presence of these adulterants (Trace, Moderate and High amounts combined). This qualitative analysis which has unfolded proved that the milk procured did not conform to the legal standards and was adulterated with toxic substances which are harmful to health.

Keywords:

Milk; Adulteration; Dairy; Qualitative analysis of milk.

Introduction:

Milk in its natural form has high food value. It supplies nutrients like proteins, fat, carbohydrates, vitamins and minerals in moderate amounts in an easily digestible form. Due to its nutritive value, milk is significant to young and old people.

Milk contains more than 100 substances that are either in solution, suspension or emulsion in water, the important being casein- the major protein of milk, lactose- milk sugar, whey and mineral salts.

The casein micelles and fat globules give milk most of its physical characteristics, and give taste and flavor to dairy products such as butter, pannier, curd, cheese etc. the composition of milk varies considerably with the breed of cow, stage of lactation, feed, season of the year, and many other factors. However, some relationships between constituents are very stable and can be used to indicate whether any tampering with the milk composition has occurred. From the view point of protection the health of the consumer, the Government of India promulgated the prevention of Food Adulteration Act' (PFA Act) in 1954. The act came into force from 1st June, 1955. It prohibits the manufacture, sale and distribution of not only adulterated foods but also foods contaminated with toxicants.

The nature of adulterants generally encountered in milk and milk products are water, fat, addition of skim milk powder, reconstituted milk, thickening agents such as starch, glucose, urea, salt, chlorine. Preservatives such as neutralizers which usually consists of sodium bicarbonate, sodium carbonate, sodium hydroxide and calcium hydroxide. Some rarities include animal fats, aflatoxins and vegetable oils.

Thus it is obvious that apart from less harmful adulterants, toxic and potentially injurious substances also are being added to milk. Despite food legislation, adulteration remains uncontrolled, furthermore legal steps laid down in the PFA Act are extremely difficult to maintain due to inadequate and untrained man power and laboratory facilities. Such is the state in the country where we are one of the largest nations of milk producers.

Here are a few examples of what adulterants can be added to milk in order to maintain its freshness and market value which ion turn is harmful to the consumer leaving them clueless of what direct effect these adulterants have on them.

Water is an adulterant in milk which is often always added to increase the volume of milk which in turn decreases the nutritive value of milk which if contaminated poses a health risk especially to infants and children.

Detergents are added to emulsify and dissolve the oil in water giving a frothy solution, the characteristic whit color of milk. Detergents cause gastro-intestinal complications.

Urea is added to milk to provide whiteness, increase the consistency of milk and for leveling the contents of solid-not-fat (SNF) as are present in natural milk. The presence of urea in milk overburdens the kidneys as they have to filter out more urea content from the body.

Hydrogen peroxide is also added to milk to prolong its lifetime, but peroxides damages the gastro intestinal cells which can lead to gastritis and inflammation of the intestine.

Starch is also used as an adulterant and if high amount of starch are added to milk this can cause diarrhea due to the effects of undigested starch in colon. Its accumulation in the body may prove very fatal for diabetic patients.

Carbonates and bicarbonates are added to milk too, this can cause disruption in hormone signaling that regulate development and reproduction. Keeping in view the above facts, the present study was conducted to detect various common adulterants in milk samples obtained from public and educational institutions.

Materials and Methods:

A standard milk adulteration kit manufactured by HIMEDIA(K088A) laboratories, Mumbai, India was used. The tests for adulteration were carried out on 10 milk samples obtained in and around the city of Karimnagar,Telangana State, India. Samples were collected ion clean, dry and sterilized glass bottles. The milk samples were tested for the following adulterants- starch, sucrose, glucose, skim milk powder, acidity, neutralizers, sodium chloride, urea, hydrogen peroxide, detergents and heat stability of milk was also tested.

Results and Discussion:

A total of 10 milk samples were tested in duplicates. All tests were carried out at room temperature (30 c). The results are summarized into categories. Group I (Table 1), shows the presence of carbohydrates, Group II (Table 2) shows the presence of salts and Group III(Table 3) is classified as other compounds where peroxides and d detergents.

ADULTERANTS	**Starch**	**Sucrose**	**Glucose**	**Skim Milk Powder**
No. Of samples positive	-	01	-l	08
No. Of samples Negative	10	09	10	02
No. Of samples positive (%)	-	10	-	80
No. Of samples Negative (%)	100	90	100	20
Cumulative percent	100	100	100	100

Table 1: Group I Adulterants

ADULTERANTS	**Acidity/ Alkalinity***	**Neutralizers**	**Sodium chloride**	**Urea**
No. Of samples positive	10	03	07	01
No. Of samples Negative	-	07	03	09
No. Of samples positive (%)	100	30	70	10
No. Of samples Negative (%)	-	70	30	90
Cumulative percent	100	100	100	100

Table 2: Group II Adulterants*All samples tested positive only for alkalinity.

ADULTERANTS	**Hydrogen peroxide**	**Detergents**
No. Of samples positive	04	06
No. Of samples Negative	06	04
No. Of samples positive (%)	40	60
No. Of samples Negative (%)	60	40
Cumulative percent	100	100

Table 3: Group III Adulterants

Test for Urea,starch,sucrose, glucose.Left hand side tube--Negetive for test, Right hand side - Possitive for test

Test for Neutralizers,sodium chloride, hydrogen peroxide.Left hand side tube Negetive for test, Right hand side - Possitive for test

The results of group I adulterants is shown in Table 1. As evident from the table all the samples tested negative for both starch and glucose. In these samples the extent of adulteration for sucrose and skim milk powder were 10% and 80% respectively. This explains that these two adulterants were used to either increase the weight or relative mass of natural milk. Presence of sucrose may indicate that it was used to mimic the natural sweetness of milk. Group II adulterants are summarized in Table 2. As evident from the table all the samples tested positive for acidity/alkalinity. In these samples the extent of adulteration with neutralizers, sodium chloride and urea were 30%, 70% and 10% respectively. These chemical are used as cheap preservative which increase the shelf life of fresh milk. Neutralizers such as carbonates and bicarbonates of various alkalis are generally used to mask the pH and acidity values of badly preserved milk passing it off as fresh milk. Sodium chloride is particularly used to interfere with lactometer reading and urea which is a toxic substance is used to give false positive measurements of protein content in milk. Group III adulterants are summarized in Table 3. In this group 40% of milk samples were positive for hydrogen peroxide. Similarly 60% of milk samples were positive for detergents. Peroxides are generally used to preserve shelf life of milk; detergents on the other hand can be due to low maintenance of milk tanks while preparation of it can be used to mask fat value of milk.

Conclusion:

It is apparent from the analyses that a large number of samples procured did not conform to the legal standards prescribed by the food safety and standards Authority of India (FSSAI). These results clearly suggest that most of the milk samples were adulterated. The extent of adulteration varied significantly with least percentage for sucrose 10% and highest for skim milk powder (80%). This portrays that most of the milk samples were prepared with added adulterants during their production and processing or added intentionally according to one’s own choice to generate money. In a country such as India where milk and milk products play an important role in different foodstuffs, this analysis carried out should bring about more awareness to the general public about the malpractices or negligence in milk production.

2.

A Study of Medicinal Plants in Siddipet, Dist Medak, Telangana.

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ABSTRACT

Plant tissue culture and biotechnology offers a greater stimulus for crop modifications by using r-DNA technology, genetic transformations and gene amplification for be herbicide resistance. Embryogenic cell suspension cultures of *Glycine max* were subjected to stepwise selection with increasing Glyphosate concentrations (0.01 to 50 mMG) for induction of tolerance and gene amplification studies. The cell lines were less adoptive in terms of herbicide resistance upto concentration 2.0 mMG. In a stepwise selection from 2.0 mMG onward the cell lines showed greater efficiency and tolerance for selection pressure when compared to other concentrations W-82/35G cell lines showed 14 fold increase in the enzyme activity and 650 folds increase in the I_{50} value than that of unselected wild type of embryogenic soybean cell lines. Enhanced EPSPS enzyme activity is due to over expression of corresponding target gene or amplification of DNA

INTRODUCTION

Plant tissue culture comprises the *In Vitro* cultures of various kinds of explants ranging from cells to tissues and organs. It facilitates experimental approaches with a large variety of objectives in developmental biology. The theory and goals of mutant (or) variant selection from tissue is reviewed by several people. Due to the presence of a large population of totipotent cells under aseptic conditions, the plant tissue culture system is considered as ideal for genetic manipulations of crop plants. The cell line selection for crop modifications has made tremendous progress in plant biotechnology by manipulation of genetic material at cellular and

molecular level. Soybean is the most important Legume Crop in terms of total production and international trade. Presently it ranks high among the Legume Crops in its nutritional value owing to its high protein content as high as 42 percent. Soybean cells grow readily when placed under culture conditions and have been studied as undifferentiated friable callus or suspension cultures. In the last decade new approaches were developed to produce cultures capable of regeneration in fertile plants via either organogenesis or embryogenesis. These culture systems usually consist of relatively large tissue masses, which are ideal as single (or) small clumps of cells for I*n Vitro* simple and complicated selection experiments. The use of herbicide to reduce loss in crop yield has become an integral part of modern agricultural practices. There is a continuous demand for new herbicide that are highly effective and safe for both animals and the environments. Most of the herbicides do not distinguish between weeds and crop plants (Widholm *et al.*, 1996). A new group of herbicides has emerged and this fulfills these needs by inhibiting specific amino acid biosynthesis pathway in plant. Modifying plants to become resistant to broad-spectrum herbicides would allow their selective use for crop protection (Dunken and Widholm 1984, Ramulu, 1996). Glyhosate (N-phosphonomethyl glycine) is highly effective broad-spectrum herbicides, a competitive inhibitor with respect to PEP and an uncompetitive inhibitor with regard to the other substrate, S-3-P, in the EPSPS reaction. This Glyphosate is lacking specificity between weeds and crops has been used a selective agents for micro-organisms and higher plants cells (Stemhurken & Amerhen 1980, Donn *et al.* 1984). This paper reports the study of selection for amplification of EPSPS gene product in soybean W-82 cell lines as suspension cultures on modified MS liquid medium.

MATERIALS AND METHODS

Germplasm of *Glycine max* (CV Jack) was obtained from Illinois agriculture Experimental Station at Urbana-Champaign, Illinois. Callus cultures were initiated and grown from hypocotyl explants on B5 (Gamborg *et al.* 1968) medium. The selection and growth studies were carried by inoculation of 0.5-1 of fresh weight of cell suspension into liquid MX medium, modified from Mursashige and Skoog (1962) with 0.4 mg/L (1.18 µM/L) 2.4-D (Dichlorphenoxyacetic acid), the only growth hormone in liquid medium. For determination of I50 value, different concentrations of Glyphosate were incorporated into liquid medium and three replicates were maintained for each concentration. The optimum growth period for

suspension culture is 14-16 days and the cultures were maintained under continuous photoperiod with 120 rpm on a rotary shaker. Depending on the tolerance of the cell line and growth response & further step wise selection were made after 15-30 days to select for highest tolerance level on Glyphosate at 35 mMG. Finally resistance cell lines W-82 at 35 mMG were selected and several sub-cultures were also made on the same concentration.

Measurement of EPSP-synthase Activity : EPSP-synthase extracts were prepared by powdering cells in liquid nitrogen and re-suspending in 2 ml g^{-1} 50 mm Hepes – KOH, 10% glycerol (v/v), 2 mM DTT, 0.1 mM EDTA, 0.01 mM $(NH_4)_6Mo_7O_{24}$ $4H_2O$, pH 7.0 with 1% polyvinylpolypyrrolidone (w/v). All subsequent operations were carried out at 0-40C. The homogenate was centrifuged at 27,000g for 10 min and the pellet discarded. After adding 2 ml of saturated ammonium sulfate per ml supernatant, the extract was held on ice 10 min. then centrifuged as above. The pellet was re-suspended in the extraction buffer, 1 ml g-1 cells. EPSP-synthase activity was measured by determining inorganic phosphate release using with minor modifications a malachite green dye assay described by Forlani *et al.* (1994). All assays were performed in the presence of 0.5 mM $(NH_4)_6Mo_7O_{24}$ $4H_2O$ to inhibit phosphatase activity. EPSP0-synthase extracts were diluted with extraction buffer or added directly to the assay solution and incubated from 1-20 min at 300 C and compared to 0 min. values. Controls contained 10 mM glyphosate or onlyone substrate, S-3-P or PEP. Release of inorganic added was generally less than 10% of the rate with both substrates present. The molar absorption coefficient of the phosphomolybdate complex was determined to be 79,000 M^{-1} cm^{-1}.

RESULTS AND DISCUSSION

The wild type cell suspension cultures of *Glycine max* showed 50 percent growth inhibition at 0.06 mMG, which is most sensitive. Growth experiments were conducted with different concentrations ranging from 0.1 to 35 mM of Glyphosate. Stepwise selections were mad3e depending upon the I_{50} value and growth plotted with log phase cells of W-82 cell suspension. The initial selection experiment with wild type cell line was a made with 0.1 mM concentration of Glyphosate 40% of growth inhibition was observed (Table-1). The results of inhibitory level of selection in certain food Legumes are in conformity with reports of Ramulu,

1994. During stepwise selection on Glyphosate medium, embrorganic cell suspension was adoptive up to 2.0 m MG. Considerable time has taken for achieving optimum growth as that of wild type cell lines. From 2.0 mMG concentration onwards, cell lines showed greater efficiency of resistance against the selection pressure. Gradual increase in concentration of glyphosate is applied in initial selection experiments and optimum growth was obtained at high concentration of glyhosate (2.0 mMG).

TABLE-1 : Growth of cell suspension culture of soybean W-82 on MX medium with various concentration of Glyphosate

Type of Cell line	*Conc. Of glyphosate in mM*	*Fresh Weight in grams Grams*	*Percentage of Inhibition*
Wild	0	5.5 ± 0.30	100
	0.1	2.2 ± 0.45	40
	0.3	1.5 ± 0.21	26.2
	0.5	1.0 ± 0.08	19.3
	1	0.8 ± 0.78	14
	3	0.8 ± 0.01	14
	10	0.8 ± 0.06	7.7
	35	0.3 ± 0.02	4.7
W-82 mMG	0	9.3 ± 0.50	100
	3	9.2 ± 0.60	98
	10	7.9 ± 0.21	87
	35	6.8 ± 0.39	73
	50	1.6 ± 0.41	17.2

Table-2 : EPSP Synthase Enzyme activity in sobbean W-82 cell lines

Name of the cell line	*EPSP Enzyme activity in Pka mg/L*	*No. folds increased*
Soybean (W-82) 0mMG	169	(1)
Soybean (w-82) 35 mMG	2366	(14)

The tolerance of W-82 cell line to herbicides is more efficient. Increasing fresh weight values and also corresponding high growth index value was observed at 19 mMG period of 32 days. When the concentration of glyphosate was doubled (35 mMG), cells were more efficiently adapted and tolerant cell lines yield good growth with cell proliferation (Table-3). The enzyme activity in wild type of cell lines showed 169 pka mol-1 and selected cell lines (35 mMG) showed 14-folds increased enzyme activity and was recorded as 2366 pka mol-1 (Table-2). Increased enzyme activity and enhancement of gene copy number were reported in certain Legumes while selecting against the Glyphosate (Ramulu, 1996). Cell lines selection on (35 mMG) I_{50} value at 39 mMG, which has increased 650-folds over the unselected control cell lines. This clearly indicates that the tolerance to herbicide in an adaptive cell line is stable and consistent in selected cell lines on (35 mMG). The time period 297 days required for the selection of the soybean cell lines for efficient tolerance to glyphosate after 10 subcultures progressively (Table-4). Biotechnological method were very effective in crop modification to understand the DNA amplification of EPSP synthase gene which confers the glyphosate resistance in tobacco cell suspension cultures was reported where the enzyme activity increases several folds (Shyr *et al*, 1992). Stepwise increase in the concentration of herbicide (Glyphosate resulted in the over production of the target enzyme, EPSP synthase due to gene amplification. Amplification of EPSP synthase gene and increased enzyme activity in several folds are well documented in several species of *Alfalfa*, *Nicotina* and *Carrot*. Stepwise selection of *Daucus carota* (L) cells against chlorosufuran showed over production of fragment of DNA, which

increased in 10 copies (Caretto *et al*, 1994). The increased enzyme activity is due to over expression of ESPS synthase gene by production of more mRNA. Stepwise selection for glyphosate resistance in *Cordialis sempervirens* suspension cultures produced high EPSP activity due to post-transcriptional changes associated with mRNA stability (Holland *et al,* 1998).

Table-3 : Step wise selection of soybean CV W-82 cell suspension on MX medium with different concentration of herbicide

Sl. No.	*Cons. of Glyphosate mM*	*No. of days for optimum growth*	*Suspension F.W. (gm)*	*Growth index value*
1	0	16	5.5	111
2	0.1	19	13.0	25
3	0.3	16	3.5	6
4	0.5	20	9.3	17.6
5	1.0	16	2.9	4.8
6	2.0	19	12.7	24.4
7	4.0	41	7.6	14.2
8	6.0	38	2.1	3.2
9	10	60	2.6	4.2
10	16	32	13.3	25.6
11	35	36	11.5	22

Table-4 : Growth inhibition value of soybean cell lines

Name of the cell line	*150 values (Con. in mM)*	*No. of days*	*No. of subcultures*
Soybean (W-82) 0 mMG	0.06	15	(1)
Soybean (W-82) 35 mMG	39	297	(10)

The possible explanations for this may be increase in target due to either in gene expression or gene amplification. The over production of target enzyme EPSP synthase in soybean embryoganic cell lines may be due to the amplification of gene (DNA) encoding corresponding EPSP synthase (Widholm *et al.* 2001).

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3.

Botanicals against *Fusarium oxysporum* f. sp. ricini causing wilt in castor

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Abstract

Fusarium oxysporum f. sp. *ricini* which causes castor wilt is one of the major disease on castor in Andhra Pradesh, which is soil and seed borne, results in the death of infected plant and therefore total yield loss. The present investigation was carried out under *in vitro* conditions to find out the possibility of using ecofriendly measures to manage the disease in cost effective manner. *In vitro* efficacy of different plant extracts tested at 5 percent and 10 per cent concentration against *F. oxysporum* f. sp. *ricini* by poisoned food technique, showed considerable diminution in the growth of pathogen. Among all the plant extracts, neem cake showed maximum inhibition of pathogen of 88.42 per cent at 5 per cent concentration, whereas henna tree showed 93.88 per cent at 10 per cent concentration and minimum was recorded by lime with 22.59 and 49.07 per cent at 5 and 10 per cent concentration respectively. This suggests botanical pesticides in plant health management programme can be used as viable alternative to chemical control.

Keywords: Castor, *F. oxysporum* f. sp. *ricini*, botanicals, poisoned food technique.

Introduction

Castor (*Ricinus communis* L.) which belongs to the family Euphorbiaceae is an important non-edible oilseed crop and plays a vital role in Indian vegetable oil economy. The world's castor production is 15.4 lakh m t (FAO, 2008). India ranks first in area 10.96 lakh ha and11.43 lakh t production of castor in the world of which Gujarat, Rajasthan and Andhra Pradesh are major castor producing states. Andhra Pradesh accounts for 2.22 lakh ha with yield of 675 kgha^{-1} (INDIASTAT, 2013). The crop is extensively cultivated in Mahaboobnagar, Ranga Reddy, Nalgonda and Kurnool districts of the state. *F. oxysporum* f. sp. *ricini* is a soil and seed borne

pathogen colonizing the xylem vessels and blocking them completely to cause wilting. Wilt caused by *F. oxysporum* f. sp. *ricini* is one of the serious disease of castor causing heavy loss upto 85 % depending on fungal inoculum and environmental condition (Dange, 2003). Soil drenching with fungicides are generally used to control of this disease, however, frequent and indiscriminant use of it leads to ill effects on environment causing soil and water pollution and development of new strain with more virulence, hence Bio-control and Botanicals has been advocated as one of promising alternative strategy to overcome these problems. The demand for products and technologies based on plants to control plant pathogens has increased in recent years due to concern about the use of hazardous pesticides. The present study was conducted to find out effective plant extracts for eco-friendly and economical management of castor wilt.

Materials and Methods

In vitro* evaluation of botanicals against *F. oxysporum* f. sp. *ricini (Table 1)

Different plants were collected and washed thoroughly in tap water followed by sterilized water and cut into small pieces. The required quantity (10 g) of leaves, flowers, bark and powder was boiled (100°C) in 20 ml of sterile water for 20 minutes. The aqueous extract was filtered through double layered muslin cloth and considered as stock solution. The volume of stock solution was made up to 50ml by adding sterile water and that solution was used at 2 concentrations 5% and 10% respectively.The antifungal mechanism of plant extracts was studied by poisoned food technique (Nene and Thapliyal, 1973). The stock solution of plant extracts (5 and 10 ml) was mixed with 95 and 90ml of sterilized molten PDA media respectively so as to get 5 and 10 per cent concentration respectively. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile petriplates. Mycelium of 5 mm size disc from periphery of actively growing culture were cut by sterile cork borer and the disc was placed on the centre of each agar plate. Suitable control was also maintained by growing the pathogen on PDA plates. Each treatment was replicated thrice and plates were incubated at 25 ± 2ºC till control plates reached the radial growth of 90 mm. The per cent inhibition over control was calculated according to the formula given by Vincent (1927). R= CD – TD/ CD × 100 Where, R = Per cent growth reduction of test pathogen, CD = Radial growth of test pathogen in control (mm), TD = Radial growth of test pathogen in treatment (mm)

Table 1. List of botanicals tested against *F. oxysporum* f. sp. *ricini*

S.No.	Botanical name	Common name	Family	Plant part used
1.	*Allium sativum L.*	Garlic	Liliaceae	Clove
2.	*Azardirachta indica A.*	Neem	Meliaceae	Leaf,cake, bark
3.	*Allium cepa L.*	Onion	Liliaceae	Bulb
4.	*Zingiber officinale*	Ginger	Zingiberaceae	Rhizome
5.	*Ocimum sanctum L.*	Tulsi	Labiateceae	Leaf
6.	*Pongamia pinnata L.*	Karanja, Kerong Pongamia, Indian Beech	Fabaceae	Leaf
7.	*Annona squamosa*	Custard Apple, Sugar Apple	Annonaceae	Leaf
8.	*Citrus sinensis*	Lime / lemon	Rutaceae	Leaf
9.	*Lantana camara*	Shrub verbenas, lantana	Verbenaceae	Leaf, stem
10.	*Capsicum annum*	Chili	Solanaceae	Fruit (pod)
11.	*Polyalthia longifolia*	Ashoka, Buddha Tree, Indian mast tree.	Fabaceae	Leaf, bark
12.	*Hyptis suaveolens*	Horehound, Mint Weed, Pignut	Lamiaceae	Leaf
13.	*Mentha Arvensis*	Pudina, Pudinhara, Mint	lamiaceae	Leaf
14.	*Parthenium hysterophorous*	Parthenium	Asteraceae	Leaf
15.	*Calotropis gigantean*	Jilledu, Giant Rubber Bush, King's Crown, Calotrope	Apocynaceae / Asclepiadaceae	Leaf, Flower
17.	*Thuja occidentalis*	Tuja, White cedar, Northern White Cedar, Yellow Cedar	Cupressaceae	Leaf
19.	*Eucalyptus citridora*	Eucalyptus	Myrtaceae	Bark
21.	Curcuma longa	Turmeric, Indian saffron	Zingiberaceae	Powder
22.	*Lawsonia inermis*	Henna tree, Mignonette tree	Lythraceae	Powder
23.	*Piper nigrum*	Black pipper	Piperaceae	Dried Unripe Fruit
24.	*Alo vera*	Aloe	Liliaceae	Leaf

Table 2. *In vitro* evaluation of plant extracts against *F. oxysporum* f. sp. *ricini*

S. No.	Botanicals	*Radial growth of *F. oxysporum* f. sp. *ricini* (mm)	*Per cent inhibition over control	*Radial growth of *F. oxysporum* f. sp. *ricini* (mm)	*Per cent inhibition over control
		5%		10%	
1	Garlic	34.75	61.38 (51.56)	31.91	64.53 (53.45)
2	Neem	25.16	72.03 (58.10)	19.91	77.87 (61.94)
3	Onion	34.08	62.12 (52.00)	31.91	64.53 (53.45)
4	Ginger	48.66	45.92 (42.64)	45.50	49.44 (44.66)
5	Tulsi	17.25	80.83 (64.01)	12.75	85.83 (67.86)
6	Pongamia,	24.25	73.05 (58.71)	16.58	81.57 (64.55)
7	Custard Apple	52.66	41.48 (40.06)	30.75	65.83 (54.21)
8	Lime	69.66	22.59 (28.34)	45.83	49.07 (44.45)
9	Mint weed	41.16	54.25 (47.58)	34.83	61.29 (52.35)
10	Chili	50.50	43.88 (41.44)	36.66	59.25 (51.35)
11	Ashoka	20.75	76.94 (61.31)	10.75	88.05 (69.78)
12	Lantana	27.50	69.44 (56.43)	24.91	72.31 (58.30)
13	Mint	29.75	66.94 (54.92)	15.91	82.31 (65.38)
14	Parthenium	26.41	70.64 (57.22)	20.00	77.77 (62.21)
15	Calotrope leaves	26.50	70.55 (57.11)	17.08	81.01 (64.46)
16	Calotrope flower	22.83	74.62 (59.75)	21.00	76.66 (61.53)

17	Tuja	25.66	71.48 (57.70)	23.08	74.35 (59.57)
18	Lantana bark	25.91	71.20 (57.55)	25.75	71.38 (57.64)
19	Neem bark	22.16	75.37 (60.35)	13.00	85.55 (68.45)
20	Eucalyptus bark	21.16	76.48 (61.31)	12.83	85.74 (67.82)
21	Ashoka bark	25.66	71.48 (57.70)	23.83	73.51 (59.01)
22	Henna tree	14.66	83.70 (66.17)	5.50	93.88 (75.67)
23	Turmeric	28.75	68.05 (55.56)	21.91	75.64 (60.43)
24	*Aloe vera*	13.08	85.46 (67.57)	11.08	87.68 (69.43)
25	Neem cake	10.41	88.42 (70.08)	9.08	89.90 (71.45)
26	*Piper nigrum*	15.33	82.96 (65.59)	14.58	83.79 (66.24)
27	Control	90.00	0.00 (4.05)	90.00	0.00 (4.05)
Mean			65.23		72.5
CD at 5%			4.98		8.89
S.Ed±			2.48		4.42
S.Em±			1.75		3.12

*Mean of three replications, Figures in the parentheses are angular transformed value

Figure 1. ***In vitro*** **evaluation of plant extracts at 5 % concentration against** ***F. oxysporum*** **f. sp.** ***ricini***

Figure 2. ***In vitro*** **evaluation of plant extracts at 10% concentration against** ***F. oxysporum*** **f. sp.** ***ricini***

Results and Discussion

All the plant extracts showed considerable inhibition of growth of *F. oxysporum* f. sp. *ricini* when compared to control. The data presented in Table 2. and Figure 1. indicated that among all plant extracts tested at 5% concentration neem cake showed maximum inhibition (88.42 per cent) against *F. oxysporum* f. sp. *ricini* test pathogen followed by *Aloe vera* (85.46 per cent), Henna tree (83.70 per cent), Piper (82.96 per cent), tulsi (80.83 per cent), ashoka (76.9 per cent), eucalyptus bark (76.48 per cent), neem bark (75.37 per cent), calotrope flower (74.62 per cent), pongamia (73.05 per cent), neem leaves (72.03 per cent), tuja (71.48 per cent), ashoka bark (71.48 per cent), lantana bark (71.20 per cent), parthenium (70.64 per cent), calotrope leaves (70.55 per cent), lantana (69.44 per cent), Turmeric (68.05 per cent), mint (66.94 per cent), onion (62.12 per cent), ginger (45.92 per cent), chili (43.88 per cent), garlic (61.38 per cent), mint weed (54.25 per cent), custard apple (41.48 per cent) and lime (22.59 per cent). However, all the treatments showed no significant difference between them except garlic, mint weed, custard apple and lime which differ significantly from other treatments. While minimum inhibition of pathogen was observed with lime. The data presented in Table 2. and Figure 2. revealed that at 10% concentration henna tree was found superior with 93.88 per cent followed by neem cake (89.90 per cent), ashoka (88.05 per cent), *Aloe vera* (87.68 per cent), tulsi (85.83 per cent), eucalyptus bark (85.74per cent), neem bark (85.55 per cent), piper (83.79 per cent), mint (82.31 per cent), pongamia (81.57 per cent), calotrope leaves(81.01 per cent), neem (77.87 per cent), parthenium (77.77 per cent), calotrope flower (76.66 per cent), turmeric (75.64 per cent), tuja (74.35 per cent), ashoka bark (73.51 per cent), lantana (72.31 per cent), lantana bark (71.38 per cent), custard apple (65.83 per cent), garlic (64.53 per cent), onion (64.53 per cent), mint weed (61.29 per cent), ginger (49.44 per cent), chili (59.25 per cent) and lime (49.07 per cent), while the lime was found least effective and all the treatments were non significant between them except chili differ significantly. Among all the botanicals at both the concentrations tested neem cake powder at 5% (88.42 per cent) and 10% (89.90 per cent) recorded highest and least was recorded by lime at 5% (22.59 per cent) and at 10% (49.07 per cent). Babu *et al*. (2008) reported that *A. indica* was superior in inhibiting *F. solani* f. sp. *melongenae* causing brinjal wilt at different concentrations 5, 10, 15 and 20% *in vitro* compared to *R. emodi, E. globulus, Artemessis annua and O. sanctum.* Similarly, the effectiveness of neem products in inhibiting the different plant pathogens was reported by Asit Dubey *et al*. (2010). Sharma *et al.* (2011) against *F. oxysporum* f. sp. *lycopersici* tomato causing wilt. Annapurna *et al.* (1989) reported

aqueous leaf and fruit extracts of neem was found effective in inhibiting the growth of *A. padwickii* in rice seeds.

Besides neem oil other oils like tulsi, lemon grass, citronella was also found effective in inhibition of test pathogen *F. oxysporum* f. sp. *ricini.* Similarly Sangeetha and Muthukumar (2011) reported that tulsi, lemon grass, palmarosa and citronella oil completely inhibited the pathogen *R. solani in vitro.* Garlic at 5% (61.3 per cent) and 10% (64.53 per cent) was found ineffective in inhibition of growth of *F. oxysporum* f. sp. *ricini,* but it is effective in inhibition of other pathogen as this can be supported by Chattopadhyay (2001) who proved garlic extract was effective against *A. carthami in vitro.*

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4.

Influence of PEG Imposed Water Stress and Exogenous Application of Brassinosteroids on Metabolites in Radish

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ABSTRACT

Brassinosteroids (BRs) are plant hormones widely distributed throughout the plant kingdom in low concentrations and with structural homology to animal and insect steroids. BRs are involved in numerous physiological processes. The effect of 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) on the metabolites content of radish (*Raphanus sativus*) subjected to water (Osmatic) stress being imposed by polyethylene glycol was studied. Brassinosteroids supplementation under desiccation stress was associated with elevated levels of soluble proteins, nucleic acids and carbohydrates. Brassinosteroids also enhanced the accumulation of the osmolyte free proline in radish seedlings challenged with drought stress. The results of present study demonstrate the protective role of brassinosteroids against PEG imposed water stress in radish seedlings.

Keywords: 24-Epibrassinolide; 28-Homobrassinolide; Polyethylene Glycol (PEG); Proline; Radish; Water Stress

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INTRODUCTION

Radish (*Raphanus sativus* L.), belonging to the brassicaceae family, is an important annual root vegetable. Radishes are good source of ascorbic acid, folic acid and potassium, vitamin B6, riboflavin, magnesium, copper and calcium. It also a rich source of two important medicinal compounds: glucosinolates and isothiocynates (Curtis, 2003; Martı´nez-Villaluenga, 2008). Grusak and Dellapenna (1999) stressed the need of 'divert research' activities in improving the nutritional quality of plants with respect to nutrient content and composition.

Water stress is the most important abiotic stress limiting the plant growth. Water stress leads to a series of morphological, physiological and molecular changes which adversely affect plant growth. Drought stress is primarily manifested as osmotic stress resulting in the disruption of homeostasis and ion distribution in the cell (Wang *et al.,* 2003). Phytohormones have been implicated in modulating the plant response to desiccation stress.

Brassinosteroids (BRs) were discovered in 1970 by Mitchell and his co-workers and were later extracted from the pollen of *Brassica napus* L. (Grove *et al*., 1979). BRs are considered ubiquitous in plant kingdom as they are found in almost all the phyla of the plant kingdom like alga, pteridophyte, gymnosperms, dicots and monocots (Bajguz, 2009). Brassinosteroids (BRs) are a new group of polyhydroxy steroidal phytohormones which regulate broad spectrum of physiological processes including seed germination, plant growth, vascular differentiation and photomorphogenetic process (Rao *et* a*l*., 2002; Sasse 2003). One of the most promising roles of BRs is their ability to confer resistance to wide array of abiotic stresses (Bajguz and Hayat 2009).

The osmotic solution is used to impose water stress reproducibly under *in vitro* conditions. Polyethylene glycol (PEG) widely used to induce water stress, is a non-ionic water polymer which is not expected to penetrate into plant tissue rapidly (Macar *et al*., 2009). As PEG does not enter the apoplast, water is withdrawn from the cell including cell wall and thus PEG solutions mimic dry soils more closely than solutions of low—MR osmotica which infiltrate the cell wall with solution (Verslues *et al*., 1998).

The present study is undertaken to understand the effect of application of 24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) on the metabolites of radish (*Raphanus sativus* L.) under water stress as imposed by PEG is being investigated.

MATERIALS AND METHODS

Chemicals and Plant Material

24-epibrassinolide (EBL) and 28-homobrassinolide (HBL) were purchased from CID tech research Inc, Mississauga, Ontario, Canada. Seeds of radish (*Raphanus sativus* L.) Pusachekthi varieties were obtained from National Seed Corporation, Hyderabad. After preliminary experiments employing a range of concentrations of polyethylene glycol (PEG 6000), a concentration of 15% was selected as the drought stress concentration. The osmotic potential of 15% aqueous solution of PEG is −2.95 bars at 25°C [8].

Seeds were surface sterilized with 0.5% (v/v) sodium hypochlorite solution from commercially available 4% NaClO and washed thoroughly with several changes of sterile distilled water. They were soaked for 24 h either in 1) Distilled water (control) 2) 0.5, 1.0 and 2.0 µM concentrations of EBL and HBL 3) 15% Polyethylene glycol (stressed control) 4) 15% Polyethylene glycol supplemented with brassinosteroids. The two brassinosteroids (EBL and HBL) were employed at 3 concentration levels viz., 0.5 µM, 1.0 µM and 2.0 µM. 20 seeds for each treatment were distributed in separate petri plates (15 cm diameter) provided

with whatman No. 1 filter papers. The seeds were allowed to germinate in the dark at 25 ± 1°C. On the seventh day fresh seedlings material (200 mg) was homogenized with 70% (v/v) ethyl alcohol and stored in deep freezer. The alcoholic homogenate was used for the metabolites content estimation.

Nucleic Acids

DNA and RNA fractions in the ethyl alcohol homogenate were separated by the method of Ogur and Rosen (1950). DNA was estimated by the procedure of Burton (1968) employing diphenylamine reagent and RNA was quantified by the method of Schneider (1957) using Orcinol reagent.

Soluble Proteins

Soluble proteins in alcohol homogenate (extract in case of enzyme assay) were precipitated by using 20% (w/v) trichloroacetic acid. The precipitate was dissolved in 5 ml of 1% (w/v) sodium hydroxide and was centrifuged at 4000 rpm for 10 min. The supernatant was used for estimation of proteins by Lowry *et al.* (1951) method.

Carbohydrates

The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of reducing sugars (Nelson, 1944). Non-reducing sugars were calculated by the formula given by Loomis and Shull (1937). The residue was used for the estimation of starch (Mc Cready *et al.,* 1950).

Free Proline

The amount of proline content was estimated as described by Bates and others (1973). Seedling material (0.5 g) was homogenized with 10 ml of 3% (w/v) sulfosalicylic acid and the homogenate was filtered through filter paper. The supernatant was taken for proline estimation.

RESULTS AND DISCUSSION

The decline in the levels of nucleic acids was found negated by the exogenous application of BRs. Even in BRs alone treatments also, the levels of nucleic acids were found higher than the untreated and unstressed controls. The growth promotion in radish seedlings by BRs in unstressed and water stressed conditions was found associated with enhanced levels of DNA and RNA (Figures 1(a) and (b)). It has been suggested by Key (1969) that phytohormones regulate the growth by affecting nucleic acid synthesis. The results obtained in the present study with BRs are in conformity with the observations made by Key. The increase in the levels of nucleic acids might be due to enhanced synthesis and reduced degradation. Mung bean seedlings, when treated with 24-epibrassinolide, are reported to exhibit elevated activity of RNA polymerase and lowered activity of RNase and DNase (Wu and Zhao, 1993). Similarly, BRs application markedly increased the DNA and RNA content in 3 week old maize plants under salinity stress (Khallal *et al*., 2009). The ameliorative influence of BRs on salinity stress induced growth inhibition in rice plants was linked to elevated levels of nucleic acids (Anuradha and Rao, 2003).

In radish seedlings under drought stress, there was remarkable decline in soluble proteins. Supplementation of BRs resulted in improvement in soluble protein content in radish seedlings growing under water stress. Brassinosteroids alone treatments also cause enhancement in soluble protein levels (Table 1). Sasse, (1990) suggested that BRs can stimulate the synthesis of particular proteins associated with growth. Supplementing the culture media with 24-epibrassinolide increased cell division rate and soluble protein content in Chinese cabbage protoplasts (Nakajima *et al*., 1996.). Sairam (1994) also obtained enhanced protein levels in wheat plants by 28-homobrassinolide under moisture stress.

Similarly, the alleviating influence of BRs on salinity stress induced inhibition of growth in rice was found associated with elevated levels of proteins (Anuradha and Rao, 2001).

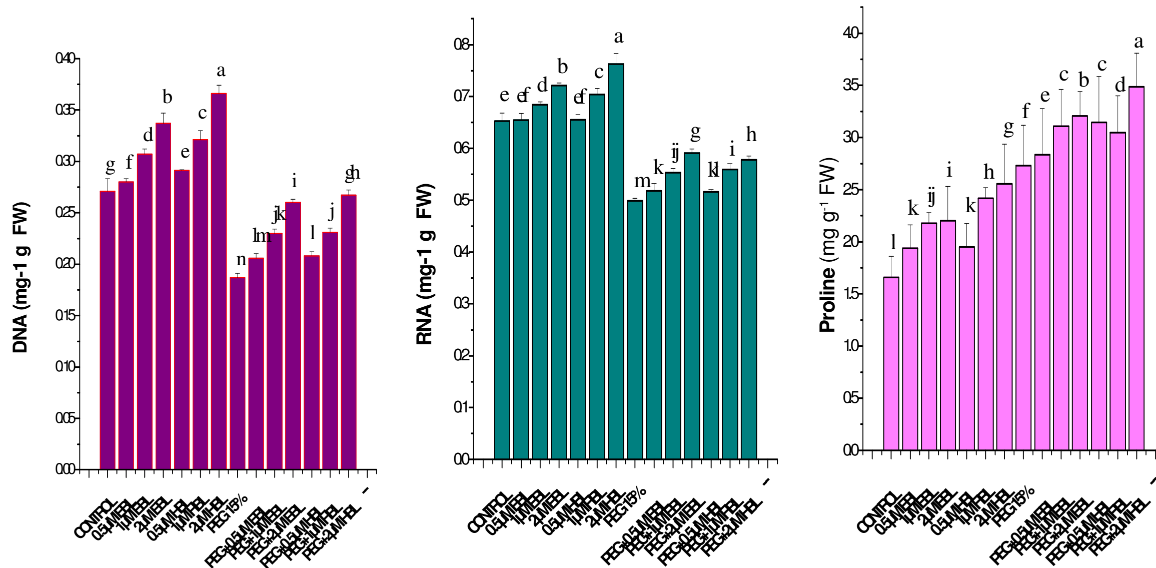

Figure 1. Effect of BRs on the levels of DNA (a), RNA (b) and Free Proline (c) levels of radish seedlings grown under PEG imposed water stress (OP = −2.95 bars). Vertical bars represent the means ±SE (*n* = 5); mean followed by the same alphabet in a column is not significantly different at *p*=0.05 according to Post Hoc test.

Exogenous application of BRs to stressed seedlings reduced the inhibitory effect of drought stress and decrease in the contents of reducing sugars, non-reducing sugars and starch was significantly restored (Table 1). Brassinosteroids alone applications also enhanced the carbohydrate fractions in radish plants. Schilling et al. (1991) reported that homobrassinolide increased sucrose content in sugar beets grown under drought stress. The increase in carbohydrate levels as observed in this study might be due to enhanced photosynthetic capacity of the plants as influenced by the 28-HBL and 24-EBL application. Infact increase in CO_2 fixation and levels of reducing sugars was reported in wheat and mustard plants by the application of brassinolide (Braun and Wild, 1984). Similarly, Vardhini

et al., (2011) reported increased carbohydrate fractions like reducing sugars and starch in the storage roots of radish by foliar supplementation of 28-HBL and 24-EBL.

Table 1. Effect of brassinosteroids alone treatments and in combination with water stress on carbohydrate and protein profile of radish seedlings.

Treatment	Reducing Sugars (mg^{-1} g FW)	Non Reducing Sugars (mg^{-1} g FW)	Starch (mg^{-1} g FW	Protein (mg $^{-1}$FW)
CONTROL	0.99 ±0.007g	1.69 ±0.041f	2.29 ±0.051g	4.72 ±3.280e
0.5µM EBL	1.14 ±0.056f	1.72 ±0.029e	2.54 ±0.058e	5.21 ±1.854cd
1µM EBL	1.52 ±0.068d	1.80 ±0.022d	2.66 ±0.042c	5.91 ±1.974c
2µM EBL	1.78 ±0.022b	1.99 ±0.044b	2.84 ±0.045a	6.72 ±2.067b
0.5µM HBL	1.23 ±0.033e	1.72 ±0.028e	2.42 ±0.074f	5.53 ±1.154c
1µM HBL	1.65 ±0.023c	1.82 ±0.034c	2.61 ±0.043d	6.11 ±1.274b
2µM HBL	1.83 ±0.032a	2.06 ±0.097a	2.81 ±0.050b	7.93 ±1.867a
15% PEG *	0.45 ±0.017n	0.72 ±0.006m	1.13 ±0.042n	1.91 ±0.807j
PEG+0.5µM EBL	0.54 ±0.015m	0.84 ±0.009l	1.30 ±0.041l	2.45 ±1.435i
PEG+1µM EBL	0.63 ±0.021k	0.96 ±0.010i	1.63 ±0.051j	3.87 ±2.969g
PEG+2µM EBL	0.80 ±0.037i	1.05 ±0.027h	1.93 ±0.026i	4.28 ±0.910ef
PEG+0.5µM HBL	0.57 ± 0.023l	0.85 ±0.009k	1.24 ±0.030m	2.16 ±1.435i
PEG+1µM HBL	0.69 ±0.007j	0.95 ±0.027j	1.62 ±0.043j	3.14 ±2.609h
PEG+2µM HBL	0.86 ±0.016h	1.13 ±0.044g	1.98 ±0.039h	4.66 ±2.144e

The values are means ±SE (n = 5); mean followed by the same alphabet in a column is not significantly different at p=0.05 according to Post Hoc test.
*15% PEG is equivalent to osmatic potential of -2.95 bars at 25° C.

Osmolytes play significant protective role in plant responses to water stress and resistance. Under water stress, proline concentration can reach up to 80% of the total amino acid pool. In the present study, osmotic stress imposed by PEG caused substantial increment free proline levels and furtherance of proline content was found in radish seedlings exposed to desiccation stress (Figure 1(c)). The biochemical defense system against abiotic stress involves the amino acid proline (an osmolyte) which acts as cellular protectors largely

accumulated in several plant species in response to abiotic stress, and scavenge ROS (Ashraf and Foolad, 2007). Farooq *et al*. (2009) also observed that application of BRs increased the free proline levels in rice under drought stress.

The findings of present investigation suggest that both the BRs (EBL/HBL) at 2 µM concentration are playing a positive role in combating the PEG-6000 induced drought stress by enhancing the levels of metabolites and Osmolytes. BRs alone treatments are analysed promising results that are substantial improvement rather than control treatment.

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5.

QUANTIFICATION AND QUALITY CHECK OF ISOLATEDGENOMIC DNA FROM TURMERIC VARIETIES OF TELANGANA STATE, INDIA.

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ABSTRACT

Genomic DNA isolation from Turmeric is difficult due to the presence of proteins, polysaccharides, and phenolic compounds of the lignin pathway that act as strong inhibitors of DNA extraction. Modified CTAB method was used to isolate the DNA.Quality and quantity of the DNA is checked using Agarose gel electrophoresis and spectrophotometer respectively.A desired protocol for such specimens can be arrived at only through trial and error. From the present study we describe a method to isolate genomic DNA from the leaves of 18 varieties of Turmeric. The quality of DNA isolated from all the 18 turmeric varieties showed equal grade. The amount of isolated DNA ranged from 676 (Duggiarala variety) to 307 (Erragunturu).

INTRODUCTION

Medicinal plants have played an essential role in the development of human culture. Many of the modern medicines are produced indirectly from medicinal plants. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese and Indian traditional medicine. Many food crops have medicinal effects, for example Garlic and Turmeric.

Turmeric (*Curcuma longa* L) belongs to the monocot family Zingiberaceae. It is an important medicinal plant as well as a spice of India. Spices are widely used as medicine and food flavorants. Although there are about 109 species listed as spices in the International Standards Organization (ISO) list, their uses are specific as each of these commodities have different roles in flavoring foods or in medicine. Over 80 species are reported in the genus

Curcuma from Indo Malayan region and about 40 of them are indigenous to India (Velayudhan*et al.,* 1999).In India it is popularly known as “Haldi” and used as a common ingredient for cooking.

The primary step in any DNA-based discrimination technique is the isolation and amplification of DNA. DNA isolation protocols for problem species need to be specially developed (Cingilli and Akçin, 2005). Genomic DNA isolation from Turmeric is difficult due to the presence of proteins, polysaccharides, and phenolic compounds of the lignin pathway that act as strong inhibitors of DNA extraction (Finkeldey*et al.,* 2010). A desired protocol for such specimens can be arrived at only through trial and error. The presence of polysaccharides and polyphenols will contaminate the DNA preparation and make it unsuitable for further analysis (Cheng et al., 1997; Karaca*et al.,* 2005). Here we describe a method to isolate genomic DNA from the leaves of 18 turmeric varieties.

Genetic polymorphism is classically defined as the simultaneous occurrence of a trait in the same population of two or more discontinuous variants or genotypes. Although DNA sequencing is a straightforward approach for identifying variations at a locus, it is expensive and laborious. A wide variety of techniques have, therefore, been developed in the past few years for visualizing DNA sequence polymorphism.The recent PCR based approach, gel free visualization of PCR products and automation at various steps are boons to the molecular marker approaches adopted for genome mapping and genetic diversity analysis in plant kingdom.Molecular techniques are very much useful not only to identify the genotypes for authentication, but also in assessing and exploiting the genetic variability (Whitkus*et al.,* 1994). In that instance, the isolation of intact, high molecular mass genomic DNA is essential for starting any molecular biology applications (Michiels*et al.,* 2003).

MATERIALS AND METHODS

Plant Material

In the present study eighteen popular cultivated genotypes of turmeric were collected and maintained from Turmeric Research Center, Kammerpally, Nizamabad, Telengana, India for studying the quality and quantity of DNA.The sampling was done from June 2013 to August 2013. The research work conducted at University College of Science, Saifabad, Osmania University, Telangana state in India.

Plant DNA isolation

DNA was isolated following a modified protocol of CTAB method(Doyle and Doyle, 1987)Two to three leaves of Turmeric plants from 20 days old (weighing ~2-3 g) were collected and were cut into small bits in a sterile porcelain mortar and liquid nitrogen was added till the leaf bits were completely immersed. The leaf bits were then ground into fine powder using a sterile porcelain pestle.Immediately after the grinding, 3-4 ml of extraction buffer (50 mMTrisHCl pH 8; 25mM EDTA, 300 mMNaCl and 1% SDS) was added to the mortar, which was then kept in a water bath maintained at 65^{0}C for about 5 min. Once the solution thaws, the contents from the mortar were transferred to 3-5 sterilemicro centrifuge tubes. The micro centrifuge tubes were incubated for 15 min at 65^{0}C. After the incubation, equal volume of Phenol: Chloroform: Iso-amyl alcohol (25:24:1) mixture (~400 µl) was added to each microcentrifuge tube. It was ensured that the pH of phenol used was ~8.0. The contents were mixed well by inversion for about 10 minutes and centrifuged at 10,000 rpm (~8000 g) for about 10 minutes in room temperature. After centrifugation, the supernatant was aliquoted from the micro centrifuge tube intolabeled sterile 1.5 ml micro centrifuge tubes. Care was taken that the intermediate layer of insoluble proteins was not disturbed. If the supernatant was not clear, the phenol: chloroform: isoamylalcohol purification step was repeated once again. To the supernatant 5 µl of RNase (10 mg/ml) was added and incubated for 30 minutes at room temperature. One more treatment with an equal volume of chloroform (400 µl) was given and centrifuged at 10,000 rpm (~8000 g) for 10 minutes at room temperature. To the clear aqueous supernatant, 1/8 volume of 3M sodium acetate (pH 5.2) and equal volume (500-600 µl) of chilled Isopropyl alcohol was added. The contents were mixed gently and centrifuged at 10,000 rpm (~8000 g) for 10 minutes at room temperature. The supernatant was drained gently and about 200 µl of 70% ethanol was added to the pellet collected at the bottom of the micro centrifuge tube. The tube was tapped gently so that the pellet was disturbed. Centrifugation is done at 10,000 rpm (~8000 g) for 10 minute at room temperature. After this the supernatant was drained and the pellet was washed with 70% ethanol once again as described above. Finally, the pellet was left for air-drying over night at room temperature with the tube cap open. After complete drying of pellet, about 50-100 µl of sterile TE buffer (10mM Tris-HCl, pH 8.0 and 1mM EDTA) was added to the tube depending on the size of the pellet, for dissolving the pellet. The DNA solution was then stored at 4^{0}C till further analysis.

Analysis of quality and quantity of DNA isolated

The quality and quantity of isolated DNA was checked by Agarose gel (0.8%) electrophoresis. Five microlitre of DNA solution was mixed with ~1-2 µl of loading dye (0.0025% bromophenol blue in 40% sucrose), loaded into the wells. DNA solutions of known concentration (50 ng/µl) were also loaded along with the samples for comparison of the concentration of the DNA samples. The samples were then subjected to electrophoresis at constant voltage (75 V) for 20-30 minutes till the dye front reached 2/3rd of the running length of the gel. The gel was then visualized under UV light in an Alpha Innotech gel documentation system (Alpha Innotech, USA) for checking the quality and quantity of DNA.

The DNA concentration is calculated by using the formula

DNA concentration (µg/ml) =OD at 20nm × dilution times× standard value (If OD is 1.00, it is equal to 50mg DNA per ml (standard) 50ml of DNA in 1ml TE buffer. It is diluted 20 times) by Jyothi Chaitanya *et al* 2014.

RESULTS & DISCUSSION:

The Deoxyribonucleic acid is a macro molecule that carries genetic information from generation to generation. It is responsible to preserve the identity of the species over millions of years. In the present investigation we have collected 18 popular cultivated Turmeric genotypes from North Telangana region were collected and maintained at University college of Science,Saifabad, Hyderabad. The samples DNA were isolated using modified CTAB method. While isolating the DNA the phenolic compounds occur in many plants and are one of the major bioactive constituents in rhubarb (Ye et al., 2007). When the tissue was grinded and the cell was broken, phenolic compounds were released and oxidized by binding covalently with the total DNA, resulting in browning effect and an overall loss of DNA activity (Porebski*et al.,* 1997). The way to remove the phenolic compounds is to prevent their oxidation in the initial extraction stage, and then separated them with the total DNA.The quality and quantity of isolated DNA was checked by Agarose gel (0.8%) and were visualized under UV light in an Alpha Innotech gel documentation system (Alpha Innotech, USA) for checking the quality and quantity of DNA. Quantification of DNA was done by using spectrophotometric measurement of UV absorption at wave lengths 260/230. Fig 1

showed the quality check of the turmeric varieties, which indicated the similarity in the quality of DNA isolated from all the 18 varieties.

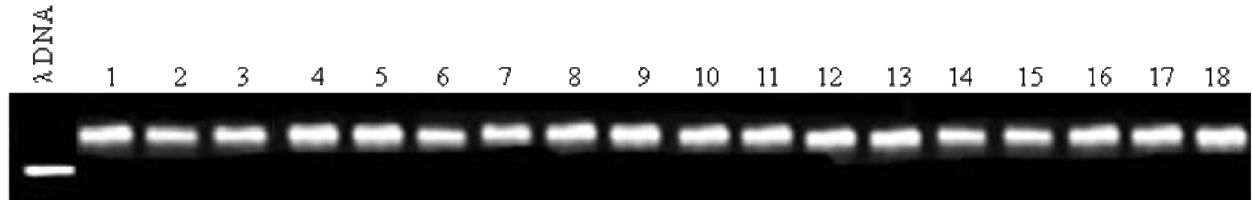

Figure 1: DNA quality check for the 18 varieties

SI.No	Genotypes	Original Designation	ng/µl at 260/230 wv	SI.No	Genotypes	Original Designation	ng/µl at 260/230 wv
1	PTB	Prathiba	358	10	TKP	Terkupeta	553
2	EGT	Eraaguntur	307	11	MDK	Mydukur	412
3	TGT	Tellagunturu	452	12	KSR	Kesari	514
4	MNP	Manapasupu	507	13	JTL	Jagityal local	536
5	SHL	Shylam	628	14	KDR	Kedaram	455
6	KTA	Kasturiavidi	474	15	CLI	CLI-Rajampet	376
7	TDP	Thudapuja	359	16	SMJ	Somajuli	429
8	ARM	Armoor local	512	17	CMP	Casampet	464
9	DGR	Duggirala	676	18	DPG	Deepanguda pasupu	538

Table1 : DNA concentration of 18 Turmeric varieties from Telangana State.

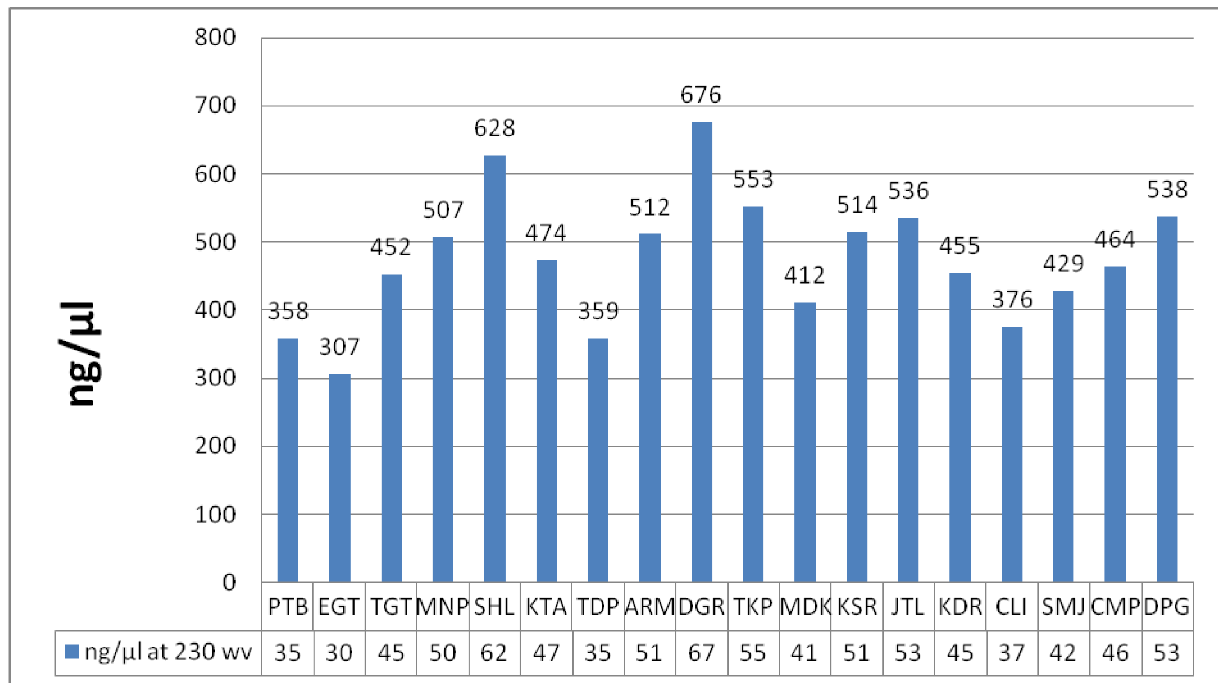

Figure 2:Graphical representation of DNA concentration of 18 Turmeric varieties

CONCLUSION

The quality of DNA isolated from all the 18 turmeric varieties showed equal grade. The amount of isolated DNA ranged from Duggiarala variety (676) to Erragunturu (307).Duggurala ,Shylam varieties showed highest range (676,628) where as Erragunturu, Prathibaverities showed lowest range(307,358).

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6.

Evaluation of Phytochemicals in Fruit Extracts of *Psidium guajava* L.

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Abstract

Psidium guajava L. plant belongs to the family Myrtaceae, have been used extensively in traditional folk medicine. This study analyzed the phytochemical constituents in extracts of guava fruit and the total phenol content was quantified. Guava fruit peel, pulp, seed extracts were prepared with ethanol, ethyl acetate, methanol, sterile distilled water. Organoleptic Characters, Extractive values, phytochemical constituents [Alkaloids, Flavonoids, Saponins, Tannins, Glycosides, and Amino acids] were analyzed in the variety of fruit extracts. The total phenolic content of fruit extracts was evaluated spectrophotmetrically according to Folin-Ciocalteu phenol method and expressed in gallic acid equivalents [GAE]. This study reveals the fruit extracts are moderate potential source of natural antioxidants.

Introduction

Large number of medicinal plants has been examined for their antioxidant properties. Natural antioxidants either in the form of raw extracts or their chemical components are very effective to avoid the destructive processes caused by oxidative stress. Sizeable evidence has collected and indicated key roles for reactive oxygen species (ROS) and other oxidants in causing various disorders and diseases. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health.

Human body has an intrinsic anti-oxidative mechanism and many of the biological functions such as the anti-mutagenic, anti-carcinogenic, and anti-aging responses originate from this property. Antioxidants stabilize or deactivate free radicals, often before they attack

targets in biological cells. Currently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products. In response to stress conditions, plants synthesize secondary metabolites mostly phenolics that improve resistance. These plant phenolics may be soluble and insoluble and complex in nature. Phenolic compounds in fruits, vegetables, spices and herbs are prominent source of antioxidants for humans.

Myrtaceae is a dicotyledonous plant family widely distributed in tropical and subtropical regions of world with 3000 species grouped into 130 genera. These species are mostly woody essential oil bearing plants, with evergreen, alternate, simple leaves [2-6 inches long, 1-2 inches wide, dull green, the odor is with aroma after crushing] usually with entire margin, coriaceous with pronounced veins. The genus *Psidium* comprises approximately 150 species of trees and shrubs, only 20 species produce edible fruits. The most common cultivated species of *Psidium* is *Psidium guajava* L. is an evergreen fruit species common in countries with warmer climate and wide range of soils. It requires annual water supply of 1000-2000 m^3 ha^{-1}, average temperature ranges from 15-30°C. This plant is phytotherapic plant used in traditional folk medicine for the treatment of various human ailments. Fruits, leaves and bark are the plant parts used. The guava leaf show resistance against pathogens, bioactive contents is cineol, tannins, triterpenes, flavonoids, resin, eugenol, malic acid, cellulose, chlorophyll, mineral salts.

P. guajava is nutritionally important since it is excellent source of vitamin C, niacin, riboflavin and vitamin A. The bark has been used for treating diarrhea in children. The leaves are useful for relief of cough, pulmonary disorders, wounds and ulcers. The fruit is tonic, cooling, laxative and antihelmintic (Shen et al., 2008). It has shown several biological activities such as antidiabetic (Oh et al., 2005), anticough, antibacterial (Jaiarj et al., 1999) and antispasmodic actions (Lozoya et al., 2002). The essential oil from guava leaves contain compounds, 1,8-cineole and trans-caryophyllene (Li et al., 1999; Chen et al., 2007; Cole and Setzer, 2007). Pharmacological studies reported important anti-proliferation, anti-oxidant and antimicrobial activities in this essential oil (Sacchetti et al., 2005; Manosroi et al., 2006).

This study analyzed the phytochemical constituents in extracts of guava fruit and the total phenol content was quantified. Guava fruit peel, pulp, seed extracts were prepared with ethanol, ethyl acetate, methanol, sterile distilled water. Organoleptic Characters, Extractive values, phytochemical constituents [Alkaloids, Flavonoids, Saponins, Tannins, Glycosides, and Amino acids] were analyzed in the variety of fruit extracts.

Apple guava (*Psidium guajava*) fruit

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Myrtales
Family:	Myrtaceae
Subfamily:	Myrtoideae
Tribe:	Myrteae
Genus:	*Psidium*
Species:	***P. guajava***
Binomial name	
Psidium guajava L.	

Materials & Methods:

Preparation of fruit extracts: The fruits were collected from local vegetable market, Nizamabad. The samples were washed in tap water, fruit peels, pulp, & seeds were separated, then dried separately at 50°C, pounded to get coarse powder and passed through sieve mesh. 100 g of coarse powder was used to prepare extracts with ethanol, ethyl acetate, methanol, sterile distilled water by maceration extraction procedure. The mixtures were made with 20% concentration in sterile 125 ml Erlenmeyer flask wrapped with aluminum foil to avoid evaporation and exposure to light for 3 days at room temperature. The flasks were placed on platform shaker at 70rpm. After 3 days of soaking in the solvent, the mixtures were transferred to 50 ml tubes and centrifuges for 10 min at 4,000rpm at 25°C. The supernatant was collected and stored until use.

The following parameters of fruit peel, pulp, seed extracts with ethanol, ethyl acetate, methanol, sterile distilled water were studied: Organoleptic Characters, Extractive values, Phytochemical analysis: the chemical tests for the screening of bioactive components were carried out with extracts using the standard procedure described below by using 1ml of each solvent extract for each test except 3ml for saponin test.

Alkaloids: extract was mixed with 1% HCl, 6 drops of Mayer's/Wagner's/ Dragendorff's reagent. Cream/brown/red/orange color indicates the presence of alkaloids.

Flavonoids [Shinoda test]: extract was mixed with Mg ribbon fragments and con. HCl drop wise, orange, red or pink or purple color indicate the presence of flavonoids.

Saponins: extract was placed in test tube and shaken vigorously. The formation of stable foam confirms presence of saponin.

Tannins: extract was mixed with 2% $FeCl_3$ solution, blue-green or black color indicates the presence of tannins.

Glycosides [Keller kiliani test]: extract was mixed with 2ml CH_3COOH containing 2 drops of 2% FeCl3. The mix was poured into another tube containing 2 ml con. H_2SO_4, brown ring indicate presence of glycosides.

Amino acids [Ninhydrin test]

Quantization of total phenols: this was done spectrophotmetrically according to Folin-Ciocalteu phenol method. An aliquot of extract was mixed with 1ml distill water and 0.5ml Folin-Ciocalteu phenol reagent. 2.5ml 20% Na_2CO_3 solution were added to the mixture, followed by incubation for 20 min in dark at room temperature. All samples were assayed in triplicate. Gallic acid was used to prepare a standard curve. The results were expressed in gallic acid equivalents [mgGAE/g]

Results

Tab 1: Organoleptic characters of *Psidium guajava* L.

Characters	Peel	Pulp	Seeds
Colour	Green	White	Creamy
Taste	Bitter	Palatable	Palatable
Odour	Leafy	Aroma	Aroma
Texture	Rough	Rough	Rough

Tab 2: Extractive values of *Psidium guajava* L.

Solvents	Peel	Pulp	Seeds
Ethanol	30	32	31
Ethyl acetate	28	29	24
Methanol	32	24	29
Distill water	18	17.2	19

Tab 3: Phytochemical constituents of *Psidium guajava* L. fruit peel

Phytochemical	Ethanol	Ethyl acetate	Methanol	Distill water
Alkaloids	+	-	+	-
Amino acids	+	+	+	-
Flavonoids	+	-	-	+
Glycosides	+	-	-	+
Saponins	-	-	+	+
Tannins	-	+	-	-

Tab 4: Phytochemical constituents of *Psidium guajava* L. fruit pulp

Phytochemical	Ethanol	Ethyl acetate	Methanol	Distill water
Alkaloids	-	+	+	-
Amino acids	+	+	+	-
Flavonoids	-	-	-	-
Glycosides	+	-	-	+
Saponins	-	-	-	-
Tannins	-	-	-	-

Tab 5: Phytochemical constituents of *Psidium guajava* L. seeds

Phytochemical	Ethanol	Ethyl acetate	Methanol	Distill water
Alkaloids	-	-	+	-
Amino acids	+	+	+	+
Flavonoids	-	-	-	-
Glycosides	-	-	-	-
Saponins	-	-	-	-
Tannins	+	+	+	-

Fig 1: Total phenols of *Psidium guajava* L. Fruit peel Extracts

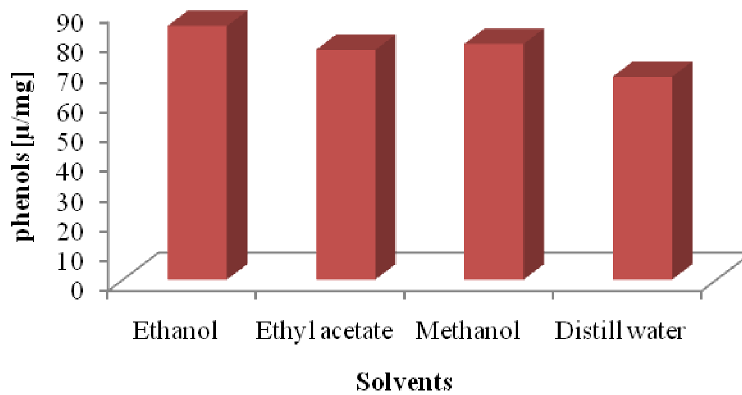


Fig 2: Total phenols of *Psidium guajava* L. Fruit pulp Extracts

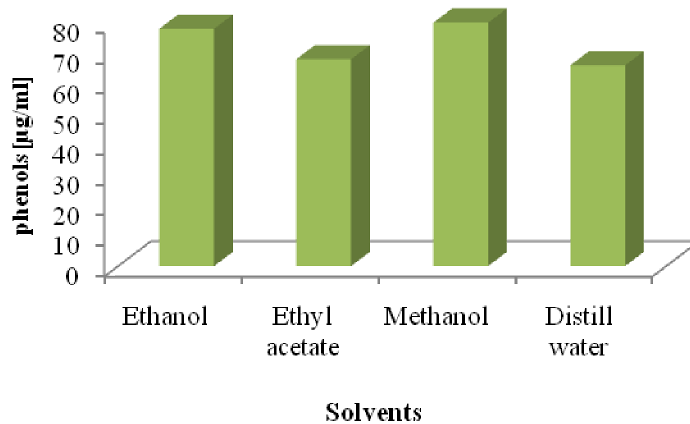
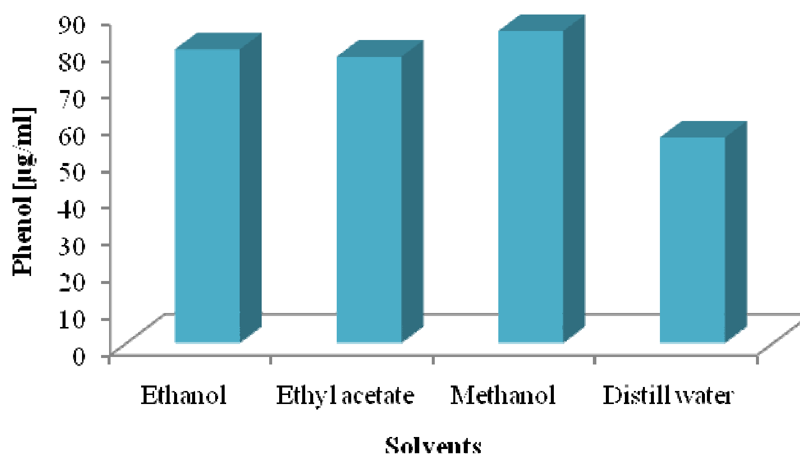


Fig 3: Total Phenols of *Psidium guajava* L. Seed extracts



The results of Organoleptic Characters, Extractive values are illustrated in table 1 and 2. The peel extractive values were high with solvent methanol, pulp, seed values with ethanol. Ethanol could be better solvent to prepare the extracts. Phytochemicals are presented in table 3, 4 and 5. The peel extract with ethanol revealed the presence of alkaloids, amino acids, flavonoids and glycosides, ethanolic extracts followed by methanol and distill water showed three phytochemicals and ethyl acetate showed two chemicals. The fruit pulp extracts with ethanol showed amino acids and glycosides, ethyl acetate and methanolic extracts revealed alkaloids and amino acids. The seeds extracts with ethanol showed positive test for amino acids and tannins, methanolic extracts showed alkaloids, amino acids and tannins. Ethanol and methanol could be better solvents to prepare extracts. The total phenols were presented in fig 1, 2, 3 and ethanol and methanol extracts of fruit peel, pulp and seeds showed high phenols.

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7.

Effect of *Rhizobium* and Herbicides (2,4-D and Pendimethalin) on growth and protein content of *Vigna radiata* (L.)

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Abstract

The effect of *Bradyrhizobium* and two herbicides combination with *Bradyrhizobium* were studied on the growth of *Vigna radiata* (L). The Vigna seeds were grown for 15 days in different concentrations (0, 100, 200 and 300ppm) of herbicides 2, 4-Dichlorophenoxy acetic acid (2,4-D) and Pendimethalin in natural conditions. The results indicated that both herbicides were able to reduce the root, shoot fresh and dry biomass, nodule number, protein and leghaemoglobin content gradually from lower to higher concentration of herbicides.

Keywords: *Bradyrhizobium*, Growth, Herbicide

INTRODUCTION

Vigna radiata (L.) is an important pulse crop in many countries including India where the diet is mostly cereal based. It contains light protein, easily digestible, and does not cause the flatulence as many other legumes do (Arif et al., 2012). This crop is versatile having short growing period and easily fits in different cropping patterns (Kumar et al., 2010). However *Vigna radiata* (L.) productivity often suffers from weed competition, thus requiring herbicides to be used widely (Zaidi et al., 2005). Use of herbicides for weed control in legume field has contributed to increased yield and improved quality (Knott, 1985). Due to extensive and injudicious application, most of the unused fractions of herbicides are known to persist within soils (Madhaiyan et al., 2006). Frequently, herbicides not only affect plant growth but also have a detrimental effect on soil micro-organisms growth and metabolism (Sawicka, 1996). The legume-rhizobia symbiosis has a unique importance in agriculture. The symbiosis results in huge quantities of nitrogen fixation throughout the world and any adverse effect on rhizobia results in reduced rates of biological nitrogen fixation. Many factors influence the growth of nitrogen fixing bacteria. Herbicides as one of them may also influence the growth of rhizobia (Singh and Wright, 2002). They have been reported to

exhibit negative effects on the growth of rhizobia (Martensson, 1992). Herbicides influence nodulation and biological nitrogen fixation in legumes either by affecting rhizobia, or their host plant or both. The magnitude of the toxic effects of herbicides, however, depends primarily on the type and dose of compounds, duration of exposure, species and age of plants, and other environmental factors (Zaidi et al., 2005).

MATERIALS AND METHODS

The field experiment was conducted during the month of April to June in 2014 to evaluate the effect of two herbicides on growth and nodulation in *Vigna radiata* (L.). The experiments were carried out in the field of the Department of Botany, C.C.S. University Meerut. Experimental field designed in eight plots of equal size, seven plots for treatment and one plot for control. Seven treatments performed in the present work were as follows:

1) Treatment with *Rhizobium* alone.
2) Treatment with 100ppm concentration of pendimethalin+*Rhizobium*
3) Treatment with 200ppm concentration of pendimethalin+*Rhizobium*
4) Treatment with 300ppm concentration of Pendimethalin+*Rhizobium*
5) Treatment with 100ppm concentration of 2,4-D+*Rhizobium*
6) Treatment with 200ppm concentration of 2,4-D+*Rhizobium*
7) Treatment with 300ppm concentration of 2,4-D+*Rhizobium*

Healthy seeds of *Vigna radiata* (L.) were selected and washed with distilled water. 2,4-D (Dicholorophenoxy acetic acid) recognised as synthetic auxin, act as plant growth regulator at lower concentration and as growth retardant at higher concentration. Pendimethalin is used as an herbicide prevents the growth of certain plants acting as a root inhibitor. Pendimethalin (30% EC Bond Herbicide) and 2,4-D (Dimethyl Amine Salt 58% WSC Weedicide Zura) were appropriately diluted with distilled water to the soil their final concentration of 100, 200 and 300ppm. The herbicides were added to the soil as pre-sowing application to moist soil, 24h before sowing.

Fifty healthy seeds of *Vigna radiata* (L.) were sown in each plot. The seed germination percentage was calculated after counting the difference between germinated (coming out of the soil) and non-germinated seeds (remaining inside, non emergent). All plants in the three pots for each treatment were removed 30 days after seeding (DAS) and were observed for the extent of nodulation. The roots were carefully washed and nodules were detached, counted, oven dried (at 60°C) and weighed. Nodules were detached from the plant root with the help of forceps. Fresh weight of

nodules was measured immediately and followed by dry weight after drying them at 60ºC for 48 h to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. The leghaemoglobin (Lb) content of fresh nodules was quantified at 50 DAS (Sadasivam and Manikam, 1996).

RESULTS AND DISCUSSION

Seed germination

The seed germination was measured at the different time-intervels i.e. 5 day after sowing (DAS), 10 DAS and 15 DAS. Germination in the control seeds was found higher than treated seeds. *Rhizobium* improves the germination percentage in the *Vigna radiata* L. as compared to pendimethiln+*Rhizobium* and 2,4-D.+*Rhizobium* (Table1). The final germination percentage reduced progressively with the increasing concentration of herbicides 100, 200 and 300ppm. Both 2,4-D and pendimethalin herbicide showed inhibitory effect on seed germination. Among the treatments 2,4-D showed maximum inhibition in seed germination. Reduction in germination by 2,4-D is the result of drastic inhibition of root growth due to its strong phytohormonal action (Audus, 1977). At high concentration 2,4-D is reported to inhibit amylase activity resulting into the suppression of root and shoot growth (Shaukat, 1976).

Shoot and Root length

Finding with the effect of *Rhizobium* and two herbicides on *Vigna radiata* L. growth are given in Table 2. In general root and shoot length of plant were found significantly higher with 100ppm treatment of pendimethalin+*Rhizobium*. *Rhizobium* showed remarkable increase in growth parameters as compared to control and 2,4-D+rhizobium. The maximum reduction in root length and shoot length occurred at 300ppm concentration of 2,4-D+*Rhizobium*. An adverse effect of herbicides on chickpea vitality and subsequently the *Mesorhizobium*-chickpea symbiosis is also reported earlier (Khan et al., 2014). On the contrary lower dose of herbicides are stimulate known to the growth in *Vigna radiata* L. and this suggests that the lower doses might have persisted in the soil for only a short time period, after which the viable cells of *Bradyrhizobium* get recovered and multiplied rapidly (Zaidi et al., 2005). This is possible because the soil environment can act as a buffer, reducing the potentially toxic effect by dilution of these chemicals (Castro et al., 1997).

Plant biomass

Fresh and dry weights of plant were found significantly higher in control plants over the *Rhizobium* and herbicides (Table 3). 100ppm concentration of pendimethalin+*Rhizobium* showed remarkable increase in fresh biomass of shoot and root and dry biomass of shoot as compared to *Rhizobium* and 200, 300ppm concentrations of herbicides with *Rhizobium*. The maximum increase in dry biomass was observed with *Rhizobium*. Higher concentrations of 2,4-D+*Rhizobium* reduced the fresh and dry biomass of *Vigna radiata* L., possibly due to premature senescence of the plant (Zaidi et al., 2005). The phytotoxic action of 2,4-D occurs largely as a result of its ability to mimic the activity of endogenous auxin. The excessively high concentration of auxin-active herbicides, in turn, alerts the regulation of plant metabolism, leading to the loss of cellular function, cellular integrity and repair capacities of plants (Nishitani and Masuda, 1981). Fox et al. (2007) reported a considerable decrease in nodulation, total plant biomass and nitrogenase activity of alfalfa (*Medicago sativa* L.), when grown in soil treated separately with different herbicides. The variable response of the tested legumes to herbicides is explained on the basis of extent of toxicity of any specific herbicide to the plants which in turn depends upon both the genetics and physiology of plants, varying from species to species (Ahemad and Khan, 2011c).

Nodulation

The number of the nodules was higher in *Rhizobium* treatment as compared to the herbicides application. Infection of roots of legumes by *Rhizobium* in takes place through the root hairs so that the process of nodulation of legumes is undoubtedly linked to the expression of the various parts of the root system. Therefore, it is possible that herbicides which induce a reduction in nodules formed per plant may do this by restricting root growth and or formation of lateral roots, and hence the number of root sites available for infection. Generally, the lower concentrations of herbicides improve the nodulation in the *Vigna radiata* L. possibly because at a sub-lethal dose, the herbicide may induce damage to xylem vessel without adversely affecting the nodular bacteroids, and hence the greengram-*Bradyrhizobium* symbiosis remained unaffected. *Rhizobium* treated plants show greater increase in values of nodules fresh and dry weight of nodules plant^{-1} as compared to herbicide treated plants and control (Table 4). The herbicides induce decline in nodulation in general that could be because of the inhibition of symbiosis process or the herbicide might have

interfered with the chemotactic motility between the legume root and the bacteria (Khan et al., 2004).

However, among the two herbicides, 2,4-D had a greater adverse effect on nodule formation, suggesting that this herbicide is highly toxic for *bradyrhizobium-Vigna radiata* (L.) symbiosis. According to Anderson et al. (2004), herbicides negatively affect the nodulation in legumes by limiting the number of available sites on host plants to cognate *Rhizobium* by decreasing the carbohydrate supply to existing nodules. Thus, herbicides are known to decrease the rhizobial survival and growth, to inactivate the biochemical signaling required to initiate nodule development in plants and to inhibit the nodule development by reducing cell division. Likewise of toxicity of herbicides on nodulation and N2 Fixation in soybean (Malik and Tesfai, 1985) and chickpea (Khan et al., 2004) have also previously been reported.

Protein content

The effect of *Rhizobium* treated plants showed a greater increase in protein content than in control and herbicide treated plants (Table 5). 300ppm concentrations of pendimethalin+*Rhizobium* and 2,4-D+*Rhizobium* showed adverse effect on protein content. The effects of herbicides are known by the type and rates of their application, health and stage of plant growth, as well as other environmental variables (Kumar, 2012). Morphological changes and disturbances in cell division due to impact of both the herbicides used individually and their combinations (Shamsi et al., 2006, Qasem 2007, Kumar et al., 2010), are now well established.

Egli et al. (1985) reported that many herbicides interfere with the protein synthesis, for instance, atrazine or diuron inhibits the synthesis of protein in *Solanum nigrum* cell suspension. This might be the unspecific disruption of the cell metabolism by large amounts of the applied exogenous compounds. There are also some reports which exhibit that there is no direct effect of herbicides on protein or nucleic acid synthesis, probably because neither of these sites is primary site of action of any commercial herbicide (Khan et al., 2006). However the high concentrations of the herbicides may affect the enzymatic reaction responsible for protein biosynthesis. Reduction in protein content following herbicide application could probably, be due to inhibition of the enzymes and functional proteins of metabolic pathways involved in protein synthesis (Nare et al., 2010; Ahemad and Khan, 2011).

Leghaemoglobin content

Leghaemoglobin content is the precise parameters to assess the actual impact of any stress factor on nodulation. Its content was significantly higher in *Rhizobium* treated plants (Table 5) and it was maximum at 100ppm concentration of 2,4-D+*Rhizobium* when compare to higher concentrations of 2,4-D+*Rhizobium*, pendimethalin+*Rhizobium* and control. Herbicides decrease leghaemoglobin content in nodules of the different legumes as recently reported by Ahmed (2014). The decline in Lb content of legumes could be due to the toxic effects of herbicide on plant organs, especially the function of nodules which consequently disrupts the legume-*Rhizobium* symbiosis and hence, the N2 fixation and in turn the overall plant growth (Evans et al., 1991). In addition, the inhibitory effect of the herbicide application may possibly be due to (i) the inhibition of enzymes involved in growth metabolisms (Zablotowicz and Reddy, 2004) and (ii) disruption of signaling between legume derived phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors (Fox et al., 2007).

Conclusions

Different symbiotic attributes of the tested legumes were also assessed under herbicide-stress which showed varying degree of toxicity to the selected legumes. Nodulation in legumes is an important growth parameter. Comparative evaluation of nodule numbers of legume species did not provide any accurate assessment on the size of nodules which varied from one legume species to another. Pyriproxyfen was observed as a highly toxic substance to seed germination, root and shoot dry mass nodulation, leghaemoglobin content and protein content.

Acknowledgements
The authors are grateful to the Department of Botany, C.C.S. University, Meerut, for providing all facilities.

Table1: Seed germination percentage of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**5 DOS**	**10 DOS**	**15 DOS**
Control	78	90	92
Rhizobium	72	86	87
2,4-D+*Rhizobium* 100ppm	40	84	85
2,4-D+*Rhizobium* 200ppm	2	72	74
2,4-D+*Rhizobium* 300ppm	2	48	62
Pendimethalin+*Rhizobium* 100ppm	74	83	84
Pendimethalin+*Rhizobium* 200ppm	52	76	80
Pendimethalin+*Rhizobium* 300ppm	62	76	78

Table 2: Root length and shoot length of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**Plant length (cm)**	**Root length (cm)**	**Shoot length (cm)**
Control	36	7	29
Rhizobium	37	7.6	29.4
2,4-D+*Rhizobium* 100ppm	31.4	6.6	24.8
2,4-D+*Rhizobium* 200ppm	18.8	3.8	15.4
2,4-D+*Rhizobium* 300ppm	16.2	3.4	11
Pendimethalin+*Rhizobium* 100ppm	37.4	8.6	28.8
Pendimethalin+*Rhizobium* 200ppm	32.8	10.8	22
Pendimethalin+*Rhizobium* 300ppm	25	6.2	18.8

Table 3: Root and shoot fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**Root**		**Shoot**	
	Fresh wt. (gm)	**Dry wt. (gm)**	**Fresh wt. (gm)**	**Dry wt. (gm)**
Control	0.333	0.2146	4.2496	2.1452
Rhizobium	0.3528	0.2254	3.7164	1.9782
2,4-D+*Rhizobium* 100ppm	0.146	0.1236	1.6764	1.116
2,4-D+*Rhizobium* 200ppm	0.0662	0.065	0.4812	0.3644
2,4-D+*Rhizobium* 300ppm	0.474	0.1155	0.397	0.3096
Pendimethalin+*Rhizobium* 100ppm	0.3528	0.2228	4.0344	2.1008
Pendimethalin+*Rhizobium* 200ppm	0.2138	0.1566	2.7648	1.6484
Pendimethalin+*Rhizobium* 300ppm	0.2082	0.134	2.1994	1.1368

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Table 4: Nodule number, volume, fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**Nodule no.**	**Volume**	**Fresh wt. (gm)**	**Dry wt. (gm)**
Control	38	0.9	0.0372	0.0198
Rhizobium	53.8	1.4	0.0596	0.0306
2,4-D+*Rhizobium* 100ppm	21	1.2	0.0508	0.0246
2,4-D+*Rhizobium* 200ppm	15.4	0.7	0.242	0.0092
2,4-D+*Rhizobium* 300ppm	5.6	0.9	0.0154	0.0058
Pendimethalin+*Rhizobium* 100ppm	16.2	1.3	0.02066	0.0212
Pendimethalin+*Rhizobium* 200ppm	7.8	0.5	0.0416	0.0122
Pendimethalin+*Rhizobium* 300ppm	9.2	0.4	0.0282	0.0186

Table 5: Protein and Leghaemoglobin content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**Protein mg/g fresh wt.**	**Leghaemoglobin mM (g.f.m.)$^{-1}$**
Control	3.0482	0.005
Rhizobium	3.9729	0.009
2,4-D+*Rhizobium* 100ppm	3.0321	0.006
2,4-D+*Rhizobium* 200ppm	2.0126	0.003
2,4-D+*Rhizobium* 300ppm	1.7041	0.002
Pendimethalin+*Rhizobium* 100ppm	2.9577	0.007
Pendimethalin+*Rhizobium* 200ppm	2.7263	0.004
Pendimethalin+*Rhizobium* 300ppm	1.5248	0.003

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8.

STUDIES ON THE MEDICINAL PLANTS OF ETHNOPAEDIATRIC IMPORTANCE IN MAHADEVAPUR RESERVE FOREST OF KARIMNAGAR EAST DIVISION OF (A.P.) INDIA

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ABSTRACT

As the subject-Ethnopaediatric medicinal plants are very poorly known, therefore the present Ethnopaediatric information may prove helpful for further scientific studies. In recent years no valuable study has been made out on Ethnopaediatric medicinal plants. As, owing to complex topography, variety of climatic parameters, and lack of developmental facilities, the forest region of Bhubaneswar of Orissa' India, has been neglected. As this area is ethno-botanically very rich, as such we have conducted ethno-botanical explorations, with a view to collect information on Ethnopaediatric medicinal plants along with the information on their uses in traditional medicines as practiced by tribal and medicine men of the region since time immemorial. The tribals are, by nature, reluctant to go to the hospital and have a great faith and insist on their own traditional system of medicine. Still the tribals use these plants frequently to cure diseases. The knowledge of this traditional medicine is intact in this region and they use this knowledge mostly for their daily requirements, due to lack of modern facilities. The tribals are very conservative in nature and they do not easily mixed up with other communities for exchange or to share their empirical knowledge. The tribal people are usually not willing to disclose their knowledge about the uses of plant wealth. In general they maintain secrecy about the use of certain medicinal plants with a belief that the medicines may lose their healing power if too many heads know about them. In the present studies, we aimed to envisage about twenty of the herbal medicinal plants such as from *Abrus*

precatorius to *Vicoa indica* which are specially prescribed for children by local tribal and village medicine men are discussed in this paper.

INTRODUCTION

During our Ethnobotanical field study in Bhubaneswar of Orissa India, the “Deshari”, “Bijunis” (Tribal Medicine Men) and other informers from different villages extending from 25-30 were consulted. The informers include responsible old experienced persons, village medicine men, who are fully aware about their forest wealth. The Tribal Medicine Men look after the welfare of the tribal society and do the needful for the prosperity of the tribal village too.

During the course of studies, besides the “Deshari” and “Bijunis”, the local inhabitants also been contacted with the help of cross enquiries within the same community of the different villages, and the collected data were confined and authenticated. The collected data was compared with the literature on medicinal plants like Watt (1888-93), Dastur (1915), Kirtikar and Basu (1935), Chopra *et. al.,* (1956, 1969), Wealth of India (1948-1976). Besides this monumental work, the recent Ethnobotanical works in Andrapradesh viz Mahadevapur (1963), Mudgal and Pal (1980), Tribedi *et al.,* (1982), Srivastava and Rout (1994) and it was found that most of the information are not recorded in above published accounts.

MATERIAL AND METHODS

The tribals who have the knowledge of medicinal plants were taken into the forest area in their village vicinity. Country yards of the villagers and protected inhabitants near dwellings were searched and information on the cultivation and protection of the wild medicinal plants were collected. Information was also gathered on parts used of the wild medicinal plants.

The vernacular names in “Kondh” given by the medicine men were checked from local flora and their botanical names with family names were identified by taking the help of books and records of local herbarium available. All the voucher specimens were deposited in the Department of Botany, Utkal University Bhubaneswar’ Orissa, India.

Enumeration of the species: The species are arranged alphabetically by their botanical names followed by vernacular names and family name. Also, it has been noticed that, most of the prescription made by “Deshari” and “Bijunis” are restricted specifically for the treatment

of children. Generally, they prescribed in the form of powders, pills, decoctions, and infusions for various ailments along with worship, devination and exorcism.

RESULTS AND DISCUSSION

1. ***Abrus precatorius* (Kaichaamulo, and Gunjankai)-Leguminosae.**

Eight to ten seeds of *Abrus precatorius* and three to four roots of *Wrightia tomentosa* are mixed and made into about fifteen grams and grinded into powder. This powder is equally divided in to six to eight doses and mixed with honey into paste and it is prescribed orally in empty stomach for fever.

2. ***Acacia catechu.* Willd. (Khair, Kingri)-Mimosaceae.**

Ten grams of crushed and dried stem bark is grinded into powder. This powder is made into five doses. Each dose is mixed with honey and given orally for throat pain.

3. ***Adhatoda vasica* Nees. = *Justicia adhatoda* Linn. = *A. zeylanica* Medicus. (Bansa, Bhansoi)-Acanthaceae.**

Five grams of fresh root is grinded into paste and mixed with honey and is given for whooping cough. Powdered leaf is applied for skin diseases. Grinded fresh leaf is applied on forehead for headaches.

4. ***Alangium salvifolium* L. (Ankul)-Alangiaceae.**

Two inches long root of *Alangium salvifolium* is powdered along with 7-8 dry fruits of *Ficus religiosa* and made into four doses. Each dose is prescribed twice daily with honey or breast milk in cold, cough and pass out phlegm.

5. ***Bambusa tulda* Roxb. (Baunsh, Dercu)-Bambacaceae.**

Dried tender culm is powdered. The powder is mixed with paste of ginger and honey. This mixture is given thrice orally per day for five days in fever.

6. ***Calanthe triplicata* Ames. (Musa kanda)-Orchidaceae.**

Paste of the fresh tuber of *Calanthe triplicata* and three seeds of *Piper nigrum* is orally prescribed thrice a day for dysentery.

7. *Cassia siamea* Lam. (Badasakund)-Ceasalpiniaceae.

The paste of the seeds of *Cassia siamea* and *Piper nigrum* seeds in 5:1 is prescribed orally with water to stop vomiting. The seeds of *C. accidentalis* is also used as substitute.

8. *Crotalaria juncia* L. (Uturuli)-Leguminosae.

Seven pieces of roots of *Crotalaria* of about 2-3 cm long are tied in a thread and given to wear as garland in malarial fever. The flowers are also used as garland in other fevers.

9. *Desmodium trifolium* (L.) D.C. (Hopkamara)-Leguminosae.

Root paste is applied in skin diseases for external use only.

10. *Diospyros melanoxylon* Roxb. (Kendu)-Ebenaceae.

Ten green fruits are boiled in about one liter of water till it comes down to one-fourth. The decoction is orally prescribed in dysentery.

11. *Elephantopus scaber* L . (Mayrichiriae)-Asteraceae.

Three to five grams of Elephantopus root and three seeds of black pepper are made into paste, which is orally given as laxative, where as the root extract as appetizer and given to children of age group below one year.

12. *Gardenia gummifera* L. (Kumbamara)-Rubiaceae.

The small bark pieces, and peacock feathers are alternately tied to a thread and made as a garland. This garland is given to wear on neck to stop vomiting after feeding milk.

13. *Justicia betonica* L. (Kataali)-Acanthaceae.

Root pulp is applied all over the body to lower down the body temperature, during the high fever.

14. *Mangifera indica* L . (Amba) Anacardiaceae.

Powder of tender fruits is given with breast milk in dysentery.

15. *Mimosa pudica* L. (Lajkali)-Mimosae.

The extract of root and leaves powder is prescribed in fever due to enlargement of spleen. The residue is also applied externally for the same purpose over the stomach.

16. *Oroxylum indicum* vent. (Papni)-Bignoniaceae.

Oroxylum bark powder is mixed with the powder of *Curcuma longa* in 3:1 ratio is orally given in interlunar night to keep away the evil eyes which is believed to cause unconsciousness with high fever.

17. *Pavetta indica* L. (Agamatt)-Rubiaceae.

The leaves are boiled in an earthen pot and given to chew with common salt in intermittent fever and cough.

18. *Solanum surattense.* Burm. F. (Chakabhedi)-Solanaceae.

Plant paste is kept in ash of cow dung for five to six hours, and which is mixed with the bark powder of *Ficus religiosa* in equal ratio and is given twice daily for ten days in hooping coughing.

Powder of roasted twig is prescribed orally with honey twice daily for seven days in cough

19. *Urena lobata* L. (Chikni)-Malvaceae.

The leaves and fruits are burnt into ash. The ash is mixed with the oil of *Linum usitatissimum* and the paste is applied locally on wounds.

20. *Vincoa indica* .D. C. (Banaalosi)-Asteraceae.

The whole plant is burnt into ash. This ash is mixed with the oil of *Brassic campestris* and the paste is locally applied on wounds and eczema below the knees.

The above twenty species belong to twenty genera belonging to eighteen families are employed in different ailments by the tribal people of Bhubaneswar of Orissa India. It is seemed that the Leguminosae family came into the first position in treating the children, and the field survey is also envisaged the same. In the country like India, where the death rate of children, particularly in rural areas, is much higher than the other developed countries in the world. Hence the scope of this type of study is very promising and important and it may give new source of drug plants in pediatric diseases.

CONCLUSION

There has been increased interest on medicinal plants in conservation view point and for economic view point of development of Indian Herbal Medicine. The recently constituted State Medicinal Plant Board can catalyze these activities. There is also a need for greater

attention to the prioritized species and medicinal plant conservation areas. A net work of the medicinal plant gardens and protected areas can add to effective conservation. With all these programmes and involvement of the people of the state is poised to take a quantum leap towards the rapid progress in herbal health care.

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9.

Microbiological Quality of some packed mineral water

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Abstract

Water is essential for the existence of life. Changes in physical, chemical and biological nature of water adversely affect the human health. Clean fresh water that is devoid of any pollutants is necessary for the consumption. Water that is fit for drinking that doesn't cause any health problems can be called as potable water. The availability of potable water to everyone has nearly become impossible and so the mineral water came into existence as an alternative.

Mineral water is packed potable water with added minerals obtained from an approved underground source. WHO stipulated the standard conditions for the potability of water. So an attempt was made to analyze some commercial mineral water samples packed in both bottles and sachets for the presence of coliforms, total heterotrophic bacterial populations and water borne pathogens on the basis of their microscopic, biochemical and physiological characteristics by adopting standard methodologies. It was found that sachets contain high bacterial load among all packed mineral water.

The heterotrophic bacterial load in marketed mineral water threatens the health of humans. So the companies which are providing mineral water with any bacterial load is to be banned and proper action is to be taken by the higher authorities to promote public health.

Key Words: mineral water, heterotrophic bacteria, pathogen, WHO.

Introduction:

The major problem faced by the mankind today is the pollution. Of which, water pollution is considered to be more dangerous. Consumption of polluted water can become hazardous to human life so purified water is essential. As per norms fixed by WHO, the suitability of water with permissible limits for drinking purpose is given in table 1.

Table1: WHO recommended characteristics of water

PARAMETER	MAXIMUM ALLOWABLE LIMIT
pH	6.5-8.5
Total Dissolved Solids	500mg/ L
Total Hardness in terms of Carbonates and bicarbonates	300mg/ L
Chlorides	250mg/ L
Dissolved oxygen	500mg/ L
Chemical oxygen demand	200mg/ L
Coliforms	<4/100ml of water
Pathogenic bacteria	Nil

Mineral water is defined as potable water which is free of microorganisms with added minerals, which is now safe for drinking. There are some regulation to control their composition, packaging, labelling and advertising of water. Here mineral water is available in both bottles and sachets; these are drawn for microbial examination. This commercially available mineral water free from coliforms but the presence of microorganisms from the water has been reported. This present study has been taken up to bring out awareness among the people about mineral water quality standards regarding the economy of bottled and sachets water.

Materials and Methods:

Mineral water samples that are sachet and bottled mineral water were collected from in and around Kothagudem, Khammam district and were brought to the laboratory for bacteriological examination. Various characteristics of bacteria are taken into consideration for identification. Enumeration is done by MPN (Most Probable Number) 3 tube method (K.R.Aneja). Other bacterial load was identified on the basis of cultural, microscopic and biochemical characteristics (fermentation of sugars, IMVIC tests) etc.

Results and Discussion:

Both sachet and bottled mineral water were found to contain no coliforms as per MPN index table. This confirms the absence of E.coli and Enterobacter aerogens. But these samples were tested for other bacterial load, which was given in below tables.

S.No	SAMPLE TESTED (BOTTLED WATER)	TOTAL BACTERIAL LOAD (CFU/ml)
1.	Sample 1	Nil
2.	Sample 2	3.0×10^1
3.	Sample 3	1.24×10^2
4.	Sample 4	Nil

S.No	SAMPLE TESTED (SACHET WATER)	TOTAL BACTERIAL LOAD (CFU/ml)
1.	Sample 1	5.7×10^1
2.	Sample 2	4.0×10^1
3.	Sample 3	1.12×10^2
4.	Sample 4	1.24×10^2

The above samples belong to different brands.

To ensure the particular genera the following microscopic and biochemical tests were conducted and their results are as follows

S. No	Organism	Agar slant Cultural characteristics	Gram stain	Lactose fermentation	Indole sucrose	MR	VP	Citrate	NR
1	Pseudomonas	Abundant thin white growth with medium turning blue	G-ve Rod	No acid & gas	No acid & gas	-ve	-ve	-ve	+ve
2	Staphylococcus aureus	Abundant opaque, golden growth	G+ve Cocci	Acid production	Acid production	-ve	+ve	_	-ve
3	Micrococcus	Soft smooth, yellow growth	G+ve Cocci	No acid & gas	No acid & gas	-ve	-ve	-ve	-ve

4	Bacillus	Abundant opaque white waxy growth	G+ve Rod	No acid & gas	Acid producer but No gas	-ve	-ve	_	-ve

MR- Methyl Red, VP- Voges Proskeur, NR- Nitrate Reduction

This table represents microbial load in the mineral water sachets

Mineral water sachets	Pseudomonas	Micrococcus	Bacillus	Staphylococcus
Sample 1	-	-	-	+
Sample 2	-	+	+	-
Sample 3	-	+	+	-
Sample 4	+	+	-	-

On the basis of above observations faecal coliforms are completely absent, but some of the brands are having some bacterial load, which is more in sachets than that of bottled water. Bacterial load contain different types of microorganisms, some of them are Pseudomonas sps, Bacillus sps, Micrococcus, Staphylococcus. As per the results mentioned above, sachet mineral water is not safe for drinking compared to bottled water. These mineral water bottles are recommended to store at low temperature conditions till its use in order to control proliferation of bacteria. The higher authorities should take up proper action to ban such sachet mineral water brands and thereby improve their quality standards.

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10.

Diversity, Conservation Status and Medicinal Importance of *Selaginella* spp.

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ABSTRACT

Selaginella is considered as the first vascular plant on the earth which existed before 300 million years. It has shown wide range of distribution with especially tropical regions of the world, rare in the temperate regions and almost absent in the alpine zone. Some spp of *Selaginella* are xerophytic, (*S.lepidophylla*), epiphytic (*S.oregana*) and cultivated as ornamentals (*S.krussiana* and *S.pygmea*). Majority of *Selaginella* spp were extinct and few are in serious threat. From ancient days *Selaginella* spp have been used traditionally to cure several diseases such as cancer, pneumonia, tonsils, kidney stones, headache, fever, skin diseases, bone fractures, snakebites and scorpion bites. The bio active compounds of *Selaginella* spp like alkaloids, tannins, flavonoids are useful medicinally as antimicrobial, antioxidant, anti-inflammatory, anti UV irradiation, anti allergy, anti hemorrhagic, antinocieptive and anti diabetic. Many *Selaginella* spp have shown high desiccation tolerance under severe environmental stress due to the presence of trehaloses. Hence these are termed as "Resurrection plants". The present paper reveals the biodiversity, conservation status and medicinal importance of few *Selaginella* spp like *S.convoluta, S.involvens, S.moellendorffi, S.rupestris, S.lepidophylla, S.unicata, S.bryopteris and S.apoda.*

Key words:- *Selaginella* ,Diversity, Conservation and Flavonoids.

INTRODUCTION

Selaginella is an ancient plant which has been exisisting since 300 million years. ***Selaginella*** has been distributed worldwide, especially found in China, Japan, Russia, U.S.A, Malaysia, Myanmar, Indonesia, Columbia, India, Nepal, Tibet, Bhutan and Srilanka. ***Selaginella*** is traditionally used to cure several diseases like wounds, fever, cancer, pneumonia, tonsils,

kidney stones, headache, hepatitis, fever, paralysis skin diseases, bone fractures, toothache, blood coagulation, diarrhea, gastric ulcers, asthma, backache, blood purification, fatigue, and to neutralize the poison of snake and scorpion bites (Dixit and Bhatt, 1974, Bouquet and Lee et al.1992, Batugal et al 2004, Setyawan and Darusman2008). Recent research proved that *Selaginella* spp have secondary metabolites like bioflavonoid(Silva et al.,1995,and Lee et al.,1996), tannins(Chickmavathi et al.,2008), glycosides(Man and Thakshiki,2002) alkaloids (Zheng et al.,2004) and Selaginellin (Zhang etal,2007:Cheng et al., 2008). ***Selaginella* spp** show anti inflammatory, anti allergic, hepato protective, anti viral, anti carcinogenic effects due the presence of flavonoids (Gayathri etal., 2005). Alkaloids of ***Selaginella* spp** have diuretic, antiplasmodic, anti inflammatory effect (Gayathri etal.,2005)and tannins show anti bacterial and anti parasitic effects(Kolodziej etal,2005).

The Diversity and conservation data of different *Selaginella* spp has been collected from **Nature Conservation System , IUCN**, USA and considered the following status globally (Global status).

- (EX) – No known individuals remaining.
- Extinct in the wild (EW) – Known only to survive in captivity, or as a naturalized population outside its historic range.
- Critically endangered (CR) – Extremely high risk of extinction in the wild.
- Endangered (EN) – High risk of extinction in the wild.
- Vulnerable (VU) – High risk of endangerment in the wild.
- Near Threatened (NT) – Likely to become endangered in the near future.
- Least Concerned (LC) – Lowest risk. Does not qualify for a more at risk category. Widespread and abundant taxa are included in this category.
- Data deficient (DD) – Not enough data to make an assessment of its risk of extinction.
- Not Evaluated (NE).

Research Methodology

The research paper entitled "**Diversity, Conservation Status and Medicinal Importance of *Selaginella* spp.**" is based largely on desk research and analysis. A detailed literature review has been undertaken to identify relevant data. The primary and secondary source of data acquired from International scientific official websites, bulletins of different Scientific

Research Institutes, Scientific Journals, Articles, Reviews, Abstracts, IUCN Red data list, Newspapers, Google search...etc.

Biodiversity of *Selaginella* spp in India-:

Above seventy species of *Selaginella* grow in the tropical and sub tropical forests along the Himalayas and in the plains of India. Majority of the species grow in the Eastern Himalayas(*S.caulensis* and *S.biformis*)few in the Northern-Western Himalayas(*S.adunca* and *S.chryoscaulos*),Central India(*S.bryopteris* and *S.rupestris*), Kodaikanal (*S.involvens, S.radiata and S.cactarum*), Malabar(*S.rodrigaesiana*), Eastern India (*S.*willdenovii and S.*multifloia*) (Panigrahi and Dixit 1966-1968, Panigrhi,1978) .

List of Endangered species with total distributions and IUCN category of *Selaginella* spp.

The present list expressed that of threatened *Selaginella* given by Chandra et al. 2008, Christopher Roy, 2012) as it concerns only the top six IUCN categories, EX (Extinct), EW (Extinct in the wild),CR(Critically endangered),EN(Endangered),VU(Vulnerable)and NT (Near threatened).The IUCN categories given here apply to political India only with Statistic summary of the categorized threatened species.

1) ***Selaginella agustyamalayana*** Endemic to South India. Perhaps a synonym of *S. cataractarum* Alston, requiring further study. (CR. R. Antony, S.Khan et al G. S. Nair - S.).
2) ***Selaginella adunca* subsp. *adunca*** -N.W.India (Himachal Pradesh; Uttarakhand; locally abundant); W. Nepal, rare. Listed by IUCN (1998) as Endangered, in error. Alston`s (1945) record from Kashmir was in error for Sri-nagar, Garhwal, Uttarakhand].

3) ***Selaginella aitchisonii* Hieron**.—Tien Shan; Sinkiang; Afghanistan; N. W. Pakistan, very rare; India (Jammu & Kashmir; very rare).Turkestan was also listed by Dixit (1992a). It appears that this is not just an ecotype of *S. sanguinolenta*, but a separate species. EN;Globally threatened.

4) ***Selaginella cataractarum* Alston** —S.India (Tamil Nadu; very rare and partly extinct). Reported in error from Kerala and Orissa by Dixit (1984, 1992a). Listed as endangered by IUCN (1998). CR; globally threatened.

5) ***Selaginella kurzii Baker***— N.E. India (Mizoram; very rare); Myanmar; Thailand; Malaysia. Reported in error from Nepal and in a wide sense from Assam, but meaning Mizoram. CR.

6) ***Selaginella miniatospora*** **(Dalzell) Baker** (syn.: S. blatteri Bole et M. R. Almeida; type not found at BLAT by the author) —S. India (Maharashtra; Goa; Karnataka; rare and very restricted). Endemic to S.W. India. Its relationship to the similar N. Indian etc. species. *S. tenuifolia* Spring requires study. NT; Glob-ally threatened.

7) ***Selaginella pulvinata (*****Hook.et Grev**.)Maxim. - N. W.India (Uttarakhand, Pithor-agarh; very rare); N. W. Nepal, very rare; N.E.India (Assam, herb. Kew (K) (Alston 1945), presumably a collection from Mishmee, northern Arunachal Pradesh, by W. Griffith); Myan-mar (Mandalay; reported erroneously from Moulmein); Tibet; China, widespread. EN.

8) ***Selaginella wattii*** **Baker** — N.E. India(Manipur; very rare, known only from the type); Myanmar. Endemic to N.E.India. Reported from Myanmar by Dixit (1984,1992a) but without details, and requiring confirmation, and not so reported by Alston(1945); reported from Mizoram, Nagaland and Bangladesh by Ghosh et al. (2004) in error for *S. chrysorrhizos*. CR; globally threatened.

Diversity, Conservation Status and Medicinal Importance of Few Selaginella spp

Selaginella apoda (L.)Spring

Distribution-: ***Selaginella apoda*** **(L.)Spring** is a lycophyte native to much of the Eastern USA, parts of NE-USA,C-USA, S-USA (Alabama, Arkansas, Connecticut, Delaware, Florida, Georgia, Illinois, Indiana, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Mississippi, Missouri, New Hampshire, New Jersey, New York, North Carolina, Ohio, Oklahoma, Texas Pennsylvania, Rhode Island, South Carolina, Tennessee, Virginia, West Virginia, Cuba, Mexico ,Guatemala, Chile) and NE-Germany.

Common name-: Meadow Spike-moss, Basket Spike moss

Conservation status-: G5-Secure (common, widespread, abundant, and lacking major threats or long-term concerns).

Medicinal importance-: The water extraction of ***S. apoda*** **(L.)Spring** show anti bacterial activity.

***Selaginella bryopteris* (L.)Bak**

Distribution-: India (throughout)

Common name-: Sanjeevani

Conservation status-: Near Treatened (NT) – Likely to become endangered in the near future.

Medicinal importance-:

Extraction obtained from the stem of ***S.bryopteris (L.)*Bak** showed antibacterial activity against ***Niesseria gonorrhea*** and the paste of leaves is used in Spermatorrhea, leucorrhoea, diuretic, stomachache and urinary tract inflammation in children (Jitender Malviya etal., 2012). Ethanolic extract of ***S.bryopteris (L.)*Bak** can cure stomachic (Pandey etal., 1993). Water extract of ***S.bryopteris (L.)*Bak** reduces cell death caused by UV irradiation (Sah etal. 2005). The extract of ***S.bryopteris (L.)*Bak** increased cell growth and protected against dead cells induced by oxidative stress (Sah etal., 2005). ***S.bryopteris (L.)*Bak** is treated as anti inflammatory and cures venal diseases (Agarwal and Singh 1999). Amentoflavone and hinkoflavne from ***S.bryopteris (L.)*Bak** have anti protozoan activity against *Plasmodium falciparum, Leismania donovani and Trypanosoma* spp (Kunert etal., 2008).

***Selaginella convoluta*(Arn.)Spring**

Distribution-: Plant found in northeastern Brazil. Cuba, Hispaniola, S-Mexico Guatemala, Peru Honduras, Colombia, Venezuela, Bolivia, Brazil, Paraguay, Argentina and British Guyana and France.

Common name-: Jerico,

Conservation status-: Apparently Secure (uncommon but not rare).

Medicinal importance-:

***Selaginella convoluta* (Arn.)Spring** is a medicinal plant. The entire plant used as aphrodisiac, diuretic and against amenorrhea. A decoction of entire plant in two cups of water is used as tea three times daily to avoid debility. ***S.convoluta* (Arn.) Spring** is used in folk medicine as an antidepressant, aphrodisiac, diuretic, analgesic, anti-inflammatory and it is used to combat amenorrhea, coughing and bleeding(**Pedro etal,**2012)**.**The flavonoids of ***S.convoluta* (Arn.) Spring** work as antiviral, leishmanicidal, antifungal, anti cancerous and also as an antioxidant

(Sousa etal, 2012). ***S.convoluta* (Arn.) Spring** extracts with the inhibition route of cell proliferation becomes the selectivity index for cancer cells.

Selaginella doederleinii Hieron

Distribution-:*S.doederleinii* Hieron is naturally distributed in India, Burma, Thailand, Laos, Cambodia, Vietnam, Malaya, Chinese, Hongkong, S-Japan, Taiwan and Japan (Hung 2006, USDA 2008).

Common name-: Chicken claw

Conservation status-:Near Threatened (NT) – Likely to become endangered in the near future.

Medicinal importance-:

***Selaginella doederleinii* Hieron** is used to treat chorioepithelioma, choriocarcinoma, nasopharyngeal cancer, lung cancer, cough, sore throat, pyodermas, respiratory tract infections, Bronchitis, Tonsillitis, Hepatitis, Cholecystitis, Cirrhosis, Ascites, Acute urinary tract infection, pneumonia, pneumonia, bloated stomach, urinary tract infections, bone fractures and rheumatic disorders. This plant act as antitoxic, antineoplastic stops the bleeding (homeostatic) and eliminate swelling. Moreover ***Selaginella doederleinii* Hieron** also efficacious for treating coughs, respiratory tract infection, pneumonia, hepatitis, diarrhea, vaginal discharge, broken bones, bleeding and cancer(Dalimarta.1999). Biflavonoids of ***Selaginella doederleinii* Hieron**, to which different bioactivities have been attributed, including anti-cancer, anti-inflammatory, anti-oxidant, anti-fungal and anti-virus activity. ***Selaginella doederleinii* Hieron** has been used as anti cancer therapeutics (Liu etal., 2011). *Selaginella doederleinii* **Hieron** can inhibit sarcoma and cervical cancer in mice and cells isolated from human liver cancer (Dalimarta.1999). The chloroform and methanol extracts of ***Selaginella doederleinii* Hieron** are toxic to larvae *Artemia salina* and Leach (Widodo 2006).

*Selaginella involvens(*Sw.*)*Spring

Distribution-:*S.involvens(Sw.)Spring* Bhutan, India, Japan, Korea, Laos, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Vietnam, Russia, Korea, China, Taiwan, Thailand, Malaysia, Indonesia, India, Sri Lanka (Germplasm Resources Information Network (GRIN) USDA&Flora of china).

Common name-: Medicinal Spike moss.

Conservation status-: Endangered (EN) – High risk of extinction in the wild.

Medicinal importance-:***S.involvens*** **(Sw.) Spring** is ant oxidative**,** anti inflammatory and anti bacterial agent. The water extracts of ***S. involvens*** **(Sw.)Spring** degrades the blood cholesterol (Gyathri etal., 2005)and have an analgesic activity (Ko etal.,2007) **.**Tannins of ***S.involvens*** **(Sw.)Spring** show anti bacterial and anti parasitic effect (Akiyama etal.,2001, Kolodziej etal.,2005) and protect the kidneys(Lu etal,.2004).Anabolic steroids of ***S. involvens*** **(Sw.)Spring** increase the muscle mass.

Selaginella lepidophylla (HOOK&GREV.)Spring

Distribution -: It is native to the Chi huahuan Desert.
Common name-: False rose of Jericho, Dinosaur plant and Resurrection moss.
Conservation status: -G**4-Apparently secure** (uncommon but not rare, but with some cause for long-term concern; typically having 101 or more occurrences, or 10,001 or more individuals).
Medicinal importance-:
It has been used as a diuretic and used to treatment of urinary and kidney infections (Ruiz-Bustos etal.,2009).It is used to chronic gastritis & gastric carcinoma (Robles-Zepeda etal.,2011). Bioflavonoid of ***S. lepidophylla*** is potential medicine for antiviral, anti microbial & anti cancer activities (Aguilar et al., 2008). Methalic extracts of ***S. lepidophylla*** contain 3-methylen hydroxyl -5-methoxy-2, 4 di hydroxyl tetra hydro furane , which shows inhibitory effect on the uterus contraction (Perez et al.,1994).***S. lepidophylla*** has show hypoglycemic property(Andra de Cetto & Heinrich ,2005)This plant has been used as an herbal medicine. An infusion (tea) is made by steeping a tablespoon of dried material in hot water and the tea used as an antimicrobial in case of colds and sore throat (Curtin et al 1997).

Selaginella moellendorffii Hieron.

Distribution -: It is a lycophyte believed to be originated from the earliest vascular plants approximately 410 million years ago (Kenrick etal., 1997).It is widely distributed in Southeast Asia and China south of the Yangtze River. As the first reported non-seed vascular plant genome (Xi Chen etal., 2014). China (Jiangxi, Shanxi, Sichuan, Hubei, Guizhou, Hunan, Zhejiang, Guangxi,
Fujian, Guangdong, Yunnan, Hongkong), N-Vietnam, C-Vietnam, Cambodia, Taiwan, Ryukyu Isl., Philippines and New Zealand

Common name -: Clubmosses, Quillwort and Gemmiferous Spike moss.

Conservation status-: **Exotic** (Non- native in the specified area, even historically).

Medicinal importance-:

S. moellendorffii Hieron, a medicinal plant has been used in traditional Chinese folk medicine for treatment of jaundice, gonorrhea, bleeding, and acute hepatitis (Shi etal., 2008), idiopathic thrombo cytopenicpurpura (Ma etal.,2001). A Chinese patent-medicine,"**Jiangnanjuanbai tablet**", has been produced from a plant extract (Ma etal., 2001), (Liu etal., 2011).Alkaloids of ***S. moellendorffii Hieron*** act as anti microbial agent(Wanget etal.,2009).Total flav0noids extracted from *S. moellendorffii* Hieron show antiviral activity against Coxsackie Virus B3 which causes of Viral Mycocarditis (Dan Yin etal.,2014). Ethyl extracts of *S. moellendorffii* Hieron are reported to be flavones, such as Amentoflavone, Robustaflavone, Biapigenin, Hinokiflavone, Podocarpus flavones A, and Ginkgetin, which have antioxidant, antivirus, and anti tumor properties((Shi etal.,2008,Ma etal., 2001&Liu etal., 2011. It act as anti metastasis at lung cancer cells (Yang etal.,2007). Gingitin that extracted from ***S. moellendorffii* Hieron** can inhibit cancer cell growth of HeLa cells (Sun etal., 2006).

*Selaginella rupestris (L.)*Spring

Distribution-: North America including one locality in Greenland. In Greenland, ***S. rupestris****is* categorized as vulnerable (Jensen and Christensen 2003). In several states of the USA, ***S.rupestris*** was listed as threatened and endangered species, (FWS 2009),Canada C-USA, N-USA, E-USA (Alabama, Arkansas, Connecticut, Delaware, Georgia, Illinois, Indiana, Iowa, Kentucky, Kansas, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, South Dakota, Tennessee, Vermont, Virginia, West Virginia, Wisconsin, Wyoming).

Common name-: Rock spike moss, Festoon pine, Bird nest moss and Northern *Selaginella.*

Conservation status-:G5 Secure (common, widespread, abundant, and lacking major threats or long-term concerns).(Wyoming Natural Diversity Database)-

Medicinal importance-:

***S.rupestris (L.)*Spring** show anti plasmodia effect to ileum & strengthening the heart (Silva etal, .1995). It is used as an age-sustaining tonic and has been credited with the property of prolonging life. A decoction is prescribed for amenorrhea, bleeding piles and prolapsed of rectum. The leaves of ***S.rupestris (L.)*Spring** are crushed and used for wounds.

***Selaginella tamariscina*(Beauv.)**

Distribution-: *Selaginella tamariscina (**Beauv.)***distributed in Asia-Temperate (Mongolia, Russian Federation Kamchatka ,China),Eastern Asia(Japan, Korea, Taiwan), Asia-Tropical(India, Thailand, Malaysia, Indonesia, Java, Philippines)(Bull. Acad. Roy. Sci. Bruxelles 10:136, 10-Jun-1999) E-Siberia, Japan, Taiwan, Manchuria, Korea, peninsular Malaysia and N-Thailand.

Common name -: Little-club-moss and Spike moss.

Conservation status-: IUCN category N/A (Not applicable, meaning not suitable for conservation activities, typically used for hybrids with no conservation value, or non-native ecosystems.

Medicinal importance-:

Selaginella tamariscina **(Beauv.)** is a traditional Chinese herbal medicine for chronic trachitis. People of China drink it every day to keep healthy and slim. The whole plant is astringent and haemostatic. A decoction is used in the treatment of traumatic bleeding, haemoptysis in pulmonary disease, gastro-intestinal bleeding, metrorrhagia, haematuria and leucorrhoea. The major constituents ***Selaginella tamariscina*** **(Beauv.)** are flavonoids (amentoflavone, hinokiflavone, and apogenin) and saccharides (e.g. trehalose of, D-glucose, D-fructose and D-rhamnose) (Cheong etal., 1998, Zheng etal & Shimada etal., 1984) show anti-bacterial, anti-hypertensive, and anti-hyperglycemic effects (Miao etal, 1996, Zheng, 2011). Moreover, ***Selaginella tamariscina*** **(Beauv.)** has been shown to have anti-tumor activities, probably via an expression of the p53 tumor suppressor gene and an induction of G1 arrest in the cell cycle against certain tumor cell lines (Lee etal, 1999). ***Selaginella tamariscina*** **(Beauv.)** extract can down-regulate the expression of MMPs and u-PA, and inhibit the invasion and metastatic activities of lung cancer cells (Yang etal., 2007). ***Selaginella tamariscina*** (**Beauv.)** extract suppresses TPA-induced invasion and metastasis through inhibition of MMP-9 in human nasopharyngeal carcinoma HONE-1 cells.

***Selaginella unicata* (Desv.)Spring**

Distribution-: Southwest China

Common name-: Blue Spike-Moss, **Peacock Fern or Rainbow Moss**

Conservation status-: Exotic

Medicinal importance-:***S.uncinata*** (**Desv.)Spring** is a Chinese herbal medicine widely distributed

throughout southwest China which has been used to treat jaundice, dysentery, edema and beriberoid diseases. Bioflavonoid from ***S. uncinata*** **(Desv.)Spring** displayed protective effect against Anoxia (Jun-Xia etal.,2011). ***S. uncinata*** **(Desv.)Spring** works against acute hepatitis, cholecystitis, nephritic Edema, Enteritis, dysentery, pulmonary tuberculosis, hemoptysis, Burns, cuts, snake bites, chronic Nephritis and pyodermas (Dictionary of Chinese Materia Medice,2001). ***S. uncinata*** **(Desv.)Spring** show anti viral activity against RSV&PIV-13 due to presence of glycosides namely uncinoside A and uncinoside B (Man &Takahashi, 2002),(Ma etal.,2003).

Conclusion:-

Selaginella is considered as the first vascular plant on the earth, existed before 300 million years, shown greater diversity in the world. From ancient period it has been using as medicine to cure various alinments traditionally. According to WHO (World Health Organization)60-80% of the world people depends on plants for their therapeutic practices,***Sellaginella*** **spp** have medicinally potential and the bioactive compounds of ***Sellaginella*** **spp** have show medicinal properties like anti bacterial, anti viral, anti fungal, anti inflammatory, anti dote, and anti diabetic. Hence need to conduct more research on it. At global level majority of ***Sellaginella*** **spp** are under serious threat, hence need follow conservation methods to protect them.

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11.

DIVERSITY OF ALGAE IN PALERU RESERVOIR OF TELANGANA STATE, INDIA

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ABSTRACT

In the present paper an attempt has been made to explore the algae from Paleru reservoir, located in kusumanchi mandal of khammam district in Telangana state. In order to study the biodiversity of the reservoir two different sites were selected for collection of algal samples. Algal samples were collected at monthly intervals for a period of one year from January, 2013 – February, 2014. In the present study 35 species under 28 genera have been identified and recorded. The genera majorly belonged to the Class Chlorophyceae, Class Cyanophyceae, Class Bacillariophyceae and Charophyceae. Chlorophycean members were found to be dominant when compared to other groups.

KEY WORDS : Algal biodiversity, Paleru reservoir, Kusumanchi mandal, Khammam district, Telangana.

INTRODUCTION

Paleru is a tributary of the river Krishna and it lies in kusumanchi mandal of khammam district. Reservoirs are large man made inland waterbodies made on various rivers principally for irrigational purpose. The flora in these water bodies remain unchanged for longer periods until any drastic environmental changes occur in these habitats. These are also free from adequate anthropogenic inputs. In India enormous work has been done on algal biodiversity with special reference to reservoirs. In the present study investigations have been made on Paleru reservoir which is one of the important reservoirs in khammam district and it is famous for fish farming . Water of the reservoir is used for irrigation purpose.

Considerable amount of work has been done in India about systematic survey, distribution peridiocity, hydrobiological studies and ecology of algae in different habitats. (Pandey,1973;Kumar,et.al.1974;Prasad and Suxena,1980; Mohan et.al.1989). A number of investigations were carried out on fresh water lakes of Peninsular and continental Antarctica.(Hirano,1965; Heywood,1977; Longton, 1973; Seaburg et.al 1979). Iyengar and Venkatraman 1951 observed seasonal succession of the coover river of Madras with special reference to diatomataceae. Cyanophycean diversity has been studied extensively throughout India. (Tiwari et.al 2001; Pattanaik and Adhikary,2002; Chatterjee and Kehri,2005). Much work has not been done on the reservoirs of Telangana to understand the biodiversity of algal groups Review of literature reveals that Paleru reservoir has not been explored so far as its biodiversity is concerned. Thus, the objective of the present work is to explore the algal members from Paleru reservoir.

MATERIALS AND METHODS :

To study algal biodiversity of Paleru reservoir two different sites at Paleru village were selected. Algal samples were collected from the selected sites at monthly intervals from January,2013- February,2014. The samples collected in sterilized bottles were brought to the laboratory and preserved in 4% formalin for further taxonomic study. Subsequently the samples were observed under binocular microscope and microphotographs were taken with the help of a camera attached to the microscope. The organisms were identified by following different monographs.(West and West, 1908; Geitler 1925; Desikachary ,1959,1987,1989;Philipose ,1967; Misra and Srivastava 2003; Rath and Adhikary 2005; Jena et.al, 2006).

RESULTS AND DISCUSSION

In the present study a total of 35 species under 28 genera were identified and recorded. 18 genera under 11 families and 9 orders belonged to the class Chlorophyceae. 1 genera ,*Chara* belonged to the class Charophyceae, family Characeae under order Charales. 12 genera belonged to the class Cyanophyceae under families Nostocaceae, Oscillatoriaceae and Scytonemataceae. 3 genera under 9 families and order Pennales belonged to the Class Bacillariophyceae. It is evident that Chlorophycean members were dominant when compared to other forms. The finding are in coincidence with the earlier workers. Thirteen algal species were documented from a reservoir in Srinagar (Gharwal), Uttaranchal(Chaturvedi and

Habib,1995.) Tarar and Bodkhe,1998; recorded 51 chlorococcalean taxa from three reservoirs of Nagpur, Maharashtra.

In the above study Chlorophycean members were found to be dominant in winter and monsoon season , Cyanophycean members were observed to be maximum in summer season, Bacillariophycean members were found maximum in summer and winter season and Charophytes were recorded in winter season only. It is confirmed that there was seasonal variation throughout the period of study. Such seasonal variations in the diversity of phytoplankton in three water supply reservoirs of Chennai, TamilNadu was studied by Rajan and Azariah,2004.

TABLE.1: LIST OF ALGAE RECORDED FROM PALERU RESERVOIR

TABLE-1

S.NO	NAME OF THE ALGA	FAMILY	ORDER	CLASS
1	*Coleochaete sentata*	Coleochaetaceae	Chaetophorales	Chlorophyceae
2	*Cladophora crispata*	Cladophoraceae	Cladophorales	Chorophyceae
3	*Oedogonium formosum*	Oedogoniaceae	Oedogonialls	Chlorophyceae
4	*Pediastrum duplex*	Hydrodictyaceae	Chlorococcales	Chlorophyceae
5	*Hydrodictyon reticulatum*	Hydrodictyaceae	Chlorococcales	Chlorophyceae
6	*Eudorina indica*	Volvocaceae	Volvocales	Chlorophyceae
7	*Pandorina morum*	Volvocaceae	Volvocales	Chlorophyceae
8	*Volvox globator*	Volvocaceae	Volvocales	Chlorophyceae
9	*Chlamydomonas recticutata*	Chlamydomonadaceae	Volvocales	Chlorophyceae
10	*Chorella vugaris*	Chlorellaceae	Chlorococcales	Chlorophyceae
11	*Scenedesmus arcuatus*	Scenedesmaceae	Chlorococcales	Chlorophyceae
12	*Spirogyra acquinoctialis*	Zygnemaceae	Conjugales	Chlorophyceae
13	*Zygnema melanosperous*	Zygnemaceae	Conjugales	Chlorophyceae
14	*Spirogyra subsala*	Zygnemaceae	Conjugales	Chlorophyceae
15	*Spirogyra triplicata*	Zygnemaceae	Conjugales	Chlorophyceae
16	*Clostridium leiblenni*	Desmidiaceae	Zygnematales	Chlorophyceae
17	*Cosmarium monoloforme*	Desmideaceae	Conjugals	Chlorophyceae
18	*Chara fragilis*	Characeae	Charales	Charophyceae
19	*Pinnularia viridis*	Naviculoidaceae	Pennales	Bacillariophyceae
20	*Navicula oblonga*	Naviculoidaceae	Pennales	Bacillariophyceae
21	*Navicula turgidus*	Naviculoidaceae	Pennales	Bacillariophyceae
22	*Oscillatoria principes*	Nostocaceae	Nostocales	Cyanophyceae
23	*Oscillatoria chlorina*	Nostocaceae	Nostocales	Cyanophyceae
24	*Nostoc microscopium*	Nostocaceae	Nostocales	Cyanophyceae
25	*Nostoc endophytum*	Nostocaceae	Nostocales	Cyanophyceae
26	*Nostoc commune*	Nostocaceae	Nostocales	Cyanophyceae
27	*Chroococcus minor*	Chroococaceae	Chroococcales	Cyanophyceae

28	*Gleocapsa sps*	Chroococaceae	Chroococcales	Cyanophyceae
29	*Spirulina major*	Oscillatoriaceae	Nostocales	Cyanophyceae
30	*Spirulina gigantea*	Oscillatoriaceae	Nostocales	Cyanophyceae
31	*Lyngbya major*	Oscillatoriaceae	Nostocales	Cyanophyceae
32	*Lyngbya gracilis*	Oscillatoriaceae	Nostocales	Cyanophyceae
33	*Anabaena planctonica*	Oscillatoriaceae	Nostocales	Cyanophyceae
34	*Scytonema cincinnatum*	Scytonemataceae	Nostocales	Cyanophyceae
35	*Phormidium molle*	Oscillatoriaceae	Nostocales	Cyanophyceae

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12.

Commercial farming of Sarpagandha (*Rauvolfiaserpentina*(L.) Benth. exKurz.), a high value medicinal herb to upliftsocioeconomic status of ruralpopulace of Moradabad district of Rohilkhand Region of Uttar Pradesh

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Abstract

Rauvolfiaserpentina (L.)Benth. exKurz. (Family - Apocyanaceae) is of great therapeutic importance, having high market demand. This high value medicinal herb is suitable for cultivation as sole as well as inter/ under storey crop due to their inherent abilities to tolerate certain degree of shade. Apart from high economic potential, cultivation and processing of this medicinal herb is highly labour intensive, and do not require sophisticated technology and high capital investment.This make the cultivation of sarpagandha, a highly desirable proposition for improving the economic status and employment generation in rural areas of Moradabad district.Since, sarpgandha is a slow growing plant and does not develop sufficient canopy to cover the ground surface fully throughout its growth period, intercropping with short duration and less competitive crops can ensure additional benefit to sarpgandha growers. In the present study two families of Manoharpur village of Moradabad district were participated in cultivation programme.At an average yield of 1270 kg/ha dried roots and at currentselling price of Rs. 260/ Kg, sarpagandha cultivation may givea gross return of Rs. 258.00 lakhs/ ha from 24months crop.Therefore cultivation ofsarpgandhais recommendedfor income and employment generation in rural areas of Moradabad.

Key words: Medicinal herb, cultivation, sarpgandha,economic and employment generation, Moradabad.

Introduction

In modern times people relies on herbal medicines and this have led to an increased demand of plant based medicines. And with this trend getting stronger every day, the demand of medicinal plants and their derivatives is truly phenomenal. Earlier India had the monopoly for the supply of serpentina roots to world market. However, due to over exploitation of forest resources Govt. of India imposed a ban on the export of roots in the year 1969 (Sarin, 1986).Against present demand of about 750 tonnes of roots (Ahemad, 1996), the availability from wild sources during 1986 was estimated to be only 60 tonnes (Sarin, 1986) which has further gone down now. In fact there is crisis of roots availability for indigenous pharmacies and for export. The rural areas account for the major percentage of people living below the poverty line. Lack of education, poor economic condition, unemployment, and poor asses to newer technologies are some of the fundamental issues that always surge the rural areas. Although, addressing effectively any of these issues can greatly benefit people of rural areas, however, the increase in income and employment generation through the better utilization of limited land resources and human man power at their disposal are considered to be the most important to bring a perceptible change in their socioeconomic status(Singh &Khanuja, 2003).Different ethnic groups use this plant to treat snake, insect and animal bite, mental illness, schizophrenia, hypertension, blood pressure, gastrointestinal diseases, circulatory disorders, pneumonia, fever, malaria, asthma, skin diseases, scabies, eye diseases, spleen diseases, AIDS, rheumatism, body pain, veterinary diseases etc. This plant is also being used to prepare fermented food products(Dey and De, 2011).

In natural habitat, sarpgandha is found growing under the shades of shrubs and trees. Therefore, for optimum growth neither full nor very low light intensities are required. Rauvolfia can sustain drought as well as temporary water logging, it grows well in medium soils, which are rich in organic matter contents. Sandy or too clayey soils do not support good plant growth and root development. It can be propagated by seed as well as through vegetative means, root cuttings.This plant has been designated as threatened with extinction, endangered and threatened, critically endangered in India (Mamgain et al., 1998; Singh et al., 2010; Sukumaran and Raj, 2008; Mao, 2009). Because of overexploitation, need for conservation and low propagation rate, there are several reports of *in vitro*propagation and manipulation of this plant (Sehrawat et al., 2001; Pandey et al., 2007; Ilahi et al., 2007).The

major bio-active alkaloids of this plant are ajmaline, deserpidine, rauwolscine, rescinnamine, reserpine, serpentinine, yohimbine etc.

Study area

Moradabad is a district of state Uttar Pradesh which is located between $28^0\ 50^/$ to $28^0\ 83\ ^/$ N latitude and $78^0\ 47^/$ to $78^0\ 78^/$ E longitude at an altitude of about 186 m above the mean sea level. The maximum and minimum atmospheric temperatures are 44.2^0C and 4^0C respectively. Rainy season starts from the middle of June and in the first week of July and extended up to September. The average rainfall varies between 800 to 1000 mm .The relative humidity is up to 90% in monsoon season and in drier part of the year it decreases to less than 20%.Commercial cultivation of sarpagandha in Moradabad district has not been studied so far and there is no comprehensive programme for monitoring the demand of this herb. Weconvinced rural people about the profitability of sarpagandhacultivation and Manoharpurvillage in MoradabadTehsil was selected for cultivation of sarpagandha which receives sufficient water from GaganRiver, a tributary of RamgangaRiver.

Methodology

Freshly collected seeds weresown in the nursery beds in the month of April-May2011. Before sowing, seeds were dipped in 10% sodium chloride solution. Those heavy seeds sink to the bottom were separated and used for sowing of nursery. We required 10 Kg seeds for producing seedlings for one hectare planting. Seedlings were planted at 60cmx30cm distancein the seed bed in the month of July after the onset of monsoon when they were 55-60 days old.We used only green manure as fertilizer.Sahu(1970) and Maheshwariet al.(1988) also suggested green manure for significant response in terms of total alkaloid production.Sarpgandha is a water loving plant. Therefore, stability of high soil moisture throughout the growing period was maintained. Fields were irrigatedat 20 days intervals during summer and30 days during winter as recommendedby Maheshwari et al. (1991).Regular weeding and hoeing operations were done for optimum plant growth and root development as recommended by Trivedi (1995).

Since, sarpgandha is a slow growing plant and does not develop sufficient canopy to cover the ground surface fully throughout its growth period, intercropping with short duration and less competitive crops can ensure additional benefit to sarpgandha growers. Experiments

conducted at Akola (Maharashtra) and Indore (M.P.) indicated additional returns from intercrops. At Indore one row of soybean during Kharif and three rows of garlic during Rabi between two rows of sarpgandha, spaced at 45cm apart, proved highly remunerative (Sahu, 1979). At Akola one row of soybean during Kharifand three rows of onion during Rabi was the most suitable intercrop combination (Maheshwari et al; 1985). Therefore we also used intercropping procedure for extra income.

Harvesting of roots was done two years after planting in the month of December 2013. Soil around plant was removed carefully up to a depth of 60-75 cm. Plants were pulled out from the soil without applying much pressure. This was done primarily to ensure removal of fine roots from the soil. After digging, the roots be freed from dirt and soil and spread in sun for drying. According toDutta et al. (1963)awell-managed crop, harvested two years after planting may yield 1200-1500Kg dried roots/ ha.

Economics of cultivation of Sarpagandha

Table 1. Economics of Sarpagandha cultivation in Manoharpur village

Item	Cost (Rs./ha)
First year expenditure(seeds cost, nursery raising, uprooting of seedlings & planting, irrigation, weeding)	48,000/-
Second year cost (seed collection, , and digging, cleaning, cutting, drying and grading of roots)	24,000/-
Total cost of cultivation	72,000/-
Total production 1270kg/ha dry roots	
Estimated income by selling price of Rs. 260/ Kg roots	3,30,200/-
the net return from 24 months crop Rs / ha	2,58,200/-

Singh et al. (1990) recorded 1170 Kg dry root yield per ha from crop grown in poor fertile sandy soil and harvested two years after planting at the Central Institute of Medicinal Aromatic
Plants, Lucknow. Singh &Khanuja (2003) also reported similar results.Sarpgandha grown in laterite soil at Bhubaneswar and harvested three years after planting yielded 2000-3500 Kg roots/ ha.

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13.

EFFECT OF SOME PESTICIDES ON THE INDUCTION AND GROWTH OF SOMATIC EMBRYOS OF *SOLANUM MELONGENA* L.

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ABSTRACT

In vitro somatic embryogenesis and regeneration was achieved from cotyledon explant of *Solanum melongena* L. Somatic embryogenesis by the addition of three different pesticides like Endosulfan and Rogor individually and in combination with Kitazin was studied at different concentrations of these chemicals in MS medium supplemented with 10 mg/l NAA. The investigation was carried out to study the induction, maturation, germination and regeneration of induced embryos in relation pesticides concentrations.

KEYWORDS: Green Roung brinjal, pesticides, somatic embryogenesis and regeneration.

Abbreviations: NAA – Naphthelene Acetic Acid, IBA – Indole Buteric Acid, MS – Murashige & Skoog's.

INTRODUCTION

The recent developments in agro technology have accelerated the use of pesticides in an enormous amount. Pesticides are the modern tools to the farmers to control pests, diseases, weeds and to increase crop yields. A lot of work has been done on the role of pesticides in providing protection to plants against weeds in terms of crop yield [1-3]. Only a little work has been established on the role of pesticides in affecting biochemical characteristics of the plants [4].

Although tissue culture of *Solanum melongena* is being carried out for more than 25 years, reports regarding somatic embryogenesis in *S. melongena* has been reported earlier in hypocotyls and leaf segments[5-6]. The study of pesticides *in vitro* level provide a suitable

source for morphogenetic developments and the analysis of molecular and biochemical events occurring during induction and maturation.

Hence, present study is an attempt to study the effect of 2 pesticides like Endosulfan, Rogor either alone and in combination with other fungicide Kitazin on the development of embryoids from the cotyledon derived embryogenic callus and their conversation into plantlets.

EXPERIMENTAL

Materials and Methods

Healthy and uniform seeds of *Solanum melongena* (L) were selected. Surface sterilization of the seeds was done with a commercial detergent teepol (Reckitt Colman, India) for 15 min, 0.1% Mercuric chloride for 10 min followed by washing four times with sterile distilled water to remove traces of $HgCl_2$. Seeds were germinated in MS basal medium. Cotyledons from 8-10 days old was considered as the best explant for the studies on basis of the percentage of response cotyledon segments were surgically excised and inoculated into culture tubes containing MS[7] media Supplemented with 10 mg/l NAA and different concentrations of Endosulfan & Rogor (25, 50, 75,100, 150 and 200 ppm) and in combination of Endosulfan with Kitazin (10 +10, 20 + 20, 30 + 30, 50 + 50, 75 + 75 and 100 + 100 ppm) and Rogor + Kitazin (10 +10, 20 + 20, 30 + 30, 50 + 50, 75 + 75 and 100 + 100 ppm). After the addition of sucrose P^H of the media was adjusted to 5.6 – 5.8 with 0.1 N NaOH in HCl. The agar was added and heated gently with constant stirring till the added agar was dissolved and autoclaved for 15 min at 102 KPa. The cultures were incubated at 25 ± 2^0 C under fluoroscent white lights (1500 lux) maintained at 16:8 hr light and dark regime. Media with normal micronutrients and without the addition of pesticide was constituted as control. All the experiments were repeated thrice. The data was tabulated and analyzed statistically.

To ascertain the embryogenic nature of differentiating structures, cultured tissues were subjected to histological study. Callus bearing somatic embryos at different developmental stages was fixed in acetic acid: alcohol (1:3) then dehydrated in alcohol – Kylol series, embedded in paraffin wax, sectioned at 10 μ thickness and stained with heamatoxylin and basic fuschin.

RESULTS AND DISCUSSION

On the observations, placement of cotyledon on the media was also important for the somatic embryo response. It was noted that embryogenesis induction was more when abaxial surface of the cotyledon was placed touching the media compared to adaxial surface. The response of cotyledon was considered as the best explant for the induction of embryogenesis. MS media supplemented with NAA (10 mg/l) was suitable for the study of somatic embryogenesis. It was also significant the both induction and maturation of somatic embryos took place on the same media.

With the addition of Endosulfan the percentage of responding increased upto 100 ppm and then followed decrease in the response in higher concentrations of chemical. Control cultures shown 90% response and number of somatic embryos induced were 33.5. Somatic embryos frequency decreased in lower concentration of Endosulfan. The decreasing trend of induction was seen up to 75 ppm, however, there was a increase (24.6) noticed especially in 100 ppm and further decrease (18.4 & 14.5) observed in higher concentrations of 150 and 200 ppm respectively (Table.1). In Rogor added media both percentage of responding as well as induction of somatic embryos gradually decreased as

TABLE 1

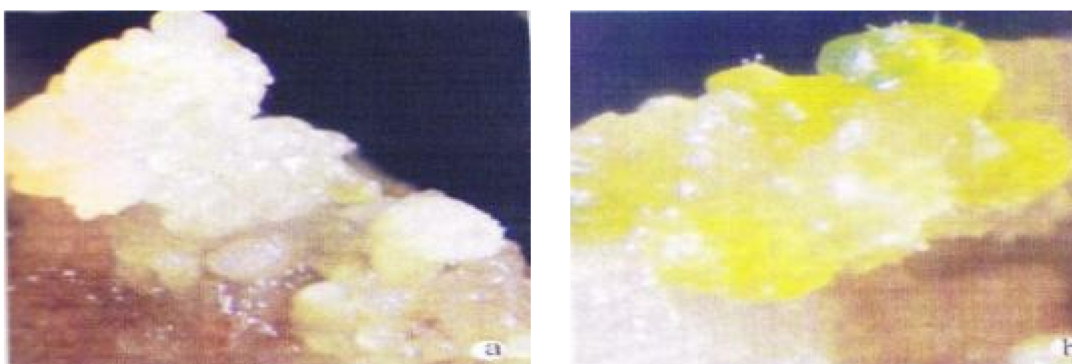
Effect of Rogor and Endosulfan both individually and in their combination with Kitazin on the induction of somatic embryos

Concentration in ppm	Percentage of response	Average number of somatic embryos
Control	90	**33.5±0.1789**
25	62.0	**20.3 ±0.1642**
50	70.4	**22.5±0.1783**
75	78.0	**19.0±0.1152**
100	90.0	**24.6±0.1243**
150	50.0	**18.4±0.2146**
200	25.0	**14.5±0.1765**
25	80.0	**36.5±0.1782**
50	65	**24.4±0.1265**

75	64	**21.5±0.1420**
100	42	**17.4±0.2126**
150	38	**16.0±0.1230**
200	25.3	**14.6±0.1765**
10+10	58.4	**18.6±0.2357**
20+20	52.0	**17.2±0.2943**
30+30	42.2	**16.0±0.1230**
50+50	35.0	**12.5±0.1157**
75+75	28.3	**12.0±0.1711**
100+100	16.0	**10.0±0.1427**
10+10	50.0	**14.5±0.1765**
20+20	42.2	**10.0±0.1427**
30+30	30.0	**8.3±0.1859**
50+50	250.	**7.5±0.2582**
75+75	180.	**7.1±0.1475**
100+100	15.0	**5.0±0.2406**

Number of cultures maintained = 30

PLATE –I



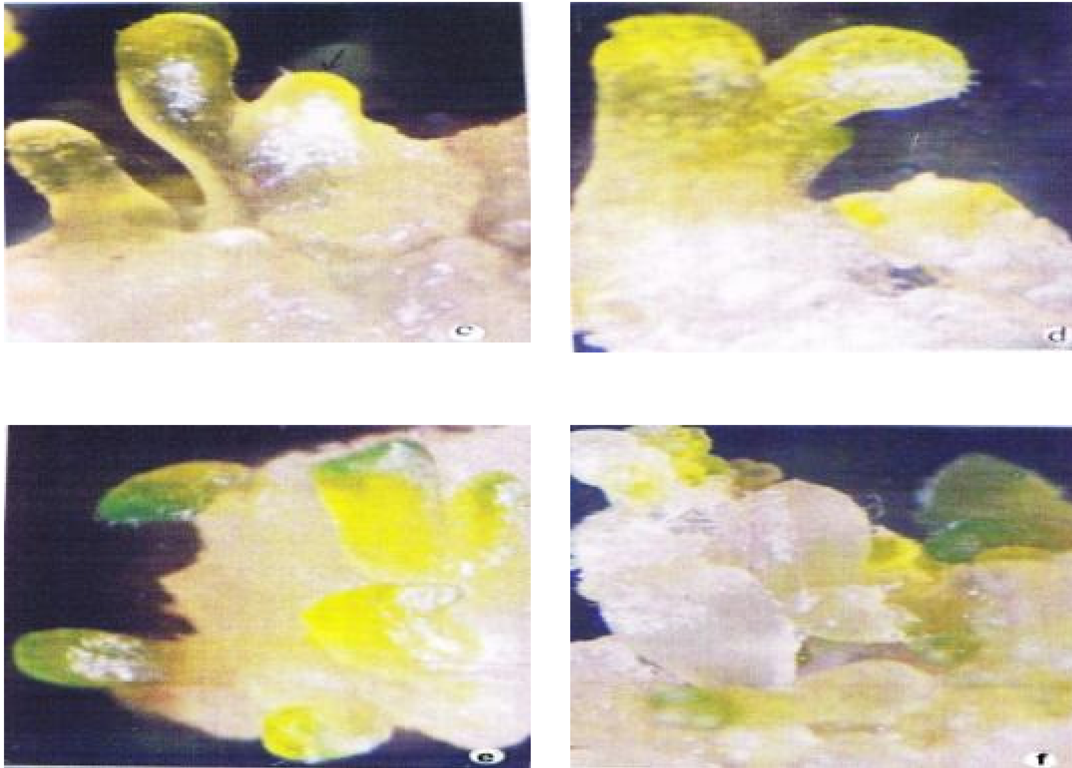


Plate-1: Somatic embryogenesis in S*olanum melongena* L.

Fig a. Induction of proliferating embroygenic mass with globular embryos were observed in control samples (10mg/l NAA).

Fig b. Globular and early torpedo embryos with hair on their surface in combined treated medium (Rogor+Kitazin-20+20ppm)

Fig c. Mature and immature torpedo staged somatic embryos were observed in medium containing Rogor at 25 ppm. One of the somatic embryo with budding can be seen (arrow).

Fig d. Cotyledonary stage somatic embryo was seen in media containing Rogor at 50 ppm.

Figs e&f. Group of embryos at various developmental stages with long suspensor observed in proliferation media containing Endosulfan+Kitazin - 20+20ppm.

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their concentrations were increased. 200 ppm Rogor containing media showed lowest percentage of response (25.3). In both combined treated media of Endosulfan + Kitazin and Rogor + Kitazin, there was a decrease in both percentage of responding and number of somatic embryos as increasing their concentrations. Both combination treatments exhibited same response with regard to induction of somatic embryos.

Induction of somatic embryos by the incorporation of pesticides (Endosulfan, Rogor) to the media decreased as increasing concentrations both individually and in their combination with Kitazin. Exceptionally, in Endosulfan added media at 100 ppm equated the percentage of response with control cultures.

Induction of somatic embryos was much affected in combined treated (Endosulfan + Kitazin and Rogor + Kitazin) samples than their individual (Endosulfan, Rogor) treated cultures. It was important to note that, when compared to control cultures all the pesticides used individually and in combination showed less responding percentage and in an induction on somatic embryogenesis.

Torpedo and heart shaped somatic embryos were record frequently in control (Plate-I & Fig.a) and both globular and early torpedo shaped somatic embryos with hair on their surface were observed in (Rogor + Kitazin 20 + 20 ppm) treated samples (Plate-I & Fig.b). Mature and immature torpedo stage somatic embryos were observed in Rogor presence at lower concentration (25 ppm) (Plate-I & Fig.c). One of the somatic embryo with budding can be seen (arrow) in same concentration of Rogor. Cotyledonary stage somatic embryo was seen in Rogor at a concentration of 50 ppm (Plate-I & Fig.d).

Group of embryos at various developmental stages with long suspensor observed in proliferation media containing Endosulfan 25 ppm. Interestingly, various developmental stages of cluster of cotyledonary embryos at the end of 6th week was obtained from combined treatment of Endosulfan + Kitazin (20 + 20 ppm) added media (Plate-I & Figs. e & f).

The individual treatment of pesticide Endosulfan 75 ppm remarkably induced series of embryos (5-6), which were confirmed their origin by histological study. All these series of embryos induced by Endosulfan showed synchronization with regard to maturity and germination. This future can be exploited for large scale production of somatic embryo. In some treated samples undescribed structures were also observed. Addition of pesticide to the media showed induction of only normal somatic embryos in all most all treated samples along with control, but there was no induction of abnormal somatic embryos were noted.

The matured somatic embryos developed from different samples were transferred to basal media MS + IBA (3 mg/l) and half MS media to view their regeneration capability. The individual pesticide treated somatic embryos only developed to plantlets and somatic embryos obtained from combined treated samples completely failed to regenerate. Basal and MS + IBA (3 mg/l) was suitable than half MS media, in which proper regeneration was not seen.

In the present study cotyledon explants gave best response. This is in conformity with other reports[8-11]. Francis Satyasealn and Jayachandran[12] also reported the importance of 2,4-D in combination with NAA in Somotic embryogenesis in *Solanum melongena*. The source of explants is important in determining regeneration of plantlets via somatic embryogenesis has much potential for its use in plant regeneration[13-18]. Similar results were obtained in brinjal with heavy metals[19].

The pesticides Endosulfan & Rogor were absolutely necessary up to non toxic certain level and their high level presence leads to abnormal morphogenetic developments proved with the observations. Especially individual treatment of Endosulfan at suitable concentration (100 ppm) used in the present study to induce series of somatic embryos *in vitro* level.

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14.

Observations on patterns of vegetation in Tropical Forests of Siwaram Wildlife Sanctuary, Telangana state, India.

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ABSTRACT

The present study deals with the phytosociological observations in the tropical deciduous forests of Siwaram wildlife sanctuary. Along with the quantitative structure, floristic composition was analyzed. The study resulted in documentation of 143 species for floristic enumeration. Tree stand density varied from 254 ha-1 with average basal area of 12.88 m2ha-1 covering tree 51 species. Shannon–Wiener index (H') ranges from 3.71-5.01. Similarity index reveals that only 62.4% of floristic composition of dry deciduous forest is similar with deciduous forest. Margalef's Species Richness Index varies from 5.40. This study takes the first step to provide the baseline data necessary for future monitoring and conservation of these important protected areas. This analysis will serve as a primary input towards monitoring and management of the vegetation in the sanctuary.

KEY WORDS: Phytosociology, Andhra Pradesh, Siwaram Wildlife Sanctuary, Crocodile, Deciduous

INTRODUCTION

Vegetation is very important natural resources which provides basis of life to fauna in one way or the other. However, conservation management requires information on plant species diversity and the forest community structure in order to chalk out necessary actions. Even though, Andhra Pradesh, one of the largest State in India represents 20 wildlife sanctuaries, does not received much attention for quantitative assessment of vegetation. Keeping in view this present study aims to understand the vegetation status and analyses threats to the siwaram sanctuary and envisages documenting plant species diversity, variation in species diversity and changing species association over space in the sanctuary.

Study Area

The Siwaram Crocodile Sanctuary is situated along the common boundaries of Adilabad and Karimanagar Districts of northern Telangana region of Andhra Pradesh, India. It was declared to conserve the fresh water crocodile (Mugger) – *Crocodylus palustris*. It is located 260 km away from Hyderabad. The sanctuary spreads between longitudes 18^0 35'-18^0 45' N and latitudes 79^0 40' – 79^0 50' E. The total area of the Siwaram Sanctuary is 29.81 Sq. Km. (Vinodkumar, C.P.2003) It is an abode for a variety of flora and its associated fauna. The Sanctuary is an ideal Crocodile Habitat with perennial water source in river Godavari in the form of lake formation and shoreline stretches adjoining the water body – an important requirement of marsh crocodile for basking and egg laying. The river Godavari divides the sanctuary into northern bank (Adilabad District) and southern bank (Karimnagar District). The Sanctuary supports the vegetation which exhibits a classical example of southern tropical dry deciduous forest. (Champion, H.G. & S.K. Seth., 1968). It harbors the important fauna like Sloth Bear, Neelgai, Cheetal, Blackbuck, Chowsingha, Langur and rhesus monkey with the endangered fauna like four horned Antelope and grey jungle fowl. Panther is also

reported in the Sanctuary. In the hot season the forests are leafless in almost all the canopies. *Tectona grandis*, *Anogeissus latifolia*, *Diospyros melanoxylon*, *Acacia sandra*, *Cleistanthus collinus* are the economically important and commonly occurring tree species. *Madhuca indica*, *Boswellia serrata*, *Steruclia urens*, *Lannea coromandelica*, *Garuga pinnata*, *Acacia leucopholea*, also occur extensively. *Cleistanthus collinus*, *Butea monosperma* occur common in the second canopy. *Dendrocalamus strictus* forms the under storey in these forests. *Holarrhena antidyssentrica*, *Zizyphus xylopyrus*, *Cassia auriculata* form the ground cover. *Butea superba* and *Acacia intsia* are common climbers. The Siwaram sanctuary is a natural aquatic ecosystem with Mugger as apex species provided with deep water-body formed in the river Godavari, the vegetation in the two sides of the river was included in the sanctuary to protect the catchment to maintain watershed of the riverine water-body. The crocodile plays a vital ecological role as a master predator in the aquatic habitats where it lives, by preying on weak and diseased fish and animals, it maintains genetic quality by its habit of selective feeding, it controls predatory fish, its presence thus actually helps to increase yields of edible fish to mankind.

MATERIALS AND METHODS

Field Sampling

Phytosociology studies were conducted in the sanctuary to understand vegetation status and forest structure. A total of 28 sample plots were laid out covering the whole sanctuary, 1.12 ha. is sampled randomly. Sampling intensity is 0.01 ha.

RESULTS

The study resulted in documentation of 143 species for floristic enumeration and phytosociological analysis, of these Herbs with 58 species followed by Trees (51 Species), Climbers (24 Sp.) and Shrubs (10 Sp.) Tree stand density varied from 254 ha-1 with average basal area of 12.88 m2ha-1. Shannon–Wiener index (H') ranges from 3.71-5.01. Similarity index reveals that only 62.4% of floristic composition of dry deciduous forest is similar with deciduous forest. Margalef's Species Richness Index varies from 5.40.

DISCUSSION

Species wise analysis shows *Diospyros melanoxylon* is found to be the ecologically dominant species (Table. No.1.) in the sanctuary with an highest IVI (22.62). This indicates a wide range of growth and adaptability of *D. melanoxylon* throughout the sanctuary. The subdominants are *Chloroxylon swietenia* and *Cliestanthus collinus* with 21.62 (IVI) and 20.78 respectively. The lowest IVI was noticed with *Casaeria elliptica* (0.95). The highest basal areas observed with *Madhuca indica* (199982.65), *Chloroxylon swietenia* (17167.94), *Diospyros melanoxlyon* (11624.66) and *Albizia amara* (7563.80). The lower basal area was 76.45 of *Limonia acidissima*. *D. melanoxylon* shows high regeneration capacity followed by *Chloroxylon swietenia, Cliestanthus collinus* etc. where regeneration of *Careya arborea, Soymida febrifuga* and *Sterculia urens* (with low IVI 1.00) is very less and made their occurrence rare. For the globalization of weeds, Siwaram sanctuary is not spared, thanks due to the regular biotic disturbance (movement of domesticated cattle, goat, sheep etc.) and vehicular movement, the deepest of the wildlife sanctuary has given room for all kind of exotic weeds. Herbaceous undergrowth is good in the interior areas of the sanctuary but an urgent action is required to control the predominance of exotic invasive like *Hyptis suaveolens* (E. N. Murthy, 2007) with highest IVI 19.21 (Table No.2.) , it poses survival

threat to indigenous flora existing in the sanctuary. The invasion by this exotic species is leading the loss of important palatable, economic and ethnomedicinal indigenous species. Besides, it is enhancing fire problem during the dry seasons. Extensive felling of large and plus trees like *Tectona* and *Anogeissus etc*. leads to gradual soil erosion that contributes to siltation in the crocodile habitat. Forests in the sanctuary is now open, became shrub land with scattered saplings of intruded exotic trees like *Peltophorum pterocarpum, Samanea saman* and *Eucalyptus* Spp. *etc*. They were once planted in the fringes of the sanctuary by the social forestry department instead it is better to plant native species to aid natural regeneration for better management and conservation of indigenous biodiversity. Another concern is that tree density in the sanctuary is very low. It was noticed that extensive human interference, particularly the removal of valuable economic tree species such as *Tectona grandis, Anogeissus latifolia, Pterocarpus marsupium, Hardwickia binata* and *Terminalia tomentosa etc.* One notable feature of this forests was that very less saplings of *Tectona grands* and *Anogeissus latifolia* were recorded with in the protected area. In overall sapling growth sapling dominance is much in *Chloroxylon swientenia* (IVI 21.70) where *Tectona grandis* (IVI 19.44) shows very less sapling density that indicates the indiscriminate harvesting of Teak (Figure. No.1) by the Natives causing to conversion of forest into non-teak mixed forest. If this trend continues uninterrupted the even prevailing *Diospyros* dominant forest could turn into a scrub forest and ultimately into open barren land. The protected area forms catchment area for a number of streams which drain finally into Godavari river, most of the areas in the protected area are subjected to erosion of top soil rich in nutrients due to clean trimming of vegetation, resulted into degradation of vegetation cover in the buffer area as well as in the core area. Removal of the vegetation from the hills around the water-body causes land sliding into the deep lake formation of Godavari, leads to gradual siltation of the aquatic body.

CONCLUSION

An accurate census of India's crocodilian species is not available, but according to approximate estimation of Andhra Pradesh forest department there are 26 child and 14 adult crocodiles in the siwaram crocodile sanctuary. As per Indian Wildlife Protection (1972) Act-Article-26/A (Forest & Wildlife Laws, 2005), there is no permission to work with in 500mt from the sanctuary boundary as sand digging, fishing and pumping of Water are not allowed from the Sanctuary. The fishermen community around the sanctuary is entirely dependent on the water body for fishing and earning their livelihood. In order to reduce the pressure of fishing in the sanctuary it is proposed to regulate the fishing and need to provide alternate sources of income generation to fishing communities besides providing fish ponds in the nearby areas. But in recent days Sanctuary is witnessing many disturbances like illicit fishing, heavy sand digging using machines with in the sanctuary boundaries. These are contributing disturbance and siltation in the crocodile pond that ultimately affect on vulnerable (IUCN Red list, 2009) Crocodile population. The status of crocodile reflects not only the health of the ecosystem and its prey species but also the effectiveness of the conservation efforts. There is an urgent need to check above irregularities to save the sanctuary. Forest and vegetation cover in and around the sanctuary is fast diminishing due to much anthropogenic activities resulting into heavy soil erosion, depleting water table around the Crocodile pond that ultimately questions the fate of the sanctuary. The evaluation of the conservation status of river corridor vegetation should be maintained on priority basis to protect the watershed. Comprehensive measures are needed to retain the sanctuary. The sanctuary is surrounded by coal mines, cement factories and thermal power stations and there are every chance of pollution and siltation are more. The proposal of setting up a 600 MW merchant power plant at Jaipur mandal of Adilabad district by the Singareni Collieries Company Limited will adversely affect the vegetation in the sanctuary and pollute the

Godavari waters with its coal residues. Government should take necessary steps to minimize the disturbance to the sanctuary by setup a environmental experts group. It is essential that India too looks at all the various conservation options, including sustainable use of wildlife if we are to effectively conserve crocodiles and their dwindling habitat. To maintain the ecology of the sanctuary it was felt necessary to initiate a project for crocodiles within its natural home for preservation by rearing. Ranching is rapidly becoming the accepted technology for using actual breeding utilization of the wild population through collection of eggs of the wild crocodile. Research and monitoring are essential components of a ranching programme (Shukla, R.K. & Singh, S. K. 2008). There is a need to develop a crocodile rearing centre at the siwaram sanctuary with the assistance of Madras Crocodile Bank to maintain and boost the wild population in their natural habitat. Any biodiversity conservation programme, however cannot succeed without the involvement of local people, (Singh, J.S. 2002) reasonable measures are needed in this 'Protected Area' to keep harmonious relationship between human activities and environment. Conservation work will naturally and inevitably benefit local people, because it will sustain resources over time, (Emma Maris, 2007). There is need to protect the fragile fresh water crocodile habitat and its prey population on priority basis, otherwise the fate of the sanctuary will be in peril. Finally this study underlines the lack of available basic information of the sanctuary. Thus, permanent sampling plots should be established in these forests to facilitate periodic evaluation of the ecological parameters. The conservation and monitoring of biological diversity has become an important issue, receiving national and international attention and is regarded as essential to carrying out the Convention on Biological Diversity (CBD) (Teder, T., 2007). It is also an important element of ecosystem management and of an adaptive management approach. (Everett R, 1994). This study takes the first step to provide the baseline data necessary for future monitoring and conservation of the protected area.

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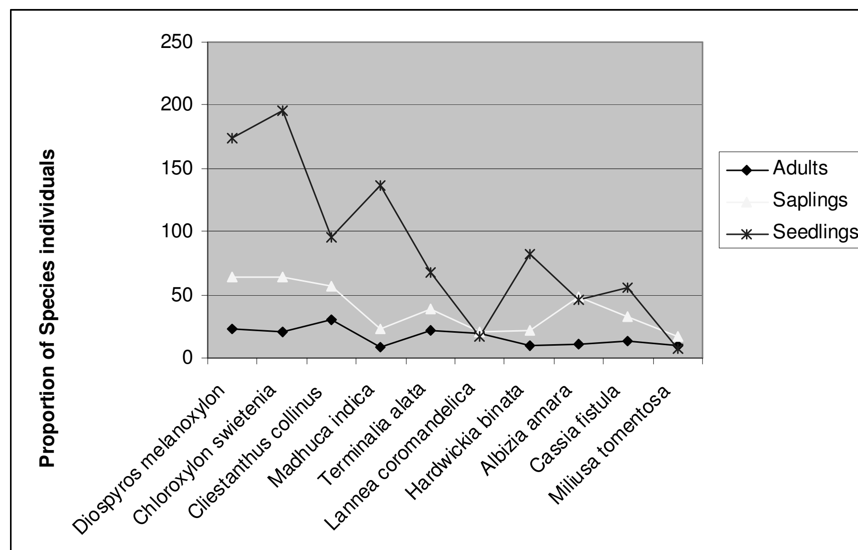
Table No. 1. Ecological Dominance of tree species (>30 cm GBH) based on IVI values

Sl. No.	Species	Relative Density	Relative Frequency	Relative Dominance	IVI
1	*Diospyros melanoxylon*	8.07	6.49	8.06	22.62
2	*Chloroxylon swietenia*	7.02	2.70	11.90	21.62
3	*Cliestanthus collinus*	10.53	5.41	4.84	20.78
4	*Madhuca indica*	2.81	3.24	13.85	19.91
5	*Terminalia alata*	7.72	4.32	4.45	16.49
6	*Lannea coromandelica*	6.67	6.49	2.29	15.44
7	*Hardwickia binata*	3.51	3.78	4.79	12.08
8	*Albizia amara*	3.86	2.16	5.24	11.27
9	*Cassia fistula*	4.56	4.32	2.33	11.22
10	*Miliusa tomentosa*	3.51	5.41	2.02	10.94

Table. No.2. shows the dominance of *Hyptis suaveolns*

Sl.	Species	Relative Density	Relative Frequency	Relative Abundance	IVI
1	*Hyptis suaveolens*	9.95	2.73	6.53	19.21
2	*Cynodon dactylon*	7.76	1.52	9.16	18.43
3	*Borreria hispida*	8.23	6.06	2.43	16.72
4	*Triumfetta rhomboidea*	4.47	6.36	1.26	12.10
5	*Oxalis corniculata*	4.83	2.73	3.17	10.73
6	*Waltheria indica*	4.37	4.24	1.84	10.45
7	*Commelina benghalensis*	3.08	0.91	6.06	10.05
8	*Alysicarpus vaginalis*	3.19	4.24	1.34	8.77
9	*Leucas aspera*	3.58	2.12	3.02	8.72
10	*Phyllanthus urinaria*	3.29	3.64	1.62	8.55

Figure: No.1. Trend line representation of population distribution pattern of top ten predominant species



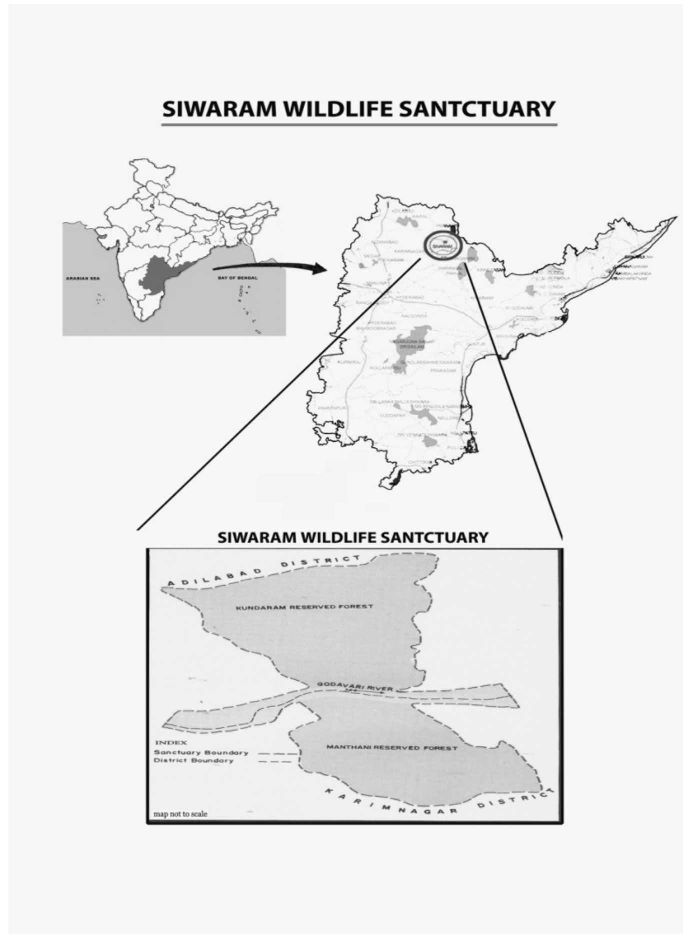


Fig. No. 2. Location map of Siwaram Wildlife sanctuary, Andhra Pradesh, India.

15.

ENUMERATION OF MEDICINAL PLANTS OF RAMAGIRI HILL FORESTS KARIMNAGAR DISTRICT, TELANGANA STATE, INDIA

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ABSTRACT

Ramagiri hill forest is a good reserve of medicinal plants in Karimnagar district of Andhra Pradesh, India. It is a sacred groove and historical site. There are 150 medicinal plant taxa recorded which represent 55 angiosperm families. Papilionaceae are the dominant family with 10 species, followed by Caesalpiniaceae eight, (seven each) Apocynaceae, Combretaceae, Cucurbitaceae and Mimosaceae, Euphorbiaceae, Malvaceae and Rubiaceae (five each). Plant habit-wise, the herbs dominante (57 species), followed by trees (50), shrubs (36), climbers (6) and parasites (1) as source of medicines. This study underscores the need to declare Ramagiri hill as a Medicinal Plant Conservation Centre.

Key words: Ethnomedicinal plants, MPCA, Ramagiri Khilla, Ramagiri hill fort, Karimnagar district, Telangana, Andhra Pradesh, India

INTRODUCTION

Plant-based traditional medical systems continue to provide the primary health care to more than three-quarters of the world's populace. WHO has estimated that over 80% of the global populations rely chiefly on traditional medicine (Akerele, 1991). Indigenous herbal treatment is a part of the culture and dominant mode of therapy in most of the developing countries. It was officially recognized that 2500 plant species have medicinal value while over 6000 are estimated to be explored in traditional, folk and herbal medicine (Huxley, 1984). More emphasis is being placed on possible economic benefits, especially of the medicinal use of tropical forest products (non-woody forest produce) instead of pure timber harvesting (Pimbert and Parks, 1995). In many developing countries, a large population especially in rural and forest areas, depends on traditional medicines for their primary health care.

Ramagiri hill forest is located in Karimnagar district of Andhra Pradesh. It not only known for its rich wealth of medicinal plants but also historical value with the fort built by Kakatiyas. During the Telugu month of Shravana (Aug.-Sep.), the fort attracts pilgrims to offer their rituals and many botanists and Ayurvedic doctors to explore the plant wealth there in.

Kapoor and Kapoor (1980) were the first to publish the medicinal plant wealth of Karimnagar district. Later, Hemadri (1990) enlisted 436 medicinal plants (mere botanical names and vernaculars only) for Karimnagar and Warangal ditricts. Ravishankar (1990) studied the ethnobotany of Karimnagar and Adilabad districts. An estimation of tribal dependency on local forest (Mahadevpur reserve) was made by Reddy (1996). Rao et al. (1998) reported plants used in ethnomedicine by the tribals of Mahadevapur. Reddy et al. (2003) reported the ethnoveterinary medicinal plants used by the Gonds of Karimangar district. Naqvi (2001) discussed briefly some of the ethnomedicinal plants from the district as part of his study of the flora. Murthy et al. (2008) recorded ethnomedicinal plants used by the tribes of Mahamuttaram and Yamanpally villages of Karimnagar district. The present report is the inventory the ethnomedicinal plants from Ramagiri Hill forests of Karimnagar district of Andhra Pradesh, India.

MATERIALS AND METHODS

Study site

Ramagiri hill forest is located 40 km away from Karimnagar, the district head quarters. It includes seven forest beats of Manthani forest range of Karimnagar East Forest Division, viz. Mydambunda, Kundaram, Lakkaram, Peddapally, Sabbitham, Kalvacherla and Maredugonda (Fig. 1). It lies between 79 0 25' E - 79 028' E long. and 18 0 34' N – 18 0 38' lat. The hills extend over 14.7 km, attaining an altitude 679 m. The hill top is plateau of surface area over 40 sq km in which a rock fort was built, called Ramagiri Hill Fort or Quilla. The total forest area of Ramagiri hill ranges is 3205.16 sq ha. Ramagiri hill is often referred as Ratnagiri or Ratnagarbha. The history of Ramagiri Hill fort began from the first century AD. This fort was once called Vajra kootami. Gowthami Puthra Shri Shathakarni (62 AD) and Pulomavi (86 AD) ruled this region. The historians believe that the fort was developed by the Mouryan emperors - Chandragupta, Bindusara and Asoka. Kakatiyas defeated Chalukya Gunda Raju and occupied the fort in 1158 AD. Later, the fort went under the rule of Bahamani Sultans (1442-1457) and till 1597 AD under the rule of Moghuls. In 1606 AD, Golconda Nawabs

occupied the fort. Muslim Kings ruled the fort till the Nizam regime. Now the glory of the fort is history and it is in ruins due to the negligence (Rajagopal, 1974; Naqvi, 2001; Rajesham, 2006).

Ramagiri hill ranges and the surrounding forests are known for its medicinal plants. Many families of herbal vendors, traditional medicine men, village vaidyas and folk healers gather the medicinal plants. Some collect the crude medicinal plants to be sold in towns like Karimnagar, Peddapally, Manthani, Jagitial, Siricilla and Huzurabad. Local students visit this place to collect the plant to prepare the herbarium specimens. There is a great scope to develop and preserve the fort area as a Medico-botanical centre.

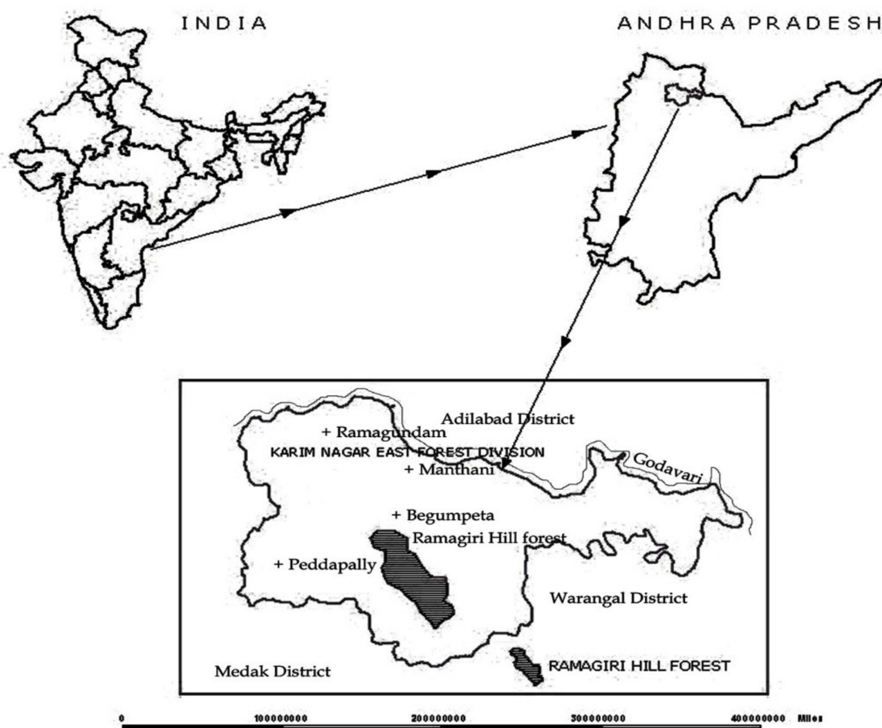

Fig. 1.The study area.

Data collection

The medicinal plant survey included repeated interviews with aged local people, herbal healers, shepherds, tribal headmen, owners of cattle herds, etc. in different seasons for two consecutive years. Field trips were conducted during 2009-2011. The information on useful plant species, parts used, local names and mode of utilization was collected. Plants used in their traditional uses were identified with the help of regional floras (Gamble and Fischer,

1915-35).The plant specimens were pressed and deposited in the Herbarium of Botany Department (KUH), Kakatiya University, Warangal, India.

KEY FINDINGS AND EMPIRICAL OBSERVATIONS

There are 150 medicinal plants recorded from the Ramagiri hill forest representing 55 Angiosperm families. The detailed list of medicinal plants are enumerated in the table1 with their local/vernacular names, habit, medicinal uses and part used etc.

Table 1: Ethnomedicinal plants of Ramagiri Hill forest, Karimnagar District, Andhra Pradesh, India

	Latin Name	Habit	Family	Vernacular Name	Medicinal Uses	Part Used
1	Abelmoschus ficulneus (L.)Wight & Arn.	H	Malvaceae	Adavi benda	Contraceptive, boils, sprains, sores	L
2	Abrus precatorius L.	C	Papilionaceae	Gurija	Aphrodisiac, anti-inflammatory, eye-troubles	Sd
3	Abutilon indicum (L.) Sweet	S	Malvaceae	Tuturu benda	Leprosy, urethritis	W
4	Acalypha indica L.	H	Euphorbiaceae	Pippenta	Antihelmenthic, hysteria, rheumatism	W
5	Acacia catechu Willd.	T	Mimosaceae	Chandra	Skin diseases, diarrhoea	B
6	Acacia chundra (Rottl.)Willd.	T	Mimosaceae	Chandra bheda	Skin diseases, toothache	B
7	Acacia farnesiana (L.)Willd.	T	Mimosaceae	Murki tumma	Toothache, gum swelling	B
8	Acacia leucophloea (Roxb.) Willd.	T	Mimosaceae	Tella tumma	Stringent	B
9	Acacia nilotica (L.)Del.	T	Mimosaceae	Nalla tumma	Toothache, gum swelling	B

10	Achyranthus aspera L.	H	Amaranthaceae	Uttareni	Piles, diuretics, easy child birth	W
11	Actinopteris radiata (Sw.)Link.	H	Actinopteridaceae	Mayur sika	Antihelmintic	W
12	Adiantum incisum Forssk,	H	Adiantaceae	Rajahamsa	Skin diseases, diabetes	W
13	Aegle marmelos (L.) Corr.	H	Rutaceae	Maredu	Dysentery, vomiting, colic, deafness, piles, jaundice	RB,L ,Fr
14	Ailanthes excelasa Roxb.	T	Simaroubaceae	Pedda manu	Dyspepsia, bronchitis, arthritis	B,L.
15	Alangium salviifolium (L.)Wang	T	Alangiaceae	Uduga	Poisioning, dog bite	L.R.Sd
16	Albizia lebbeck (L.) Benth.	T	Mimosaceae	Dirisena	Snake-bite, scropion sting	B
17	Aloe vera (L.)Burm.f.	H	Liliaceae	Kalabanda	Piles, menorrhagia	L
18	Alternanthra sessilis (L.)R.Br.	H	Amaranthaceae	Ponaganti	Snake-bite	W
19	Andrographis paniculata (Burm.f.)Nees	H	Acanthaceae	Nalavemu	Fevers, antihelmintic	W
20	Anisochillus carnosus (L.f.)Wall.	H	Lamiaceae	Bhutankusham	Diaphoretic, expectorant	W
21	*Annona squamosa L.	T	Annonaceae	Seethapalam	Paste of seed to kill lice	Sd
22	Anogeissus acuminata (DC.)Guill. &Perr.	T	Combretaceae	Peruleni chettu	Wound healing	B
23	Anogeissus latifolia (DC.) Bedd.	T	Combretaceae	Chiru manu	Snake-bite, scorpion sting	B
24	*Argemone mexicana L.	H	Papaveraceae	Brahma dandi	Syphlis gonorrhoea, leprosy, eczema, eye trouble	W

25	Aristolochia bracteolata L.	H	Aristolochiaceae	Gaddapaku	Antihelminthic, amenorrhoea	W
26	Aristolochia indica L.	H	Aristolochiaceae	Nalla eeshwari	Snake-bite, arthritis	W
27	Asparagus recemosus Willd.	C	Liliaceae	Pilli teegalu	Stomach-ache	L
28	Azadiracta indica Juss.	T	Meliaceae	Vepa	Fevers, antiseptic, malarial fever, febrifuge	W
29	Balanites roxburghii Planch.	T	Simaroubaceae	Gara	Leprosy, wounds, antheimantic, snakebite	B,Fl, Sd
30	Baliospermum montanum (Willd.)Muell.-Arg.	H	Euphorbiaceae	Danthi	Purgative, stimulent	L,R,Sd
31	Barleria prionitis L.	S	Acanthaceae	Mulugorinta	Sprematorrhea, ootitis	SD
32	*Basella rubra L.	C	Basellaceae	Batchali teega	Catarrha affections bilious, vomting	W
33	Bauhinia racemosa Lam.	T	Caesalpiniaceae	Arechettu	Headache, malaria	L
34	Boerhavia diffusa L.	H	Nyctaginaceae	Galli jeru	Urinary disorders, anthelmentic, fever	W
35	Buchanania lanzan Spreng.	T	Anacardiaceae	Charapappu	Urinary dissorders	Fr
36	Butea monosperma (Lam.)Taub.	T	Papilionaceae	Moduga	Leucorrhoea	B,Fl
37	Butea monosperma (Lam.)Taub. var. lutea Maheshw.	T	Papilionaceae	Tella moduga	Tonic after delivery	B
38	Butea superba Roxb.	C (Liana)	Papilionaceae	Tiga moduga	Leucorrhoea	B,Fl
39	Caesalpinia bonduc (L.) Roxb.	S	Caesalpiniaceae	Gachapoda	Emmenagogue, gastic tonic	Sd

40	Calotropis gigantean (L.) R.Br.	S	Asclepiadaceae	Tella jilledu	Alterative, tonic, spasmodic, expectorant, eye trouble	Sd
41	Calotropis procera (Ait.)R.Br.	S	Asclepiadaceae	Jilledu	Alterative, tonic, spasmodic, expectorant, eye trouble	W
42	Capparis zeylanica L.	S	Capparaceae	Are donda	Sedative, diuretic	R
43	Caralluma adscendens var. attenuata (Wight) Gravely &Mayur.	H	Asclepiadaceae	Kundeti kommu	Analgetic, toothache	W
44	Cordiospermum helicacabum L.	C	Sapindaceae	Budda teega	Diuretic, laxative, emetic, rheumatism, piles	W
45	*Carica papaya L.	T	Caricaceae	Boppayee	Dyspepsia, psoriasis, chronic eczema	Fr, St
46	Carissa spinarum L.	S	Apocynaceae	Kalimi	Fevers, stomachc, digestive	Fr
47	Carissa carandas L.	S	Apocynaceae	Kalimi	Digestive, carminative	Fr
48	Senna auriculata (L.) Roxb.	S	Caesalpiniaceae	Tangedu	Diabetes, bed wetting	Fr, L
49	Senna fistula L.	T	Caesalpiniaceae	Rela	Laxative, diabetes, gout, rhematism	Fr, S
50	Senna occidentalis (L.) Link	H	Caesalpiniaceae	Kaasinta	Asthma, skin deseases, laxative	W
51	Senna sophera (L.) Roxb.	H	Caesalpiniaceae	Chennangi	Antiseptic	W
52	Senna tora (L.) Roxb.	H	Caesalpiniaceae	Kasivinda	Leprosy, psoriasis, plague, gout, sciatia, pains	W
53	Cassytha filiformis L.	P/C	Lauraceae	Pachi teega	Bilious affectious,	W

					urethrilis, skin diseases	
54	*Catharanthus roseus (L.)G.Don	H	Apocynaceae	Billa gannera	Cancer, blood pressure	W
55	Catunaregum spinosa (Thunb.) Tirv.	S	Rubiaceae	Konda manga	Emetic	Fr
56	Cleome gynandra L.	H	Cleomaceae	Vamintaku	Ear diseases, wounds, ulcers	L
57	Cleome viscosa L.	H	Cleomaceae	Kukka vaminta	Infalmnation of middle ear, applied to wounds	L
58	Clerodendrum phlomidis L.f.	S	Verbenaceae	Takkali	Gonorrhoea, pherperal diseases	L
59	Coccinia grandis (L.) Voigt	C	Cucurbitaceae	Donda teega	Cooling effect	Fr
60	Cocculus hirsutus (L.)Diels	C	Menispermaceae	Dusara teega	Acute gonorrhoea, rhemnatism, syphilis	L
61	Cochlospermum religiosum (L.) Alston	C	Cochlospermaceae	Konda gogu	Diarrhoea, dysentery	B
62	Commelina benghalensis L.	H	Commelinaceae	Venna veduru	Laxative, diabetes, gout, rhematism	W
63	Crotalaria verrucosa L.	H	Papilionaceae	Telleshwari	Scabies	W
64	*Cucumis sativus L.	C	Cucurbitaceae	Dosa kaya	Throat affections, sun stroke	Fr
65	Curculigo orchioides Gaertn.	H	Hypoxidaceae	Nela tadigadda	Polyurea,aphrodisiac,scropion bite,menstural disorders	W
66	*Datura innoxia Mill.	H	Solanaceae	Tella ummetha	Diarrhoea, poisonous bites	W
67	*Datura metal L.	H	Solanaceae	Nala ummetha	Aphrodisiac, insanity	L
68	Dodonaea viscosa	Sh	Sapindaceae	Bandarae	Broken bones, wounds	L

	(L.)Jacq.					
69	Echinops echinatus Roxb.	H	Asteraceae	Brahma dandi	Nervine tonic, diuretic aphrodisiac	W
70	Eclipta prostrata (L.)L.	H	Asteraceae	Gunta galagara	Skin diseases, hepatic tonic, bites	W
71	Euphorbia nivulia Buch.-Ham.	T	Euphorbiaceae	Bonta jemudu	Rhumatism	Latex
72	Evolvulus alsinoides (L.)L.	S	Convolvulaceae	Vishnu kranta	Fevers, dysentery, anthelmintic	W
73	Ficus benghalensis L.	T	Moraceae	Marri	Rhematism, toothaches	B
74	Flacourtia indica (Burm.f.)Merr.	T	Flacourtiaceae	Porika	Snake-bites, gout, rheumatism	Sd
75	Gardenia gummifera L.f.	T	Rubiaceae	Bikki	Antiseptic, authelmintic, bleeding piles	L,Fr, Sd
76	Gisekia pharnaceoides L.	H	Aizoaceae	Irshi-rashkura	Diuretic	W
77	Gloriosa superba L.	H	Liliaceae	Nabi chettu	Abortificient, leprosy, gonorrhoea	W
78	Gmelina arborea Roxb.	S	Verbenaceae	Gummadi teku	Galactogogue, gonorrhoea, fevers, indigestion	B
79	Gymnema sylvestris (Retz.)Roem. &Schult.	H	Asclepiadaceae	Poda patri	Stomachic, diuretic, diabetes	L
80	Haldina cordifolia (Roxb.) Ridsdale	T	Rubiaceae	Batta ganepu	Tonic after delivery	B
81	Oldenlandia umbellata L.	H	Rubiaceae	Chiruveru	Snake bite, asthama	W
82	Helictris isora L.	S	Sterculiaceae	Nul tada	Febrifuge	Sd
83	Hemidesmus indicus (L.)R.Br.	H	Asclepiadaceae	Pala sgandhi	Nutritional disorders, leucorrhoea,	R

					rhematism	
84	Hybanthus enneaspermus (L.) F. Muell.	H	Violaceae	Rathna purusha	Aphrodisiac	W
85	Holorrhena antidysenterica (Roth)DC.	T	Apocynaceae	Kodisha pala	Anthelmintic, carminative	B
86	Ichnocarpus frutescens (L.)R.Br.	C	Apocynaceae	Pala teega	Purifies the blood,skin diseases,syphilis,eleph antiasis	L
87	Indigofera tinctoria L.	H	Papilionaceae	Neeli chettu	Sedative, piles, diuretic, dropsy	W
88	*Lawsonia inermis L.	S	Lythraceae	Gorintaku	Burning feet, small pox, rheumatism, wounds	L,Sd
89	Hygrophila auriculata (Schumach.)Heine	H	Acanthaceae	Neeru golimidi	Aphrodisciac, diuretic, dropsy	Sd
90	Leucas aspera (Willd.)Link	H	Lamiaceae	Tummi kura	Insecticide, scabies, snake bite	W
91	Lepidagathis cristata Willd.	H	Acanthaceae	Mulla banthi	Fevers	W
92	Luffa acutangula (L.)Roxb.	C	Cucurbitaceae	Beera	Expectoraut, splenitis, haemorrhoides, leprosy	Fr,Sd
93	Luffa acutangula var. amara (L.)Roxb.	C	Cucurbitaceae	Chedu beera	Diabetes, dropsy	Fr
94	Luffa cylindrica (L.)M.Roem.	C	Cucurbitaceae	Venna beera	Cool, demulcent	Fr
95	Madhuca longifolia var. latifolia (Roxb.) A. Chiov.	T	Sapotaceae	Ippa	Asthma, epistaxis, gives vigour, vitality	B,L,Fr
96	Mangifera indica L.	T	Anacardiaceae	Mamidi	Atonic dyspepsia, constipation, bleeding	Fr

97	Dregea volubilis (L.f.)Benth. ex Hook.f.	C	Asclepiadaceae	Penujittu	Cooling, alterative, gonorrhoea	L, St
98	*Martynia annua L.	S	Martyniaceae	Telu gondi	Used in scorpion stings	Fl
99	*Melia azadirach L.	H	Meliaceae	Turekapa	Anthelintic, nervous headaches	Fl, Fr
100	*Mimosa pudica L.	H	Mimosaceae	Ati pati	Piles, fistula, scorpion sting, menstrual disorders	Sd
101	Mitragyna parvifolia (Roxb.) Korth.	T	Rubiaceae	Batta ganapa	Stomachache, stimulant, emetic	B
102	*Moringa oleifera Lam.	T	Moringaceae	Munaga	Rheumatism, gout, syphlilis, paralysis	B,Fl, Sd
103	Moringa concanensis Nimmo ex Dalz.	T	Moringaceae	Chedu munaga	Blood purifier	Sd
104	Mucuna pruriens (L.)DC.	C	Papilionaceae	Duli dundi	Aphrodisiac, leucorrhoae, spermatorrhoea	L
105	*Murraya koengii (L.) Spreng.	S	Rutaceae	Karivepa	Stimulant	L
106	*Musa x paradisica L.	S	Musaceae	Arati	Haemoptysis, diabetes	Fr
107	Momordica charantia L.	C	Cucurbitaceae	Kakara	Diabetes, leprosy, piles, jaundice	Fr
108	Momordica dioica Roxb.	C	Cucurbitaceae	Karkotaki	Diabetics	Fr
109	*Nerium indicum Mill.	S	Apocynaceae	Ganneru	Conjuctivities, syphilis	Fl
110	*Ocimum tenuifloiun L.	H	Lamiaceae	Tulasi	Expectorant, antiseptic	W
111	*Opuntia dillenii (Ker-Gawl)Haw.	S	Cactaceae	Naga jamudu	Whooping cough, gonorrhoea	W

112	Pergularia daemia (Forssk.)Choiv.	C	Asclepiadaceae	Dishtapu teega	Asthama, leprosy	L,St
113	Phoenix sylvestris (L.)Roxb.	T	Arecaceae	Eetha	Opthalmia, opacity of cornea	Fr
114	Phyllanthus amarus Schumm.and Thonn.	H	Euphorbiaceae	Nela-usiri	Jaundice, gonorrhoea, insect bites	Fr
115	Phyllanthus emblica L.	T	Euphorbiaceae	Usiri	Asthma, menstrual disorders	Fr
116	Plumbago zeylanica L.	S	Plumbaginaceae	Chiramulamu	Piles, skin diseases	W
117	Pterocarpus marsupium Roxb.	T	Papilionaceae	Teddagi	Toothace, boils	B
118	Pongamia pinnata (L.)Pierre	T	Papilionaceae	Kanugu	Skin diseases, pyorrhea	B
119	*Punica granatum L.	S	Punicaceae	Danimma	Diarrhoea, anthelmentic	Fr,Sd
120	Rivea hypocrateriformis (Desr.)Choisy	C	Convolvulaceae	Teega boddi	Piles, constipation	W
121	Sapindus emarginatus Vahl	T	Sapindaceae	Kunkudu	Migrain, abortifacient	B,Fr, Sd
122	Sarcostemma acidum (Roxb.)Voigt	S/C	Asclepiadaceae	Atukudu teega	Wounds, cuts, leprosy	W
123	Ledebourea hyacinthina Roth	H	Hyacinthaceae	Adavi ulligadda	Rheumatic pains	W
124	Sida acuta Burm.f.	H	Malvaceae	Parasukamp	Gen. Debility, boils, absciss	W
125	Sida cordifolia L.	H	Malvaceae	Bhoomi bala	Paralysis, anaemia	W
126	*Solanum americanum Mill.	H	Solanaceae	Kamanchi	Heart diseases, hiccough	Fr,L
127	*Solanum virginianum L.	H	Solanaceae	Nalavakudu	Cough, urinary tract infections	Fr

128	Soymida febrifuga (Roxb.)Juss.	T	Meliaceae	Somi	Fevers, vaginal infections	B
129	Sphaeranthus indicus L.	H	Asteraceae	Boadataram	Eye trouble, tonic, lice killer	W
130	Strychnos nux-vomica L.	T	Loganiaceae	Vishmushti	Paralysis, fevers	Fr,L
131	Strychnos potatorum L.f.	T	Loganiaceae	Chilla	Urinary treat infections, eye diseases	Fr
132	Syzigium cumini (L.)Skeels	T	Myrtaceae	Neredu	Diabetes, Diarrhoea	B,Fr, Sd
133	Tamarindus indica L.	T	Caesalpiniaceae	Chintha	Oedema, piles	Fr,L
134	Tephrosia purpurea (L.) Pers.	T	Papilionaceae	Vempali	Diabetes, spleen, level disorders	W
135	Terminalia alata Roth	T	Combretaceae	Nalla maddi	Bactericidal, ulcer	B
136	Terminalia arjuna (DC.)Wight & Arn.	T	Combretaceae	Eru maddi	Heart diseases, urinary tract infections	B,Fr, Sd
137	Terminalia bellerica (Gaertn.)Roxb.	T	Combretaceae	Tanikya	Urinary calculi, asthma	B
138	Terminalia catappa L.	T	Combretaceae	Badam	Diabetes, back pain	B
139	Terminalia chebula Retz.	T	Combretaceae	Karakkaya	Piles, jaundice	B
140	Tinospora cordifolia (Willd.)Hook.f. & Thoms.	C	Menispermacea e	Tippa teega	Fevers, gout	W
141	Trianthema portulacastrum L.	H	Aizoaceae	Galijeru	Night blindness, urinary disorders	W
142	*Tridax procumbens L.	H	Asteraceae	Nallalam	Antiseptic, cut, wounds, burns	W
143	Tylophora indica (Burm.f.)Merr.	C	Asclepiadaceae	Mekameyami aku	Asthma, emetic	B,L
144	Ventilago denticulata	C	Rhamnaceae	Erra sulugudu	Tonic, post delivery	B

	Willd.				treatment	
145	Vitex negundo L.	S	Verbenaceae	Vavila	Sciatica, arthritis, eye trouble	W
146	*Withania somnifera (L.) Dunal	H	Solanaceae	Aswaghanda	Reducing sugar, body strength, aphrodisiac	R,Sd
147	Wrightia tinctoria R.Br.	S	Apocynaceae	Doddapala chettu	Diarrhoea, dysentery	B
148	Ziziphus mauritiana Lam.	T	Rhamnaceae	Ganga regu	Aphrodisiac, diuretic	B,Fl, L
149	Ziziphus oenoplia (L.)Mill.	S	Rhamnaceae	Pariki	Digestive tonic, cut wounds	B, Fr
150	Ziziphus xylopyrus (Retz.)Willd.	S	Rhamnaceae	Gotti	Skin diseases	Fr

* Planted/exotic/runningwild; B=Bark; C=Climber; Fl=Flower; Fr=Fruit, H=Herb; L=Leaves; R=Root;Sd=Seed; P= Parasite; S=Shrub; St=Stem; T=Tree; W=Whole plant.

The Papilionaceae are the dominant family (Fig. 2) with 10 medicinal species followed by Caesalpiniaceae with eight, Apocynaceae, Combretaceae, Cucurbitaceae, Mimosaceae with seven, Euphorbiaceae, Malvaceae and Rubiaceae with five species. The rest of the families contribute one or two medicinal species only. It is a thero-phanerophytic climate (Naqvi 2001). Herbs (57) dominate in their medicinal use followed by trees (50), shrubs (36), twiners (6) and parasites (1) (Fig.3).

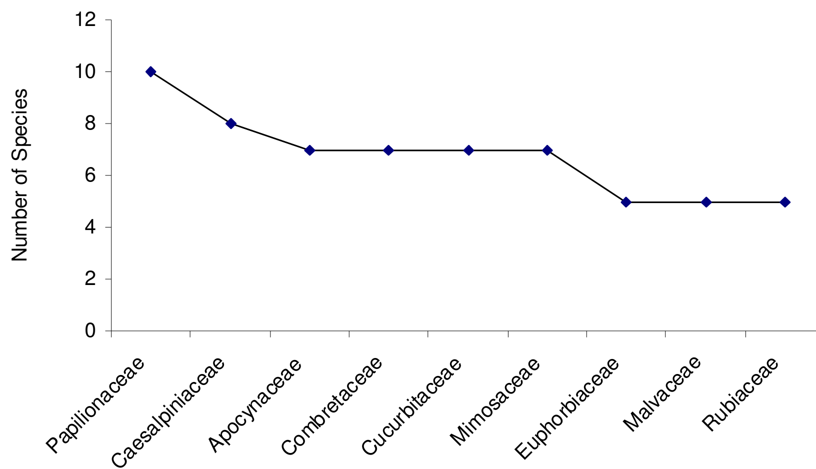

Fig. 2. Dominant angiosperm families contributing medicinal plants.

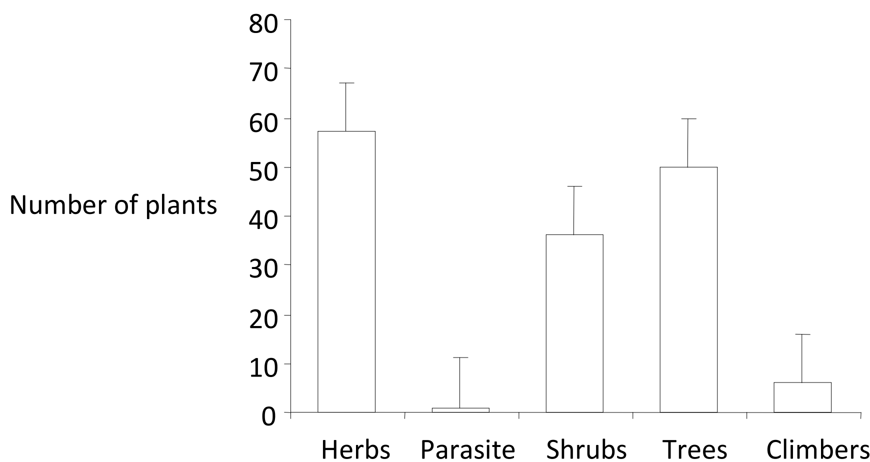

Fig. 3 Habit-wise analysis of the medicinal plants.

The local people and the herbal physicians use the following phytodrugs for common human ailments: (i) Asthama: Dishtapu teega (Pergularia daemia, leaves and stem), Usiri (Phyllanthus emblica: fruits), Tani (Terminalia bellerica: stem bark) and Mekameyani aku (Tylophora indica: leaves). (ii) Blood Purifier: Barre sughandi pala (Hemidesmus indicus var. pubescens: whole plant), Pala teega (Ichnocarpus frutescens: leaves) and Chedu munaga (Moringa concanensis: stem bark). (iii) Bone fracture: Bandarae (Dodonaea viscose: leaves) and Venna bera kaya (Luffa cylindrical: fruit) (iv) Diabetes: Tangedu (Senna auriculata: leaves and fruits), Rela (Senna fistula: fruits and stem bark), Poda patri (Gymnema sylvestris: leaves), Chedu beera (Luffa acutangula var. amara). Arati (Musa paradisica: fruits), Kakara (Momordica charantia: fruits), Boda kakara (Momordica dioica: fruits), Neredu (Syzygium cumini, seeds: stem bark) and Vempali (Tephrosia purpurea: whole plant). (v) Diarrhoea: Konda gogu (Cochlospermum religiosum: stem bark), Tella ummetta (Datura innoxia, whole plant) and Dodda palachettu (Wrightia tinctoria: stem bark). (vi) Eye troubles (including conjunctivitis and opacity of cornea): Gurija (Abrus precatorius: seeds), Brahmadandi (Argemone Mexicana: whole plant), Tella and Erra jilledu (Calotropis gigantean: C. procera, all parts), Eetha (Phoenix sylvestris: fruits), Chilla (Strychnos potatorum: fruit) and Vavila aku (Vitex negundo: leaves) (vii) Fever: Nela vemu (Andrographis paniculata: whole plant), Vepa (Azadirachta indica: all parts), Kalimi (Carissa spinarum: fruits), Vishnu kranti (Evolvulus alsinoides: whole plant), Jegi (Soymida febrifuga: stem bark) and Tippa teega (Tinospora cordifolia: whole plant). (viii) Skin diseases: Chandra (Acacia catechu: bark), Chandra bheda (Acacia chundra, bark), Kasintha (Senna occidentalis: whole plant), Pachiteega (Cassytha filiformis, whole plant), Gunta galagara (Eclipta prostrate: whole plant), Pala teega (Ichnocarpus frutescens: leaves), Chitramulam (Plumbago zeylanica: whole plant), Kanugu (Pongamia glabra: bark) and Gotti (Ziziphus xylopyrus: fruit), and (ix) Toothache and gum swelling: Chandrabheda (Acacia chundra: bark), Murki tumma (Acacia farnesiana: bark), Kundeti kommu (Caralluma adscendens var. attenuate: whole plant), Marri (Ficus benghalensis: bark) and peddegi (Pterocarpus marsupium: bark).

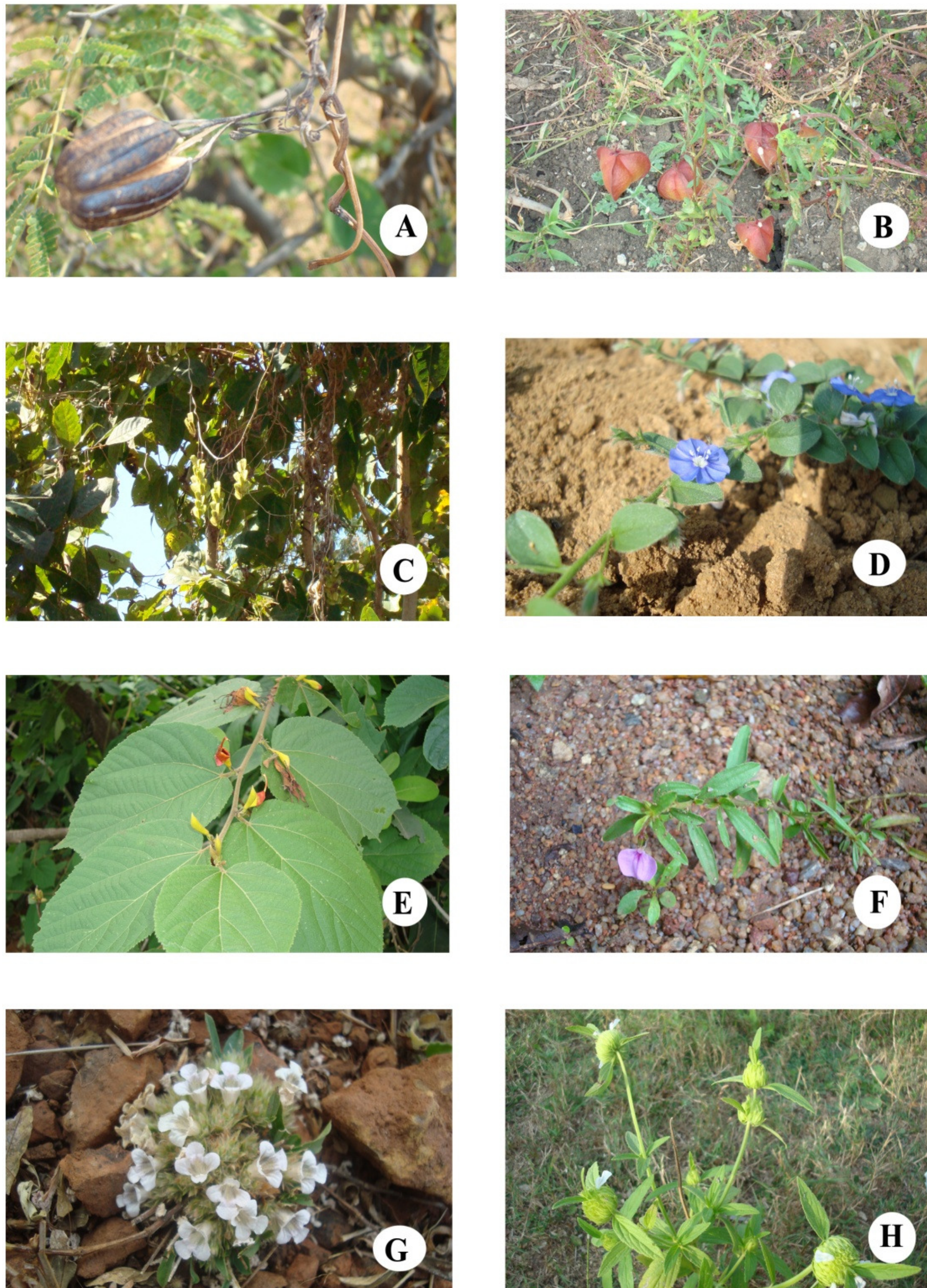

Fig. 4. Medicinal plants of Ramagiri hill forest.

A) Aristolochia indica; B) Cardiospermum halicacabum; C) Mucuna pruriens; D) Evolvulus alsinoides; E) Helicteres isora; F) Hybanthus enneaspermus; G) Lepidathis cirstata; H) Lecuas asper.

Fig. 5 Architectural variations in entrance gates of Ramagiri Fort

Apart from the medicinal plants, beedi leaves, gum karaya, chilla, musti, brooms, ippa and copri are the major non-timber forest produce (NTFPs) collected and sold by the

tribal and non-tribals people. Koyas are the main ethnic tribe who are though settled cultivators, depend largely upon the nearby forests for non-timber products. Nayakpods, the other important ethnic tribe, are also primarily agriculturists and podu (shifting) cultivators. They also collect forest produce. Lambadas, a gypsy non-local tribe, are largely workers and, at places, settled agriculturists. An estimate of their dependence on the local forest was made by Reddy (1996) and Rao et al. (1998). Most of the local communities are benefited by collection of beedi leaf (Diospyros melanoxylon), flower and seeds of Mahua (Madhuca longifolia) and broom grass (Thysanolaena maxima).

CONCLUSION

The plants are used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals of hitherto unmet therapeutic needs. Ramagiri hill forest needs immediate attention from the standpoint of conservation. Karimnagar district is rapidly developing in all spheres through rapid expansion of urban limits, indiscriminate open-cast coal mining, granite mining, agriculture, irrigation dams, thermal power stations and cement industries which have bearing on forest ecosystem, and forest cover in the district. Againt this backdrop, Ramagiri forest should be announced as Medicinal Plants Conservation Center and need to be developed as ecotourism place along with Kondagattu and Singarayakonda hill forests and Mahadevpur reserved forests which are the important sources of medicinal plants in Karimnagar district of Andhra Pradesh, India.

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16.

Phytochemical studies, anthelmintic activity of leaf extracts of *Annona reticulata* Linn.

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Abstract: *Annona reticulata* is traditionally used for various ailments. Fresh leaves are used as anthelmintic and bark powder is used for diaorrhea and dysentery. In the present study preliminary phytochemical screening was performed for chloroform, methanolic, ethanolic and aqueous extracts and results showed the presence of carbohydrates, alkaloids, tannins and steroids. These extracts were screened for their anthelmintic activity. Traditional plant based remedies continue to be an important therapeutic aid for treating worm infections. Experiments were conducted to evaluate the possible anthelmintic activity of the above mentioned extracts of leaves of annona reticulata . 10mg/ml, 20mg/ml, 50mg/ml concentrations of all extracts were tested for antihelmentic activity against the earthworm *pheritima posthuma*. The results were expressed in terms of time of paralysis and time for death of worms. 50mg/ml concentrations methanolic and aqueous extracts had shown good anthelmintic activcity as compared to chloroform and ethanolic extracts.

Keywords: Annona reticulata, Anthelmintic activity

Introduction:

Many plant species have been used to treat various ailments in folk medicine. A survey by WHO says 80% populations rely almost exclusively on traditional medicine for their primary healthcare needs (1,2). Natural remedies from medicinal plants are found to be safe and effective. The phytochemicals found in plants are known to provide protection against insect attacks and plant diseases. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, cardiac glycosides, steroids and saponins (3). *Annona reticulata* is small

deciduous or semi-evergreen tree in the plant family Annonaceae. It is best known for its fruit, called Custard-Apple, a name it shares with fruits of other species from the same genus: *A.Cherimola* and *A.Squamosa*. *Annona reticulata* L. is a highly apparent plant in ayurvedic system of medicine for the treatment of various ailments. The plant is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, antibacterial infection, dysuria, fever, and ulcer. Ethanolic extracts of leaves and stem are reported to have an anticancerous activity. The aqueous leaf extract has also been reported to ameliorate hyperthyroidism (4). Leaf can be used for destroying lice (5). Previously reported phytochemical constituents from the plant are anonaine, roemerine, norcorydiene, corydine, norisocorydine, Carvone, linalool, samoquasine A, squamocin-I, squamocin-B, squamocenin, motrilin, Kaurenoic acid, phenolic and nonphenolic alkaloids , two crystalline alkaloids – muricine, muricinine, (2,4-cis and trans)-squamolinone, (2, 4-cis and trans)-9-oxoasimicinone, bullacin [6,7] etc. Helminthiasis is a macroparasitic disease of humans and animals in which a part of the body is infested with parasitic worms. More than half of the population of the world suffers from infection of one or the other (8). Anthelmintics are the drugs or agents that destroy or cause the expulsion of parasitic intestinal worms. Increasing problems of development of resistance in helminthes (9,10) against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. The plants are known to provide a rich source of botanical anthelmintics. The present investigation was intended to screen the phytochemicals in various leaf extracts of *annona reticulata* and study their anthelmintic activity.

Materials and Methods:

1.Collection of plant materials:

Leaves of *annona reticulata* were collected from gannervaram village, karimnagar district. The identity of the plant was confirmed by Dr.Naqvi, taxonomist SRR Govt.Degree college, karimnagar.

2.Standard reference drug :

The standard anthelmetic drug used is Albendazole obtained from NIHAL TRADERS Pvt Ltd**.**

3.Worm collection:

Healthy adult Indian earthworms , pheritima posthuma, due to its anatomical and physiological resemblance with the intestinal roundworm parasites and human beings were used in present study. All earthworms were approximately equal size (15cm). They were collected from local moist place , washed and kept in water.

Methods:

1.Preparation of extracts :

The plant material (leaves) were shade dried and powdered by a mechanical grinder. The dried powders were extracted with chloroform, methanol, ethanol and water by cold maceration for 2 days. The obtained extracts were filtered by using whatmann no.1 filter paper. The solvents were then distilled off.

2.**Anthelmintic activity:**

The anthelmentic activity was performed according to the method of ghosh etal on adult indian earthworm *pheritima posthuma* as it has anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. 6 groups of approximately equal sized indian earthworms consisting of 5 earthworms in each group were released in to desired formulations. 1st group served as control, received only normal saline, 2nd group served as standard, received standard drug Albendazol(10mg/ml). 3rd ,4th ,5th group served as test which received 10mg/ml, 20mg/ml, 50mg/ml concentrations of chloroform extracts. Group 6, 7, 8 served as test-2 which received 10mg/ml, 20mg/ml, 50mg/ml concentrations of methanolic extract. 9th,10th and 11th group served as test-3 which received 10mg/ml, 20mg/ml, 50mg/ml of ethanolic extract. Group 12, 13 and 14 served as test-4 which received 10mg/ml, 20mg/ml, 50mg/ml of concentrations of aqueous extracts. Observations were made for the time taken to paralysis or death of individual worms. Time taken for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colour.

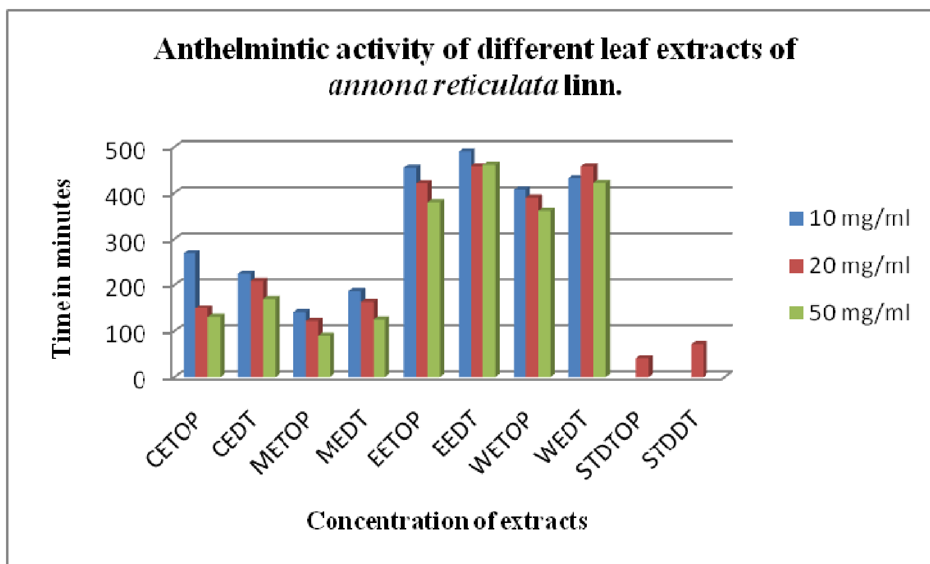
RESULTS OF PHYTOCHEMICAL SCREENING

Preliminary Phytochemical screening of different leaf extracts *Annona reticulata* linn

Name of the phytochemical constituents	Chloroform	Methanol	Ethanol	Aqueous
Carbohydrates	+	+	-	+
Proteins	-	-	-	-
Amino acids	-	-	-	-
Volatile oils	-	-	-	-
Steroids	+	+	-	-
Alkaloids	+	+	-	-
Glycosides	-	-	-	-
Flavanoids	-	-	-	-
Tannins	-	+	-	-

Anthelmintic activity of different leaf extracts of *annona reticulata* linn

Plant extract	Conc(mg/ml)	Time of paralysis (minutes)	Death time (minutes)
Saline	-	-	-
Albendazole	20	40 ± 0.70	72 ± 1.41
Chloroform	10 20 50	270 ± 2.91 150 ± 7.90 130 ± 1.22	224 ± 1.00 208 ± 1.41 170 ± 1.58
Methanol	10 20 50	140 ± 1.22 121 ± 2.00 90 ± 1.41	187 ± 1.58 164 ± 0.70 124 ± 1.41
Ethanol	10 20 50	456 ± 1.22 420 ± 4.24 380 ± 2.00	490 ± 1.41 459 ± 3.16 462 ± 0.71
Water	10 20 50	407 ± 2.96 390 ± 5.52 362 ± 1.58	431 ± 1.00 459 ± 0.70 421 ± 0.70



CETOP-Time of paralysis for Chloroform extract. CEDT-Death time for chloroform extract.
METOP-Time of paralysis for methanolic extract. MEDT- Death time for methanolic extract.
EETOP- time of paraslysis for ethanolic extract. EEDT- Death time for ethanolic extract.
WETOP-Time of paralysis for aqueous extract. WEDT: Death time for aqueous extract.

Discussion: From the present study it is known that all the extracts have shown positive response to certain degree of anthelminthic activity, where as methanolic extract had shown most potent anthelmintic activity compared to other extracts. Chloroform extract also exhibited good activity. Further studies are required to identify the active phytoconstituents that are responsible for the anthelmintic activity and study its pharmacological action.

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17.

Biotechnology: A new era for plant pathology and plant protection

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Plant biotechnology ushers in a new era for plant scientists working to maintain healthy plants, optimize crop yields, and minimize pesticide usage. One of the ultimate aims of agricultural biotechnology is to feed an expanding world population. A recent survey by The Economist shows that the world population has increased by 90% in the past 40 years while food production has increased by only 25% per head. With an additional 1.5 billion mouths to feed by 2020, farmers worldwide will have to produce 39% more grain (The Economist, March 25, 2000). These survey results aptly describe the food production challenges facing the global community of farmers and consumers in the new millennium and the dimension of the debate on the risks and benefits of developing genetically engineered crop plants to meet the increasing global food demand while preserving the environment.

Genetic engineering has the potential to provide a cornucopia of beneficial plant traits, particularly an enhanced ability to withstand or resist attack by plant pathogens. New approaches to plant disease control are particularly important for pathogens that are difficult to control by existing methods. The percentage of crop losses caused by plant pathogens, insect pests, and weeds, has steadily increased to 42% worldwide, accounting for $500 billion dollars worth of damage (Oerke et al., 1994). In the United States alone, crop losses due to plant pathogens amount to $9.1 billion dollars, while worldwide, plant diseases reduce crop productivity by 12% (Food and Agriculture Organization, 1993). Worldwide, pesticide applications costing $26 billion dollars annually are applied to manage pest losses. Genetically engineered plants resistant to plant pathogens can prevent crop losses and reduce pesticide usage. This feature article provides a current perspective on four major areas of research and application of plant genetic engineering for resistance to plant pathogens.

Enhancing resistance with plant genes: Scientists from all over the world are investigating the biochemical nature of, and the signals involved in, a plant's reactions to pathogen invasion and disease development. Plant resistance genes and the genes involved in resistance reactions are being identified and engineered into crop plants to protect them against plant diseases. This rapidly advancing field of investigation is described in this feature under 'Enhancing a plant's resistance with genes from the plant kingdom'

Pathogen derived resistance: Plants can be protected from diseases with transgenes (genes that are engineered into plants) that are derived from the pathogens themselves, a concept referred to as pathogen-derived resistance. For example, plant viral transgenes can protect plants from infection by the virus from which the transgene was derived. Genetic engineering of plants for viral resistance is a thriving area of research and is described in this feature with special emphasis on research being done at Cornell University, Geneva, NY, under Genetic engineering: A novel and powerful tool to combat plant virus diseases.

Antimicrobial proteins: Another area of investigation involves peptides and proteins with antimicrobial properties that when produced by plants have the potential to strengthen plant resistance to fungal and bacterial plant pathogens. Fungi, insects, animals, and humans all contain genes encoding antimicrobial compounds. This use of antimicrobials to improve plant resistance to pathogens is described in this feature with special emphasis on research being done at Cornell University, Geneva, NY, under Using antimicrobial proteins to enhance plant resistance.

Plantibodies: Although plants have mechanisms to protect themselves against pathogen attack, in contrast to animals, there is no "immune system" *per se* in plants. With the advent of genetic engineering, plants can be engineered to express an antibody against a protein crucial for pathogenesis resulting in a level of immunity or resistance to the pathogen. This promising approach is described under Plantibodies: an animal strategy imported to the plant kingdom to fight back pathogens.Biotechnology is now a lightning rod for visceral debate, with opposing camps making strong claims of promise and peril. The debate involves not only scientific but also political, socio-economic, ethical, and philosophical issues (Wambugu 1999, Hails 2000, Ferber 1999, Trewavas 1999, Sagar *et al.* 2000).

This feature article provides a glimpse of the application of biotechnology to plant improvement. The dawn of a new era in plant pathology and plant protection is upon us. Biotechnology has rewritten the scope of scientific investigation, broadened the avenues to resistant plants, and challenged us to take safe and careful steps. Like any other new technology, much still needs to be done before the full potential of agricultural biotechnology is realized. As more and more plant biotechnology products become available, studies to evaluate the risks associated with biotechnology must be intensified. Findings from such studies must be easily accessible to the general public. The risks associated with this technology must be addressed and the benefits should be kept in mind. We are confronted with biotechnology's vast perspective and this astounding view has expanded the very foundation of our understanding of life.

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18. INDUCED GLYPHOSATE RESISTANCE IN SOYBEAN CELL SUSPENSION CULTURES

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ABSTRACT

Plant tissue culture and biotechnology offers a greater stimulus for crop modifications by using r-DNA technology, genetic transformations and gene amplification for be herbicide resistance. Embryogenic cell suspension cultures of *Glycine max* were subjected to stepwise selection with increasing Glyphosate concentrations (0.01 to 50 mMG) for induction of tolerance and gene amplification studies. The cell lines were less adoptive in terms of herbicide resistance upto concentration 2.0 mMG. In a stepwise selection from 2.0 mMG onward the cell lines showed greater efficiency and tolerance for selection pressure when compared to other concentrations W-82/35G cell lines showed 14 fold increase in the enzyme activity and 650 folds increase in the I_{50} value than that of unselected wild type of embryogenic soybean cell lines. Enhanced EPSPS enzyme activity is due to over expression of corresponding target gene or amplification of DNA

INTRODUCTION

Plant tissue culture comprises the *In Vitro* cultures of various kinds of explants ranging from cells to tissues and organs. It facilitates experimental approaches with a large variety of objectives in developmental biology. The theory and goals of mutant (or) variant selection from tissue is reviewed by several people. Due to the presence of a large population of totipotent cells under aseptic conditions, the plant tissue culture system is considered as ideal for genetic manipulations of crop plants. The cell line selection for crop modifications has made tremendous

progress in plant biotechnology by manipulation of genetic material at cellular and molecular level. Soybean is the most important Legume Crop in terms of total production and international trade. Presently it ranks high among the Legume Crops in its nutritional value owing to its high protein content as high as 42 percent. Soybean cells grow readily when placed under culture conditions and have been studied as undifferentiated friable callus or suspension cultures. In the last decade new approaches were developed to produce cultures capable of regeneration in fertile plants via either organogenesis or embryogenesis. These culture systems usually consist of relatively large tissue masses, which are ideal as single (or) small clumps of cells for I*n Vitro* simple and complicated selection experiments. The use of herbicide to reduce loss in crop yield has become an integral part of modern agricultural practices. There is a continuous demand for new herbicide that are highly effective and safe for both animals and the environments. Most of the herbicides do not distinguish between weeds and crop plants (Widholm *et al.*, 1996). A new group of herbicides has emerged and this fulfills these needs by inhibiting specific amino acid biosynthesis pathway in plant. Modifying plants to become resistant to broad-spectrum herbicides would allow their selective use for crop protection (Dunken and Widholm 1984, Ramulu, 1996). Glyhosate (N-phosphonomethyl glycine) is highly effective broad-spectrum herbicides, a competitive inhibitor with respect to PEP and an uncompetitive inhibitor with regard to the other substrate, S-3-P, in the EPSPS reaction. This Glyphosate is lacking specificity between weeds and crops has been used a selective agents for micro-organisms and higher plants cells (Stemhurken & Amerhen 1980, Donn *et al.* 1984). This paper reports the study of selection for amplification of EPSPS gene product in soybean W-82 cell lines as suspension cultures on modified MS liquid medium.

MATERIALS AND METHODS

Germplasm of *Glycine max* (CV Jack) was obtained from Illinois agriculture Experimental Station at Urbana-Champaign, Illinois. Callus cultures were initiated and grown from hypocotyl explants on B5 (Gamborg *et al.* 1968) medium. The selection and growth studies were carried by inoculation of 0.5-1 of fresh weight of cell suspension into liquid MX medium, modified from Mursashige and Skoog (1962) with 0.4 mg/L (1.18 μM/L) 2.4-D (Dichlorphenoxyacetic acid), the only growth hormone in liquid medium. For determination of

I50 value, different concentrations of Glyphosate were incorporated into liquid medium and three replicates were maintained for each concentration. The optimum growth period for suspension culture is 14-16 days and the cultures were maintained under continuous photoperiod with 120 rpm on a rotary shaker. Depending on the tolerance of the cell line and growth response & further step wise selection were made after 15-30 days to select for highest tolerance level on Glyphosate at 35 mMG. Finally resistance cell lines W-82 at 35 mMG were selected and several sub-cultures were also made on the same concentration.

Measurement of EPSP-synthase Activity : EPSP-synthase extracts were prepared by powdering cells in liquid nitrogen and re-suspending in 2 ml g^{-1} 50 mm Hepes – KOH, 10% glycerol (v/v), 2 mM DTT, 0.1 mM EDTA, 0.01 mM $(NH_4)_6Mo_7O_{24}$ $4H_2O$, pH 7.0 with 1% polyvinylpolypyrrolidone (w/v). All subsequent operations were carried out at 0-40C. The homogenate was centrifuged at 27,000g for 10 min and the pellet discarded. After adding 2 ml of saturated ammonium sulfate per ml supernatant, the extract was held on ice 10 min. then centrifuged as above. The pellet was re-suspended in the extraction buffer, 1 ml g-1 cells. EPSP-synthase activity was measured by determining inorganic phosphate release using with minor modifications a malachite green dye assay described by Forlani *et al.* (1994). All assays were performed in the presence of 0.5 mM $(NH_4)_6Mo_7O_{24}$ $4H_2O$ to inhibit phosphatase activity. EPSP0-synthase extracts were diluted with extraction buffer or added directly to the assay solution and incubated from 1-20 min at 300 C and compared to 0 min. values. Controls contained 10 mM glyphosate or onlyone substrate, S-3-P or PEP. Release of inorganic added was generally less than 10% of the rate with both substrates present. The molar absorption coefficient of the phosphomolybdate complex was determined to be 79,000 M^{-1} cm^{-1}.

RESULTS AND DISCUSSION

The wild type cell suspension cultures of *Glycine max* showed 50 percent growth inhibition at 0.06 mMG, which is most sensitive. Growth experiments were conducted with different concentrations ranging from 0.1 to 35 mM of Glyphosate. Stepwise selections were mad3e depending upon the I_{50} value and growth plotted with log phase cells of W-82 cell suspension. The initial selection experiment with wild type cell line was a made with 0.1 mM

concentration of Glyphosate 40% of growth inhibition was observed (Table-1). The results of inhibitory level of selection in certain food Legumes are in conformity with reports of Ramulu, 1994. During stepwise selection on Glyphosate medium, embrorganic cell suspension was adoptive up to 2.0 m MG. Considerable time has taken for achieving optimum growth as that of wild type cell lines. From 2.0 mMG concentration onwards, cell lines showed greater efficiency of resistance against the selection pressure. Gradual increase in concentration of glyphosate is applied in initial selection experiments and optimum growth was obtained at high concentration of glyhosate (2.0 mMG).

TABLE-1 : Growth of cell suspension culture of soybean W-82 on MX medium with various concentration of Glyphosate

Type of Cell line	*Conc. Of glyphosate in mM*	*Fresh Weight in grams Grams*	*Percentage of Inhibition*
Wild	0	5.5 ± 0.30	100
	0.1	2.2 ± 0.45	40
	0.3	1.5 ± 0.21	26.2
	0.5	1.0 ± 0.08	19.3
	1	0.8 ± 0.78	14
	3	0.8 ± 0.01	14
	10	0.8 ± 0.06	7.7
	35	0.3 ± 0.02	4.7
W-82 mMG	0	9.3 ± 0.50	100
	3	9.2 ± 0.60	98
	10	7.9 ± 0.21	87
	35	6.8 ± 0.39	73
	50	1.6 ± 0.41	17.2

Table-2 : EPSP Synthase Enzyme activity in sobbean W-82 cell lines

Name of the cell line	*EPSP Enzyme activity in Pka mg/L*	*No. folds increased*
Soybean (W-82) 0mMG	169	(1)
Soybean (w-82) 35 mMG	2366	(14)

The tolerance of W-82 cell line to herbicides is more efficient. Increasing fresh weight values and also corresponding high growth index value was observed at 19 mMG period of 32 days. When the concentration of glyphosate was doubled (35 mMG), cells were more efficiently adapted and tolerant cell lines yield good growth with cell proliferation (Table-3). The enzyme activity in wild type of cell lines showed 169 pka mol-1 and selected cell lines (35 mMG) showed 14-folds increased enzyme activity and was recorded as 2366 pka mol-1 (Table-2). Increased enzyme activity and enhancement of gene copy number were reported in certain Legumes while selecting against the Glyphosate (Ramulu, 1996). Cell lines selection on (35 mMG) I_{50} value at 39 mMG, which has increased 650-folds over the unselected control cell lines. This clearly indicates that the tolerance to herbicide in an adaptive cell line is stable and consistent in selected cell lines on (35 mMG). The time period 297 days required for the selection of the soybean cell lines for efficient tolerance to glyphosate after 10 subcultures progressively (Table-4). Biotechnological method were very effective in crop modification to understand the DNA amplification of EPSP synthase gene which confers the glyphosate resistance in tobacco cell suspension cultures was reported where the enzyme activity increases several folds (Shyr *et al*, 1992). Stepwise increase in the concentration of herbicide (Glyphosate resulted in the over production of the target enzyme, EPSP synthase due to gene amplification. Amplification of EPSP synthase gene and increased enzyme activity in several folds are well documented in several species of *Alfalfa*, *Nicotina* and *Carrot*. Stepwise selection of *Daucus carota* (L) cells against chlorosufuran showed over production of fragment of DNA, which

increased in 10 copies (Caretto *et al*, 1994). The increased enzyme activity is due to over expression of ESPS synthase gene by production of more mRNA. Stepwise selection for glyphosate resistance in *Cordialis sempervirens* suspension cultures produced high EPSP activity due to post-transcriptional changes associated with mRNA stability (Holland *et al,* 1998).

Table-3 : Step wise selection of soybean CV W-82 cell suspension on MX medium with different concentration of herbicide

Sl. No.	*Cons. of Glyphosate mM*	*No. of days for optimum growth*	*Suspension F.W. (gm)*	*Growth index value*
1	0	16	5.5	111
2	0.1	19	13.0	25
3	0.3	16	3.5	6
4	0.5	20	9.3	17.6
5	1.0	16	2.9	4.8
6	2.0	19	12.7	24.4
7	4.0	41	7.6	14.2
8	6.0	38	2.1	3.2
9	10	60	2.6	4.2
10	16	32	13.3	25.6
11	35	36	11.5	22

Table-4 : Growth inhibition value of soybean cell lines

Name of the cell line	*150 values (Con. in mM)*	*No. of days*	*No. of subcultures*
Soybean (W-82) 0 mMG	0.06	15	(1)
Soybean (W-82) 35 mMG	39	297	(10)

The possible explanations for this may be increase in target due to either in gene expression or gene amplification. The over production of target enzyme EPSP synthase in soybean embryoganic cell lines may be due to the amplification of gene (DNA) encoding corresponding EPSP synthase (Widholm *et al.* 2001).

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19.

Effect of *Rhizobium* and Herbicides (2,4-D and Pendimethalin) on growth and protein content of *Vigna radiata* (L.)

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Abstract

The effect of *Bradyrhizobium* and two herbicides combination with *Bradyrhizobium* were studied on the growth of *Vigna radiata* (L). The Vigna seeds were grown for 15 days in different concentrations (0, 100, 200 and 300ppm) of herbicides 2, 4-Dichlorophenoxy acetic acid (2,4-D) and Pendimethalin in natural conditions. The results indicated that both herbicides were able to reduce the root, shoot fresh and dry biomass, nodule number, protein and leghaemoglobin content gradually from lower to higher concentration of herbicides.

Keywords: *Bradyrhizobium*, Growth, Herbicide

INTRODUCTION

Vigna radiata (L.) is an important pulse crop in many countries including India where the diet is mostly cereal based. It contains light protein, easily digestible, and does not cause the flatulence as many other legumes do (Arif et al., 2012). This crop is versatile having short growing period and easily fits in different cropping patterns (Kumar et al., 2010). However *Vigna radiata* (L.) productivity often suffers from weed competition, thus requiring herbicides to be used widely (Zaidi et al., 2005). Use of herbicides for weed control in legume field has contributed to increased yield and improved quality (Knott, 1985). Due to extensive and injudicious application, most of the unused fractions of herbicides are known to persist within soils (Madhaiyan et al., 2006). Frequently, herbicides not only affect plant growth but also have a detrimental effect on soil micro-organisms growth and metabolism (Sawicka, 1996). The legume-rhizobia symbiosis has a unique importance in agriculture. The symbiosis results in huge quantities of nitrogen fixation throughout the world and any adverse effect on rhizobia results in reduced rates of biological nitrogen fixation. Many factors influence the growth of nitrogen

fixing bacteria. Herbicides as one of them may also influence the growth of rhizobia (Singh and Wright, 2002). They have been reported to exhibit negative effects on the growth of rhizobia (Martensson, 1992). Herbicides influence nodulation and biological nitrogen fixation in legumes either by affecting rhizobia, or their host plant or both. The magnitude of the toxic effects of herbicides, however, depends primarily on the type and dose of compounds, duration of exposure, species and age of plants, and other environmental factors (Zaidi et al., 2005).

MATERIALS AND METHODS

The field experiment was conducted during the month of April to June in 2014 to evaluate the effect of two herbicides on growth and nodulation in *Vigna radiata* (L.). The experiments were carried out in the field of the Department of Botany, C.C.S. University Meerut. Experimental field designed in eight plots of equal size, seven plots for treatment and one plot for control. Seven treatments performed in the present work were as follows:

1) Treatment with *Rhizobium* alone.
2) Treatment with 100ppm concentration of pendimethalin+*Rhizobium*
3) Treatment with 200ppm concentration of pendimethalin+*Rhizobium*
4) Treatment with 300ppm concentration of Pendimethalin+*Rhizobium*
5) Treatment with 100ppm concentration of 2,4-D+*Rhizobium*
6) Treatment with 200ppm concentration of 2,4-D+*Rhizobium*
7) Treatment with 300ppm concentration of 2,4-D+*Rhizobium*

Healthy seeds of *Vigna radiata* (L.) were selected and washed with distilled water. 2,4-D (Dicholorophenoxy acetic acid) recognised as synthetic auxin, act as plant growth regulator at lower concentration and as growth retardant at higher concentration. Pendimethalin is used as an herbicide prevents the growth of certain plants acting as a root inhibitor. Pendimethalin (30% EC Bond Herbicide) and 2,4-D (Dimethyl Amine Salt 58% WSC Weedicide Zura) were appropriately diluted with distilled water to the soil their final concentration of 100, 200 and 300ppm. The herbicides were added to the soil as pre-sowing application to moist soil, 24h before sowing.

Fifty healthy seeds of *Vigna radiata* (L.) were sown in each plot. The seed germination percentage was calculated after counting the difference between germinated (coming out of the soil) and non-germinated seeds (remaining inside, non emergent). All plants in the three pots for each treatment were removed 30 days after seeding (DAS) and were observed for the extent of nodulation. The roots were carefully washed and nodules were detached, counted, oven dried (at 60°C) and weighed. Nodules

were detached from the plant root with the help of forceps. Fresh weight of nodules was measured immediately and followed by dry weight after drying them at 60ºC for 48 h to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. The leghaemoglobin (Lb) content of fresh nodules was quantified at 50 DAS (Sadasivam and Manikam, 1996).

RESULTS AND DISCUSSION

Seed germination

The seed germination was measured at the different time-intervels i.e. 5 day after sowing (DAS), 10 DAS and 15 DAS. Germination in the control seeds was found higher than treated seeds. *Rhizobium* improves the germination percentage in the *Vigna radiata* L. as compared to pendimethiln+*Rhizobium* and 2,4-D.+*Rhizobium* (Table1). The final germination percentage reduced progressively with the increasing concentration of herbicides 100, 200 and 300ppm. Both 2,4-D and pendimethalin herbicide showed inhibitory effect on seed germination. Among the treatments 2,4-D showed maximum inhibition in seed germination. Reduction in germination by 2,4-D is the result of drastic inhibition of root growth due to its strong phytohormonal action (Audus, 1977). At high concentration 2,4-D is reported to inhibit amylase activity resulting into the suppression of root and shoot growth (Shaukat, 1976).

Shoot and Root length

Finding with the effect of *Rhizobium* and two herbicides on *Vigna radiata* L. growth are given in Table 2. In general root and shoot length of plant were found significantly higher with 100ppm treatment of pendimethalin+*Rhizobium*. *Rhizobium* showed remarkable increase in growth parameters as compared to control and 2,4-D+rhizobium. The maximum reduction in root length and shoot length occurred at 300ppm concentration of 2,4-D+*Rhizobium*. An adverse effect of herbicides on chickpea vitality and subsequently the *Mesorhizobium*-chickpea symbiosis is also reported earlier (Khan et al., 2014). On the contrary lower dose of herbicides are stimulate known to the growth in *Vigna radiata* L. and this suggests that the lower doses might have persisted in the soil for only a short time period, after which the viable cells of *Bradyrhizobium* get recovered and multiplied rapidly (Zaidi et al., 2005). This is possible because the soil environment can act as a buffer, reducing the potentially toxic effect by dilution of these chemicals (Castro et al., 1997).

Plant biomass

Fresh and dry weights of plant were found significantly higher in control plants over the *Rhizobium* and herbicides (Table 3). 100ppm concentration of pendimethalin+*Rhizobium* showed remarkable increase in fresh biomass of shoot and root and dry biomass of shoot as compared to *Rhizobium* and 200, 300ppm concentrations of herbicides with *Rhizobium*. The maximum increase in dry biomass was observed with *Rhizobium*. Higher concentrations of 2,4-D+*Rhizobium* reduced the fresh and dry biomass of *Vigna radiata* L., possibly due to premature senescence of the plant (Zaidi et al., 2005). The phytotoxic action of 2,4-D occurs largely as a result of its ability to mimic the activity of endogenous auxin. The excessively high concentration of auxin-active herbicides, in turn, alerts the regulation of plant metabolism, leading to the loss of cellular function, cellular integrity and repair capacities of plants (Nishitani and Masuda, 1981). Fox et al. (2007) reported a considerable decrease in nodulation, total plant biomass and nitrogenase activity of alfalfa (*Medicago sativa* L.), when grown in soil treated separately with different herbicides. The variable response of the tested legumes to herbicides is explained on the basis of extent of toxicity of any specific herbicide to the plants which in turn depends upon both the genetics and physiology of plants, varying from species to species (Ahemad and Khan, 2011c).

Nodulation

The number of the nodules was higher in *Rhizobium* treatment as compared to the herbicides application. Infection of roots of legumes by *Rhizobium* in takes place through the root hairs so that the process of nodulation of legumes is undoubtedly linked to the expression of the various parts of the root system. Therefore, it is possible that herbicides which induce a reduction in nodules formed per plant may do this by restricting root growth and or formation of lateral roots, and hence the number of root sites available for infection. Generally, the lower concentrations of herbicides improve the nodulation in the *Vigna radiata* L. possibly because at a sub-lethal dose, the herbicide may induce damage to xylem vessel without adversely affecting the nodular bacteroids, and hence the greengram-*Bradyrhizobium* symbiosis remained unaffected. *Rhizobium* treated plants show greater increase in values of nodules fresh and dry weight of nodules plant^{-1} as compared to herbicide treated plants and control (Table 4). The herbicides induce decline in nodulation in general that could be because of the inhibition of symbiosis process or the herbicide might have interfered with the chemotactic motility between the legume root and the bacteria (Khan et al., 2004).

However, among the two herbicides, 2,4-D had a greater adverse effect on nodule formation, suggesting that this herbicide is highly toxic for *bradyrhizobium-Vigna radiata* (L.) symbiosis. According to Anderson et al. (2004), herbicides negatively affect the nodulation in legumes by limiting the number of available sites on host plants to cognate *Rhizobium* by decreasing the carbohydrate supply to existing nodules. Thus, herbicides are known to decrease the rhizobial survival and growth, to inactivate the biochemical signaling required to initiate nodule development in plants and to inhibit the nodule development by reducing cell division. Likewise of toxicity of herbicides on nodulation and N2 Fixation in soybean (Malik and Tesfai, 1985) and chickpea (Khan et al., 2004) have also previously been reported.

Protein content

The effect of *Rhizobium* treated plants showed a greater increase in protein content than in control and herbicide treated plants (Table 5). 300ppm concentrations of pendimethalin+*Rhizobium* and 2,4-D+*Rhizobium* showed adverse effect on protein content. The effects of herbicides are known by the type and rates of their application, health and stage of plant growth, as well as other environmental variables (Kumar, 2012). Morphological changes and disturbances in cell division due to impact of both the herbicides used individually and their combinations (Shamsi et al., 2006, Qasem 2007, Kumar et al., 2010), are now well established.

Egli et al. (1985) reported that many herbicides interfere with the protein synthesis, for instance, atrazine or diuron inhibits the synthesis of protein in *Solanum nigrum* cell suspension. This might be the unspecific disruption of the cell metabolism by large amounts of the applied exogenous compounds. There are also some reports which exhibit that there is no direct effect of herbicides on protein or nucleic acid synthesis, probably because neither of these sites is primary site of action of any commercial herbicide (Khan et al., 2006). However the high concentrations of the herbicides may affect the enzymatic reaction responsible for protein biosynthesis. Reduction in protein content following herbicide application could probably, be due to inhibition of the enzymes and functional proteins of metabolic pathways involved in protein synthesis (Nare et al., 2010; Ahemad and Khan, 2011).

Leghaemoglobin content

Leghaemoglobin content is the precise parameters to assess the actual impact of any stress factor on nodulation. Its content was significantly higher in *Rhizobium* treated plants (Table 5) and it was

maximum at 100ppm concentration of 2,4-D+*Rhizobium* when compare to higher concentrations of 2,4-D+*Rhizobium*, pendimethalin+*Rhizobium* and control. Herbicides decrease leghaemoglobin content in nodules of the different legumes as recently reported by Ahmed (2014). The decline in Lb content of legumes could be due to the toxic effects of herbicide on plant organs, especially the function of nodules which consequently disrupts the legume-*Rhizobium* symbiosis and hence, the N2 fixation and in turn the overall plant growth (Evans et al., 1991). In addition, the inhibitory effect of the herbicide application may possibly be due to (i) the inhibition of enzymes involved in growth metabolisms (Zablotowicz and Reddy, 2004) and (ii) disruption of signaling between legume derived phytochemicals (luteolin, apigenin) and *Rhizobium* Nod D receptors (Fox et al., 2007).

Conclusions

Different symbiotic attributes of the tested legumes were also assessed under herbicide-stress which showed varying degree of toxicity to the selected legumes. Nodulation in legumes is an important growth parameter. Comparative evaluation of nodule numbers of legume species did not provide any accurate assessment on the size of nodules which varied from one legume species to another. Pyriproxyfen was observed as a highly toxic substance to seed germination, root and shoot dry mass nodulation, leghaemoglobin content and protein content.

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Treatment	5 DOS	10 DOS	15 DOS
Control	78	90	92
Rhizobium	72	86	87
2,4-D+*Rhizobium* 100ppm	40	84	85
2,4-D+*Rhizobium* 200ppm	2	72	74
2,4-D+*Rhizobium* 300ppm	2	48	62
Pendimethalin+*Rhizobium* 100ppm	74	83	84
Pendimethalin+*Rhizobium* 200ppm	52	76	80
Pendimethalin+*Rhizobium* 300ppm	62	76	78

Table1: Seed germination percentage of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	5 DOS	10 DOS	15 DOS
Control	78	90	92
Rhizobium	72	86	87
2,4-D+*Rhizobium* 100ppm	40	84	85
2,4-D+*Rhizobium* 200ppm	2	72	74
2,4-D+*Rhizobium* 300ppm	2	48	62
Pendimethalin+*Rhizobium* 100ppm	74	83	84
Pendimethalin+*Rhizobium* 200ppm	52	76	80
Pendimethalin+*Rhizobium* 300ppm	62	76	78

Table 2: Root length and shoot length of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	Plant length (cm)	Root length (cm)	Shoot length (cm)
Control	36	7	29
Rhizobium	37	7.6	29.4
2,4-D+*Rhizobium* 100ppm	31.4	6.6	24.8
2,4-D+*Rhizobium* 200ppm	18.8	3.8	15.4
2,4-D+*Rhizobium* 300ppm	16.2	3.4	11
Pendimethalin+*Rhizobium* 100ppm	37.4	8.6	28.8
Pendimethalin+*Rhizobium* 200ppm	32.8	10.8	22
Pendimethalin+*Rhizobium* 300ppm	25	6.2	18.8

Table 3: Root and shoot fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	Root		Shoot	
	Fresh wt. (gm)	Dry wt. (gm)	Fresh wt. (gm)	Dry wt. (gm)
Control	0.333	0.2146	4.2496	2.1452
Rhizobium	0.3528	0.2254	3.7164	1.9782
2,4-D+*Rhizobium* 100ppm	0.146	0.1236	1.6764	1.116
2,4-D+*Rhizobium* 200ppm	0.0662	0.065	0.4812	0.3644
2,4-D+*Rhizobium* 300ppm	0.474	0.1155	0.397	0.3096
Pendimethalin+*Rhizobium* 100ppm	0.3528	0.2228	4.0344	2.1008
Pendimethalin+*Rhizobium* 200ppm	0.2138	0.1566	2.7648	1.6484
Pendimethalin+*Rhizobium* 300ppm	0.2082	0.134	2.1994	1.1368

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Table 4: Nodule number, volume, fresh and dry weight of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**Nodule no.**	**Volume**	**Fresh wt. (gm)**	**Dry wt. (gm)**
Control	38	0.9	0.0372	0.0198
Rhizobium	53.8	1.4	0.0596	0.0306
2,4-D+*Rhizobium* 100ppm	21	1.2	0.0508	0.0246
2,4-D+*Rhizobium* 200ppm	15.4	0.7	0.242	0.0092
2,4-D+*Rhizobium* 300ppm	5.6	0.9	0.0154	0.0058
Pendimethalin+*Rhizobium* 100ppm	16.2	1.3	0.02066	0.0212
Pendimethalin+*Rhizobium* 200ppm	7.8	0.5	0.0416	0.0122
Pendimethalin+*Rhizobium* 300ppm	9.2	0.4	0.0282	0.0186

Table 5: Protein and Leghaemoglobin content of *Vigna radiata* (L.) with *Rhizobium* and different concentrations of herbicides.

Treatment	**Protein mg/g fresh wt.**	**Leghaemoglobin mM $(g.f.m.)^{-1}$**
Control	3.0482	0.005
Rhizobium	3.9729	0.009
2,4-D+*Rhizobium* 100ppm	3.0321	0.006
2,4-D+*Rhizobium* 200ppm	2.0126	0.003
2,4-D+*Rhizobium* 300ppm	1.7041	0.002
Pendimethalin+*Rhizobium* 100ppm	2.9577	0.007
Pendimethalin+*Rhizobium* 200ppm	2.7263	0.004
Pendimethalin+*Rhizobium* 300ppm	1.5248	0.003

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20.

Availability of Muga Silkworm host plant species in district Bageshwar and their economic potentiality

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Abstract

Bageshwar is located at 29°50' N latitude and 79° 46' E longitudes with elevation of 850m in State Uttarakhand. District covers an area of 2,286 sq. km of which around eighty percent covers under forest where various tropical to temperate plant species are found in abundance in which precious muga silkworm host plant species like *Persea. bombycina* Kost. (Som) and *Litsea monopetala* Roxb. (Soalu) are also found profusely. Muga silkworm (*Antheraea assamensis* Helfer) is a multivolitine and polyphagous in nature. Its primary host plants are *Persea bombycina* Kost. (Som) and *Litsea monopetala* Roxb. (Soalu) and some secondary, tertiary food plants are *Cinnamomum tamala* (Bach-Ham.) T Nees & Eberm., *Gamelina arborea* Linn., *Zizyphus jujuba* Mill. etc. Apart from sericulture these plant species serve in many ways to mankind. The extract of different parts of these plant like flowers, fruits, seeds, bark, leaves and root etc. having medicinal value in pharmaceutical industry. An attempt has already been made towards the use of these plants for human need as earlier these species are being used by local tribe of the area namely Bhotias against specific reason. The basic mean of the present study is to extend the respective knowledge to the general mass so that the nature wealth of the area will not only lead to the prosperity of the livelihood but also provide accurate as well as sustainable use of existing nature grown plant species in the hilly tracts of Uttarakhand.

INTRODUCTION

Among the silk producing countries, India is the only country which produces all kind of natural silks and is a home of golden glittering muga silkworm. Muga silk is exclusively cultivated in north -eastern part of the country because of conduce climatic conditions to the muga insect and a wide distribution of host plant species in the region. The annual production of golden muga silk was nearly around 119 MT. (CSB Annual Report 2012-2013), which is nearly stagnant for last two decades because of industrialization, pollution and natural calamities in the region. The world wide consumption of golden muga is increasing day by day which is showing remarkable gap between its consumption and its production. Therefore it is also introduced in Uttarakhand due to its diverse weather conditions i.e. sub-tropical, tropical, sub-temperate and temperate. Trial for muga rearing and grainage had started from 2010 onwards in Bageshwar district of Uttarakhand, which proved very good results in comparison to North eastern states in India.

Bageshwar is located at confluence of river Saryu and Gomti in hilly region at 29°50’ N latitude and 79° 46’ E longitudes with elevation of 850m in State Uttarakhand. Distt. covers an area of 2,286 sq. km of which around eighty percent covers under forest where various tropical to temperate plant species are found in abundance in which precious muga silkworm host plant species like *Persea. bombycina* Kost. (Som) and *Litsea monopetala* Roxb. (Soalu) and some secondary host plant species are also found abundantly. Due to availability of nature grown host plant species in forest as well as conducive weather for cultivation of muga make a strong path for introducing golden glittering muga silk in the distt.

Muga silkworm (*Antheraea assamensis* Helfer) is a multivolitine and polyphagous and reared in open weather on host plant species. It consume voraciously on leaves of various forest plant species and covers four to five crop cycle in a year. As per eating priority of this insect its host plant species have been categories in primary, secondary and tertiary. Accordingly, the host plant species like *Persea bombycina* Kost. (Som) and *Litsea monopetala* Roxb. (Soalu) are kept as primary and subsequently, species like *Cinnamomum tamala* (Bach-Ham.) T Nees & Eberm. are kept in secondary and species like *Gamelina arborea* Linn. and *Zizyphus jujuba* Mill. etc. in tertiary.

Apart from the use of the aforesaid plant species under sericulture these plant species serve in many ways to mankind. The extract of different parts of these plant like flowers, fruits, seeds, bark, leaves and root etc. having medicinal value in pharmaceutical industry. An attempt has already

been made towards the use of these plants for human need as earlier these species are being used by local tribe of the area namely Bhotias against specific reason.

EARLIER WORK

Brandis (1972) followed by and Thangavelu (1988) have reported the natural distribution of one of the muga host plant *L. monopetala* Roxb. in Dehradun valley and adjoining hilly areas upto an altitude of 800 m. Raja Ram *et al.* (1993) and Paliwal, D.P. *et al.* (2001) made significant contribution to the taxonomy and identification of muga food plants. Ramkrishna *et al.* (2000) worked on medicinal values of non mulberry host plants. D.P. Paliwal *et al.* (2009) had done studies on floral biology, seed and seedling of muga silkworm host plant species *Persea bombycina* Kost. and also worked related to availability of muga host plants and capacity to rear muga seed in Bageshwar of Uttarakhand. D. P. Paliwal et al (2010) had done work on scope of muga silk production in Uttarakhand state as a new resource for muga seed supplementation to north eastern states.

There are several other workers who made significant contribution to Muga culture and its host plants however most of the previous work is of general nature covering a large area, only few works have been done in a reasonable demarcated area like Bageshwar district.

METHODOLOGY

The mention investigation was carried out in many parts of Bageshwar district. Regular visits and surveys were carried for field work and collection. Tribal, local villagers and journals are source of information.

PRIMARY HOST PLANTS

***Persea bombycina* Kost. Syn.** *Machilus bombycina* King.

Family: Lauraceae

Local Name: Som, Kaul or Kau

Description of plant :

Evergreen, monoecious, middle sized tree with spreading branches. Leaves are simple, exstipulate, petiolate, alternate and size and shape variable. Blade 2.5-5.0 x 0.8-2.0 inch. Inflorescence axillary or terminal panicles. Flower colour greenish white, turns yellow with age. Fruit fleshy, drupe, globose or oblong, 6-8 mm, dark purple when mature.

Fls. & Frts. : Feb.-May

Chemical constituents : The flower oil constituents caryophyllene oxide, (E)-nerolidol, 11-dodecenal and 11-dodecenoic acid. Fruit oil constituents the furanoid forms of trans and cis-linalool oxide (Choudhury *et al.,*1997).

Economic utility :

Medicinally, extract of leaves is given in mouth ulcers and bark extract is useful in asthma (Sinha, 1996).

Litsea monopetala **Roxb. Syn.** *Litsea polyantha* Juss.

Family: Lauraceae

Local Name: Soalu, Katmara or Karkawa

Description of plant : Small or mediam sized evergreen tree. Leaves simple, alternate, rarely sub-opposite, elliptic-oblong, usually rounded at both ends, pubescent beneath. Blade 8-30 x 5-11 cm. Inflorescence umbels in clusters. Flowers small, pale greenish yellow, sessile or subsessile. Fruit 10mm long, ovoid, black.

Fls. & Frts. : Feb.-Aug.

Chemical constituents : β-sitosterol and actinodaphnine have been isolated from the bark of the plant (Rastogi and Mehrotra, 1990).

Economic utility: The bark is mildly astringent, stomachic and stimulant; after being bruished, applied to contusions. Water extract of the bark is given with sugar to treat diarrhoea and dysentery. Powder of the bark is applied to body for pains arising from blows or bruises or from hard work; it is also applied to fracture in animals. (Sinha, 1996)

SECONDARY & TERTIARY HOST PLANTS

Cinnamomum tamala **(Bach-Ham.) T Nees & Eberm**

Family: Lauraceae

Local Name: Tejpata, Tamal.

Description of plant : A small to medium-sized tree. Leaves simple, opposite, or sub-opposite. ovate-lanceolate or oblong, coriaceous, glabrous, acuminate, triple nerved. Blade 7.0-15.0 cm long. Inflorescence panicles, subterminal and corymbiform, crowded. Flowers small, pale yellow, in axillary and terminal. Drupe 13 mm long, ovoid, fleshy, black.

Fls. & Frts. : March-April

Chemical constituents : The chief constituent of the leaf is an essential oil rich in eugenol (about 78%), iso-eugenol d- a-phellandrene and cinnamaldehyde (Ghani, 2003).

Economic utility: The leaves are stimulant, carminative and diuretic. Essential oil from leaves possesses antibacterial and antifungal properties It is also used in flatulence, stomache and dysentery (Asolkar *et al.,* 1992). Leaves are extensively used as a spice in North India.

***Gamelina arborea* Linn.**

Family: Verbenaceae

Local Name: Gambhari

Description of plant : A medium-sized deciduous tree. Leaves 10-20 cm long, broadly ovate, acuminate, entire. Inflorescence terminal recemes. Flowers complete, bisexual, large, yellow, tinged with brown, actinomorphic, hypogynous, pentamerous. Drupe 2-2.5 cm long, ovoid or pyriform, orange-yellow when ripe.

Fls. & Frts. : Feb.- June

Chemical constituents : Leaves contain alkaloids and luteolin, apigenin etc. Fruits are reported to contain butyric and tartaric acids (Ghani, 2003). A new lignan, arboreol has been isolated from the plant (Rastogi & Mehrotra, 1993).

Economic utility: Juice of the young leaves is used as a demulcent in gonorrhoea and cough. Flowers are astringent; useful in leprosy and blood diseases. Fruit decoction is used in fever and bilious affections. Flower is used in blood diseases while plant is used in snake bite and scorpion bite. Dasmula, an Ayurvedic preparation consists of root as one of the ingredients Roots are used for the treatment of septic wounds (Pandey, 1981, Maheshwari and Singh, 1991), Sinha, 1996, Nairand Jayakumar, 1998). Wood posseses good timber value and used as mortar and for making plough parts and drums.

***Zizyphus jujuba* Mill. Syn.** *Zizyphus mauritiana* Lam.

Family: Rhamnaceae

Local Name: Bogori or ber

Description of plant : A small tree or shrub usually upto 30 ft. in height, monoecious, almost evergreen. Leaves simple, cauline and ramal, alternate, petiolate, strongly 3 nerved, stipules modified to prickles. Inflorescence cyme, axillary, nearly sessile. Flowers regular, complete, actinomorphic, hermaphrodite, greenish yellow in colour. Drupe 6 mm across, ellipsoidal, acuminate with fleshy, mealy aromatic acids, red or orange when ripe.

Fls. & Frts. : Sept - Dec.

Chemical constituents : Peptide alkaloids zizyphinine and zizyphine A - E along with abyssinines A and B have been isolated from stem bark (Rastogi & Mehrotra, 1993).

Economic utility: The fruits have emollient and expectorant properties (Kirtikar and Basu, 1935). They are also considered to be cooling and an anodyne and a tonic. They are used externally in poultices and are applied to wounds. The leaves are laxative and prescribed in scabies and throat troubles (Anon., 1976). These fruits are eaten by villagers. The wood of this plant is very hard and durable and is used in making agricultural implements. The leaves of this plant are used as a fodder for sheep and goats.

RESULT AND DISCUSSION

In above studies five host plant species of muga silkworm are described with their family, local name, plant description, chemical constituents and economic utility. Muga host plants not only provide foliage to the silkworm but also have other economic potential. This flora should be protected and harnessed for the use of rural mass so that economic standard of rural mass can be raised and villagers can get the opportunity of employment at homested which ultimately prevent migration from the villages.

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21.

Study of Rate of Litter Decomposition in Dewghat Forest, Koraon Range of Allahabad (U.P.)

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ABSTRACT

Rate of litter production in a forest can be considered as an estimate of net production of plant community (Golley, 1978). Litter fall and leaf litter decomposition were studied in Dewghat forest, south parts of Allahabad district, stand dominated by Tendu patta (*Diospyrous melanoxylon*) common man's cigarette, of the total litter fall 78% was contributed by leaves. The trend of litter fall of different components was in the order : Leaves with petiole > Twig > Floral parts + seeds/ Fruits > Bark. Total annual litter fall was 934gm^{-2} of which there has been a seasonal variation in litter production. *D. melanoxylon* produced 687 gm^{-2}. The total litter was found to be maximum in spring and minimum in rainy seasons. Annualy 77.7% of *D. melanoxylon* leaf litter was decomposed. Maximum rate of leaf litter decomposition was observed in spring season. Formation of human find product of humification improves the fertility and quality of soil. It enhances the water retaining capacity of soil with sustained supply of minerals.

Keywords : Litter production, leaf litter decomposition, *D. melanoxylon.*

INTRODUCTION :

Litter fall is the major pathway for the return of organic matter and nutrients from aerial parts of the plant community to soil surface, and fertility. Litter production and decomposition rates have great importance in maintaining the fertility of soil. A substantial portion of nutrients accumulated by plants is returned to the soil as litter fall followed by its decomposition, i.e. the integrity of an ecosystem is maintained by these transfer of matter and nutrients. In tropical deciduous Dewghat forest ecosystem, maintenance of soil organic pool achieved by high and rapid circulation of nutrients through the fall and decomposition of litter. Standing crop of litter acts as an input output systems of nutrients and the rate at which forest litter falls, and subsequently decays, regulate energy flow, primary productivity and nutrient cycling in forest ecosystems. Nutrient cycling rates in forests are usually inferred from a comparison of nutrient concentration and amounts of litter fall, forest floor litter and crown drips. The present study deals with litter fall and leaf litter decomposition in a Tendu forest stand at Dewghat, Koraon Range (U.P.). The present findings supported the works of Singh, K.P. (1968), Pant and Tiwari (1992), Sharma and Ambast (1989), Sunderapandian and Swamy (1999), Odiwe et al., (2003), Wright et al., (2004), Pragason and Parthasarthy (2005).

STUDY AREA :

The Dewghat forest is located in Koraon Range in south side of Allahabad district. It lies from 25°15' - 35°13' N latitude and 81°45' -- 82°15' E longitude. The forest has specific geographical situation, comprises of treasures of medicinal plants used by all sections of society. The climate is hot and humid in summer and dry-cool seasons in winters. The annual rainfall ranges from 100-160 cm mainly received with June to September month of the year. Average maximum and minimum temperatures ranges from 30-35°C in summer and 20-30°C in winter respectively.

MATERIAL AND METHODS

Litter fall was measured in 10 traps (50cm×50cm each) randomly placed in forest floor. The trap fitted with nylon net base at bottom to facilitate drainage, were set up 30 cm above the ground (Visalakshi, N. 1993). Litter from each trap was collected fortnightly / monthly intervals from January 2004 to December 2005. On each sampling date, litter from each trap was packed

in separate polythene bags. The samples were brought to laboratory and were sorted species wise into leaves, twigs, bark and floral parts + seeds and /or fruits. The sorted material was oven dried at 80°C for 72 hrs and weighed. Leaf litter decomposition in Tendu patta (*D. melanoxylon)* was studied by enclosing 10g air dried fresh litter samples in nylon bags. A total of 120 bags (40 bags in each of three different locations, i.e. upper crust, slopes and low lying area) were put in the forest floor on 29 June, 2004. On each sampling date, at an interval of about 30 days, 3 litter bags were recovered and brought to laboratory in separate polythene bags. The material from each samples was washed under a five jet of water by using a fine mesh screen to remove all adhering soil particles. The washed material was oven dried 80°C to constant weight. Bulk samples fresh air dried litter samples were oven dried at 80°C to constant weight to adjust initial dry weight in the bags.

RESULTS :

Litter Fall :

Maximum leaf litter fall followed by April 2004 (Table-1), occurred during March (198±111 gm^{-2} month^{-1}). Total amount leaf fall amounted to 729 gm^{-2} which was distributed as : *Diospyrous melanoxylon* 74.6%, *Butea Monsperna* 7.6%, *Pongamia pinata* 7.2%, *Lyonia ovalifolia* 4.1%, *Spaium insigne* 3.3%, *Rhus semialata* 2.2%, *Pyrus pashia* 1.9%, *Rhododendron arboreum* 1.1%, *Syzgygium cumini* 0.5% and others 0.5%. Total annual twig litter fall was highest in November (22.4± 22.3gm^{-2}) . Twig litter fall was 118 gm^{-2} (Table-1), bark litter was highest in April (6.1±4.3 gm^{-2}) and its annual production was 28.7 gm^{-2} (Table-1). Floral parts and seed / fruits were maximum during April (9.8±6.2 gm^{-2}) and again during December (9.8±8.3 gm^{-2}). The total production of this category was 57.7 gm^{-2} (Table-1). The total litter fall amounted to 934 gm^{-2} yr^{-1}(Table-1). The peak in litter fall observed during March.

Table 1: Litter fall in the Tendu (*D. Melanoxylon*) forest stand during study period, January 2004 through December 2005 (monthly values for litter components are mean ± S.E.)

Period	Litter components (gm^{-2} $month^{-1}$)				Total monthly litter fall (gm^{-2} $month^{-1}$)	Share of *D. melanoxylon* (% Total)
	Leaves	Twigs	Barks	Floral parts + seeds/fruits		
Jan	10.6±10.2	3.8±3.5	1.4-1.0	7.1-6.9	23	72.0
Feb	76.2±65.4	9.3±5.6	1.6±1.2	8.4±5.2	95	76.0
Mar	198.8±111.2	9.5±4.2	0.7±0.3	2.0±0.8	211	95.9
Apr	155.0±81.5	17.4±8.4	6.1±4.3	9.8±6.2	188	87.0
May	82.0±70.2	10.2±6.5	4.2±2.7	2.7±2.1	99	63.7
Jun	50.0±46.5	4.6±3.1	1.7±0.9	2.2±1.4	58	43.2
Jul	18.2±16.1	5.4±4.1	2.6±1.7	3.3±2.2	29	51.2
Aug	20.1±17.2	0.5±0.2	3.2±2.7	0.6±0.4	24	49.2
Sep	29.7±23.2	7.2±5.2	1.5±1.1	2.4±1.7	41	48.5
Oct	33.0±27.4	11.1±8.1	1.3±1.1	4.8±3.8	50	50.6
Nov	22.4±21.8	2.4±22.3	1.9±1.8	4.6±4.2	51	58.5
Dec	33.7±31.6	17.4±15.4	2.5±1.7	9.8±8.36	63	65.1
Annual	729	118	28	57	934	

Litter fall by Species :

The dominant species *D. melanoxylon* showed peak litter fall during March and April which amounted to respectively 95.9% and 87% of total (Table 1). Species other than *D. melanoxylon* showed maximum litters fall during May and June.

Leaf litter decomposition : The pattern of leaf litter decomposition indicated most rapid weight loss during the rainy season (29 June to 29 September 2004) and the minimum during the spring season (Table 2) .

Table 2- Seasonal variation in leaf litter decomposition in Tendu Patta (D. melanoxylon)

Season	Weight loss in leaf litter	
	% Season $^{-1}$	% day $-^{1}$
Rainy (29 Jun to 29 Sept 2003	52.9	0.58
Winter (30 Sept 2003 to 29 Jan 2004)	19.4	0.16
Spring (30 Mar to 29 May 2004)	1.4	0.02
Summer (30 Mar to 29 May 2004)	2.2	0.04
Rainy (30 May to 29 Sept 2004)	18.7	0.15
Winter (30 Sept to 29 Jan 2005)	4.2	0.03

DISCUSSION :

Litter fall rates of all tropical vegetation types have been reviewed by proctor (1984), while Bray & Gorham (1964) reviewed litter fall rates in vegetation type throughout the world. The total litter fall studied did not indicate seasonal equatibility (Table 1). However the rate of total litter fall on per day basis was higher is spring season. The litter fall in summer was followed by winter and rainy seasons. Pragasan and Parthasarthy (2005) recorded maximum litter fall during summer season in case study of dry evergreen forest of South India. The distribution of rainfall and litter fall have an inverse relationship, i.e. low rainfall period and high

litter fall and vice versa. This is conformity with the results of tropical forest kodyer (Sundarpandian & Swamy 1999), tropical dry evergreen forest in India (Meher-Homji 1974), tropical mixed dry forest stand (Rai & Srivastava 1982) and *Alnus nepalensis* plantation stands in Eastern Himalya (Sharma & Ambasht 1987). However, the process of litter fall continues to occur throughout the year due to evergreen nature of vegetation, this is conformity with the behaviour to Tropical dry evergreen forest on the Coranamandel coast of India (Venkateswan and Parathasarthy, 2003). However the range in the litter production within different climatic zones is rater wide. Thus the results reviewed by Jenson (1974), indicate a range in total annual litter fall of 1.5 - 9.9t ha^{-1} in coll temperate regions and 5.5 - 15.3t ha^{-1} in tropical region. The value of total litter fall recorded for the present Dewghat forest stands (9.3t ha^{-1} yr^{-1}) is higher than the equatorial forest. However it fall within the range of coll temperature region (Jenosn 1974) and tropical region.

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