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Contents

Cell-based phenotyping for abiotic stress tolerance

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Abstract: To match predicted population growth, annual food production should be doubled by 2050. This is not achievable by the current agronomical and breeding practices, due to impact of climate changes and associated abiotic stresses on agricultural production systems. The overall losses in food and fiber production due to abiotic stresses such as salinity, drought or flooding exceed US$170 billion p.a. and represents a major threat to global food security. To the large extent this is a result of past trends in breeding for higher yield on expense of tolerance, as the abiotic stress tolerance has been present in wild progenitors of modern crops but was lost during their domestication. In this talk, I argue for a need for a major shift in our paradigm of crop breeding, focusing on the climate resilience, and call for a broader use of wild relatives as a major tool in this process. I also show that, while molecular tools are currently in place to harness a potential of climate-resilient genes present in wild relatives, a complex polygenic nature of tolerance traits remains a major bottleneck in this process. I show that the whole plant-based phenotyping will be not able to resolve this issue and argue for a need to shift towards cell-based phenotyping platforms allowing to assess in plants operation of key genes. I then use several case studies to show how novel electrophysiological and imaging techniques can be used to overcome the above limitations and allow discovery of the candidate genes and/or QTLs conferring abiotic tolerance traits.

Key words: Abiotic tolerance, Food security, Phenotyping

Heavy metals: Contamination, localization and histochemical staining in plant tissues

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Abstract: Plants live in diverse environment that provide essential nutrients as well as non nutrient metals inclusive of mercury, cadmium, lead and chromium and the level of those heavy metals in the soil ranges from low to high depending on the nature of environmental conditions. Histochemical localization of mercury, cadmium, lead and chromium in stem and root tissues of some medicinal plants - *Bacopa monnieri* (Brahmi) and *Boerhavia diffusa* (Punarnava/Thazhuthama) and in *Vigna mungo* is done by staining with safranin, dithizone and tolune blue in routine manner. Localization of the heavy metals could be observed as various specifically coloured masses in the vascular cells and other tissues of root and stem variably upon the stains. Safranin stained tissues showed dark red deposits in *B. monnieri*. When dithizone is used to stain the tissues, it is interestingly observed that these deposits are dark orange/brown coloured in the same plant and also in the stem and root tissue of *V. mungo*. Localization is mainly observed in vascular tissues ie. xylem and among other permanent tissues. Stem tissues of *V. mungo* seedlings and *B. diffusa* showed anatomical modification in the epidermal cells of the treated stem as multicellular uniseriate (rarely multiseriate) trichomes. While bluish-yellow/ bluish-green coloured deposits are observed to localize heavy metals such as cadmium and chromium and mercury and lead respectively in the root and stem tissues stained with toluene blue upon *B. diffusa*.

Keywords: Dithizone, Heavy metals, Localization, Safranin, Trichome.

Modern trends in plant taxonomy

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Taxonomy is the science of grouping Organisms into different categories in relation to their physical Characters. Without taxonomy, no one would be sure of the identity of organisms they were keen on, or whether they belonged to the same or different species as the organisms studied by others.However, this discipline is currently in a crisis: there is a lack of funding for taxonomy, the number of taxonomists is noticeably decreasing and taxonomical studies have a low impact factor.The common interest about biodiversity conservation, the revolution of internet and web pages, the advances in molecular techniques, the development of statistics in phylogeny, and the new taxonomic funding initiatives and global projects are given some light; taxonomy is getting fashionable again, and we could be witnessing the start of a 'taxonomic revolution. The morphological characters single-handedly should not be measured in systematic classification of plants, the entire knowledge of taxonomy is achievable with the principles of various disciplines like Cytology, Genetics, Anatomy, Physiology, Geographical Distribution, Embryology, Ecology, Palynology, Phenology, Bio-Chemistry, Numerical Taxonomy and Transplant Experiments.These have been set up to be useful in solving some of the taxonomical puzzles by providing additional characters. It has changed the face of classification from *alpha* (classical) to *omega* (modern kind). Thus the latest systematic has evolved into a better taxonomy. *Chemotaxonomy*: Chemotaxonomy can be defined as the application of chemical data to the problems of systematics.The occurrence and distribution of various chemical compounds provide as taxonomic evidence. Not all chemical compounds provide valuable taxonomic clues. Only certain compounds particularly with a low molecular weight and which are secondary in origin are of taxonomic significance.*Cytotaxonomy*:Cytotaxonomy makes use of cytological characters in the solution of taxonomic problems. Cytological characters such as Chromosome number, structure, behavior etc. are of great help in solving many phylogenetic troubles.*Numerical taxonomy*: Numerical taxonomy is also called as taxometrics.It is defined as the method of catagorizing of taxonomic units by numerical methods.*Molecular Taxonomy*:Molecular Taxonomy is the division of phylogeny that analyses hereditary molecular differences, chiefly in DNA sequences, to achieve information and to establish genetic relationship between the members of different taxonomic categories.The results of a molecular phylogenetic analysis are articulated in the form of a tree called phylogenetic tree. Different molecular markers like allozymes, mitochondrial DNA, microsatellites, RFLP, RAPD, AFLPs, single nucleotide polymorphism, microchips or arrays are used in analysis. *Embryology:*It is bring into being that various parts such as ovules, embryosac, endosperm, sporogenous tissue, embryo, anther, pollen grain greatly vary in different taxa and they can be used in taxonomy.*Palynology in relation to taxonomy:* Palynology has also been found helpful in taxonomy. Some significant features in pollens such as their shapes, size, number and position of furrows, sculpturing pattern on the exine, are measured for the characterisation of taxa.*Serotaxonomy:*The study of antigen-antibody reaction is called serology. The matter capable of motivating the formation of an antibody is antigen. A specific protein molecule formed by plasma cell in the immune system is antibody. The antibodies combine chemically with specific antigen and this combination elevates an immune response.

Key words: Plant taxonomy, Cytotaxonomy, *Serotaxonomy,* Palynology, Molecular taxonomy

Morphological and physiological changes in snake gourd (*Trichosanthes cucumerina* L.) under chromium stress

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Abstract

Background: The aim of the present study is to assess various morphological and physiological parameters to explore the effect of different levels of heavy metal chromium (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) in *Trichosanthes cucumerina* L. seedlings. **Methods:** Various morphological (shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index percentage) and physiological (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid content). **Findings:** The plant growth characteristics such as shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index were adversely influenced by application of chromium in *T. cucumerina* seedlings. Likewise, the toxicity induced by Cr was resulted in significant decrease in contents of photosynthetic pigments chlorophyll and carotenoids.

Key words: Chromium, Heavy metal, Reactive oxygen species, Stress.

1. Introduction

Toxic metal induced by environmental pollution has dramatically increased because of various human activities during and after industrial revolution. Various human activities promote the enrichment of world-wide agricultural soil with a myriad of chemical pollutants. Soil contamination, due to heavy metals such as mercury (Hg), cadmium (Cd), lead (Pb), chromium (Cr), and arsenic (As) was a major environmental challenge worldwide [1].

Chromium is a non-essential element for plants and is known to be a toxic metal that can cause severe damage to plant growth and development. Discharge of sewage and industrial effluent into surface water bodies directly or indirectly cause accumulation of Cr and other toxic

metals in soil and causing toxicity to plants [2]. Cr induced oxidative stress involves inhibition of seed germination and induction of lipid peroxidation in plants that causes severe damage to the morphological, physiological and biochemical properties of plants. Oxidative stress induced by Cr initiates the degradation of photosynthetic pigments causing decline in growth. Cr in high concentration can disturb the chloroplast ultrastructure there by disturbing the photosynthetic process. Cr can affect antioxidant metabolism in plants. Various studies reported that Cr toxicity in plants triggers the production of reactive oxygen species (ROS) and lipid peroxidation. These overexpressed ROS can desynchronize the antioxidant defence systems (enzymatic and non-enzymatic) besides malformation of chloroplast ultrastructure, and degradation of photosynthetic carbon assimilation mechanism that ultimately leads to the reduction in plant growth and biomass [3]. In this study, a pot experiment was conducted using snake guard plant (*Trichosanthes cucumerina* L.) grown in Cr polluted soil conditions to assess various morphological and physiological parameters to explore the effect of different levels of Cr toxicity in *Trichosanthes cucumerina* L.

2. Materials and Methods

2.1 Experimental area

The experiment was carried out at botanical garden K. A. H. M. Unity Women's College, Manjeri, Malappuram, Kerala from January – June 2022 under natural conditions. The climate is generally hot and humid. The range of temperature varying between 32 and 28 ^{0}C. The average rainfall is 290 mm.

2.2 **Plant material**

Trichosanthes cucumerina L. commonly known as snake gourd. Seeds of Manusree variety were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India.

2.3 **Experimental setup**

The pot experiment was conducted under the green house conditions for 6 months. Snake gourd was selected as experimental crop. 3-5 seeds were grown in plastic grow bags containing soil, weighing about 4 Kg. After germination, the seedlings were thinned and these with best

growth performance were retained (3 plants per pot). Irrigation was done regularly using tap water.

2.4 Chromium treatment

After 21 days of sowing, the soil was treated with Potassium dichromate ($K_2Cr_2O_7$) solution of different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) along with an untreated control. The experiment was conducted in triplicates. After Chromium treatment all pots including control were equally watered measuring 200 ml of tap water. The growth of plants was monitored daily to fix the day of stress showing severe conditions in plants. On the second day, toxic limits of chromium were found in plants treated with 0.15, 0.2 and 0.25 g/kg soil. In the same day all treated plants including control were separated out from the experiments for analysis. The collected plant samples were washed with running tap water to get rid of soil or contaminants at the root zone and kept immediately in to plastic bags and the samples were refrigerated for analyzing various morphological and physiological parameters.

2.5 Morphological studies

The shoot length, root length, number of leaves, leaf area, fresh weight and dry weight were measured. Tolerance index percentage (TI) is calculated using root length of control and treatments.

$$TI = \frac{observed\ value\ of\ root\ length\ in\ solution\ with\ metal}{observed\ value\ of\ root\ length\ in\ solution\ without\ metal} \text{ X } 100$$

2.6 Physiological parameters

The total chlorophyll and carotenoid content in leaves were estimated following the method on Arnon (1949) [4] using 80% acetone was used as the extracting medium.

2.7 Statistical analysis

The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean±standard error (S.E.).

3. Results

The present study has been aimed to investigate the toxic effects of different concentrations of chromium stress induced *Trichosanthes cucumerina* L. seedlings and evaluated the various morphological and physiological parameters.

3.1 Morphological parameters

Shoot length of Cr (0.05, 0.1, 0.15, 0.2, 0.25 g/kg soil) treated snake gourd plants were significantly decreased by Cr treatment compared with the untreated plants. Compared to control plants, maximum decrease of shoot length was clearly visible in the plants treated with 0.25g (26%) as compared to other treatments of Cr. However, the percentage of decrease in shoot length of plants treated with 0.05g was less (11%) as compared to control seedlings than other stress treatments. Moreover, shoot length of the plants subjected to 0.1, 0.15 and 0.2 g Cr stress also decreased as compared to control snake gourd plants (16, 18 and 20% respectively). The root length was also decreased with increasing Cr concentration in snake gourd plants and maximum decrease (20%) was observed when subjected to 0.25 g Cr as compared to control plants. Likewise, the declines in root length were found by 4%, 10% and 14% in snake gourd plants subjected to 0.1, 0.15 and 0.2 g Cr stress, respectively as compared to control seedlings. However, there was only 3% variation in snake gourd plants treated with 0.05 g Cr as compared to untreated plants (Table 1; Fig. 1).

Number of leaves per plant was counted to analyze the morphological variations of different Cr treatments in snake gourd seedlings. In the case of snake gourd plants subjected to 0.2 and 0.25 g/kg soil Cr, the number of leaves per plant was slightly reduced (20%) as compared to control plants. However, there is no noticeable reduction on the number of leaves per snake gourd plants after subjected to 0.05, 0.1 and 0.15 g/kg soil Cr. With increase in the Cr stress concentration (0.05-0.25 g) in the soil induced a decline in the leaf area of snake gourd plants and the deleterious effect of Cr became more severe with increasing Cr level. The reduction in leaf area was less in snake gourd plants subjected to 0.05 and 0.1 g Cr stress (9 and 11% respectively) as compared to control plants and the decline in leaf area was maximum in plants subjected to 0.2 and 0.25 g Cr (21 and 27%) as compared to control plants. Moreover, snake gourd plants treated with 0.15 g Cr showed 14% reduction in leaf area as compared to control plants (Table 1; Fig. 1).

Snake gourd plants subjected to 0.05g and 0.1g Cr stress showed 19% and 23% reduction in fresh weight as compared to control plants. Likewise, fresh weight of snake gourd plants subjected to 0.2 and 0.25 g Cr was highly decreased (37 and 54%, respectively) as compared to untreated plants. Compared to control plants, maximum decrease of dry weight was recorded in the plants treated with 0.2 and 0.25 g Cr (29 and 36%, respectively) as compared to other treatments. Whereas, other treatments of Cr induced reduction in dry weight was less as compared to untreated snake gourd plants. There were significant differences in tolerance index (TI) in snake gourd plants exposed to different Cr concentrations. Compared with the control plants, the TI changed little when snake gourd plants were treated with 0.05 and 0.1 g/kg soil Cr, while TI was decreased obviously after 0.15, 0.2 and 0.25 g Cr treatments (10, 14 and 20%, respectively) as compared to untreated snake gourd plants (Table 1).

Table 1: Shoot length (cm), root length (cm), number of leaves, leaf area (cm^2), fresh weight (g) and tolerance index (%) of snake gourd plants treated with different concentrations of chromium (0, 0.05, 0.1, 0.15, 0.2 and 0.25 g/kg). Values given are mean of 3 independent experiments, each with a minimum of 3 replicates (i.e. n=9) ± S.E.

Cr treatments (g/kg soil)	Shoot length (cm)	Root length (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Tolerance Index (%)
Control	27.9±1.93	42.3±2.43	5±0.29	25.4±1.67	7.68±0.32	0.93±0.04	100±6.23
0.05	24.7±2.12	41±1.62	5±0.87	22.9±2.06	6.19±0.29	0.85±0.02	96.93±4.03
0.1	23.3±1.96	40.5±1.57	5±0.34	22.4±1.76	5.87±0.21	0.68±0.04	95.74±6.06
0.15	22.6±2.27	37.9±1.13	5±0.64	21.8±1.46	4.94±0.19	0.67±0.05	89.52±4.86
0.2	22.1±1.74	36.1±2.17	4±0.16	19.8±1.93	4.79±0.21	0.65±0.03	85.37±6.35
0.25	20.4±2.37	33.5±1.66	4±0.26	18.3±2.16	3.46±0.18	0.59±0.02	79.16±3.51

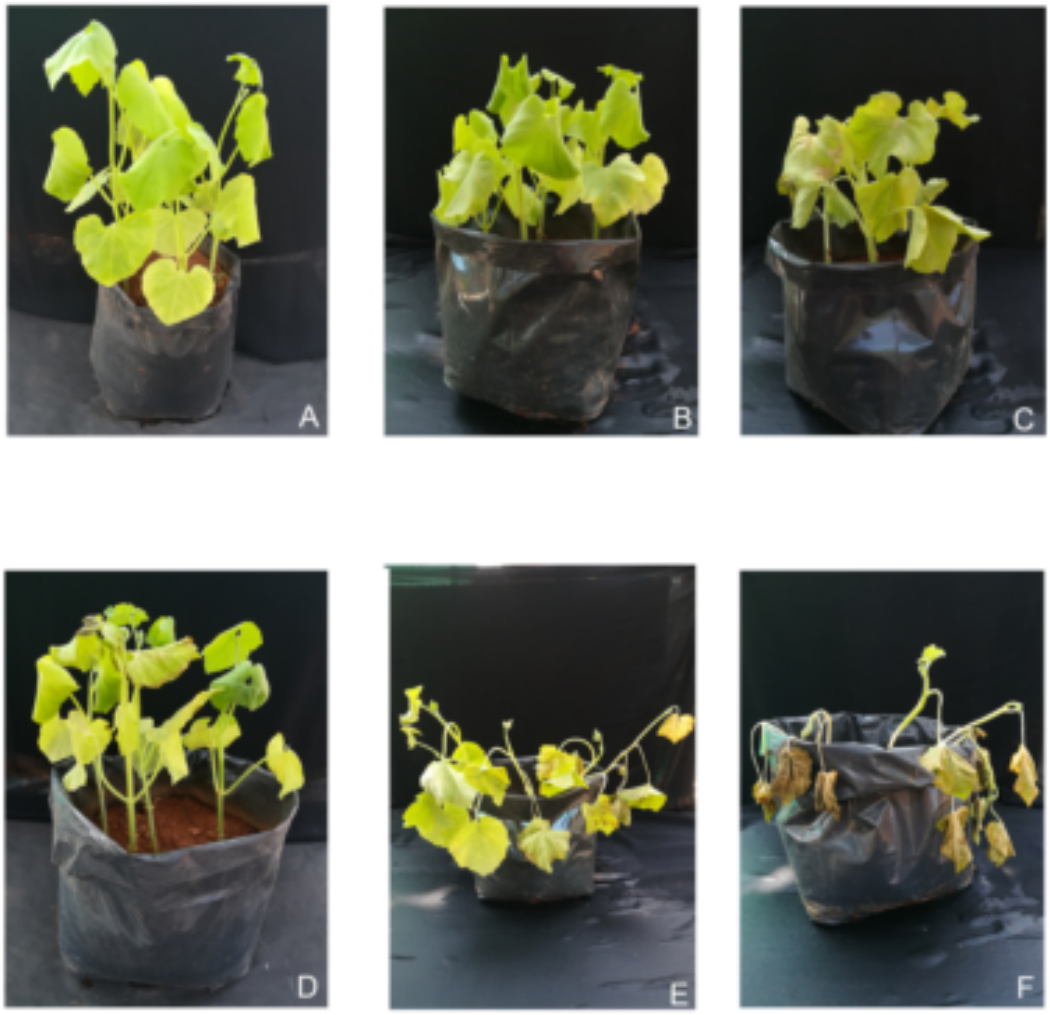

Figure 1: Effects of different concentrations of chromium (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) in snake gourd seedlings. A-Control, B-0.05 g/kg soil, C-0.1 g/kg soil, D-0.15 g/kg soil, E-0.2 g/kg soil, F-0.25 g/kg soil.

3.2 Physiological parameters

Chlorophyll *a*, *b* and total chlorophyll content in snake gourd plants exposed to various concentrations of Cr was decreased as compared to control leaves. The chlorophyll *a* content was declined with increasing Cr concentration in snake gourd plants and maximum decrease (90%) was observed when subjected to 0.25 g Cr as compared to control plants. Snake gourd plants treated with 0.1 and 0.15 g Cr showed 60 and 72% reduction in chlorophyll *a* content as compared to control plants. Likewise, the declines in chlorophyll *b* content were found by 13, 25

and 45% in snake gourd plants subjected to 0.05, 0.1 and 0.15 g Cr stress, respectively as compared to control seedlings and the decline in chlorophyll b content was maximum in plants subjected to 0.2 and 0.25 g Cr (70 and 82%) as compared to untreated leaves. Moreover, total chlorophyll content was also reduced by various concentrations of Cr treatments (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) and maximum reduction was recorded after exposure to 0.2 and 0.25 g/kg soil Cr (76%) as compared to untreated snake gourd plants (Fig. 2).

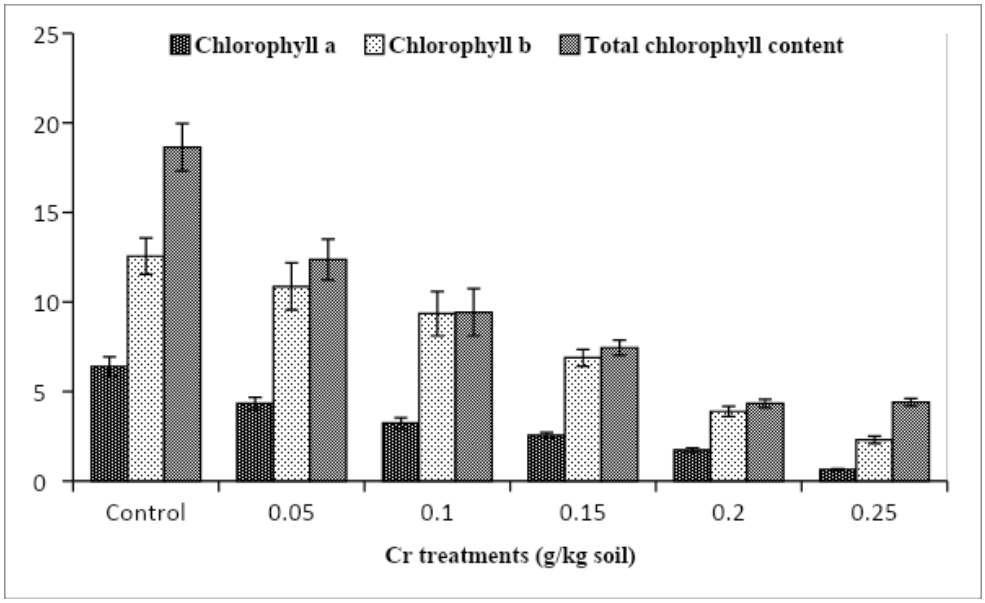

Figure 2: Chlorophyll a, b and total chlorophyll content (mg/g DW) of snake gourd plants treated with different concentrations of chromium (0, 0.05, 0.1, 0.15, 0.2 and 0.25 g/kg). The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

The carotenoid content in leaves was also decreased in snake gourd plants subjected to 0.05-0.25 g Cr stress and the maximum reduction (80-83%) was found when treated with 0.2 and 0.25 g Cr. The declines in carotenoid content were found by 45% in snake gourd plants subjected to 0.15 g Cr stress as compared to control seedlings. However, the reduction in carotenoid content was less upon exposure to 0.05 and 0.1 g Cr stress as compared to control plants (Fig. 3).

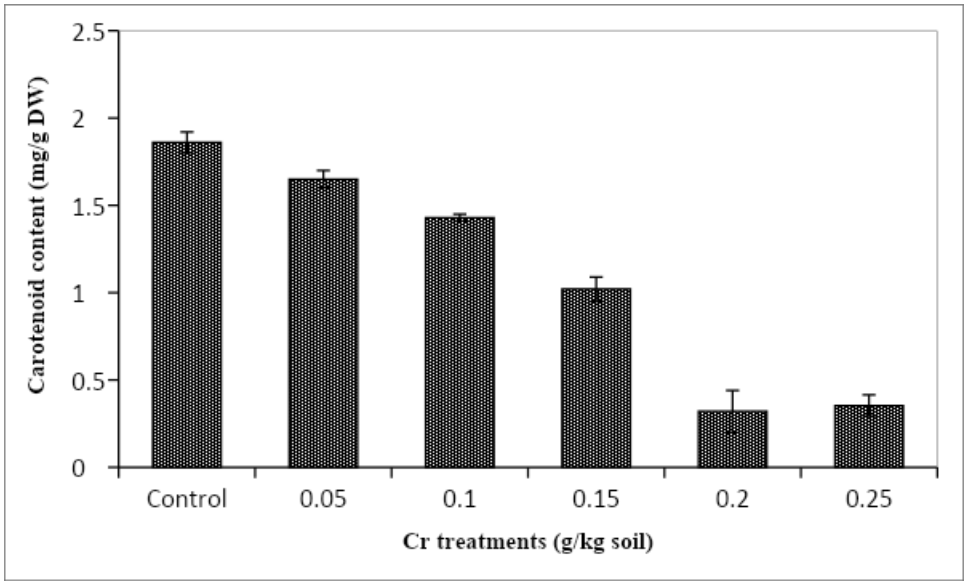

Figure 3: Carotenoid content (mg/g DW) of snake gourd plants treated with different concentrations of chromium (0, 0.05, 0.1, 0.15, 0.2 and 0.25 g/kg). The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

4. Discussion

Various morphological and physiological parameters to analyze the toxic effects of different concentrations of Cr (0.5-0.25g/kg soil) in *T. cucumerina*, were recorded. The results showed that the plant growth characteristics such as shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index were adversely influenced by application of Cr in different concentrations (0.05, 0.1, 0.15, 0.2 and 0.25 g/kg soil) in 21 days old snake gourd seedlings. However, the decrease in these morphological parameters of snake gourd plants treated with 0.05 g/kg soil Cr was less lethal than other stress treatments. The growth parameters of the plants subjected to 0.1, 0.15 and 0.2 g/kg soil Cr was significantly decreased.

Chromium stress imposition also resulted in decreased root and shoot length of radish seedlings at 1.5 mM concentration [5] and the growth inhibition by Cr can be due to chromosomal aberrations which lead to inhibition of cell division [6]. Previously reports states that the reduction in shoot length might be due to Cr induced ultrastructural damages to leaf mesophyll cells [7,8]. Similarly the morphological traits of cauliflower under Cr stress, plant height, root length,

number of leaves, leaf area and dry weight were decreased as compared to control plants [14]. The reduction in growth and biomass might be attributed to the Cr interaction with essential nutrients and reduced their uptake by plants [10].

Moreover, there were significant differences in tolerance index also observed in snake gourd plants exposed to different Cr concentrations. Little change in TI was recorded in snake gourd plants treated with low concentrations (0.05 and 0.1 g) of Cr. However, tolerance index is decreased in the higher concentrations of Cr treated snake gourd plants as compared to control plants. Likewise, the toxicity induced by Cr resulted in significant decrease in contents of total chlorophyll, Chlorophyll *a* and chlorophyll *b.* About 1.5 times decrease in total chlorophyll content was decreased in 1.5 Mm Cr stress than control in *Raphanus sativus* [5]. Similarly, Cr stress significantly decreased the photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids as compared to control plants in cauliflower plants [11]. The decrease in photosynthetic pigments might be due to ultrastructural changes in chloroplast [7] or production of ROS under Cr stress [9]. Collectively, this study provided an insight into the various morphological, physiological and biochemical aspects of the toxic effects of different concentrations of Cr (0.5-0.25g/kg soil) in snake gourd plants.

5. Conclusions

The toxicity induced by heavy metal chromium in snake gourd seedlings were measured by analysing various morphological and physiological parameters The plant morphological characteristics such as shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index were decreased by Cr treatments in snake gourd seedlings. Likewise, the toxicity induced by Cr resulted in significant decrease in photosynthetic pigments chlorophyll and carotenoid contents.

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Potassium mediated alleviation of iron toxicity in okra seedlings

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Abstract

Background: The objective of this study was to evaluate the effects of different concentrations of iron (Fe) on various growth parameters and to assess the effectiveness of different concentrations of potassium in mitigating the negative effect of iron toxicity in *A. esculentes* (L.) Moench. **Methods**: After 21 days of sowing, the soil was treated with ferric chloride ($FeCl_3$) solution of different concentrations (0.5, 1, 1.5, 2 and 2.5 g/kg soil) along with an untreated control and various growth parameters were measured. After fixing the stress concentration that imparting 50% of growth inhibition, the treatment with potassium in the form of potassium chloride (0.02, 0.04, 0.06 and 0.08 g/kg soil) was done in okra plants and various growth parameters were analyzed. **Findings**: Plant growth characteristics were significantly affected by application of excess iron in 21 days old okra seedlings. The potassium mediated Fe stress alleviation also evaluated in okra seedlings. The seedlings were treated with 0.04 g/kg soil K, reduced the toxic effects of iron and lead to the growth enhancement in terms of growth parameters in okra plants.

Keywords: Iron, Heavy metal, Potassium, Stress, Toxicity.

1. Introduction

Macronutrients are the nutrients required by the plants in large amounts and these include iron, zinc, boron etc. Iron (Fe) plays an important role in various physiological and biochemical processes in plants and it is important for the maintenance of chloroplast structure and function in plants. However, excess iron application or iron toxicity in plants induces various morphological and biochemical alterations. They include the root hair morphogenesis, yellowing of leaves and ultrastructural disorganization of mitochondria and chloroplast, antioxidants accumulation. Fe stress induces the generation of reactive oxygen species which reduces the

activity of cytoplasmic enzymes and damage to cell structures [1,2]. The antioxidant defence system protecting the plant from heavy metal induced oxidative damage [3].

In plants, a balance of inorganic nutrients is required for adapting growth under stressful environments and thus the sufficient availability of nutrients may reduce the metal toxicity in plants. Potassium (K) plays a major role in numerous physiological functions related to plant health and resistance to biotic and abiotic stress. Potassium application reduces malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content, enhanced protein, proline synthesis, secondary metabolites and enzymatic and non-enzymatic antioxidants in plant cells which is finally responsible for plant's survival [4]. In this study, field experiment was conducted in okra plants (*Abelmoschus esculentus*) grown in high concentrations of Fe treated soils. The objectives of the study were to explore the effects of different levels of Fe on the growth parameters and find the optimal K concentration for application to okra plants. This study could provide useful information for sustainable agricultural production and environmental management in Fe polluted soils.

2. Materials and methods

2.1 Plant material

Abelmoschus esculentus (L.) Moench also known as okra, is a hairy annual plant of the Malvaceae family. Seeds of Varsha uphar variety were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India. The experiment was carried out at botanical garden K. A. H. M. Unity Women's College, Manjeri, Malappuram, Kerala from January - June under natural conditions. The climate is generally hot and humid. The range of temperature varying between 32 and 28ºC. The average rainfall is 290 mm.

2.2 Experimental setup

The pot experiment was conducted under the green house conditions for 6 months. Okra was selected as experimental crop. 6-8 seeds were grown in plastic grow bags containing soil, weighing about 4 kg. After germination, the seedlings were thinned and these with best growth performance were retained (3 plants per pot). Irrigation was done regularly using tap water.

2.3 Soil conditions

The soil analysis was conducted at District soil testing laboratory, Malappuram, Kerala. The conditions of soil that used for the experiment was presented below:

Parameters	Value/Content
PH value	5.30
Total soluble salt (TSS)	0.001
Carbon content (%)	0.29
Phosphorus (Kg/ha)	6
Potassium (Kg/ha)	197
Calcium (ppm)	0.0
Magnesium (ppm)	0.0
Sulphur (ppm)	12.5
Boron (ppm)	1.0
Iron (ppm)	25.1
Manganese (ppm)	13.3
Zinc (ppm)	4.8
Copper (ppm)	0.2

2.4 Iron (Fe) treatment

After 21 days of sowing, the soil was treated with ferric chloride ($FeCl_3$) solution of different concentrations (0.5, 1, 1.5, 2 and 2.5 g/kg soil) along with an untreated control. The experiment was conducted in triplicates. After iron treatment all pots including control were equally watered measuring 200 ml of tap water daily. The growth of plants was monitored daily to fix the day of stress showing severe conditions in plants. On the second day, toxic limits of iron were found in plants treated with 1.5, 2 and 2.5 g/kg soil. In the same day all treated plants including control were separated out from the experiment pots for analysis.

2.5 **Fe stress alleviation by potassium treatment**

After fixing the stress concentration that imparting 50% of growth inhibition, the treatment with potassium in the form of potassium chloride was done in okra plants. The soil was treated with potassium chloride solution (0.02, 0.04, 0.06 and 0.08 g/kg soil) along with an untreated control. The samples were collected and analysed after two days of potassium treatment with Fe.

2.6 Growth parameters

The shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index percentage was recorded. For dry weight measurements, the weighed seedlings were kept in a hot air oven at 100ºC for one h and further kept in oven set at 60ºC. Drying and weighing were repeated at regular intervals (24 h) until the values of dry weight became constant. Tolerance index percentage is calculated according to the following formula:

2.7 **Statistical analysis**

$$TI = \frac{\text{observed value of root length in solution with metal}}{\text{observed value of root length in solution without metal}} \times 100$$

The data is an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean±standard error (S.E.) and oneway ANOVA, Tukey's studentized range test ($p \leq 0.05$) used for the statistical analysis.

3. **Results**

The present study has been aimed to investigate the protective effects of different concentrations of potassium (K) on iron (Fe) stress induced *Abelmoschus esculentus* (L.) Moench seedlings and evaluated the various morphological and biochemical parameters. The results obtained from various experiments were presented below.

3.1 **Effect of different concentration of iron stress in okra plants**

Compared to control plants, maximum decrease of shoot length was clearly visible in the plants treated with 2.5 g/kg soil Fe (14% as compared to control) than other stress treatments. However, the decrease of shoot length was less upon exposure to 0.5 g/kg soil Fe (3%) as compared to control plants. Moreover, the shoot length of the plants subjected to 1, 1.5, and 2

g/kg soil Fe stress also decreased as compared to control okra plants (5, 7 and 10% respectively). The root length was also decreased with increasing Fe concentrations in okra plants and maximum decrease (19%) was observed when subjected to 2.5 g/kg soil Fe as compared to control plants. In the case of okra plants subjected to various concentrations of Fe (0.5, 1, 1.5 and 2 g/kg soil), the number of leaves per plant was reduced (25%) as compared to control plants. Moreover, 50% reduction in the number of leaves was noticed in okra plants after subjected to 2.5 g/kg soil Fe treatment. Moreover, the reduction in leaf area was less in okra plants subjected to 0.5 and 1 g Fe stress (4-8%), as compared to control plants and it was maximum in plants subjected to 2 and 2.5 g/kg soil Fe (19-24%), as compared to control plants. Moreover, okra plants treated with 1.5 g/kg soil Fe showed 13% reduction in the leaf area as compared to control plants (Table1: Plate 1).

Likewise, the reduction in fresh weight was maximum in plants subjected to 2 and 2.5 g/kg soil Fe (53 and 66%, respectively) as compared to control plants. Moreover, okra plants treated with 1.5 g/kg soil Fe showed 44% reduction in fresh weight as compared to control plants. Dry weight of Fe stressed okra plants were decreased compared with the control plants and maximum decrease of dry weight was recorded in the plants treated with 1.5, 2 and 2.5 g/kg soil Fe (52, 62 and 72% respectively as compared to control plants). Also, there was significant difference in tolerance index (TI) of okra plants exposed to different Fe concentrations. Compared with the control, the TI was slightly decreased when okra plants were treated with 0.5 & 1 g/kg soil Fe (5-7% as compared to untreated control plants), while TI was decreases obviously after 1.5, 2 and 2.5 g/kg soil Fe treatments (10, 16 and 19% respectively) as compared to untreated okra plants (Table 1).

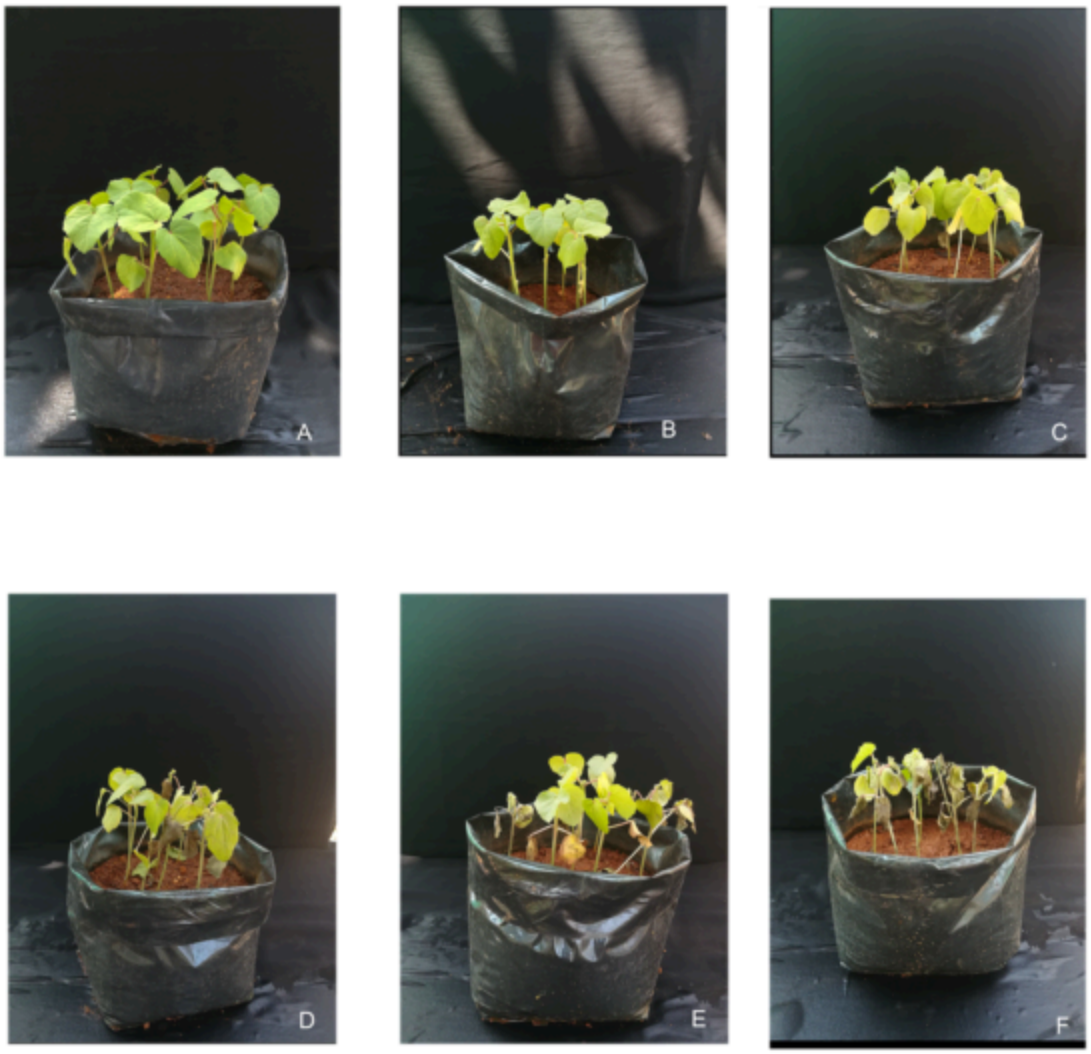

Plate 1: Effect of different concentrations of Fe (0.5, 1, 1.5, 2 and 2.5 g/kg soil) in in okra plants A – control, B – 0.5 g/kg soil, C,- 1 g/kg soil, D – 1.5 g/kg soil, E- 2 g/kg soil, F- 2.5 g/kg soil.

Table 1: Growth parameters of okra plants subjected to different concentrations of Fe (0.5, 1, 1.5, 2 and 2.5 g/kg soil). Different alphabetical letters indicate statistically significant differences at p≤0.05 following oneway ANOVA (Tukey's studentized range test).

Fe Treatments (g/kg soil)	Shoot length (cm)	Root length (cm)	Number of leaves	Leaf area (cm^2)	Fresh weight (g)	Dry weight (g)	Tolerance index (%)
Control	15.2±0.24[a]	9.5±0.23[a]	4±0.28[a]	10.6±0.23[a]	1.87±0.03[a]	0.29±0.01[a]	100±3.1[a]
0.5	14.7±0.23[b]	9±0.15[a]	3±0.15[b]	10.2±0.42[a]	1.49±0.02[b]	0.23±0.01[b]	94.73±2.6[2]
1	14.5±0.18[c]	8.8±0.18[b]	3±0.1b	9.7±0.25[b]	1.13±0.12[c]	0.17±0.11[c]	92.63±1.2[b]
1.5	14±0.22[d]	8.5±0.26[b]	3±0.21[b]	9.2±0.16[c]	1.05±0.14[c]	0.14±0.01[c]	89.47±1.4[c]
2	13.6±0.37[e]	8±0.14[c]	3±0.18[b]	8.6±0.29[d]	0.88±0.04[d]	0.11±0.01[d]	84.21±2.4[d]
2.5	13±0.83[e]	7.7±0.18[d]	2±0.08[c]	8.1±0.17[e]	0.64±0.33[e]	0.08±0.01[e]	81.05±1.7[e]

3.2 Potassium (K) mediated modulation of iron (Fe) stress in okra plants

To analyze the alleviation potential of different concentrations of potassium (0.02, 0.04, 0.06 and 0.08 g/kg soil) towards iron (1.5 g/kg soil – standardized concentration of Fe which imparts 50% growth inhibition) induced stress effects in okra, various growth parameters were recorded.

After two days of iron treatment of fixed concentration (1.5 g/kg soil) and different concentrations of K treatments (0.02, 0.04, 0.06, 0.08 g/kg soil), iron stress (1.5 g/kg soil) reduced the shoot length in okra plants by 8% as compared to the control. However, 0.0 2 g/kg soil K treated Fe stressed plants, the reduction in shoot length was less relative to control plants. 0.04 g/kg soil of Potassium treated plants showed slight increase in shoot length as compared to

control plants. However, higher concentrations of K treatments (0.06 and 0.08 g/kg soil) highly reduced the shoot length of okra plants than control plants (Figure 1: Plate 2).

In the case of root length also, there was a small reduction in decreasing the root length of plants treated with 0.02 g/kg soil (5%) concentration of potassium. Surprisingly, the root length was slightly increased upon subjected to 0.04 g/kg soil K as compared to untreated plants. However, the higher concentrations of K reduced the root length of okra plants which were treated with 1.5 g/kg soil Fe as compared to control plants. Maximum rate of alleviation of Fe stress induced decline in number of leaves among various concentrations of K treated okra plants was shown by plants treated with 0.04 g/kg soil K. Moreover, under Fe toxicity, 0.04 g/kg K treatment slightly increased the leaf area as compared to control okra plants. All other treatments of K under Fe stress reduced the leaf area of okra plants and maximum reduction was observed upon subjected to 0.08 g K (8%) (Figure 1: Plate 2).

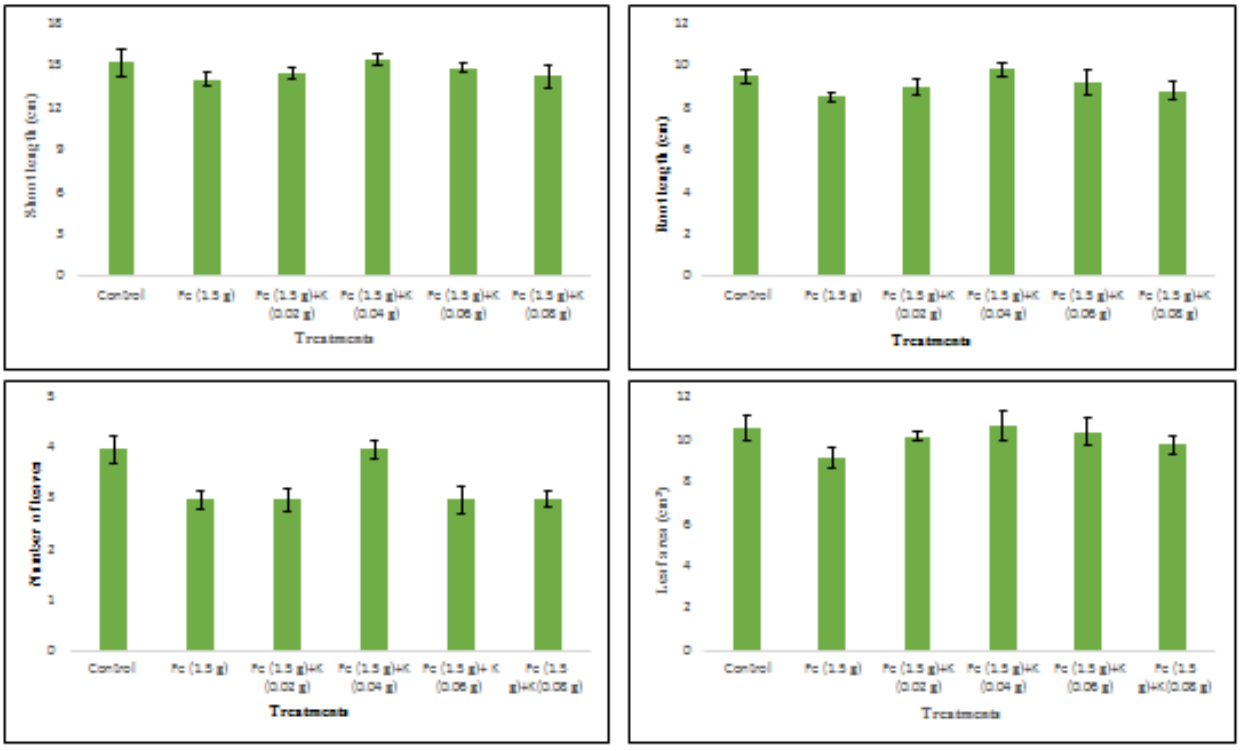

Figure 1: Shoot length, root length, number of leaves and leaf area of okra plants subjected to Fe and K treatments. The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

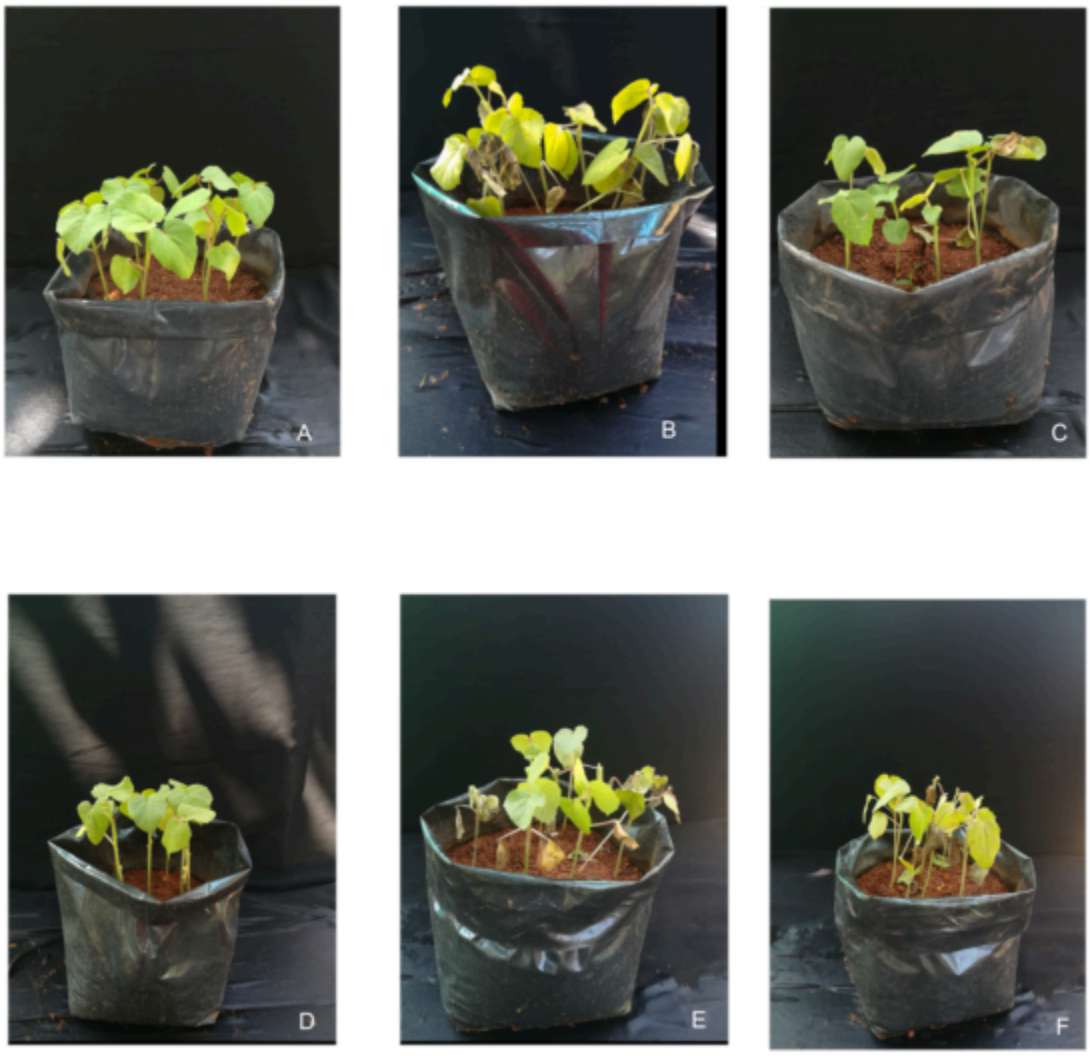

Plate 2: K mediated modulation of Fe stress in morphology of okra plants. A- control, B- Fe (1.5 g/kg soil), C- Fe (1.5 g/kg soil)+ K(0.02 g/kg soil), D – Fe(1.5 g/kg soil)+ K (0.04 g/kg soil), E- Fe(1.5 g/kg soil)+ K (0.06 g/kg soil), F- Fe(1.5 g/kg soil)+ K(0.08 g/kg soil).

Iron stress reduced the fresh weight in okra plants (44%) as compared to the control. Likewise, K treatment also reduced the fresh weight under iron toxicity, except upon subjected to 0.04 g K treatment in okra plants, where it was slightly enhanced as compared to control plants. The maximum reduction in fresh weight was recorded at 0.06 and 0.08 g/kg soil K treatments (10 and 29% respectively) under Fe stress. Relative to control plants, 0.04 g/kg soil K treated plants showed a 7% increase in dry weight under Fe toxicity as compared to control plants.

However, higher concentrations of K treatment (0.08 g/kg soil) slightly reduced the dry weight of okra plants than control plants. Moreover, different concentrations of K supply increased tolerance index of okra plants as compared to Fe stressed plants. However, the tolerance index was maintained by 0.04 g/kg soil K as in untreated plant. While tolerance index was slightly decreased in 0.06 and 0.08 g/kg K treatments (3 and 7% respectively) as compared to untreated okra plants (Figure 2).

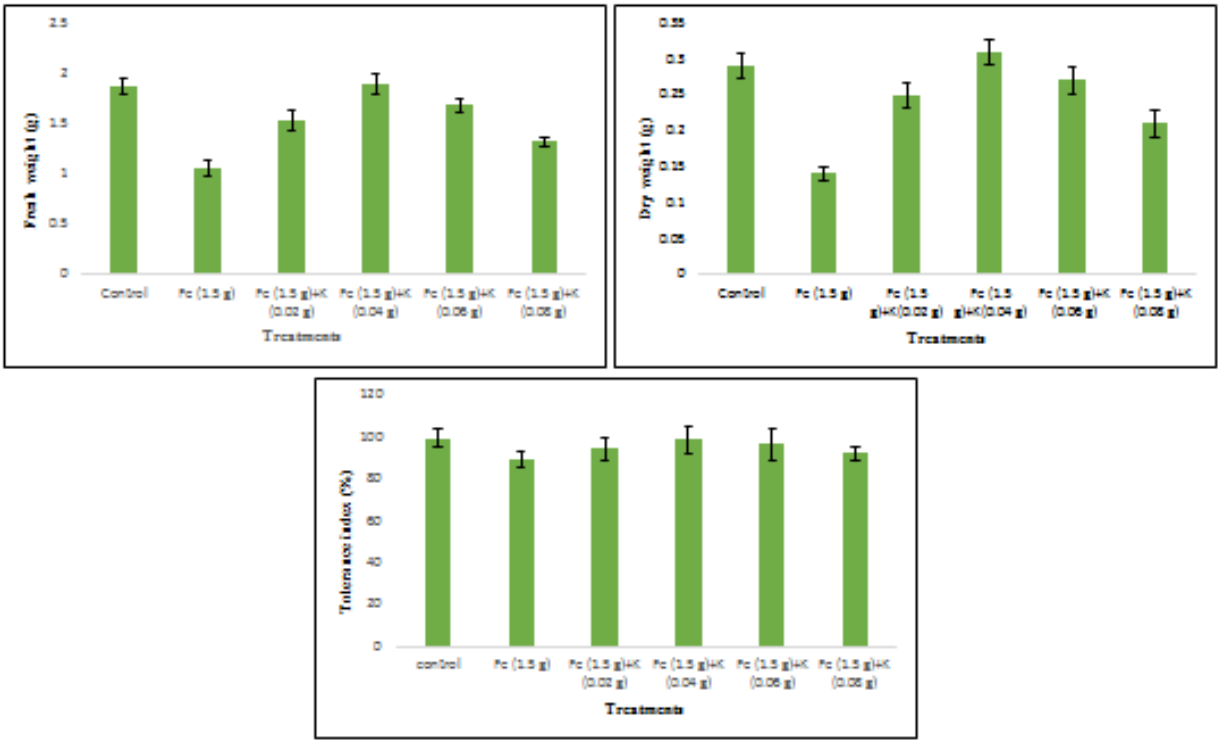

Figure 2: Fresh weight, dry weight and tolerance index of okra plants subjected to Fe and K treatments. The vertical bars represent S.E. of the mean value of recordings from 3 independent experiments each with a minimum of 3 replicates (i.e. n=9).

4. Discussion

The results showed that the plant growth characteristics were adversely influenced by application of excess iron in 21 days old okra seedlings. The effects of excess iron were measured by various growth parameters such as root length, shoot length, number of leaves per plant, dry weight, fresh weight and leaf area were found to be more sensitive to excess iron concentration. In the present study, it was found that the Fe toxicity severely affected the

morphological parameters in okra plants and the maximum decrease of shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was clearly visible in the plants treated with 2 and 2.5 g/kg soil Fe than other stress treatments. However the decrease of shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was less upon exposure to 0.5, 1, 1.5 g/kg soil Fe and 1.5 g/kg soil Fe concentration fixed as 50% growth inhibition as compared to control in okra plants.

Previously it was experimentally proved that growth parameters were significantly affected by iron toxicity in rice plants. Extreme reduction of root growth occurred under iron treatments and the iron content in the root and shoot of rice plants increased significantly by increment of iron concentration in the root medium [5]. Moreover, excessive iron greatly decreased root and shoot length of the hybrid rice [6]. Likewise, Fe toxicity significantly reduced the growth and metabolism of four different varieties of rice plants and at severe stress level, decrease of root length is observed in all the varieties under excess Fe stress [7].

The protective effect of different concentrations of potassium on iron stress (0.5, 1, 1.5, 2, 2.5 g/kg soil) induced okra plants were evaluated and results showed that the plant growth characteristics were adversely influenced by application of excess iron in 21 days old okra seedlings. In the present study, it was found that the Fe toxicity severely affected the morphological parameters in okra plants and the maximum decrease was clearly visible in the plants treated with 2 and 2.5 g/kg soil Fe than other stress treatments. However the decrease of shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was less upon exposure to 0.5, 1, 1.5 g/kg soil Fe and 1.5 g/kg soil Fe concentration fixed as 50% growth inhibition as compared to control in okra plants.

Potassium supplementation induced marked increase in growth under critical iron concentration. The present study revealed that the potassium mediated modulation of okra seedlings under Fe toxicity reduced the toxic effects of iron stress. Different concentration of K (0.02, 0.04, 0.06 and 0.08 g/kg soil) applied towards Fe (1.5 g/kg soil) induced stress effect in *Abelmoschus esculentus*. Root length, shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was slightly enhanced upon subjected to 0.04 g/kg soil K, revealing the alleviation potential of K towards Fe toxicity in okra plants. But high concentration of K (0.06 and 0.08 g/kg soil) reduced these growth parameters which were treated with 1.5 g/kg soil

Fe. However, 0.02 g/kg soil K treated Fe stressed plants the shoot length, root length, number of leaves, leaf area, fresh weight, dry weight and tolerance index was slightly reduced.

Potassium participates in physiological and biochemical processes that can alleviate the toxicity induced by heavy metals. Naciri *et al* [8] experimentally proved that K significantly reduced heavy metal cadmium translocation from root to shoot and improved root and shoot growth parameters in tomato plants. Moreover, Shamsi *et al* [9] reported that K supplementation alleviated the reduction of growth and nutrients uptake in Cd treated soybean plants. In this study, the optimum concentration of potassium was 0.04g/kg soil under high concentrations of iron in soil for okra cultivation which can alleviate the toxic effects of iron. However, higher concentrations of potassium are negatively affected the morphological as well biochemical parameters of okra plants. Moreover, the application of K in soil could be generalized as promising strategy to alleviate Fe toxicity in okra at the initial crop establishment stage.

5. Conclusion

By analysing the morphological parameters through this investigation, it was observed the iron stress severely affected the plant growth and biomass, which can lead to plant death. The higher concentrations of iron lead to significant alterations in the normal plant growth there by causing chlorosis, leaf withering, defoliation and necrosis as visible symptoms. The seedlings treated with 2.5 g/kg soil Fe showed extreme leaf withering, chlorosis, defoliation and overall deformities of the plant and ultimately the death of plants. It helped to fix the concentrations of 1.5 g/kg soil iron which caused an average range of heavy metal toxicity and that can be reduced using appropriate control measures. The morphological parameters such as root length, shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index are adversely affected by the application of iron stress in okra plants. The potassium mediated Fe stress alleviation also evaluated in okra seedlings. The seedlings were treated with 0.04 g/kg soil K, reduced the toxic effects of iron and lead to the growth enhancement in terms of root length, shoot length, leaf area, fresh and dry weight of okra plants.

6. Acknowledgement

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Comparative anatomy of invasive plants in the family Verbenaceae

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Abstract

Background: Verbenaceae, family of plants, in the order Lamiales, a worldwide but mainly tropical grouping of 30 genera and some 1,100 species, some of which are important for their flowers. The genus *Lantana*, Verbenaceae as described by Linnaeus in 1753 contain seven species, six from South America and one from Ethiopia. *Lantana* is mostly native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. *Lantana camara* L. One of the species, commonly known as wild or red sage, is the most widespread species of this genus, growing luxuriantly at elevations up to 2000 m in tropical, sub-tropical and temperate regions. *Stachytarpheta jamaicensis* is a species of plant in the family Verbenaceae, native throughout the Caribbean. It has many common names including blue porter weed, blue snake weed, bastard vervain, Brazilian tea, Jamaica vervain, and light-blue snakeweed. **Methods:** The objective of this study is to compare the stem, root, leaf anatomical characters of both *Lantana camara* and *Stachytarpheta jamaicensis*. Stomatal index of the selected plants was also made. Cross-sections were obtained, by hand, for microscopic characterization of root, stem and leaf of two plants. **Findings**: The analysis showed that characters responsible for the invasiveness is the presence of sclerenchymatous tissues in the cortical region of stem and root. Presence of trichomes noted in both plants. Study of stomatal index showed that it is higher in *Lantana camara* than *Stachytarpheta jamaicensis.* These anatomical features are useful for diagnosis of the species and provide support to their quality control.

Key words: Invasive plants, *Lantana,* Stomatal index, Trichomes, Anatomy

1. Introduction

Invasive species are the plant which grow, develop and spread uncompromisingly outside its native range. Invasive species are not dreadful originally; it is just that they depend on certain ecological characteristics which initiate its invasion, when they grow on favourable environmental condition (Mantri et al., 2002[1]). These plant deliberately or accidentally introduced into new area through transportation, animals, birds, tourists, wind and water dispersal etc. (Dogra et al., 2009[2]).

Verbenaceae, family of plants in the order Lamiales, a worldwide but mainly tropical grouping of 30 genera and some 1,100 species, some of which are important for their flowers. Members of the family, sometimes known as Verbena or Vervain, have opposite or whorled leaves that are usually undivided. The flowers are aggregated in spikes, clusters, or racemes and usually consist of a tube flaring into four or five almost equally cut lobes. The type genus, Verbena, contains some 200 to 250 species, almost all of them native to the Western Hemisphere. Outstanding among the 30 *Petrea* species, all tropical American, is a woody evergreen vine called purple wreath, or sand paper vine (*P. volubilis*). It bears long, hanging clusters of violet-blue pansy like flowers and has oval leaves so rough as to be likened to sandpaper.

The genus *Lantana,* Verbenaceae as described by Linnaeus in 1753 contain seven species, six from South America and one from Ethiopia. Lantana from the Latin lento, to bend probably derives from the ancient Latin name of the genus Viburnum which it resembles a little in foliage and inflorescence. *Lantana* is mostly native to subtropical and tropical America, but a few taxa are indigenous to tropical Asia and Africa. It now occurs in approximately 50 countries where several species are cultivated under hundreds of cultivar names. The recorded number of *Lantana* species varies from 50 to 270 specific and sub specific entities, but it appears that a better estimate is 150 species.

Stachytarpheta jamaicensis is a species of plant in the family Verbenaceae, native throughout the Caribbean. *S. jamaicensis* is a perennial woody herb which reproduces solely by seed. Mature seeds remain within the dry, brittle fruiting spike (Holm et al., 1997[3]). It has many common names including blue porter weed, blue snake weed, bastard vervain, Brazilian tea, Jamaica vervain, and light-blue snakeweed. It is unclear whether *S. indica* is a separate species. It is usually found along country roadsides and it grows also well as a ruderal plant on disturbed terrain. It is an invasive species in some places. *Stachytarpheta* species are

generally agreed to be native to tropical America but were already known in Asia in the 18th Century. *S. jamaicensis* is now widespread in Central America, the Caribbean, East and Southern Asia and the Pacific, but occurs less frequently in Africa.

Anatomical information on invasive species are very scanty, in this study. In this study, anatomical, morphological, of *Lantana camara* L. (Invasive plant) and *Stachytarpheta jamaicensis* (L.) vahl (invasive) are compared with a view to report the anatomical characters in the invasive species responsible for invasiveness and to correlate these characters with their functions in the invasive species. Hence, we aimed to compare the morphology, anatomy of stem, root and leaf of the selected invasive plants in the family Verbenaceae (*Lantana camara* and *Stachytarpherta jamaicencis)* and also to compare the stomatal index of this plants.

2. Materials and methods

2.1. Collection of plant material

The fresh plant of *Lantana camara* L. and *Stachytarpheta jamaicensis* (L.) vahl were taken from college campus and place near the college.

2.1.1. *Lantana camara* L.

Family : Verbenaceae

Common name : Red sage

Local names : *Konda, kattuchinda, kaniya*

Much branched scandent shrubs; stem 4-angled, armed with short thorns. Leaves simple, opposite, 3-6 x 2-4 cm, ovate or elliptic-ovate, apex acute to shortly acuminate, base round to obtuse, margin serrate, scabrous above, puberulous below, veins impressed above; petiole to 1.5 cm long. Inflorescence terminal and axillary condensed spikes; peduncle 3-4 cm long, shortly prickly. Flowers are sessile, orangish-red, changing to deep red on ageing; bracts closely imbricating. Calyx is truncated, Corolla salver-shaped; tube 0.8-1 cm long, slender, cylindric, bent and inflated over stamens; lobes 5, obscurely 2- lipped. Stamens 4,

included. Ovary 2- celled; ovules 1 in each cell; style slender; stigma subcapitate. Drupe 2-3 mm across, globose, purple on ripening; seeds reticulate.

2.1.2. *Stachytarpheta jamaicensis* (L.)vahl

Family : Verbenaceae

Commom name : Blue snake weed ,Blue porter weed

Local name : *Kattupunnithu, Narivalan*

Sub shrubs; branches subtertragonous. Leaves 3.5-7 x 2-4 cm, obovate, base cuneate and decurrent on petiole, margin coarsely crenate-dentate, apex obtuse or rounded; petiole to 2 cm long. Spikes terminal, 10-25 cm long, c. 4 mm across. Bracts. 7 mm long. Calyx is 6 mm long, 4-toothed, puberulous. Corolla hypocrateriform, bluish-pink; tube 8-10 mm long, slightly curved; limb c. 8 mm across. Style included. Fruit 5 mm long, oblong.

2.2. Chemical used for the study

1. Safranin
2. Glycerine

2.3 Equipments used for the study

1. Normal compound microscope
2. Research microscope
3. Measuring scale

2.4 Method of study

Present study include analysis of morphological and anatomical difference between two species of *Lantana camara* L. *and Stachytarpheta jamaicensis* L.vahl

2.4.1 Study of morphological variation

Five plants each of the two species taken for study the five morphological characters.

Table 1. Characters observed for morphological studies.

Sl. No.	Characters
1	Stem girth
2	Internode length
3	Leaf length
4	Leaf breadth
5	Petiole length

2.4.2 Study of leaf architecture

Table 2. Characters observed for leaf morphological studies

Sl. No.	Characters
1	Leaf orientation
2	Nature leaf apex
3	Nature of leaf base
4	Nature of margin
5	Type of venation

2.4.3 Anatomical evaluation

Thin transverse section of the plant part include stem, root, leaves were taken by hand. Hand sections were stained with diluted aqueous safranin washed and mounted glycerine and observed under the microscope.

2.4.4 Determination of Stomatal Index

Stomatal index is the percentage of the number of stomata formed by the total number of epidermal cells, including the stomata each stoma being counted as one cell. Taken leaf fragment of about 5 x 5 mm in size in a test tube containing sodium hydroxide solution and heat in a boiling water bath for about 15 minutes or until the fragment become transparent. Transfer the fragment to a microscopic slide and examine under microscope. Calculate the result as follows

Stomatal index = $\frac{S}{E+S}100$

S = the number of stomata in a given area of leaf and

E= the number of epidermal cells in the same area of leaf

3. Result

3.1 Morphological characters of habit

Five morphological characters of plant were studied and compared quantitatively. (Table 3). The study showed that maximum stem girth and internode length were seen in the *Stachytarpheta jamaicensis*. Leaf length, Leaf breadth and petiole length are seen as maximum also in *Stachytarpheta jamaicensis.*

Table 3: Comparative morphological (quantitative) characters *Lantana camara* and *Stachytarpheta jamaicensis*

Sl. No	Characters	*Lantana camara*	*Stachytarpheta jamaicensis*
1	Stem girth	0.1±1.4	1.3 ± 3.5
2	Internode length	2.8±4.3	2±5.6
3	Leaf length	3.4±6.4	4±6.5
4	Leaf breadth	2.5±3.5	2±4.2
5	Petiole length	1.2±2.4	1±2.3

3.2 Leaf architecture

Leaf architecture has been studied and compared on the basis of five characters and result are showed in the table (Table 4). The study showed that plants shows various morphological feature.

Table 4: Comparison of leaf Characters

Si. No	Characters	*Lantana camara*	*Stachytarpheta jamaicensis*
1	Leaf orientation	Ovate, symmetrical rough	Obovate, symmetrical, membranous
2	Nature of margin	Serrate	Dentate-crenate
3	Nature of apex	Acute	Acuminate
4	Nature of base	Round to obtuse	Decurrent
5	Type of venation	Unicostate reticulate	Unicostate reticulate

3.3 Stem anatomical characters

Table 5: Stem anatomical characters of *Lantana camara and Stachytarpheta jamaicensis.*

Sl. No.	Characters	*Lantana camara*	*Stachytarpheta jamaicensis*
1	Shape in cross section	Quadrangular outline, glandular and unicellular trichome present.	Circular in outline and glandular trichome present
2	Nature of epidermal cells	Single layerd with cuticle	Single layered epidermis with cuticle
3	Nature of hypodermis	1-2 layered collenchymatous cell	2-3 layered collenchymatous
4	Nature of cortex	3-4 parenchymatous cell Discontinues large patches of sclerenchymatous patches seen.	5-6 layered parenchyma cells and discontinues small sclerenchymatous patch seen.
5	Vascular bundle	xylem vessel present, collateral, open	Circular in outline, collateral, open
6	Nature of pith	Parenchymatous pith and cell large and small rounded	Parenchymatous pith rounded large and small cells

3.4 Leaf anatomical characters

Table 6: Leaf anatomical characters

Sl. No.	Characters	*Lantana camara*	*Stachytarpheta jamaicensis*
1	Nature of upper epidermis	Uniseriate, unicellular and multicellular trichomes	Uniseriate, with thick cuticle
2	Nature of margin	Dentate	Dentate - crenate
3	Vascular bundle	Collateral, open, form flattened arch in the V form with two accessory bundle located dorsally.	Single bundle with 4-6 row of xylem and surrounded phloem
4	Mesophyll Palisade cells Spongy cells	Elongated, compactly arranged, one layer of palisade cells, 2- 5 layers of spongy cells	Cells elongated and compactly arranged, rounded cells

5	Lower epidermis	Uniseriate with barrel shaped cells	Rounded cell or oval shaped and cells are small
6	Stomata	Present both upper and lower surface	Present both upper and lower surface

3.5 Root anatomical characters

Table7: Root anatomical characters

SI. No	Characters	*Lantana camara*	*Stachytarpheta Jamaica*
1	Shape in cross section	Circular in outline	Circular in outline
2	Nature of epidermis	Single layered	Single layered
3	Nature of cortex	Composed of parenchymatous cells, presence of more number of sclerenchymatous cells as seen as ring	Parenchyma cells, less number of sclerenchyma cells seen as ring
4	Vascular bundle	Collateral, open, radial arrangement, medullary ray present.	Collateral, open, radial
5	Pith	Absent	Pith Absent

Table 8: Stomatal index of *Lantana camara* and *Stachytarpheta jamaicensis*

Plants	*Lantana camara*		*Stachytarpheta jamaicensis*	
Leaf Surface	**Adaxial**	**Abaxial**	**Adaxial**	**Abaxial**
No. of stomata	17	11	4	5
No of epidermal cells	36	26	19	21

Table 9: shows stomatal index

SI. NO	Name of the species	Stomatal index	
		Upper	**Lower**
1	*Lanata camara*	Adxial- 32	Abaxial -29
2	*Stachytarpheta jamaicensis*	Adaxial- 21	Abaxial - 19

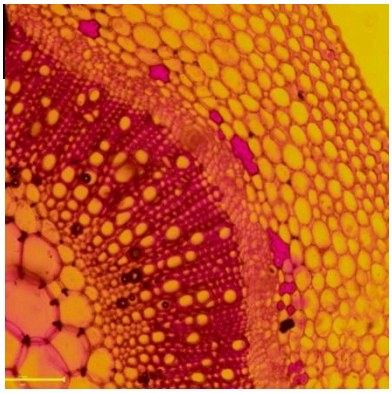
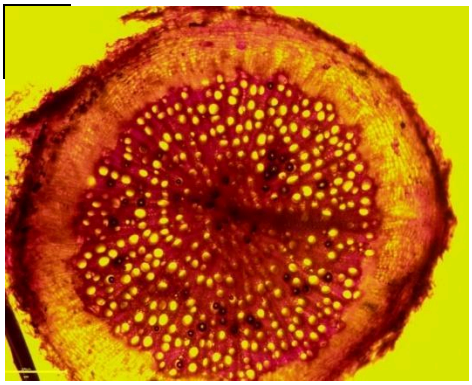
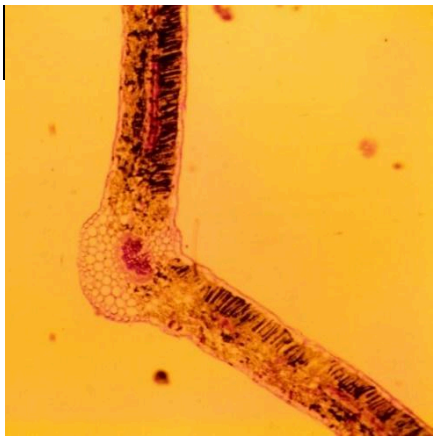
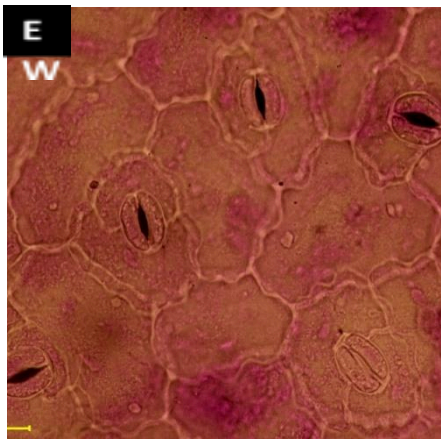
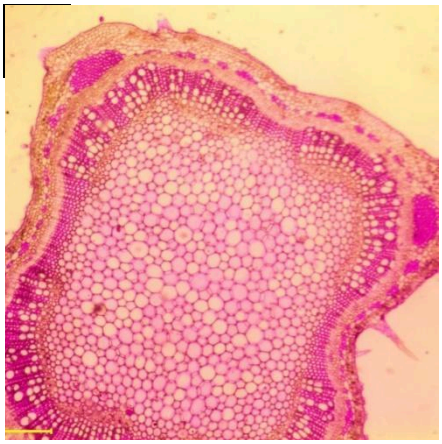
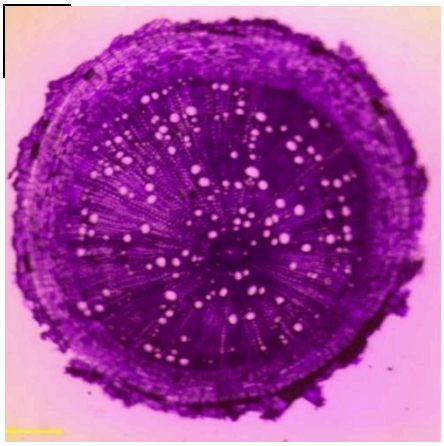






Fig. 2: Microscopic characters of *Stachytarpheta jamaicensis* (L.)Vahl**, A**- Showing habit, **B**-T.S of portion of stem, **C**- T. S of root, **D**- T. S of leaf, **E**-Adaxial surface of leaf

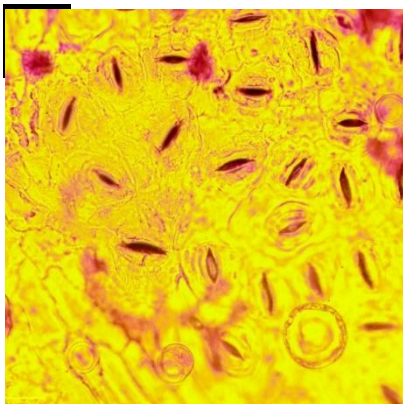


Fig.6: Microscopic characters of *Lantana camara* L., **A -** Showing habit of *Lantana camara*, **B-** T.S of stem entire, **C-**T.S of root entire, **D-** C.S. of leaf entire, **E-** Stomata at adaxial surface

4. Discussion

The characters responsible for the invasiveness in the study understanding these traits may improve the ability to predict, prevent and Mange invasion (Burns, 2006[4]). Characters abundant in *Lantana camara* and traces of some of these characters are noted in *Stachytarpheta jamaicensis*. The notable important character is the presence of sclerenchymatous tissues in the stem and root.

In the transverse section of stem of both plants *Lantana camara* and *Stachytarpheta jamaicensis,* the stem is quadrangular in outline while in *stachytarpheta* it is circular in outline. Presence of trichomes is seen in both plants. Both glandular and elongated unicellular trichomes present in *Lantana camara* glandular trichome with less number found in *Stachytarpheta jamaicensis*. Large number of parenchymatous cells found in both species, which are abundant in *S. jamaicensis.* The notable character sclerenchymatous mass of cells found in cortex of both sections, which is found more and a group of cells in *Lantana camara.*

While looking in to the root both transvestite sections are in circular outline and the epidermis is replaced by periderm, derived from the cork. Sclerenchyma mass of cells is seen as a ring in the cortex in both transverse sections, which is abundant in *Lantana camara.* There is presence of xylem tissues which occupies large area, just below the phloem tissue. Presence of both unicellular and multicellular trichrome are present in the leaf anatomical study of *Lantana camara* while those absent in *Stachytarpheta jamaicensis*.

A difference in stomatal size seen between plants. High stomatal index is seen in *Lantana camara and* less number of stomata which are seen in *Stachytarpheta jamaicensis.*

The characters noted for the invasive species are occurrence of vessel in the pillar of the abundant sclerenchymatous tissue, parenchymatous cells for effective conduction of water and nutrients, short and wide, narrow vessels long but coiled trichrome for light piping, high Stomata size with low stomatal index to reduce excess evaporation. These characters are responsible for their aggressiveness and xerophytic nature studied were made in two invasives plants, (*Chromolaena odorata,Tithonia)* and two non-invasive species (*Agaeratum conyzoides and Aspilia africana*)

Lantana camara contain important metabolites that is glycosides, saponins, tannins, flavonoids, phenolic compounds, quinones, cumarines, reducing sugars, phlobatanins, terpenoides etc. used for infectious disease and medicinal preparations.

A difference in stomatal size seen between invasive and non-invasive Species. In invasive species high stomatal size a corresponding diffusion of carbon dioxide in and water vapour out of the leaf, this implies that the invasive species have higher growth rate than the non-invasive species as a consequence of higher photosynthetic capacity. The Prolific amount of growth and reproduction in invasive plants may be achieved by greater net photosynthetic and/ or resource-use efficiency (McDowell, 2002[5]).

In anatomical study, the cross sections through the internodal area of erect stems showed that the aerenchyma consists of lacunae which decreasing in their sizes toward the endodermis. The central cylinder distinguished by non-definiteness xylem tissues, and large lacuna at the center of the stem (Al-Mandeel, F. A, 2013[6]).

A greenhouse experiment to evaluate the competition effects of *Stipa gigantea* Link (Poaceae), a functionally similar species, and *Lupinus luteus* L. (Fabaceae), a dissimilar one, over the invasive *Cortaderia selloana*. Based on results, suggest that *Cortaderia* is a weak competitor compared to a similar native species, and its advantage must lie in other traits such as its facility to generate large amounts of propagules and its efficiency in early stages of seedling growth. Functionally similar species may be an adequate choice for restoration in order to outcompete invasive plants. (Fagúndez, J. & Lema, M, 2019[7]).

5. Conclusion

From this study observed the morphological, anatomical features of both the Plants are entirely different but both are coming under the same family Verbenaceae. Leaf and stem morphology shows great variations. In *Stachytarpheta jamaicensis* leaf lamina is broader while comparing with *Lantana camara.* It will help the plant to increase the rate of photosynthesis. The characters are abundant in *Lantana camara*. The notable important characters are the presence of sclerenchymatous tissues in the stem and root. Presence of both unicellular and multicellular trichomes are present in the leaf anatomical study of *Lantana camara* while those absent in *Stachytarpheta jamaicensis*. The high Stomata sizes

recorded for the invasive species suggests that the invasive species have higher growth rate that as a consequence of higher photosynthetic capacity

The Characters responsible for invasiveness in the invasive species are the presence of: large stomatal size, low stomatal index, large trichomes, more sclerenchyma Cells, more parenchyma cells, large vessel and prominent medullary rays.

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GC-MS analysis of bioactive compounds in methanol extracts of three traditional rice varieties (*Oryza sativa* L.) in Kerala

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Abstract

Background: *Oryza sativa* L. is a major cereal food crop and staple food in most of the developing countries which act as the rich source of nutrients and medicinal secondary metabolites. **Methods**: Present study revealed the diversity of phytochemical compounds in three traditional rice varieties, Sona Masuri, Thulasi and Supriya, of Kerala. Methanol extracts of seeds prepared using soxhlet apparatus and the compounds analyzed by GC-MS. **Findings**: The extract analysis showed the presence of the highest number of bioactive compounds in Supriya followed by Thulasi and Sona Masuri. Most important compound in Sona Masuri and Thulasi was 9,12-Octadecadienoic acid (z,z)-, while Oxacycloheptadec-8-en-2-one analyzed as the major notable compound in Supriya. These phytochemical compounds are with different biological activities, in turn leading the production of new pharmaceutical medicines in future.

Key words: *Oryza sativa* L., retention time, phytochemical compound, GC-MS analysis, Soxhlet apparatus.

1. Introduction

Rice, *Oryza sativa* L. is a perennial monocot plant in the family Poaceae and originated in India, Thailand and southern China. It is now cultivated on an estimated 3% of the world's agricultural land in wet tropical, semi-tropical and warm temperate areas around the world for the production of cereal grain; hence serves as a primary source of calories for over half the world's population [1]. There are two major ecotypes of *O. sativa,* namely *'indica'* adapted to the tropics, and '*japonica*', adapted to the temperate regions and tropical uplands and they show difference in seed shape. The seed of the ecotype, '*indica'*, is characteristically long and slim whereas *'japonica'* appears short and round [2].

Although there are no scientific reports available on the exact number of traditional varieties found in Kerala, it is documented that nearly 2000 traditional varieties with nutritional or medicinal values are predominantly cultivated [3]. Some of the important aromatic and medicinal rice varieties include Chennellu, Njavara, Gandhakasala, Jeerakasala, Chomala, Kayama, Kothampala etc.; while Thekkan, Chettadi, Kuttadan, Jyothi, Uma, Jaya etc. have high nutritional value [4,5]. All these varieties grow in various agro-climatic conditions of the state. But most of the varieties have disappeared from Kerala due to reduced cultivation and by various unfavourable abiotic conditions. It can be overcome by *ex-situ* and *in-situ* conservation methods, in turn confirming the germplasm conservation of rice diversity.

According to the American Diabetes Association, carbohydrates are the body's main source of energy. Rice is a good source of carbohydrate and each variety contains different quantities. In addition, the cereal provides proteins, dietary fibres, macronutrients, micronutrients etc. [6]. Together with nutrients, some of the traditional rice varieties are rich with different medicinal secondary compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis is commonly used to detect and analyze the presence of the volatile secondary compounds. It combines the features of Gas Chromatography and Mass Spectrometry to identify different substances within the sample even if it is in minor quantity [7]. Previous study reports that Njavara, one of the major nutritional and medicinal rice varieties, included about 109 compounds having different biological activities including anti-microbial, anti-oxidant, anticancerous etc. [8]. Using advanced methods, the detection of medicinally important secondary metabolites in traditional rice varieties of Kerala is very significant in the pharmaceutical industry of the coming years.

The present study focused on three traditional rice varieties of Kerala, Supriya, Sona Masuri and Thulasi. Supriya (PTB 61) is a high yielding rice variety developed at Mannuthi Rice Research Centre, Thrissur, Kerala; which yields six and half to seven tons per hectare. A Premium Aromatic Rice Tulsi Amrit (Thulasi) is the non- Basmati variety having short to medium grain rice with intermediate amylose and gelatinization temperature. Moreover, Sona Masuri is also one of the fine varieties of non-basmati rice with lightweight and low starch content. Both Sona Masuri and Thulasi have less carbohydrate content than other traditional varieties in Kerala; so it plays a significant role in the diet of diabetic patients [9]. The knowledge on secondary metabolites of these three economically important rice varieties is very interesting and will contribute more in the future of the pharmaceutical industry.

2. Materials and methods

2.1. *Collection of plant material*: The fresh seeds of selected rice varieties, Sona Masuri and Thulasi were collected from Chandragiri Modern Rice Mill, Thirurangadi, Malappuram, Kerala, India. Supriya seeds were procured from Regional Agricultural Research Station (RARS) Pattambi, Kerala, India.

2.2. *Preparation of extract*: The seeds were dehusked carefully and powdered well using motor and pestle. About 30 gm of seed powder extracted with 250 ml methanol at a temperature between 60 and 65 ^{0}C using Soxhlet apparatus. The extract was concentrated by a rotary vacuum evaporator to obtain viscous semi-solid mass which was subjected to GC-MS.

2.3. *GC-MS analysis*: The detection of phytochemical compounds in seed extracts carried out using the GC-MS instrument (Shimadzu, Model Number - QP2010S). The Rxi-5Sil MS column showed 30 meter length, 0.25mm ID and 0.25µm thickness. In the gas chromatography part, the temperature programme, oven temperature, was 70°C raised to 260°C at 6°C/min and injection volume was 1 µl. The samples dissolved in methanol were run fully at a range of 50-650 m/z and the results were compared using NIST 11 & WILEY8 Spectral Library Programme.

3. Results

Different types of volatile secondary metabolites were detected in methanolic extracts of Sona Masuri, Thulasi and Supriya in which Supriya showed the highest number of phytochemical compounds followed by Thulasi and Sona Masuri. Most of the analyzed compounds have different biological activities including antibacterial, anticancerous, antiinflammatory, antioxidant, antifibrotic properties etc. In GC-MS analysis, 9, 12-Octadecadienoic acid (z,z)- showed the highest area % of 61.55 and 74.66 in Sona Masuri and Thulasi respectively (Table 1 & 2). The phytochemical compounds in Supriya had lower area % compared to other varieties; among the compounds, Oxacycloheptadec-8-en-2-one had the highest value, 18.82 (Table 3). In case of RT, it was lowest for dodecanoic acid (20.56) in Supriya; while methylpalmitate (28.40) showed lowest RT in Sona Masuri and Thulasi (Table 1, 2 & 3). The study revealed that Supriya is highly medicinal compared to other varieties due to the presence of diverse secondary metabolites (Figure 1, 2 & 3).

Table 1. Phytochemical compounds detected in methanol extract of dehusked Sona Masuri.

RT	Area %	Phytochemical compounds	Bioactivity
28.4	2.56	Methylpalmitate	Anti-inflammatory & antifibrotic [25]
31.6	4.96	9,12-Octadecadienoic acid, methyl ester	Anticancerous [21]
31.7	4.94	10-Octadecenoic acid, methyl ester	Antimicrobial [22]
33.4	61.55	9,12-Octadecadienoic acid (z,z)-	Anti-inflammatory & cancer preventive [14]
35.1	0.67	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	-
38.9	6.29	Dicapryl phthalate	Plasticizer, dye carrier, adhesive, hepatotoxin & mucous membrane irritant [24]
39.3	6.50	Oxalic acid, hexyl pentadecyl ester	-
39.8	4.47	Oxalic acid, 3,5-difluorophenyl nonyl ester	-
40.1	6.72	Oxalic acid, pentadecyl propyl ester	-
40.8	1.43	Adipic acid, decyl ester	-

Table 2. Analyzed phytochemical compounds in methanol extract of dehusked Thulasi.

RT	Area %	Phytochemical compound	Bioactivity
28.4	2.23	Methylpalmitate	Anti-inflammatory & antifibrotic [25]
31.6	4.99	Methyl octadeca-9,12-dienoate	Antitumor [26]
31.7	5.44	Elaidinsaeure methyl ester	-
32.2	0.29	Methyl isostearate	-
32.9	1.62	Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant & haemolytic [26]
33.5	74.66	9,12-Octadecadienoic acid (z,z)-	Anti-inflammatory & cancer preventive [14]
34.0	1.75	Solasonine	-
38.9	3.01	Dicapryl phthalate	Plasticizer, dye carrier, adhesive, hepatotoxin & mucous membrane irritant [24]
39.3	1.42	Oxalic acid, hexyl pentadecyl ester	-
39.4	0.38	1-heptanol, 2-propyl-	-
42.8	3.69	9-Octadecenamide	-
43.1	0.82	Squalene	Anti-inflammatory, anti-atherosclerotic, skin aging & adjuvant activities [26]

Table 3. Phytochemical compounds present in methanol extract of dehusked Supriya.

RT	Area %	Phytochemical compound	Bioactivity
20.56	05.82	Dodecanoic acid	Antimicrobial, antifungal, antiinflammatory & anticancerous [23]

23.97	00.37	Tetradecanoic acid, methyl ester	Antibacterial & antifungal [27]
24.94	03.25	Tetradecanoic acid	Antimicrobial, antioxidant, antiinflammatory, anticancerous, hypercholesterolemic, larvicidal & repellent [28, 29, 30]
28.16	04.51	Hexadecanoic acid, methyl ester	Antimicrobial, antipasmodic, antiinflammatory, antioxidant, hypocholesteromic, antiandrogenic & hemolytic 5-Alpha reductase inhibitor [30, 31]
28.71	00.42	Dibutyl phthalate	Antibacterial, antibacterial & antifungal [32, 33]
29.22	13.03	Hexadecanoic acid	Antimicrobial & anticancerous [28, 34]
31.36	07.69	9,12-octadecadienoic acid, methyl ester	Antimicrobial [35]
31.48	06.96	Elaidinsaeure methylester	Antiinflammatory, antiarthretic, anticarcinogenic & hepato protective [34]
32.52	18.82	Oxacycloheptadec-8-en-2-one	Antimicrobial & antiasthmatic [17]
32.62	13.67	Cis-vaccenic acid	Antimicrobial [36]
34.76	01.37	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	Antibacterial [37]
38.48	11.63	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antioxidant, hypocholesterolemic, nematicide, anti-androgenic & hemolytic 5-alpha reductase inhibitor [38]
41.13	06.70	9,12-octadecadienoic acid(z,z)-, 2-hydroxymethyl) ethyl ester	Antiasthmatic [17]
41.19	05.78	Oleoyl chloride	Antibacterial, antimicrobial, antifungal & endotoxin neutralizing activity [39, 40, 41, 42]

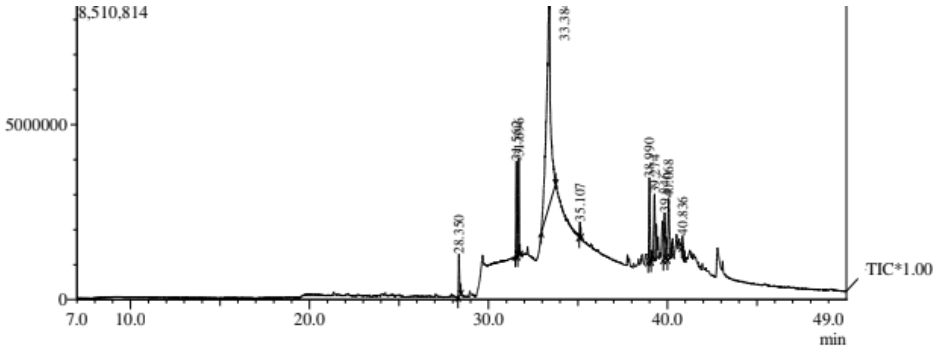

Figure 1. Chromatogram of phytochemical compounds present in hexane extract of seeds of *O. sativa* var. Sona Masuri.

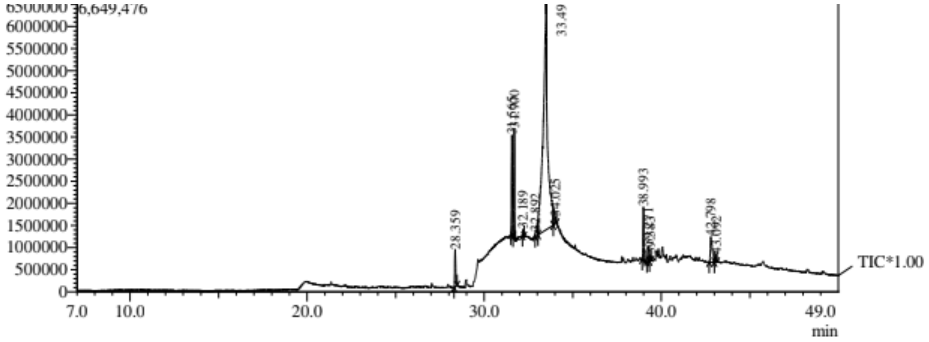

Figure 2. Chromatogram of phytochemical compounds present in hexane extract of seeds of *O. sativa* var. Thulasi.

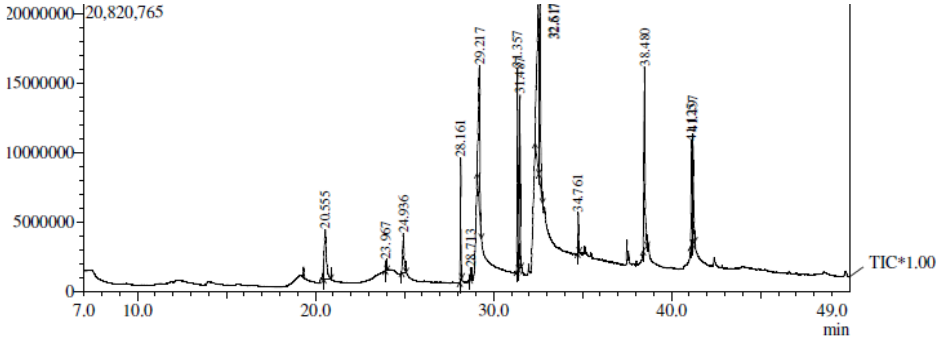

Figure 3. Chromatogram of phytochemical compounds present in hexane extract of seeds of *O. sativa* var. Supriya.

4. **Discussion**

In Kerala, many traditional rice varieties show medicinal values due to the presence of significant phytochemical compounds. GC-MS is an integrative technique for separation, identification and quantification of volatile compounds in plant extracts [10]. Quantification is an important step for data analysis and different softwares is being used for the calculation of retention time corresponding to specific peaks [11]. For the analysis of plant volatile compounds, the different solvents including ethanol, hexane, methanol, ethyl acetate etc. are commonly used [12]. GC-MS analysis of hexane extract of dehusked Njavara revealed the presence of 109 phytochemical compounds [8]. Similarly, Ashokkumar et al. detected volatile compounds including fatty acids, terpenes, alkanes, alkenes, alcohols, phenols, esters and amides in South Indian traditional rice varieties including Kichili samba, Seeraga samba, Kaiviral samba, Mappilai samba, Karuppu kavuni, Kattuyanam and Kuzhiyadichan [13].

In the present investigation, the methanol extracts of dehusked seeds of Supriya, Sona Masuri and Thulasi were used for analysis and detected 14, 12 and 10 compounds in Supriya, Thulasi and Sona

Masuri respectively. Many of above compounds are pharmaceutically important and their medicinal efficacies have been reported by many researchers including antioxidant, anticancerous, antibacterial, antiinflammatory and antiviral activities that will improve the drug designing in pharmaceutical industries (Table 1, 2 & 3); whereas some compounds show the industrial applications also. The compound, 9,12-Octadecadienoic acid (z,z)-, showed highest area percentage in Sona Masuri and Thulasi which has antiinflammatory and cancer preventive potential [14]; therefore, the rice varieties are useful to produce anticancerous medicines. The same compound is also reported from different plants like *Solena amplexicaulis*, *Adenophorae radix*, *Albizia adianthifolia, Pterocarpus angolensis* etc. [14,15,16]. The area percentage of Oxacycloheptadec-8-en-2-one is higher in Supriya compared to other compounds. The compound in the rice variety is with better potential for antimicrobial and antiasthmatic activities and can be used in pharmaceutical industry [17]. Similarly, Oxacycloheptadec-8-en-2-one is detected from hexane and dichloromethane (DCM) extracts of *Ageratum conyzoides* and essential oils of *Trollius europaeus* and *Arisaema amuremse* that makes the plant highly medicinal [18,19,20].

5. Conclusion

The rice varieties Supriya, Sona Masuri and Thulasi are the sources of different bioactive compounds that justify the pharmaceutical usage of the varieties. GC-MS analysis shows the presence of 14, 12 and 10 phytochemical compounds with different biological activities in methanol extract of Supriya, Sona Masuri and Thulasi respectively. In Sona Masuri and Thulasi, 9, 12-Octadecadienoic acid (z,z)- is the major compound; while Supriya consisted of Oxacycloheptadec-8-en-2-one as the significant phytoconstituent. Further investigations in this varieties will lead to the development of medicines in future.

6. Acknowledgement

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Toxic effects of cadmium on growth parameters of *Vigna unguiculata* (L.) Walp.

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Abstract

Background: This study was designed to examine the effect of cadmium(Cd) stress in cow pea plants by analysing various growth parameters. **Methods**: The seedlings of *Vigna unguiculata* (L.) Walp. were subjected to the treatment of different levels of cadmium chloride (CdCl2) solution (25, 50, 75,100 and 125mM) along with an untreated control. The expression of Cd stress in diverse morphological parameters such as root length, shoot length, number of leaves, leaf area, fresh weight, dry weight and tolerance index percentage was evaluated. **Findings**: The reduction in plant growth of *V. unguiculata* seedlings exposed to Cd treatment was recorded. All the evaluated plant growth characteristics are adversely influenced by the application of Cd. The high concentrations severely affect the plants resulting in ultimate death of plants. The study helped to fix the concentration of 50 mM of Cd which can cause an average range of heavy metal toxicity and that can be reduced using appropriate control measures.

Keywords: Cadmium, Growth parameters, Heavy metal stress.

1. Introduction

Plants have to cope with a variety of abiotic stresses during their life. Abiotic stresses include salinity, drought, high or low temperature, pH, and heavy metal toxicities. The effects of metal and metalloid toxicity are increasing worldwide, mainly due to anthropogenic causes. Metals and metalloids are a natural part of our planet. But, the soil contamination due to their high concentration can result in toxic effects to many of its life forms[1]. A heavy metal is defined as any element exhibiting high density and that exerts its harmful effects even when available in trace amounts. The present agricultural practice with extreme use of agrochemicals like pesticide and fertilizers result in contamination of agricultural soils [2]. Although some metals are essential micronutrients responsible for many regular

processes in plants, their excess level can cause harmful effects by directly influencing the growth, senescence and energy synthesis processes [3].

Among heavy metals, cadmium(Cd) is a non-essential and toxic metal,rapidly taken up by roots and accumulated in various plant tissues [4]. Cd stress in plants causes many negative impacts in plant cells and produces reactive oxygen species which induces oxidative stress and ultimately effects various growth parametrs in plants [5,6]. Cow pea is a nutrient rich vegetable and it has a vital role in human diet. Depending on its importance, it is essential to investigate is responses under heavy metal induced oxidative stress. Henceforth, this study was done in the purpose of evaluating heavy metal toxicity of *V. unguiculata* under various concentrations of Cd. This study was designed to evaluate the effect of various concentrations of Cd on growth parameters of *V. unguiculata* seedlings. This study will be helpful to find out the Cd tolerability levels of *V. unguiculata* and appropriate control measures for alleviating the toxic effects.

2. Materials and methods

2.1 Plant material and experimental setup

Vigna unguiculata (L.) Walp. also known as black-eyed pea is one of the important leguminous vegetable. Seeds of Anaswara variety were collected from Regional Agricultural Research Station (RARS) of Kerala Agricultural University, Pattambi, Kerala, India. The experiment was carried out at Botanical garden, KAHM Unity Women's College, Manjeri, Malappuram, Kerala from January-May under natural conditions. Malappuram district located at 75 ^{0}E - 77 ^{0}E longitude and 10 ^{0}N - 12 ^{0}N latitude. The pot experiment was conducted under the green house conditions for 5 months. Cow pea was selected as experimental crop. Before sowing, the seeds were thoroughly washed with water and 6-8 seeds were shown in plastic grow bags containing equal amount of soil, weighing about 1 kg. Each bag was filled with potting mixture (soil + cow dung and coir pith in 1:1:1 ratio).

2.2 Cadmium treatment

After germination, the seedlings were thinned and those with best growth performance were retained (3 plants per pot). Irrigation was done regularly using tap water. On 10th day of germination, the soil was treated with cadmium chloride ($CdCl_2$) solution (200 ml/kg soil) of different concentrations namely 25, 50, 75, 100 and 125 mM along with an untreated control. The experiment was conducted in triplicates. On the second day, toxic limits of cadmium were found in plants treated with 75, 100 and

125 mM/kg. In the same day, all treated plants including control were separated out from the pots for analysis.

2.3 Determination of growth parameters

The shoot length was measured with the help of scale in cm in the day fixed (2nd day). The shoot length was measured from the point where the root and shoot joints to top of the shoot. The root length was measured from the point where the root and shoot joints to the end of the root. Number of leaves per plant was counted for each concentration including control. Total number of leaves for each concentration was added. Each leaf from the plant excluding the petiole was cut and placed on the graph paper to draw the leaf shape. The leaf area was found by counting the all squares in each leaf diagram for each concentration.

After harvesting the seedlings, the fresh weight was recorded using electronic weighing balance and samples were dried in an oven at 60^0C for 24 hours to record dry weight and tolerance index percentage was calculated.

$$TI = \frac{observed\ value\ of\ root\ length\ in\ solution\ with\ metal}{observed\ value\ of\ root\ length\ in\ solution\ without\ metal}$$

$$TI = \frac{observed\ value\ of\ root\ length\ in\ solution\ with\ metal}{observed\ value\ of\ root\ length\ in\ solution\ without\ metal} \text{ X } 100$$

3. Results

3.1 Shoot length

Shoot length of Cd (25, 50, 75, 100 and 125 mM) treated cow pea plants were significantly decreased by Cd treatment compared with the untreated plants. Compared to control plants, maximum decrease of shoot length was clearly visible in the plants treated with 125 mM (21%) as compared to other treatments of Cd. However, the percentage of decrease in shoot length of plants treated with 25 mM was less (4%) than other stress treatments. Moreover, shoot length of the plants subjected to 50, 75 and 100 mM cadmium stress also decreased as compared to control cow pea plants (6, 10 and 15% respectively) (Table 1; Plate 1).

3.2 Root length

The root length was also decreased with increasing Cd concentration in cow pea plants and maximum decrease (42%) was observed when subjected to 125 mM $CdCl_2$ as compared to control

plants. Likewise, the declines in root length were found by 10, 20 and 30%, respectively in cow pea plants subjected to 50, 75 and 100 mM Cd stress. However, there was no significant variation in cow pea plants treated with 25 mM $CdCl_2$ as compared to untreated plants (Table 1; Plate 1).

3.3 Number of leaves per plants

Number of leaves per plant was counted to analyze the morphological variations of different Cd treatments in cow pea seedlings. In the case of cow pea plants subjected to 100 and 125 mM Cd, the number of leaves per plant was highly reduced (45 and 55% in 100 and 125 mM Cd concentrations, respectively) as compared to control plants. Likewise, 18% reduction was noticed on the number of leaves per cow pea plant after subjected to 25 and 50 mM $CdCl_2$. Cow pea plants treated with 75 mM Cd showed 27% reduction in the number of leaves per plant (Table 1; Plate 1).

3.4 Leaf area

With increase in the Cd stress concentration (25 - 125 mM) in the soil induced a decline in the leaf area of cow pea plants and the deleterious effect of Cd became more severe with increasing Cd level. The reduction in leaf area was less in cow pea plants subjected to 25 and 50 mM Cd stress (11 and 19% respectively) as compared to control plants and it was maximum in plants subjected to 100 and 125 mM Cd (40 and 53% respectively) as compared to control plants. Moreover, cow pea plants treated with 75 mM Cd showed 28% reduction in leaf area as compared to control plants (Table 1; Plate 1).

3.5 Fresh weight

Cow pea plants subjected to 25 mM Cd stress showed only negligible reduction in fresh weight as compared to control plants. Likewise, fresh weight of cow pea plants subjected to 75, 100 and 125 mM cadmium was highly decreased (15, 18 and 21%, respectively) as compared to untreated plants. About 8% reduction in fresh weight of cow pea plants were noticed upon exposure to 50 mM cadmium stress (Table 1).

3.6 Dry weight

Dry weight of Cd stressed cow pea plants (25, 50, 75, 100 and 125 mM) were decreased by Cd treatments compared with the control plants. Compared to control plants, maximum decrease of dry weight was recorded in the plants treated with 75, 100 and 125 mM (50-55%) as compared to other treatments of Cd. Whereas, 50 mM Cd induced reduction in dry weight was less (20%) as compared to untreated cow pea plants. Moreover, 35% reduction in dry weight was recorded when subjected to 50 mM heavy metal stress in cow pea plants (Table 1).

3.7 Tolerance index (TI)

There were significant differences in tolerance index (TI) in cow pea plants exposed to different Cd concentrations. Compared with the control, the TI changed little when cow pea plants were treated with 25 mM Cd, while TI was decreased obviously after 50, 75, 100 and 125 mM Cd treatments (10, 20, 30 and 41% respectively) as compared to untreated cow pea plants (Table 1).

Table 1: Shoot length (cm), root length (cm), number of leaves, leaf area (cm^2), fresh weight (g), dry weight (g) and tolerance index (%) of cow pea plants subjected to different concentrations of $CdCl_2$ (0, 25, 50, 75, 100 and 125 mM).

Treatments	*Shoot length (cm)*	*Root length (cm)*	*Number of leaves*	*Leaf area (cm^2)*	*Fresh weight (g)*	*Dry weight (g)*	*Tolerance index (%)*
Control	*30.30*	*9.96*	*11*	*33.20*	*16.70*	*1.76*	*100*
25 mM	*28.83*	*9.76*	*9*	*29.50*	*16.25*	*1.15*	*97.99*
50 mM	*28.40*	*8.96*	*9*	*26.60*	*15.31*	*1.41*	*89.97*
75 mM	*27.43*	*7.93*	*8*	*23.80*	*14.21*	*0.90*	*79.54*
100 mM	*25.83*	*6.93*	*6*	*19.80*	*13.58*	*0.83*	*69.50*
125 mM	*23.73*	*5.80*	*5*	*15.40*	*13.21*	*0.79*	*58.17*

Plate 1: Effects of different concentrations of $CdCl_2$ (0, 25, 50, 75, 100 and 125 mM) in cow pea seedlings.

4. **Discussion**

The results showed that the plant growth characteristics were adversely influenced by application of Cd in 10 days old cow pea seedlings. Leaf morphological characteristics are among those traits that are very sensitive stress condition particularly to heavy metals toxicity is known to reduce leaf chlorophyll index, leaf area expansion, greenness, and many other leaf metabolic processes [7]. Higher

levels of Cd reduced leaf area in cow pea plants probably due to restriction of cell division and cell expansion. The reduction in major nutrient element such as potassium and nitrate could also result in reduced leaf area due to heavy metal application [8]. Moreover, sensitivity of various enzymes in chlorophyll biosynthesis process is probably involved in reduction of reduced leaf greenness under heavy metal toxicity [7].

In the present study, the application of heavy metals has been shown to reduce many plant growth parameters including shoot length, root length, leaf area, number of leaves, fresh weight, dry weight and tolerance index when compared to control plants. Cadmium is the major heavy metal with toxic effect on many biological systems. The Cd toxicity can have adverse effects on membranes function, enzyme activity that generally resulting harmful oxidative stress [9]. Even a single application or limited amounts of Cdm can cause considerable growth reduction. Plants can resist the heavy metal stress through the synthesis of various enzymatic antioxidants, non-enzymatic antioxidants, osmolytes and chelating agents. Since Cd can accumulate in plants and enter human body through food chain, causing persistent poisoning and endangering human health, it is of essential to find out appropriate control measures for alleviating these toxic effects. In this study, by the treatment of different levels of $CdCl_2$, an optimum concentration of cadmium which can cause an average range of heavy metal toxicity before killing the plant is observed and that toxicity can be mitigated through appropriate control measures.

5. Conclusion

By evaluating these parameters in 10 days old seedlings, It was observed that cadmium stress severely affect the plant growth and thus affects the biomass which can lead to plant death. The higher concentrations of cadmium lead to significant alterations in the normal plant growth thereby causing chlorosis, leaf rolls, necrosis and stunting as visible symptoms. The seedling treated with 125 mM showed extreme leaf curling, chlorosis, wilting and overall deformities of the plant and ultimately the death of plant. It helped to fix concentration of 50 mM of cadmium which can cause an average range of heavy metal toxicity and that can be reduced using appropriate control measures.

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7. References

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Pollination Biology of *Merremia vitifolia* Hallier f. (Convolvulaceae)

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Abstract

Background: The purpose of present investigation is to unravel the mechanism of pollination in the plant *Merremia vitifolia* ,commonly known as Grape Glory which is common in our area. **Methods:** Flowering phenology and floer morphology was studied by simple observation. Pollen production was calculated by Cruden method. Pollen histochemistry was studied by using IKI solution, drop of Sudan Black B and Coomassie Brilliant Blue solution. Pollen-ovule ratio was also calculated. Pollen morphology studied accurately by acetolysis. Pollen fertility was studied by acetocarmine-glycerin technique and pollen viability was assessed by Tetrazolium. *In-vivo*ans*in-vitro* pollen germination also studied.Biochemical analysis of stigma also studied by IKI solution, Sudan black and Coomassie Brilliant Blue solution. Role of wind in pollination was studied by hanging Vaseline coated slides around the flower. Pollinators and their behaviour were observed through observation from the time of anthesis to flower closing. Breeding system and seed germination rate also studied. **Findings:**Flowers are produced in terminal cymose inflorescence and flower takes 10-12 days from initiation to bloom. Pollengrains are roughly spherical in equatorial view. Pollen cotain protein as it intensely stained with Coomassie Brilliant Blue and is estimated 240mg per ml in 0.03g anther sample. Pollen viability was peak at 1 pm. Stigma stained with IKI, Sudan Black and Coomassie Brilliant Blue which indicates the presence of starch,lipid and protein. Stigma is receptive at the time of anthesis. Wind has no role in pollination. Cross pollination lead to fruit set and seed set.

Keywords: *Merremia vitifolia,* Breeding behaviour, Pollination biology, Viability, Histochemistry, Flowering phenology.

1.Introduction:

Flowers have served as an excellent offering to God, an invaluable aid for personal make up, and a source of inspiration to poets. For plants it is the organ of sexual reproduction. Flowers exhibit a great variation in size, color, shape and insertion of floral whorls. Floral diversification is the outstanding characteristic of angiosperm radiation. In a broad sense, abundant evidencesnow confirms that pollinators play a major role in this diversification[1].

Convolvulaceae, commonly known as the Bindweed or Morning glory family, is a family of about 60 genera and more than 1650 species of mostly herbaceous vines, but also trees, shrubs, and herbs, and also including the sweet potato and some other tubers. The Convolvulaceae have a cosmopolitan distribution but 90% of the species found in the tropics. The genus *Convolvulus* is present around the Mediterranean basin. *Calystegia* is very diverse on the West Coast USA with some species growing in Europe. *Ipomoea* and *Merremia* are most diverse in Tropical America but they are also common in Africa and Asia.*Merremia* is a genus of Convolvulaceae. *Merremia vitifolia* Hallier f. in Engl. is a climber or twining shrub which is global in distribution. They are commonly known as Grape Glory or Wood roses. They mainly include climbing or creeping shrub with cord like stem rooting at the node. The plant generally found in river banks, roadsides, grass lands, degraded forests and also in the plain. The flower is medium sized, lemon yellow and found in clusters. The floral traits suggests exclusively insect mediated pollination syndrome (entomophily). Little is known about the phenology, floral biology, anthesis, stigma receptivity and pollination biology of this particular species. Present study has been undertaken to study the pollination biology of *Merremia vitifolia.*

2.Materials and methods:

Present study was undertaken on *Merremia vitifolia* plant growing at back side of the campus, KAHM UWC Manjeri.

2.1 Flowering phenology: The initiation, peak and end of flowering was recorded properly. Time of anthesis, anther dehiscence, pollen viablity, stigma receptivity was recorded. Anthesis and anther

dehiscence observed in field using hand lens by the method of Reddi and JanakiBai(1981)[2], Mathur and Mohan Ram (1986)[3] and Ramasubbu et al., (2009) [4].

2.2 Pollen production per anther/ flower: pollen production was calculated by the method described by Cruden(1977)[5]. Pollen morphology was identified by acetolysis method described by Erdtman[6].

2.3 Pollen histochemistry: presence of starch, lipid and protein were diagnosed with IKI solution, Sudan Black and Coomassie Brilliant Blue. Protein estimated by using Folin-Ciocalteau reagent to the method of Lowry *etal.,* (1951)[7].

2.4 Pollen viability: Pollen viability was checked by using 0.2% Tetrazolium solution.

2.5 Stigma receptivity:stigma receptivity was studied by cytochemical localization of esterases on surface of stigma by the method described by Shivanna and Rangaswami (1992)[8].

2.6 Stigma histochemistry: The presence of starch, lipid, and protein was checked by using IKI solution, Sudan Black and Coomassie Brilliant Blue.

2.7Pollinators and behaviour: Pollinators, non-pollinating visitors were observed continuously. Their number, visiting time, foraging nature was recorded by using stop watch.

3. Results:

Merremia vitifolia Hallier f. is a climber or twining shrub which is global in distribution. In India it is found in Assam, Andaman and Nicobar Islands, Gujarath, Meghalaya, Odisha, Maharashtra, Karnataka, Kerala and all districts of Tamil Nadu. The plant generally found in river banks, roadsides, grass lands, degraded forests and also in the plain. The flower is medium sized, lemon yellow and found in clusters. The floral traits suggests exclusively insect mediated pollination syndrome (entomophily).

3.1 TAXONOMIC TREATMENT OF *M. vitifolia*

M. vitifolia is a perennial climber. Stem brownish black or purplish, hispid, terete. Leaves simple, alternate, 5-18 × 4-15cm long, base cordate, palmately 5-lobed, lobes broadly triangular or ovate-lanceolate, sparsely fulvous hairy on both sides, basally 7- ribbed, Margin coarsely serrate or sub-entire, apex acuminate to obtuse. Inflorescence 1-3 or several flowered, peduncle 2-5cm long, bracts subulate, pedicel 1-1.6cm long, thicker distally. Sepals oblong or ovate-oblong, 1.4-1.8cm long, enlarged

in fruit, leathery, shiny, pitted adaxially, pellucid glandular, apex obtuse or acute, 1.7×0.9 cm long. Corolla lemon yellow, petals fused forming infundibuliformis corolla, 3.5-5cm long. Stamens 5, differently sized (3 long and 2 short), epipetalous. Anthers creamy white, 5mm long, filament white, slender, terete, basifixed, dithecous, extrose, split longituidinally. Ovary superior, tetracarpellarytetralocular, syncarpous, placentation axile, ovules 4. Style terete, white, 1.7cm long, glabrous, narrowing towards tip.Stigma bicapitate, wet type, 1mm long and 3mm wide, creamy white. Fruits dry dehiscent capsule, straw colored, 1-1.2cm across, globose. Seeds black brown, trigonous-ovoid, glabrous, 7mm long.

3.2 Flowering phenology

Under normal climatic conditions of the Unity campus *M. Vitifolia* started flowering from second week of December 2018 and reached its peak during January. The flowers are produced in terminal cymoseinflorascence. Approximately 6-10 flowers are produced in each inflorescence, but maximum three flowers opens at a time.

Flowering declined towards the end of February. The flower takes 10-12 days from initiation to full bloom. Flower opening starts from 8.20 am in the morning and lasts up to 9 am. The life span of individual flowers is 7-9 hours. Anther dehiscence commenced after 30 minutes to 1 hour of anthesis. On the second day of flower closing the corolla tube along with stamens will fall off and the calyx cup along with gynoecium persist for about a week and gradually develops to fruit. Fruit development takes approximately 10-12 days.

3.3 Flower morphology

The flowers are born in axillary cymes with divaricating branches. Each inflorescence with 2-3 flowers and 5-8 buds of different stages. Flowers are pedicellate, lemon yellow, 5.8cm long and 4.7cm wide. The flowers are infundibuliformis, actinomorphic, bisexual, and without having any particular odour. The stamens are epipetalous, creamy white and free. There are 3 long (1.4cm long) and 2 short (5mm long). The filaments slightly bend inward. The anthers are 5 mm long, basifixed, dithecous and extrose. The ovary is superior and tetralocular, syncarpous, containing single ovule in each carpel on axile placentation. The style tipped with creamy white bicapitate stigma, 1mm long and 3 mm wide when fully grown.

3.4 POLLEN GRAINS

Pollen grains are roughly spherical in equatorial view. Pollen grains are non-porate and hexacolpate type (SuraponSaensouk and PiyapornSaensouk, 2018)[9]. Rarely pentacolpatepollen grains also observed. The average diameter of pollen grains was 120 µm. The total pollen production in a flower is 3968. The pollen ovule ratio is 992: 1.

3.4.1 Pollen histochemistry

Pollen grains not stained with IKI solution indicating that it does not contain carbohydrate. Pollen grains stained intensely with Coomassie Brilliant Blue indicating the presence of protein. Pollen grains intensely stained with Sudan Black B indicating the presence of lipids.

3.4.2 Estimation of total protein content of anthers

0.03 g anther sample contain 240mg protein per ml.

3.4.3 Pollen fertility

A small proportion of pollen grains were found to be fertile during the first hours of anthesis as tested through acetocarmine-glycerine technique. From the third hour of anthesis the pollen fertility was found to be higher. After the fourth hour 100% pollen were found to be fertile. After that pollen viability found to decrease.

3.4.4 Pollen viability

The pollen viability at the time of flower opening was too low. It was found to be increasing during each hour and get maximum during the peak sunny time, 1pm. Then it gets gradually decreased in successive hours.

3.4.5 Effect of organic and inorganic nutrients in *in-vitro* pollen germination

An *in-vitro* pollen germination study showed that the pollen of *Merremia vitifolia* is special and was not giving pollen tube in the given media.

3.4.6 *In-vivo* pollen germination

The test using cotton blue and Lactophenol does not show any pollen germination under microscope.

3.5 PISTIL

The pistil is differentiated into ovary, style and stigma. The ovary is superior and is divided into four equal halves by the formation of septum. It is tetracarpellary, tetralocular and syncarpous. The ovules are arranged on axile placenta. Ovules 4. Style is creamy white, glabrous and cylindrical. Stigma is bicapitate and creamy white with many ridges and furrows.

3.5.1 Stigma receptivity

The stigma treated with Hydrogen peroxide instantly releases oxygen bubbles, showing that the stigma is highly receptive at the time of anthesis.

3.5.2 Biochemical analysis of stigma

Stigma stained with IKI solution indicates the presence of starch in stigma. Stigma stained with Coomassie Brilliant Blue R indicates the presence of protein in the stigma. Stigma stained with Sudan Black B indicates the presence of lipid in stigma.

3.6 Pollination biology

3.6.1 Role of wind in pollination

The possibility of wind pollination was studied by hanging Vaseline coated slides at various heights around the flower. Microscopic examinations of the slides indicate that pollination of *M. vitifolia*does not take place through wind (no anemophily).

3.6.2 Pollinators and their behavior

The study on *Ipomoea habeliana* found that ants are most frequent floral visitors[10].foraging patterns of floral visitors affect the relationship between physical distance and genetic variability[11]. Flowers of *Merremia vitifolia* were regularly visiter by insects. *Halictus* bees were found to be prolific forager during the first hours of anthesis. They are the most frequent pollinators. Other frequent visitors include carpenter bee (*Xylocopa sp*.), several species of ants like Monomorium, black carpenter beetle, and winged ants. The flower is not visited by any butterflies and moths.

Non-pollinating visitors includes white fly(*Hemiptera*), crickets(*Orthoptera*), spiders(LynX spider), leaf hoppers, white flies, grass hoppers and some species of ants.

Breeding behavior

M. vitifolia does not give successful fruit setting on self pollination. Fruit setting is observed in flowers which are cross pollinated from other flowers of the same plant and also by the pollen from other flower of other plant.

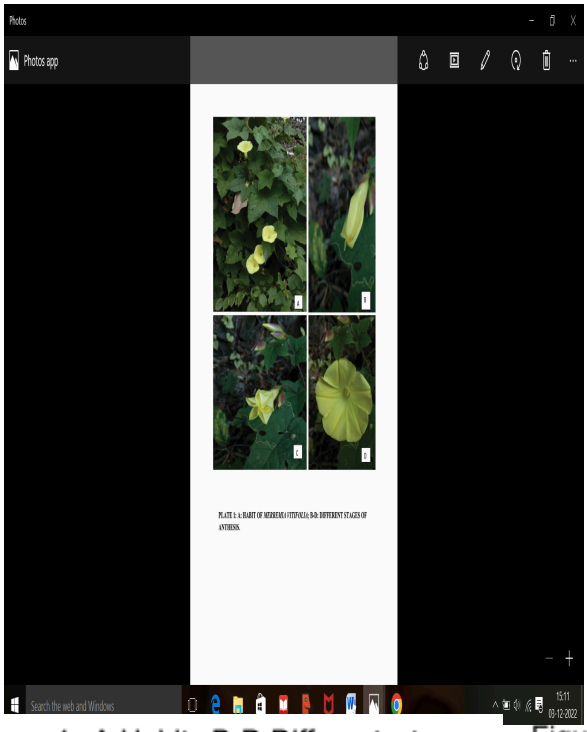

Figure.1: A:Habit; B-D:Different stages of anthesis.

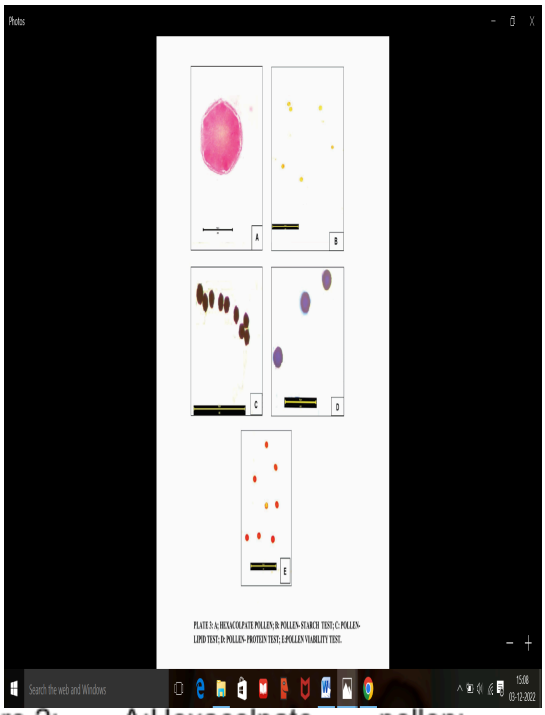

Figure.2: A:Hexacolpate pollen; B:Pollen starch test; C:Pollen lipid test; D:Pollen protein test; E:Pollen viability test.

Figure.3: A:Stigma receptivity; B:Stigma protein test; C:Stigma lipid test; D:Stigma starch test.

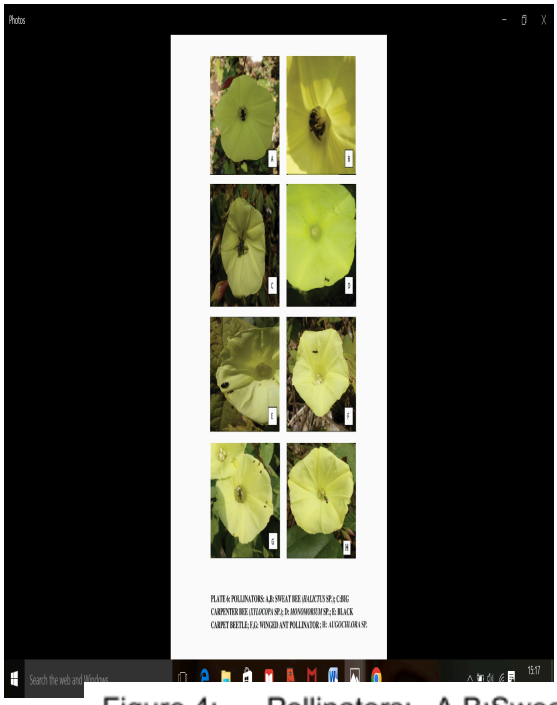

Figure.4: Pollinators; A,B:Sweat bee (*Halictus* sp.); C:Big carpenter bee (*Xylocopa* sp.); D:*Monomorium* sp.; E:Black carpet beetle; F,G:Winged ant pollinator; H:*Augochlora* sp.

Figure.5:Non-pollinating visitors; A:Cricket (*Orthoptera* sp.); B:White fly (*Hemiptera*sp.); C:Lynx spider; D:Leaf hopper(*Hemiptera*); E:Nymph of a katydid (*Orthoptera*); F:Short horned grass hopper (*Orthoptera*).

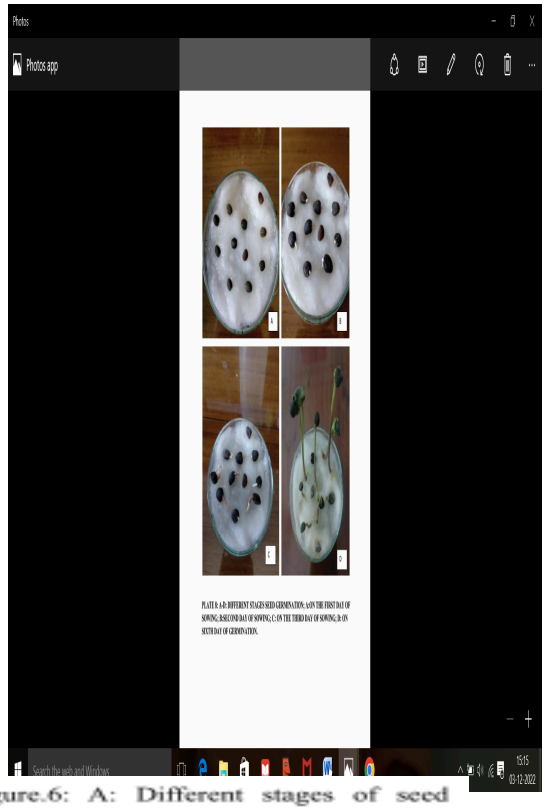

Figure.6: A: Different stages of seed germination; A:first day of sowing; B:Second day of sowing; C:Third day of sowing; D:Sixth day of germination.

3.7 Seed germination

The seeds of *M. vitifolia*are extremely hard with mechanically resistant coats that protect embryo from damage. The seeds sown in petridishes with moist cotton do not gave germination. Seeds that are scarified with conc. H_2SO_4 for 30 minutes also do not germinated. Seeds that are soaked in conc. H_2SO_4 for overnight also resulted the same. Then the seed coat at the hilum part is removed mechanically by using sterilized razor. The seeds treated in this way gave germination just next day of sowing in moist cotton. Seeds of this type also sowed in the soil. The seeds in the soil also gave germination, but not as much growth in the seeds of moist cotton. They show repressed growth. Seeds of moist cotton produced leaves after seven days of germination. The germination rate is 80%.

4. Discussion:

The present work is the first comprehensive study on the pollination biology of *Merremia vitifolia.* The basic knowledge on reproductive biology is not only essential for evolutionary and systematic studies(Anderson, 1995) [12] but also important for effective conservation strategies(Holsinger, 1991; Bernardello *et al.,* 1999) [13][14]for endangered species.

Under favourable climatic conditions of Korambayil Ahmmed Haji Memorial Unity Womens College, *M. vitifolia* started flowering on second week of December, 2018 and reached its peak during January. Flowering declined towards the end of February. It is essential to have a basic knowledge about the floral morphology for the studies of pollination biology and breeding systems. The flowers of *M. vitifolia* were born in terminal cymose inflorescence. Flowers are companulateor infundibuliformis, 5.8cm long and 4.7cm wide. It is lemon yellow in color and without having any particular odour. Each inflorescence has 2 or three open flowers at a time and the flowers are bisexual, actinomorphic and pedicellate. Flower opening starts at 8.20am in the morning and were completely closed by approximately 4pm in the evening. Anther dehiscence takes place sometime after anthesis. In pollinated flowers, the calyx cup was persistent and the fruit emerges out. Pollen grains are non-porate and hexacolpate type. The average dimeter of pollen grains was 120µm. The average number of pollen grains per flower was calculated as 3968, the number of ovule as 4 and the pollen-ovule ratio as 992:1.

Acetocarmine-glycerine technique indicates that pollen fertility at the time of anthesis was low and it is maximum at 1pm. Then the viability decreases. *In vitro* and *in vivo* pollen germination tests did not give any result.

Pollen grains stained deeply with Coomassie Brilliant Blue indicate the presence of protein, pollen also stained with Sudan Black B indicate the presence of lipids. Pollen grains do not stain with IKI solution which indicates the total absence of starch in them.

Pistil has a long style and bicapitate stigma. Stigma was receptive at the time of anthesis itself. The stigmatic surface is with many ridges and grooves. The style is erect. The stigma is the recipient of pollen grains.

There is a great variation in the morphology of stigma. Angiosperm species that produces trinucleate pollen typically have dry stigmas, whereas binucleate pollens often interact with wet type stigmas (Shivanna.,Heslop Harrison, 1977) [15].

The present study showed that wind has no role in pollination and the pollination occurs exclusively by insects. The major pollinator is found to be *Halictus* bees. Many other insects visit the flower but they not contributing anything to pollination.

Fruit setting occur only on cross pollination. Seeds are with very tough seed coat and germination rate is 90% on moist cotton.

5.Conclusion:

Merremia vitifolia Hallier f. is a perennial climber belongs to the family Convolvulaceae. Lemon yellow flowers are born on cymose inflorescence. No previous studies on pollination biology of *Merremia vitifolia* have been conducted. The present study was carried out for a period of four months (December-March 2018-19), to observe the reproductive parameters of *M. vitifolia* in the back yard of Korambayil Ahmed Haji Memorial Unity Women's College. The flowers appeared from December 2018 to February 2019 and attracted many bees and insects. Flower opening starts at 8.20am in the morning and lasts about 9 am. The life span of individual flower is approximately 8 hours. Stamens fall off in the closed flowers whereas gynoecium persists for about two or three days and gradually develops into fruit. Anther dehiscence commenced after anthesis. Pollen grains at the time of flower anthesis have low viability but attain maximum viability at peak sunny noon. Acetocarmine-Glycerine technique shows that a high proportion of pollen grains are fertile at that time.Receptivity of stigma is the critical factor for the successful completion of the post-pollination events. The test by using hydrogen peroxide shows that the stigma is receptive at the time of anthesis itself. Wind has no role in pollination of this species. The plant promotes cross pollination and fruit development in self pollinated flower is poor or completely absent. 90% fruits contain four fully viable seeds; some are with one or more non-viable seeds. The seeds have a very tough and hard seed coat, and thus it takes many months to germinate in natural soil conditions. However, 90% of treated seeds were germinated in moist cotton.

6. Acknowledgment:

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Phytolith morphotype diversity in some economically valuable Cucurbitaceae members

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Abstract

Background: Phytoliths play an essential role in better survival of plants in adverse environmental conditions. The variation in biosilicification potential is responsible for differential phytolith accumulation in plant parts. **Methods**: The present study is focused on the phytolith diversity among nine species of Cucurbitaceae. The species specific phytolith morphotype analysis was done by using concentrated sulphuric acid and nitric acid (1:1) extraction method. From each selected plant, phytoliths isolated from different tissues including stem, leaf, fruit pulp and fruit peel. **Findings**: The rate of accumulation and phytolith morphotypes in different tissues showed considerable variations. Among the four tissue types selected, stem and leaves showed higher accumulation of phytoliths in most of the species compared to fruit pulp and peels. Highest phytolith accumulation showed in *C. pepo* in contrast to *M. charantia*. In the analysis, the decreasing order of total morphotype number in the species was as follows *C. pepo* < *T. tricuspidata* < *L. cylindrica* < *M. scabrella* < *C. grandis* < *L. cylindrica* < *B. hispida* < *C. sativus* < *M. charantia*. Among the species, *C. pepo* showed 25 different morphotypes of phytoliths; while a lesser number of 12 morphotypes observed *M. charantia*.

Key words: Phytolith, Cucurbitaceae, morphotypes, biosilicification, taxonomic key

1. Introduction

Silicon is the seventh most abundant element in the universe and the second most abundant element on the planet after oxygen that makes about 25 percent of the Earth's crust. Plants assimilate silicon through roots as silicic acid and accumulate in large concentrations similar to macronutrients. Inside the plant, it transports to active growing points where it complexes with an organic compound in the cell wall and makes them stronger. Silicon enhances plant upright growth, yield, resistance to microbial diseases and tolerance against abiotic stresses like temperatures, salinity, heavy metal and aluminum toxicity [1]. In case of plants, silica depositions commonly present in epidermis, bulliform cells, trachieds, vessels, cork cells, trichomes, inflorescence bracts etc.; while most of arborescent and herbaceous plant species show silica accumulation in the epidermis or outermost covering of seeds and fruits [2].

By biosilicification, the microscopic amorphous silica structures, phytoliths, are produced within and between plant cells by precipitation and polymerization of silica. This biological process occurs in cell lumens, intercellular spaces and cell-walls under the control of different silicification mechanisms. The biogenic silica particles show a narrow particle size distribution for specific structural motifs. During the early developmental phases of juvenile organs, there is little or no Si deposition with the exception of specialized silica cells [3]. The Si uptake by plants depends on the conversion of Si in soil into plant-available silicic acid. The biosilicification potential of hundreds of plants has been reported, making way towards classifying plants as Si accumulators and Si non-accumulators. Phytoliths are often preserved in fossils due to their inorganic nature which helps to resist the destructive forces and survive as sediments in diverse environments of land and ocean. It provides pieces of evidence for the distribution of taxa and helps in drawing more reliable inferences regarding palaeovegetation and in reconstructing the palaeoenvironments [4]. Moreover, the recent studies in plant taxonomy reveal the role of phytoliths in the identification of taxa at different levels of taxonomic hierarchy [5].

The family Cucurbitaceae includes 975 species across 98 genera in which most of them are succulent climbing vines with simple, palmate and exstipulate leaves. The members are commonly used as medicines, vegetables, fodders and other sundry products [6]. Usually, the plants show high accumulation of phytoliths in leaves, stems and fruits [7]. Recently, about forty phytolith morphotypes were reported in leaves, stem and fruits of 11 species of Cucurbitaceae. Presence of phytoliths has also been applied in archeological and paleoecological studies of some fossilized members of this family. Similarly, the better density of phytoliths was reported in Poaceae, Compositae, Mimosaceae,

Cyperaceae, Commelinaceae, Marantaceae, Arecaceae, Zingiberaceae, Bromeliaceae, Orchidaceae etc. [8]. Presence of phytoliths also observed in lower plants including pteridophytes and bryophytes, which show heteromorphic alternation of generation. In the past, the classification system had utilized all other taxonomic markers except phytolith tools. But, recent studies in different families use phytolith markers to construct taxonomic keys as a significant identification tool for taxa. Here, the present study focuses on phytolith morphotype variation in fruits of selected species in Cucurbitaceae which can be used as an important taxonomic tool for future studies.

2. Materials and methods

In the present study, nine species of Cucurbitaceae including *Cucurbita pepo* L., *Benincasa hispida* (Thunb.) Cogn., *Cucumis sativus* L., *Trichosanthes tricuspidata* Roxb., *Mukia scabrella* Arn., *Momordica charantia* L., *Coccinia grandis* (L.) Voigt., *Lagenaria siceraria* (Molina.) Standl. and *Luffa cylindrica* (L.) M. Roem. were collected from Malappuram District, Kerala, India. The stem, leaf and matured fruits of all selected plants were selected for phytolith analysis. The tissues were separated and air dried properly. Further, dried samples were cut into 1 cm square pieces and soaked in an acid mixture of con.HNO_3 and con.H_2SO_4 in 1:1 proportion for 15 days. To isolate the phytoliths, the acid mixture centrifuged and the acid decanted after fifteen days. The residue was suspended in distilled water and repeated the centrifugation up to 4 cycles in order to completely remove the acid from the residue. The final residue is suspended in rectified spirit and stored in storage vials for further study. A drop of the phytolith alcohol mixture was placed on a clean slide and made a thin film. It was warmed under a spirit lamp and observed under stereomicroscope (Magnus MSZ-TR).

3. Result

A wide diversity of phytolith morphotypes was observed among 9 species of Cucurbitaceae. In *C. pepo*, the maximum number of morphotypes was observed; while the least diversity was shown by *C. sativus* and *M. charantia*. About 25 different types of phytolith morphotypes were observed in different tissues of *C. pepo* (Table 1 & 2). The rate of occurrence of phytoliths was higher in stem and leaf tissues compared to fruit pulp and peels. Most frequently observed morphotypes among 9 species were elongate tubular, rectangular, elongate smooth, blocky, cylindrical long, globular or spherical, irregular and rondal. In fruit pulp of *M. scrabella*, stem of *M. charantia* and leaf of *L. cylindrica*, only one type of

morphotype was observed. However, the silicified vessels are noticed in fruit pulp of *B. hispida* (Figure 1 & 2).

Table 1. Diversity of phytolith morphotypes in different species of Cucurbitaceae under study

Sl. No.	Species	Tissue types	Phytolith shapes
1.	*Cucumis sativus* L.	Leaf	Triangular, Rectangular, Narrow Elongate, Rondal, Irregular and Blocky
		Pulp	Long Angular, Elongate Smooth, Rectangular
		Stem	Rondal Pyramidal, Long Cell Smooth Parallel Sides, Rondal Elongate, Long Cell Angular
		Peel	Long Cell Deeply Intended, Oblong
2.	*Luffa cylindrica* (L.) M. Roem.	Leaf	Elongate Tubular, Elongate Smooth
		Pulp	Narrow Elongate, Elongate Tubular, Prickle, Elongate Faceted
		Stem	Saddle, X- Shaped, Cordate, Elongate Faceted, Rectangular, Elongate Tubular
		Peel	Rectangular, Circular Crenale
3.	*Cucurbita pepo* L.	Leaf	Rectangular, Saddle, Elongate Narrow, Spherical, Elongate Tubular, Circular
		Pulp	Rectangular, Long Cell Deeply Intended, Small Prickle, Blocky Irregular, Blocky, Achene, Cuneiform, Elongate Smooth
		Stem	Diatom, Narrow Long Cell, Cylindrical Long Cell, Globular Facetate, Blocky, Cylindroid Scorbiculate
		Peel	Elongate Smooth, Elongate Cylindrical, Cylindroid Bulbous, Blocky, Elongate Narrow
4.	*Benincasa hispida* (Thunb.) Cogn.	Leaf	Elongate Cylindrical, Blocky, Elongate Psilate
		Pulp	Lacunate, Prickle, Salcate, Silicified Vessel
		Stem	Tubular Salcate, Bullliform, Saddle
		Peel	Bulliform, Lanceolate, Tubular Salcate, Globular Granulate
5.	*Momordica charantia* L.	Leaf	Papillate, Rondal, Spherical
		Pulp	Elongated Smooth, Elongate Tubular, Cuneiform, Irregular
		Stem	Smooth Cylindrical, Long Cell
		Peel	Lanceolate, Sheet of Smooth Long Cell, Tabular Sinuate

Table 2: Diversity of phytolith morphotypes in different species of Cucurbitaceae under study

Sl. No.	Species	Tissue types	Phytolith shapes
6.	*Lagenaria siceraria* (Molina.) Standl.	Leaf	Narrow Elongate, Cylindrical, Blocky, Broad Elongate
		Pulp	Blocky, Rondal, Spherical, Irregular
		Stem	Narrow Elongate, Rondal, Saddle, Cylindrical, Irregular, Achene, Spherical, Tubular
		Peel	Papillate, Elongate Smooth, Spherical, Large Prickle/Thin Chloridoid
7.	*Trichosanthes tricuspidata* Roxb.	Leaf	Long Cell Smooth Parallel, Blocky, Narrow Long Cell, Elongate Cylindrical, Rondal, Bulliform
		Pulp	Plate Wavy, Rectangular, Long Cell Smooth Parallel
		Stem	Rondal Rounded Keeled, Polygonal, Chloridoid, Rectangular, Elongate Smooth, Thick Shank, Bulliform, Dendritic Long Cell, Thin Chloridoid
		Peel	Rectangular, Plate Wavy, Elongated Smooth, Long Cell Intended
8.	*Mukia scabrella* Arn.	Leaf	Bulliform/Deltoid, Elongate Blocky, Narrow Long Cell, Tubular Sulcate
		Pulp	Elongate Smooth, Narrow Cylindrical, Cylindrical Long, Smooth Cylindrical Long
		Stem	Clavate Cylindrical Long Cell, Bulliform, Smooth Cylindrical Long Cell, Irregular, Papillate, Cylindroid/Rectangular
		Peel	Fusiform, Smooth Cylindrical Cell, Blocky, Narrow Long/Smooth Cell
9.	*Coccinia grandis* (L.) Voigt.	Leaf	Elongated Smooth, Bulliform, Acicular, Blocky, Narrow Elongated
		Pulp	Elongate Rondal, Oblong, Globular, Elongate Smooth
		Stem	Elongate Smooth, Blocky, Rondal, Tubular Rondal, Small Prickle, Acicular
		Peel	Oblong/Sinuous, Smooth Long Cell

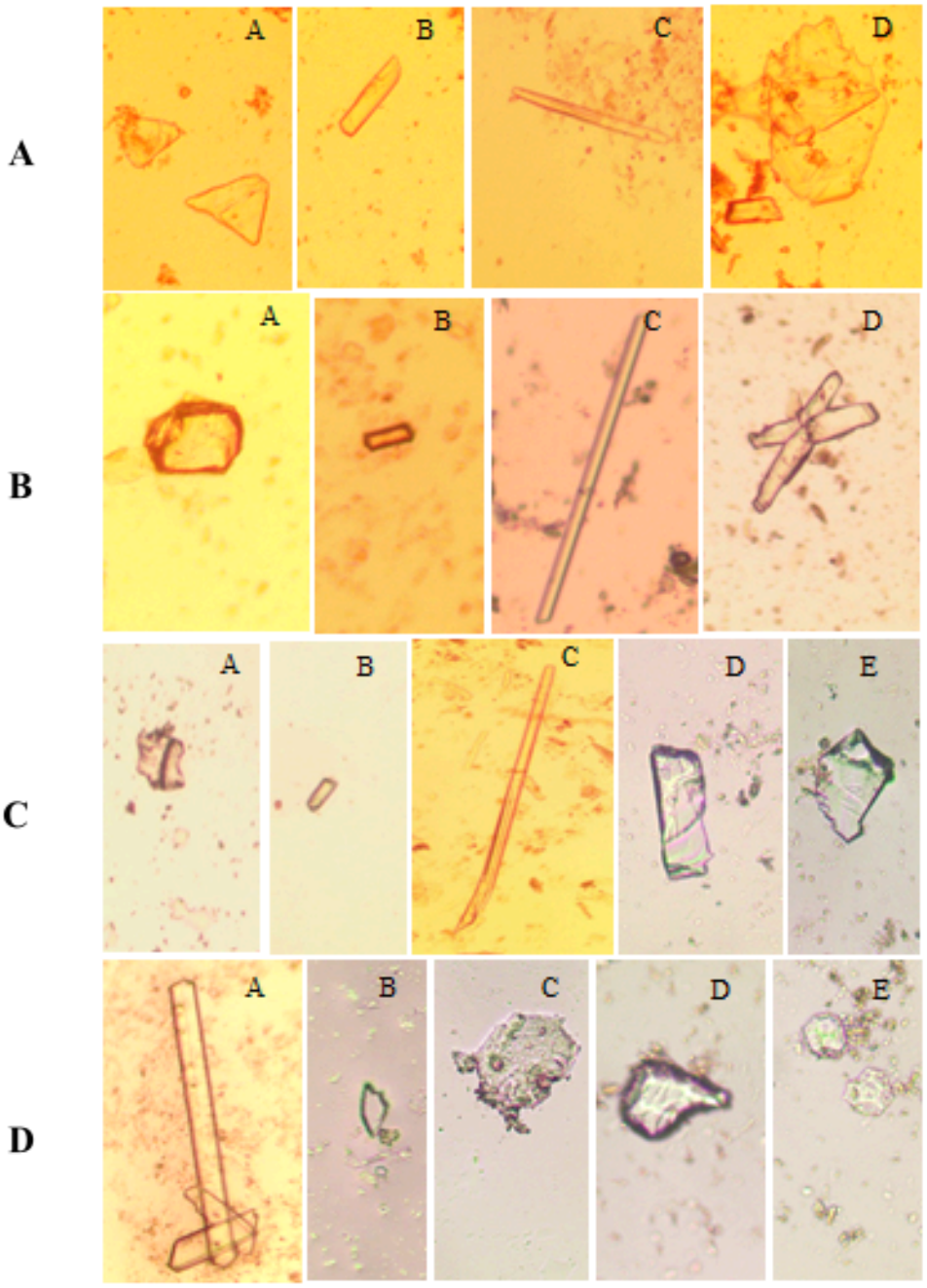

Figure 1.Phytolith morphotypes in different species of Cucurbitaceae. A. *C. sativus* (A-D), B. *L. cylindrica* (A-D), C. *C. pepo* (A-E), D. *B. hispida* (A-E).

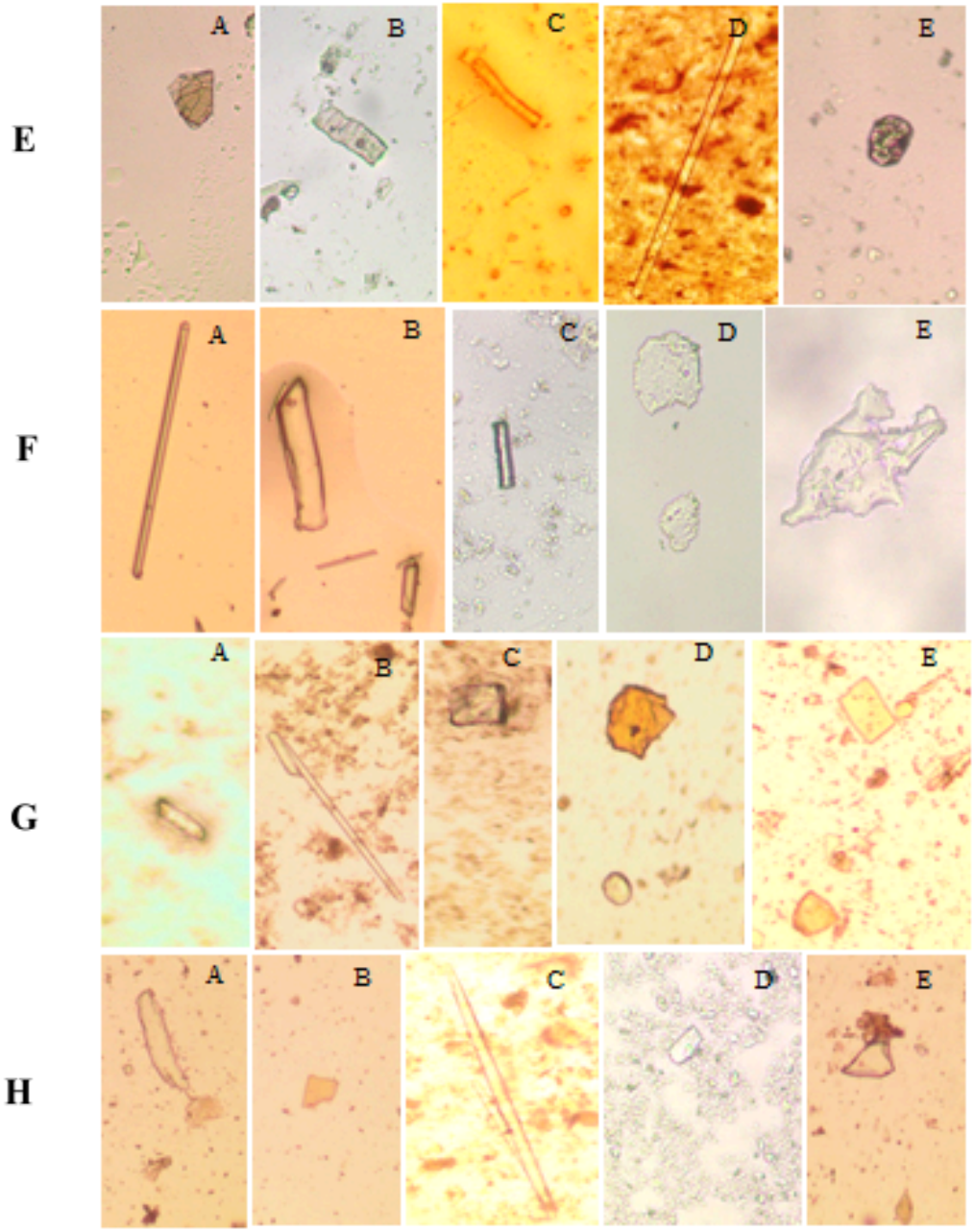

Figure 2.Phytolith morphotypes in different species of Cucurbitaceae. E. *M. charantia* (A-E), F. *L. siceraria* (A-E), G. *T. tricuspidata* (A-E), H. *M. scabrella* (A-E).

4. Discussion

Silica has long been known to be present in plants and considered to be important for normal growth and development of the plants. Silica is deposited mostly in plant cell walls and sometimes as bodies in the lumen of the cell. The more water absorbed by a plant, the greater the amount of silica deposited. Silica bodies in monocotyledonous and dicotyledonous families were studied recently. Present investigation is mainly focused on the identification of silica body types present in the leaves of some Cucurbitaceae plants. Silica is deposited in large quantities in the leaf epidermal system. The deposition of silicon in epidermal cells of grass species is thought to be an important mechanism that plants use as a defense against pests and environmental stresses.

Nine plants were selected for the present study. The isolation of silica bodies using concentrated sulphuric acid and nitric acid provided a clear idea about the shapes, which is similar to the procedure for isolation and characterization of silica bodies in Graminae [9]. The presence of hairs throughout the plant is a better identifying character for Cucurbitaceae plants. In the study, the phytoliths were identified in different tissues of nine species including *Benincasa hispida* (Thunb.) Cogn., *Cucurbita pepo* L., *Cucumis sativus* L., *Trichosanthes tricuspidata* Roxb., *Mukia scabrella* Arn., *Momordica charantia* L., *Coccinia grandis* (L.) Voigt, *Lagenaria siceraria* (Molina.) Standl. and *Luffa cylindrica* (L.) M. Roem.

From each selected plant, phytoliths are isolated from four types of tissues including stem, leaf, fruit pulp and fruit peel. The rate of occurrence and shape of phytoliths in different tissues of species were varried. In *L. cylindrica, C. pepo, L. siceraria, T. tricuspidata, M. scabrella* and *Coccinia grandis*, phytoliths were higher in stem tissues, whereas *B. hispida* showed lesser number of phytoliths in stem. Leaf tissues of *C. sativus* and *B. hispida* showed higher numbers of phytoliths compared to that of other species. In contrast, the least number of phytoliths observed in leaves of *C. pepo* and *M. charantia*. Among the four tissue types selected, stem and leaves showed higher accumulation of phytoliths in the majority of species. Moreover, fruit pulp and peels showed lesser phytolith accumulation in *C. sativus, L. cylindrica*, *L. siceraria, T. tricuspidata, M. scabrella* and *C. grandis.* Interestingly, the pulp of *M. charantia* showed higher rate of phytolith accumulation than stem, leaf and peel.

Different shapes of phytoliths are a very attractive feature of the Cucurbitaceae family. In the study, *C. pepo* showed 25 different morphotypes of phytoliths; while a lesser number of morphotypes were observed in *C. sativus* and *M. charantia*. The decreasing order of number of morphotypes in Cucurbitaceae species were as follows *C. pepo* < *T. tricuspidata* < *L. cylindrica* < *M. scabrella* < *C.*

grandis < *L. cylindrical* < *B. hispida* < *C. sativus* < *M. charantia*. The species, *C. pepo* included different morphotypes like Rectangular, Saddle, Elongate Narrow, Spherical, Elongate Tubular, Circular, Long Cell Deeply Intended, Small Prickle, Blocky Irregular, Blocky, Achene, Cuneiform, Elongate Smooth, Diatom, Narrow Long Cell, Cylindrical Long Cell, Globular Facetate, Cylindroid Scorbiculate, Elongate Cylindrical, Cylindroid Bulbous and Blocky that indicates the highest capacity of this plant to absorb and accumulate soil silica. Similarly, the lesser potential of silica absorption and deposition was observed in *C. sativus* and *M. charantia*. Most of the selected members in this family are edible vegetables and others are medicinal. The consumption of these vegetables will not cause the accumulation of silica in the body of living organisms. But silica inhalation is very dangerous to human beings. Silica is one of the essential elements in living organism to strengthen the connective tissues of the brain, nerve cells and spinal cord thereby improving memory and helping to prevent memory loss. Silica also helps to stabilize the pancreas's release of insulin.

5. Conclusion

The family Cucurbitaceae included both vegetable and medicinal plants and most of the members are climbers that possess trichomes or epidermal hairs in large numbers. Plant roots absorb essential or non-essential elements from the soil in which Si plays a significant role in plant survival. Absorbed silicon accumulated in different parts of the plant body as different shapes. Each species of Cucurbitaceae showed specific morphotypes of phytoliths. It makes the species entirely unique. Moreover phytolith character helps to identify the species by preparing taxonomic keys.

6. Acknowledgement

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PEG induced drought stress in ash gourd (*Benincasa hispida* (thumb.) Cogn.) seedlings and its mitigation with salicylic acid

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Abstract

Background: Drought is, one of the environmental stresses, plays crucial role in reduction in plant production on majority of agricultural fields in the world. This present study mainly focuses to evaluate the effect of drought stress in ash gourd seedlings and also mitigate its effect with salicylic acid. **Methods:** The 15 days old seedlings were treated with various concentrations of PEG as such 0%,5%,10% and 15%. Anti-stress treatment was given with salicylic acid in seedlings which shown 50% decline in growth (15% PEG) with a concentration of 0.50mM. **Findings**: PEG caused negative influence on plant growth which was mitigated by the salicylic acid pre-soaking treatment. This was accompanying with some physiological processes modification such as enhancing in photosynthetic pigments and metabolites like protein. Moreover, these findings indicate that salicylic acid can be used to improve the plant growth and development under drought conditions. **Novelty and application**: This study discovers that salicylic acid can be applied to improve the growth, photosynthetic pigments and some organic osmolytes of ash gourd plant grown under drought stress conditions. This study will help the farmers to use the SA seed-presoaking doses so as to increase the drought stress tolerance.

1.Introduction

Stress in plants is a condition in which the plant growing in non-optimal or poor state that negatively influences the plant growth, crop productivity, reproductive capacity or death if it exceeds the plant tolerance limits. Abiotic stress factors includes various environmental issues that disturbs plant growth such as light, water-logging, temperature, salinity, drought and heavy metal toxicity, whereas biotic stress factor is a biological harm like pathogen and pest attack, which a plant faces during its life

period. Drought is one of the most severe environmental stresses affecting plant productivity. About 80-95% of the fresh biomass of the plant body is comprised of water, which plays a vital role in various physiological processes including many aspects of plant growth, development, and metabolism [1],[4]. The effects of drought in agriculture are aggravated due to the depletion of water resources and the increased food demand from an alarming world population growth [19]. Drought stress in crop plants is characterized by reduced leaf water potential and turgor pressure, stomatal closure, and decreased cell growth and enlargement [7]. Drought stress reduces the plant growth by influencing various physiological as well as biochemical functions such as photosynthesis, chlorophyll synthesis, nutrient metabolism, ion uptake and translocation, respiration, and carbohydrates metabolism ([14] ; [7]; [18]).

This work mainly focuses on preliminary study on effects of drought and drought tolerance of seedlings of ash gourd, (*Benincasa hispida)* which belongs to the family cucurbitaceae. Here, PEG-6000 (polyethylene glycol) was used to induce drought stress. Simulation of drought stress by PEG induces drought stress on the plants. It is reported that PEG induced significant water stress in plants and not having any toxic effects [6].

The application of plant growth regulators known to be involved in the survival from stress related effects. Among the plant growth substances, salicylic acid, cytokinin and abscisic acid have been reported to play a key role in drought tolerance. Under water stress conditions, plant growth regulator treatments can significantly increase the water potential and the chlorophyll content. Salicylic acid (SA) is a phenolic compound involved in the regulation of growth and development of plants, and their responses to biotic and abiotic stress factors [20]. Exogenously sourced SA to stressed plants, either through seed soaking, adding to the nutrient solution, irrigating, or spraying was reported to induce major abiotic stress tolerance mechanisms [12] .

The main objectives of present study includes; to study the effect of drought stress on the growth of ash gourd seedlings, to analyze the different morphological, physiological and biochemical activity of the plant under various concentrations of polyethylene glycol, PEG and to mitigate the effects of drought stress by treating with salicylic acid.

2. Materials and methods

2.1 Plant material

Seeds of Ash gourd (*Benincasa hispida*) were bought from Anakkayam seed farm centre at Agricultural research station, Kerala Agricultural University. These seeds were washed with a soap solution (Teepol solution,50% diluted) and later it was surface sterilised with 0.1%(w/v) aqueous solution of mercuric chloride for 5 minutes with constant stirring and washed with distilled water. These seeds were then sowed in 7 polythene bags in such a way that, five seeds per bag. Total four replicates were done for obtaining morphological and biochemical data and were watered regularly twice in a day for 15 days until the seedlings are in a size to induce the stress.

2.2 Drought treatment

15 days old seedlings were treated with different concentrations of 100 ml PEG 6000(Polyethylene glycol) in 5%,15% and 20% concentrations along with control for 4 days. The watering was done at a low rate during this time interval in drought induced seedlings. After the 4 days of drought treatment, the seedlings were uprooted carefully and various parameters such as morphological and biochemical analysis were done.

2.3 Anti-stress treatment

The drought treatment which caused 50% decline in growth (15% PEG) was selected to treat with the growth regulator salicylic acid. Concentration of salicylic acid taken was 0.50 mM which is ideal for crop plants to impose drought tolerance. In this study, seed treatment is opted. Seeds were soaked in 0.50mM salicylic acid for 24 hr and then sowed in the pots. After 15 days, they were treated with PEG to induce drought (15%PEG) along with control. After 4 days, they are uprooted for further studies.

2.4 Morphological/Physiological studies

2.4.1 Determination of shoot length and root length

The length of the shoot and root was measured using a graduated scale and was expressed in centimetres.

2.4.2 Determination of leaf area index

Leaf area index can be measured by using a graph paper. The leaf was traced in a graph paper and calculate the area by adding squares inside it.

2.4.3 Determination of dry weight percentage

Samples were weighed in pre weighed containers using electronic balance (CAY 220). Fresh weight obtained was recorded and the weighed samples were then placed in hot air oven at 100°C for 1 hour, followed by at 60°C overnight. Dry weight of each sample was taken on the next day and drying and weighing were repeated until values became constant and dry weight percentage was calculated.

2.5 Biochemical studies

2.5.1 Estimation of pigment composition

Pigment composition of leaves was estimated according to the method of Arnon (1949). Fresh leaves of the control as well as the experimental plants were washed with water and blotted between sheets of filter paper. To estimate the chlorophyll and carotenoids, 80% acetone was used as the extracting medium. One gram of fresh leaf sample was weighed in an electronic balance and weight of each recorded, crushed using chilled mortar and pestle in 5ml of 80% acetone(w/v). Then the homogenate was centrifuged at 5000 rpm for 10 minutes at 4°C and the supernatant was collected. The residue was again washed with 80% acetone and centrifuged. The process was repeated till the pellet become colourless. The final volume of the pooled supernatant was noted. The absorbance was read at 470,646,663 and 750 nm against the solvent blank (80% acetone) in UV-VIS double beam spectrophotometer (ELICO 159). Then the amount of chlorophyll and carotenoid present in the extract was calculated as µg/g fresh weight.

2.5.2 Analysis of metabolites

2.5.2.1 Estimation of Total protein

Protein content of the plant material was estimated using Folin-Ciocalteau reagent according to the method of Lowry et al.,1951. **Extraction**: Fresh tissue weighing 0.5g was macerated in 20% trichloroacetic acid using mortar and pestles. The homogenate was then centrifuged at 600 rpm for 30 minutes and the supernatant was discarded. 5 ml of 0.1 N NaOH was added to the pellet and it was

B

saved for the estimation of protein. **Estimation**: To 0.5 ml of the extract, 5 ml of copper reagent C was added (reagent C: mixture of reagents A and B in the 50:1 ratio; Reagent A: 2% Na_2CO_3 in 0.1 N NaOH; Reagent B: equal volume of 1% CuSO4 and 2% sodium potassium tartrate. The tubes were shaken well and allowed to stand in dark for 10 minutes at room temperature. Then 0.5 ml of properly diluted Folin-Ciocalteau was added to the solution and mixed thoroughly. The tubes were kept in dark for 30 minutes for colour development. The absorbance was read in colorimeter. Bovine Serum Albumin Fraction V powder used as standard. The calculations were made in µg/g fresh weight.

Figure.1: Seedlings in various stages of growth: A: 15 days old seedlings B: control (18th day), C: 17 days old seedlings D: 5% PEG treated at 18th day

3. Results

3.1 Standardization of growth and concentration

The seedlings of ash gourd (*Benincasa hispida*) which is treated with various concentrations of PEG along with control were used to elucidate the growth parameters. Pre-treated seedlings with salicylic acid were also analysed for the morphological studies. Various parameters such as shoot length, root length, leaf area index, fresh weight and dry weight were analysed. Analysis and further studies were done on 18th day after germination.

Table 1: Shoot length, root length, leaf area index, fresh weight and dry weight of seedlings grown under different concentrations of PEG and salicylic acid treatment

Concentration of PEG	**Shoot length (cm)**	**Root length (cm)**	**Leaf area Index(cm^2)**	**Fresh weight (g)**	**Dry weight (g)**
Control	7.1098±0.63552	6.3258±0.37558	325.65±0.25487	7.158±0.6125	0.561±0.2365
5%	6.9023±0.58972	4.7636±0.48561	308.48±0.36945	7.252±0.3684	0.494±0.3668
10%	6.3455±0.45583	4.2574±0.56798	312.23±0.25687	6.458±0.5963	0.382±0.5968
15%	5.2287±0.88965	3.9254±0.35654	259.36±0.58967	6.358±0.3621	0.312±0.4896
SA treatment with 15% PEG	7.3225±0.48984	6.7896±0.56896	330.9±0.59874	7.024±0.6354	0.5063±0.5689

The effect of drought on various morphological parameters is shown in table 1. Among the drought induced plants, those treated with 15% PEG, showed approximately 50% reduction in different morphological parameters when compared to control. Upon increasing concentration of PEG from 0-15%, we can see a gradual decrease in the values of characters studied. From the table 1, a highest leaf area could be seen in SA treated plants with a value of 330 cm^2**.** The lowest rate can be observed in 15% PEG with a value of 259 cm^2. This also indicates the mitigation effects of SA. Usually the plant growth is measured in terms of height, weight (both fresh weight and dry weight) etc. and growth is based on two processes, cell division and cell enlargement. Fresh weight and dry weight decreased on increasing

concentrations of PEG. An increased fresh weight and dry weight could be seen in control plants as compared to SA treatment. Inducing drought with 15% PEG on 15 days old seedlings emerged from seeds soaked in 0.50mM salicylic acid, shoot length, root length, leaf area index, fresh weight and dry weight got increased than that of plants treated with 15% PEG alone, and is approximately equivalent to control plants. These results indicated that salicylic acid played as drought ameliorating agent in ash gourd seedlings and helped somewhat in stress tolerance.

Figure.2 18 day old seedlings under different concentrations of PEG: A: Control, B: 5% PEG , C: 10% PEG , D: 15% PEG , E: SA treated(15% PEG

3.2 Pigment composition

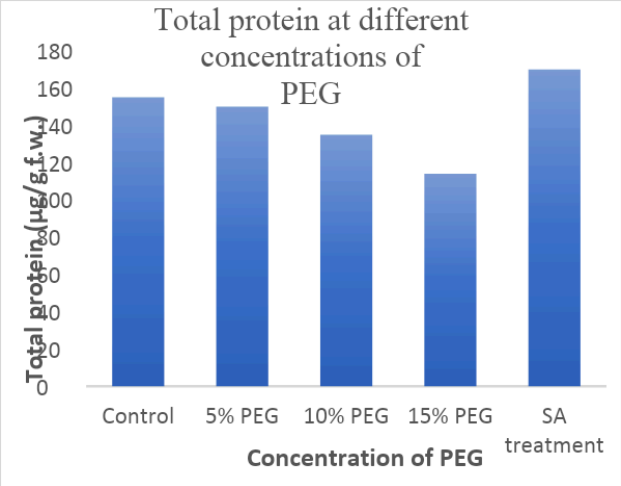
Table 2: The pigment composition (µg/g. fresh weight) of seedlings in different concentration of PEG and SA treatment.

Concentration of PEG	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Carotenoid**
Control	40.4501±.7585	15.6874±.5896	56.1375±.7857	15.876±.4344
5%	45.3695±.6547	18.3654±.5879	63.7349±.6531	35.476±.6745
10%	43.6872±.5489	14.6398±.4523	58.3273±.5634	17.452±.5748
15%	38.4789±.3256	12.6548±.6547	51.1337±.45647	11.549±.6342
SA treatment (15% PEG)	50.9876±.45678	16.7564±.4536	67.7443±.6578	65.943±.4276

Another plant response to drought stress is the change in photosynthetic pigment content (table 2). The contents of both chlorophyll a and b can change under drought stress. Photosynthetic pigments play important roles in harvesting light. Carotenoids play fundamental roles and help plants to resist drought stress. Amount of chlorophyll a has decreased in increasing the concentration of PEG and an increased amount of chlorophyll a can be observed in seedlings which are treated with salicylic acid. In the case of chlorophyll b, is also decreased along with increasing the concentration of PEG. Carotenoids can be observed as highest amount in salicylic acid treatment and it also decreases along with increasing concentrations of PEG.

3.3 Analysis of protein

The amount of carbohydrate was higher in salicylic acid treated plants when compared to control. The rate of protein can be obtained as decreasing along with increasing the concentrations of PEG. The highest value for protein content is observed in salicylic acid treated and lowest value can be observed in 15% PEG.



4. Discussion

Drought stress has negatively influences on the growth of crop plants and thus decreasing the crop productivity. Thus adopting methods for mitigating the effects of drought stress was very essential for the proper crop growth and production. Drought stress is one of the major factors, which inhibit the growth of a plant. It is well reported that injury in plants takes place due to drought stress at vegetative and reproductive stages of development [5]. Creation of drought stress by application of PEG on the growth of plant showed remarkable deviation from normal levels of moisture. PEG enhances the solute potential in plant, which causes drought stress [25].

In the present study, the application of PEG affects the growth of the ash gourd seedlings in various ways. Similar observation was also observed that PEG decreased the overall growth of plant by inducing drought stress [21]. The plants which are treated with salicylic acid shows an increased value for all morphological and biochemical parameters. Growth is a pattern of change in size, volume, or weight

which comprises the stages of cell division, elongation, and differentiation which were affected under drought conditions due to reduced loss of turgor, less energy and reduced enzyme activities ([16];[7];[22]). In this study, the shoot length has a lowest value of 5.22 cm which is treated with 15% PEG. A highest value of 7.32 can be observed in plants treated with salicylic acid along with 15% PEG.

SA treatment also significantly increased shoots and roots fresh weight and dry weight. A stimulation of shoot and root growth by the SA treatment under water stress in cucumber was also reported [3]. SA increased fresh weight and dry weight of shoots and roots in water stress conditions in muskmelon plants [17].

Due to drought stress the comparable results such as reduction in shoot weight, flower fresh weight and dry weights of marigold (*Tagetes erecta* L.) plants were reported [2]. Reduction in leaf area due to loss of turgor and reduced leaf numbers were reported in rice [7]. Drought stress significantly decreases shoot and root dry weights in Asian red sage (*Salvia miltiorrhiza* L.) [18]. Leaf area is a determinant factor in radiation interception, photosynthesis, biomass accumulation, transpiration and energy transfer by crop canopies. It is also important with respect to crop-weed competition and soil erosion [15]. In root length also, a lowest value can be seen in 15% PEG treatment with a value of 3.92 cm and a highest value in plants treated with salicylic acid with a value of 6.78 cm. On the other hand, the external application of SA appeared to ameliorate the influence of drought stress on the growth vigour of shoots and roots of both the controlled and the droughted plants. Leaf, stem and root growth rate are very sensitive to drought stress because they are dependent on cell expansion ([13] [11]).

Photosynthesis is considered to be a vital plant metabolic pathway. The conservation of plant growth under drought stress requires maintenance the right photosynthetic rate [26]. The obtained results showed that drought led to a significant reduction in the photosynthetic pigment contents in the leaves (Table 2). It was observed that the leaves became yellow when they had minimum water potentials in a certain period. These results are in conformity with those obtained with *Plectranthus tenuiflorus* [14]. Drought stress affected photosynthesis by closing of stomata, transfer of CO_2 in chloroplasts and a decrease in cell water potential, which could result in a marked reduction in the plant's productivity [8]. Moreover, the results in Table 2 indicated that the pre-soaking seeds in SA caused significant increase in the photosynthetic pigment contents in stressed as well as nonstressed ash gourd leaves. Furthermore, in other investigations, the application of SA improved the pigment contents in maize, mustard and wheat, under stress conditions.

The reduction in protein concentration was observed, as reduction is directly proportional to the PEG concentration in nutrient medium. The reduction in protein content was may be due to enhancement of hydrolysis of protein [24] or low synthesis of protein [9].

5. Summary and conclusions:

Drought has been considered as one of the most acute abiotic stresses presently affecting agriculture. Drought stress can significantly reduce photosynthesis and stomatal conductance, inhibit photosynthetic pigments synthesis and ultimately lead to reduction in growth of plants [10].

Cucurbits being warm season crops are mainly cultivated during summer season in arid regions, during April to June the environmental temperature increases up to 42 and goes beyond 45 which drastically reduced the yield of cucurbits. In this study, drought caused negative influence on plant growth which was mitigated by the salicylic acid pre-soaking treatment. This was accompanying with modification of some physiological processes such as enhancement in photosynthetic pigments and metabolites like protein. Moreover, these findings indicate that salicylic acid can be used to improve the plant growth and development under drought conditions.

This study discovers that salicylic acid can be applied to improve the growth, photosynthetic pigments and some organic osmolytes of ash gourd plant grown under drought stress conditions and it helps to ameliorate the effects of drought. This study suggests the use of SA treated seeds for cultivation by farmers to reduce the effect of drought stress and thus increases the production and yield.

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Evaluation of yield and nutritional status of oyster mushroom [*Pleurotus florida* (mont.) Singer] grown on different substrates

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Abstract

Background: This study was conducted to examine the effect of different substrates and to evaluate the yield and nutritional composition of mushroom obtained from each. **Methods**: The gathered substrates, sugarcane bagasse, lemongrass, vetiver roots, rubber sawdust, and neem cake were used for this. Statistics were applied to daily data on the weight of mushroom production on various substrates. **Findings:** Rubber sawdust from the three-month project work produced the highest yield, followed by lemongrass, sugarcane bagasse, and vetiver roots. Neem cake produces no yield, and no mycelial growth is seen in this. On nutrient analysis of mushrooms harvested from lemongrass and rubber sawdust, there were no much differences noticed. The availability of substrates at affordable prices in the specific location where oyster mushrooms are produced determines their characteristics. **Novelty and application** can create nutrient-dense mushrooms from various substrates and aid in their environmentally friendly disposal. Here used substrates which are available at free of cost or at cheap rate can be recommended for low-cost mushroom production for common man. The fact that diverse substrate wastes are recycled into vermicompost after mushroom harvesting has become interesting.

1. Introduction

A mushroom, or toad stool, is the fleshy, spore - bearing fruiting body of a fungus, typically produced above ground on soil or on its food source. Mushrooms are fleshy fungi which constitute a major group of lower plant kingdom. A mushroom is a general term applied to the fruiting bodies of the fleshy fungi and as such belongs to different groups of fungi. The majority of these mushrooms (fleshy fungi) belong to Hymenomycetes of Basidiomycotina, characterized by the presence of spore - bearing layer known as Hymenium. Mushroom is a common fungal fruiting body that produces

basidiospores at the tip of club like structures called basidia, which are arranged along the gills of the mushroom.

Sufficient food supply is a country's most precious asset. Mushroom provides a rich addition to the diet in the form of proteins, carbohydrates, valuable salts and vitamins. As food, the nutritional value of mushrooms lies between meat and vegetables. Mushrooms provide a high protein and low caloric diet. They are the number one diet to be recommended to heart patients.

In *Pleurotus* sp., exposure to light (artificial or sunlight) is necessary for initiation of fruiting body primordia, and thus, light is given for at least 15 minutes per day [10]. The raw materials which can be applied for oyster mushroom cultivation are cheaply available in farmer's yards and easily cultivated in various climatic conditions as a fast-maturing crops.

Because of low-cost production technology and high biological efficiency, *Pleurotus* species are well-known and widely cultivated around the world, mostly in Asia, America, and Europe. The taste, nutritional value, and therapeutic qualities of oyster mushrooms are also contributing significantly to their growing popularity. Species of *Pleurotus* can grow in a wide variety of temperatures and effectively break down agricultural waste. In contrast to other edible mushrooms, *Pleurotus* species grow quickly, and disease attacks on their fruiting bodies are rare.

Proteins, minerals (P, Ca, Fe, K, and Na), and vitamins (thiamine, riboflavin, folic acid, and niacin) are all abundant in *Pleurotus* species. In addition to its nutritional importance, their medical potential in the treatment of cancer and diabetes has been highlighted. A large variety of metabolites with antitumor, antigenotoxic, antioxidant, antihypertensive, antiplatelet-aggregating, antihyperglycemic, antibacterial, and antiviral activity are present in a variety of mushroom species. The use of certain oyster mushroom species in medicine is significant. *Pleurotus ostreatus* also has anticancer potential, while *Pleurotus cystidiosu*s is a potent antioxidant. To thrive in their natural habitat, mushrooms require substances that are antibacterial and antifungal. As a result, a variety of mushroom species may yield antibacterial chemicals that are useful to humans [8].

The present study was conducted to compare the effects of different agro - wastes on the growth, yield, and nutritional composition of oyster mushroom *Pleurotus florida*. The final aim was to find the best substrates for effective cultivation of oyster mushroom with best nutritional composition and cost effective.

2. Materials and methods

2.1 Mushroom shed

The first step in the cultivation technique is the preparation of the mushroom shed. A mushroom shed should be kept free from insects and rodents. The shed should also be almost air tight, allowing only a minimum air to filtrate. The shed floor should be kept wet at all times and the walls sealed with a layer of plastic to conserve moisture and maintain humidity. Spray the mushroom bag with water three times a day. But in an area where water resources are limited, water should be added once in the morning and one in the afternoon as long as the humidity well maintained. After the fruiting bodies have appeared, watering should be continued, but avoid wetting the fruiting body of the mushroom directly as they will absorb water like sponges.

2.2 Sources of mushroom

The oyster mushroom spawns (*Pleurotus florida* Mont. Singer.) were purchased from IRTC (Integrated Rural Technology Centre), Mundur, Palakkad, Kerala.

2.3 Collection of substrates

The various substrates used were sugarcane bagasse, vetiver roots, lemon grass, rubber sawdust and neem cake. These substrates were collected from nearby areas of Manjeri, Malappuram, Kerala.

2.4. Sterilization of substrates

Sterilization of substrates was done by autoclaving. Autoclaving is an easiest method and suggested for minimizing labour and expenditure. In this method, the substrates to be autoclaved are subjected to gradual temperature increase under high pressure at 15 pound per square until 121°C is reached and then steamed for around 15 minutes. Each of the substrates thus autoclaved would be free from microorganisms and other infectants.

2.5 Preparation of bags

The bag preparation was by poly bag method. Here, polythene bag having 1 ft breadth and 2 ft height was sterilized by diluted Dettol and perforated (whole size 0.4 diameter) at a distance of 4 inch all over the body surface. The perforation was done by narrow sterilized needle. Made the polythene bags and the substrates dry. All the substrates were packed on the same day. Substrates

were placed inside the bag in 4-5 layers intermittent with spawn and made tight by pressing with hand palm. The sealed portion of the polythene bag was tied at the end so that the bottom portion of the bed became round in shape and convenient for keeping them on stands or hung them from top. After the completion of one layer, 30-40 grams spawn grains were spread over the substrate layer. Then other layer of substrate is prepared and spawn grains are spread over them similarly. In this way, 4 / 5^{th} portion of the transparent polythene bag is filled up by the spawned substrate. Then the bag was closed tightly by tying with rubber band. About two bags were prepared from a packet of spawn. Then all the bags or beds were kept on ropes that are hanging in chains with a support from the top of the mushroom shed, where humidity has been artificially raised by hanging wet jutes at different place. Sprinkling of water was done regularly at the morning and afternoon to develop fruiting bodies.

Weight of packed bags of substrates

Sl. No.	Substrate	Weight of packed bags in (kg)	
1.	Sugarcane Bagasse	2.49	2.33
2.	Roots of Vetiver	2.11	2.05
3.	Lemongrass	3.10	3.01
4.	Rubber sawdust	3.86	3.8
5.	Neem cake	3.50	3.46

2.6 Fruiting body development

Periodical observation is done to see spawn growth, mycelial development started within 20 days. After the mycelial running was visible, clear markings were done along the substrates and narrow longitudinal cuts were made by using sterile surgical blades. The fruiting body development started within 7-10 days of bag opening. Thereafter, the mushrooms were grown on the same beds for 2-3 times in flushes for about 7-8 days interval.

2.7 Harvesting

Mushroom harvesting was generally done at morning. The maturity of fruiting body was identified by seeing edges of the caps that start to fold or curl upward. Plucking was done by giving gentle twist at the base of fruiting body. The adhering substrate particles are removed by and picking or cutting and the mushrooms are made ready for marketing or food preparation. After harvesting of mushrooms weighing of the harvested mushrooms was done from each bed and it was used for analysing the production rate from each substrate.

Two samples of mushrooms with rubber sawdust and lemongrass as substrates were sent to Signature solutions training and research centre, Perinthalmanna, Malappuram Dist. Kerala, to analyse their nutritional composition.

2.8 Nutritional analysis

2.8.1 Determination of carbohydrates

Weigh accurately 2-3 gm of ground sample and transferred into 1000 ml round bottom flask, Add 200 ml of 2.5% dilute HCl, Connect the flask into a water condenser and reflux for 2.5 hours, Cool and neutralize with 50% sodium hydroxide solution (use a litmus paper), Filter (using ordinary filter paper) into a 250 ml volumetric flask and make up to the mark with distilled water. Fill the solution into a 50 ml burette.

Preliminary Titration:

Pipette 5 ml each of Fehling A and B into 250 ml conical flask, Mix and add about 30 ml water and a few boiling chips or glass beads, Heat the flask to boiling, Add 10 drops of methylene blue indicator, Continue the addition of solution drop wise until the blue colour disappears to a brick-red end point. Note down the titre value.

Final Titration:

Pipette 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration, Heat the flask to boiling within 3 minutes and complete the titration, Perform the titration duplicate and take the average.

Determination of Fehling Factor

Accurately weigh around 4.75 gms of AR grade sucrose, Transfer to 500 ml volume flask with 50 ml distilled water, Add 6 ml conc. HCl and allow to stand for 24 hours, Neutralize with NaOH solution and make up to volume, Transfer to a burette and perform the titration of Fehling solution following the similar procedure as above.

2.8.2. Determination of protein

Weigh quickly about 1-2 gm of the sample and transfer to a 500 or 800 mL Kjeldahl flask taking care to see that no portion of the sample clings to the neck of the flask, Add 0.7 gm of copper sulphate, 15 gm of Potassium Sulphate and 40 mL of concentrated sulphuric acid, Add two to three glass beads, Place the flask in an inclined position on the stand in the digestion chamber and digest, Heat the flask gently at low flame until the initial frothing ceases and the mixture boils steadily at a moderate rate, During heating rotate the flask several times. Continue heating for about an hour or more until the colour of the digest is pale blue, Cool the digest and add slowly 200 ml of water, Cool, add few glass beads and carefully pour down the side of the flask sufficient Sodium Hydroxide solution (450gm/L) to make the contents strongly alkaline (about 110 mL) before mixing the acid and alkaline layer (colour of the solution should be changed into dark), Connect the flask to a distillation apparatus incorporating an efficient flash head and condenser, Pipette 50/100 ml of 0.1 N sulphuric acid into a beaker/conical flask, To the condenser fit a delivery tube which dips just below the surface of the pipetted volume of standard acid contained in a beaker/conical flask receiver, Mix the contents of the digestion flask and boil until 150 mL have distilled into the receiver, Add 5 drops of methyl red indicator and titrate with standardized 0.1 N Sodium Hydroxide solution, Carry out blank titration simultaneously.

2.8.3 Determination of total fat content

Weigh accurately sufficient amount of ground sample into an extraction thimble and plug it from top with cotton, Take the initial weight of soxhlet flat bottom flask, Put the thimble into the soxhlet extraction chamber, Connect the extraction chamber over the flask, Add solvent into the chamber until it siphoned off into the flask, After siphoning stops, again add the solvent into the half level of the chamber, Connect water condenser over the chamber, Heat the flask (heating mantle, temperature set as 5°C) for about 4-5 hours for complete extraction of fat. If the sample containing high amount of fats, more time usually 8 hours is required, After the extraction completed,

disconnect the flask and evaporated off the solvent in a heating water bath, Remove the traces of the residual solvent by keeping the flask in a hot air oven for about 30 minutes, Cool the flask and weigh.

2.8.4. Determination of energy/calorie

Energy or calorie contained in the mushrooms were identified using the equation, Energy as Kcal = [(carbohydrates x 4) + (protein x 4) + (fat x 9)] x 0.99

2.8.5. Determination of fibre content

Weigh accurately about 2 - 2.5 gm ground sample into a thimble and extract for about 1 hour with petroleum/diethyl ether in a soxhlet extractor, Transfer the material in the thimble to a 1 litre round bottom flask, If the sample is not containing any fat/ oil, skip the oil extraction steps, instead directly transfer the weighed sample into the 1 litre flask, Add 200 ml dilute sulphuric acid into the flask and connect a water condenser, Reflux for 30 minutes (Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid), Remove the flask and filter through nylon filter bag, Wash the bag with water till acid free, Semi-dry the contents of the bag in a hot air oven and transfer again into the 1 litre flask, Add 200 ml dilute sodium hydroxide solution into the flask and connect a water condenser, Reflux for 30 minutes (Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask and out of contact with the acid), Remove the flask and filter through nylon filter bag, Wash the bag with water till alkali free, Completely dry the extract in an oven and transfer the extract into a silica crucible, Take the weight of crucible + dried extract. Ash the crucible in a muffle furnace heated to 550°C for 2 hours, Cool in a desiccator and weigh.

2.8.6 Determination of Calcium content

Total calcium content was determined by EDTA Titrimetric method.

Mix the sample pre-treated, if so required and transfer a suitable volume (50 to 100 mL) to 250 mL conical flask or a beaker, Add 5 mL of concentrated nitric acid and evaporate on a hotplate at a slow boil to the lowest volume possible (about 15 to 20 mL) before precipitation or salting occurs, Add 5 mL of concentrated nitric acid, cover with a watch glass and heat to obtain a gentle refluxing action, Continue heating and adding concentrated nitric acid as necessary until digestion is complete as shown by a light coloured clear solution, Do not let sample dry during digestion, Add 1 to 2 mL of concentrated

nitric acid and warm slightly to dissolve any remaining residue, Wash down beaker walls and watch glass with water and then filter, if necessary. Transfer the filtrate to a 100 mL volumetric flask. Cool, dilute to mark and mix thoroughly, Take a portion of this solution for the determination of calcium.

Sample preparation

Because of the high pH used in this procedure, the titration should be performed immediately after the addition of the alkali and indicator, Use 50mL of sample or a smaller portion diluted to 50 mL so that the calcium content is about 5 to 10 mg, Analyse hard waters with alkalinity higher than 300 mg/LCaCO3 by taking a smaller aliquot and diluting to 50 mL or by neutralization of the alkalinity with acid , boiling for one minute and cooling before beginning the titration, Add 2.0 mL of sodium hydroxide solution or a volume sufficient to produce pH of 12 to 13, Stir. Add 0.1 to 0.2 gm of the indicator murexide - sodium chloride mixture selected, Add EDTA titrant slowly with continuous stirring to the proper end point. Check the end point by adding 1 to 2 drops of titrant in excess to make certain that no further colour change occurs.

2.8.7 Determination of Iron content

Total iron content was determined by Phenanthroline method

Pipette out appropriate portions of standard iron solution into 125 ml conical flasks to contain from 10 to 100 µg of Fe. For the reagent blank, pipette out 10 ml of water to a separate conical flask. Dilute the contents of each conical flask to about 50 ml by adding water. To each flask, add 1 ml $NH_2OH.HCl$ solution and 2 ml conc HCI. Add a few boiling chips and boil the solution until the volume is reduced to about 20 ml. Cool to room temperature and quantitatively transfer to 100 ml volumetric flasks. Add 10 ml ammonium acetate buffer solution first and add 10 ml 1, 10-phenanthroline solution to each flask. Dilute to 100 ml with water and mix thoroughly and allow to stand for 10 to 15 min. Measure the absorbance of the iron complexes at 510 nm against the reagent blank. Construct a calibration curve by plotting absorbance values against micrograms of iron in 100 ml of the final solution.

3. Results

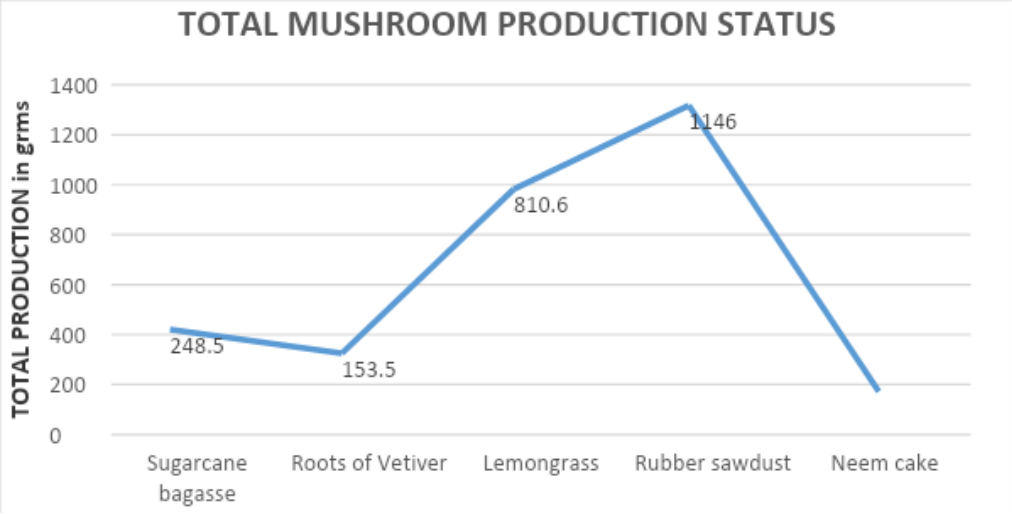
During cultivation, the fruiting body initiation was noticed on 26^{th} day, 28^{th} day, 33^{rd} day and 35^{th} day after bag preparation on lemongrass, roots of vetiver, sugarcane bagasse, rubber-sawdust respectively, and there was no fruiting body development on neem cake.

3.1 Mushroom production status

Among all substrate, rubber sawdust proved the best substrate for the effective cultivation of oyster mushroom [*Pleurotus florida* (Mont.) Singer]. There were no mycelial growth and fruiting body production, when neem cake used as a substrate.

Table 3.1 Total Mushroom Production Status

Sl. N0.	**Substrate used**	**Total production** (in grams)
1.	Sugarcane bagasse	248.5
2.	Roots of vetiver	153.5
3.	Lemongrass	810.6
4.	Rubber sawdust	1146
5.	Neem cake	Nil



4.2 Nutritional analysis

Table 4.2a_Mushroom with Lemongrass as substrate

Sl. N0.	**Parameters**	**Result**
1.	Energy	21 Kcal/100g

2.	Carbohydrates	3.5 g/100g
3.	Protein	3.5 g/100g
4.	Fat	BDL
5.	Fibre	0.7 g/100g
6.	Calcium	4.1 mg/100g
7.	Iron	0.9 mg/100g

Table 4.2b Mushroom with rubber sawdust as substrate

Sl. No.	Parameters	Result
1.	Energy	21 Kcal/100g
2.	Carbohydrates	3.6 g/100g
3.	Protein	3.4 g/100g
4.	Fat	BDL
5.	Fibre	0.8 g/100g
6.	Calcium	3.5 mg/100g
7.	Iron	0.6 mg/100g

BDL = Below Detectable Limit

4. Discussion

For the cultivation of mushrooms, a variety of substrates including Sugarcane bagasse, Vetiver roots, Lemongrass, Rubber sawdust, and Neemcake are used. The findings indicates that rubber sawdust produces the highest output, followed by lemongrass, sugarcane bagasse, and vetiver roots. Neemcake produces no yield, and no mycelial growth is seen in this. On nutrient analysis of mushrooms harvested from lemongrass and rubber sawdust, there were no much differences noticed. While they have roughly identical amounts of carbohydrate, energy and protein, samples from lemongrass were superior to those of rubber sawdust in the amount of calcium and iron.

Considering these results, my research demonstrates that rubber sawdust and lemongrass can both be used to cultivate mushrooms more successfully than other substrates. According to the production status, rubber sawdust yields the most, and the nutritional analysis shows, lemon grass produces results that are comparatively superior. The neem cake produces no yield, and the fruiting body from vetiver roots is comparatively small.

In my investigation, there was no mycelial growth in neem cake and no yield. Aqueous extracts of neem cake inhibit the growth of some fungi's mycelial spores [7]. This finding demonstrated that the antifungal action of neem cake, might be a reason for the inhibition of mycelial growth. Adding neem cake and citrus lemon to the substrate increased the yield of the *Pleurotus florida* and P. *ostreatus* oyster mushroom strains [5]. These results led to the conclusion that lowering the incidence of microbes in compost by using a concentrated form of a certain medicinal plant increases the yield of oyster mushrooms. Using neem extract in low amount in combination with other substrates may resist microbial attack and results in better production.

Oyster mushrooms grew best on sugarcane bagasse among several other substrates; whereas, in my experiment, lemongrass and rubber sawdust produced better results than sugarcane bagasse [6]. In my research, rubber sawdust and lemon grass show roughly equal nutritional qualities in the case of carbohydrate, energy and protein, with the calcium and iron contents being better in lemongrass than rubber sawdust. The effects of various sawdust substrates on *Pleurotus* growth, the best nutritional composition containing mushroom was grown on fig tree sawdust [1]. Results suggests that the nutrient composition of mushroom is dependent on the substrate in which they grow.

In order to determine the best substrate, oyster mushrooms were grown on a variety of substrates, including mustard straw, paddy straw, and sugarcane bagasse, at the Horticulture Demonstration and Training Center (HDTC). The quantity of primordial, fruiting bodies, and fresh weight or production of oyster mushrooms in cylindrical block systems were all significantly impacted by the different substrates. In all flushes, sugarcane bagasse produced the most primordial, fruiting bodies, and fresh weight while mustard straw produced the least of these [2]. In my experiment, less primordial and fruiting bodies are produced from sugarcane bagasse when compared to other substrates I used.

Rice straw gives higher yield when evaluating mushroom cultivation on different substrates [9]; [3] My findings shows that lemon grass is also a good substrate like rice straw, eventhough rubber

sawdust produces better yield. Hard wood sawdust produces mushrooms with more nutritional value whereas soft wood sawdust promotes better development [9]. My study demonstrates that rubber is a hard wood, and that rubber sawdust has a good yield and nutritional value.

The numerous lingo-cellulosic substrates used in mushroom growing can be easily recycled, and the wasted substrate from my work can be composted to make a high-quality fertiliser for outside plants. Some findings shows that the mushrooms, that transform low-quality waste streams into high-quality food, and the spent mushroom substrates has numerous applications, including compost, a growing medium for other fungi that produce mushrooms, animal feed, an improvement in animal health, the production of biofuels, and building and packaging materials [4] . The efficiency and sustainability of agricultural output can both be improved by this variety of uses.

5. Conclusions

The gathered substrates, such as sugarcane bagasse, lemongrass, vetiver roots, rubber sawdust, and neem cake were pasteurised using an autoclave by placing the substrates in a gunny sack. By using diluted dettol, the polythene bag was sterilised. For the spawn running and oyster mushroom production, the spawn and chosen substrates were filled in an organised and sequential fashion. By creating a pathway for watering, the humidity and temperature are maintained, which also helps to keep the environment aseptic. Statistics were applied to daily data on the weight of mushroom production on various substrates. Although rubber sawdust from the three-month project work produced the highest yield. The fact that diverse substrate wastes are recycled into vermicompost after mushroom harvesting has become interesting.

Oyster mushroom cultivation is often carried out by seasonal growers in a regular home without the installation of any environmental control equipment. It may grow on different agricultural waste materials both with and without fermentation. Although the cultivation process is quite straightforward, multiple technologies are employed to prepare the substrate and pasteurise or disinfect it. Additionally, the substrates that are employed in various locations vary. The availability of substrates at affordable prices in the specific location where oyster mushrooms are produced determines their characteristics. The substrates are frequently waste products of nature. As a result, there is no standard way to grow oyster mushrooms across the nation. Because we can use industrial and agricultural wastes such dried leaves, sawdust, sugarcane bagasse, tea powder, and paper scraps

for culture, growing mushrooms is less expensive. The poor farmers might embrace its high labour intensity, short duration, and ability to save land. To meet the demands of a balanced diet, they might step forward to raise edible mushrooms on a commercial scale as well as at home. It is a very nutrient-dense diet that contains different amounts of glucose, protein, fat, fibre, calcium, iron, and other minerals according on the substrate utilised. Due to the simple method of cultivation, low production costs, strong demand for their delectable taste, nutritious value, and improved market price, mushroom production can be a significant source of revenue. So that social entrepreneurs are likewise advised to do it. Nowadays, there is a lot of interest in oyster mushrooms in both academic and business circles. Mushroom cultivation becomes one of the most profitable agri business that may create nutrient-dense food from various substrates and aid in their environmentally friendly disposal.

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Anatomical and histochemical characterization of three endangered species in Fabaceae

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Abstract

Back ground: The study of plant tissues and cells is to to learn more about the way these plants are constructed and how they work. The anatomical and histochemical studies are helpful for taxonomic plant identification and phytochemical analysis. *Methods.* The present study deals with the Anatomical and histochemical characterization of three endangered species in the Westernghats of family Fabaceae– *Prioria pinnatum (*Roxb. Ex DC.) Breteler, *Humboltia bourdilloni* Prain *and Humboltia vahiliana Wight*'. These plants are critically endangered plant species of Western ghats in Kerala. Stem, leaves, rachis & pulvinus leaf base were characterized using anatomical and histochemical analysis. *Findings*: Macroscopic characterization of the selected species showed almost similar morphological charecters and some differences also. All species have compound leaves. Microcopic study revealed the presence of similar characters like uniseriate epidermis, amphivasal vascular bundle etc. Two species of Humboltia possess unicellular non-glandular trichomes in the stem. Sclerenchymatous bundle cap is present in all. Secretory glands are observed in the leaf bases of *Prioria pinnatum* and *Humboltia bourdilloni*. Transverse section of petiole of *P. pinnatum* and *H. vahiliana* showed the presence of a vascular bundle in the centre region of pith. All species have paracytic stomata. Presence of starch grains are observed in cortex medullary rays and pith in all species. Both Humboltia species have winged petiole. In histochemical view, these three species show similarities. Presence of starch, proteins, lipids, essential oils, phenols and Alkaloids are present. Starch granules are commonly found in all species.

Key words: Anatomy, Fabaceae, Endangered, Alkaloids, Phenols , Starch, Lipids

1. Introduction

Plants are an incredibly important kingdom of organisms. Plant anatomy is the study of plant tissues and cells in order to learn more about the way these organisms are constructed and how they

work. Fabaceae**,** also called Leguminosae**,** pea family of flowering plants (angiosperms), within the order Fabales. Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. Fabaceae has traditionally been divided into three subfamilies: Caesalpinioideae, Mimosoideae, and Faboideae (or Papilionoideae), each of which have been considered a separate plant family in the past. The subfamily Caesalpinioideae is a heterogeneous group of plants with about 160 genera and some 2,000 species. The selected three endangered plants are comes under the sub family–Caesalpinioideae[2].

Plant anatomy is also known as Phytotomy. Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve taxonomic problems and for the explanation of phylogenetic relationship. Anatomical evidence can be useful in systematic in several ways. When morphological characters prove to be of no help in the preliminary identification of herbarium material, anatomical study may prove to be helpful. Anatomical data has proved to be very useful in understanding evolutionary trends and interrelationship of taxa at and above the species level and at higher taxonomic categories. They are most useful in determining relationship between different genera, families, orders and other taxonomic categories. Plant anatomy provides characters such as trichomes, stomata, cuticular pattern, leaf venation, wood anatomy, growth rings etc. to aid in species identification and in performing physical matches of evidence. Plant anatomy can be important as a forensic tool in criminal investigations. The Knowledge of the preparation of plant fragments, the analysis of these fragments and the interpretation of the data obtained all must be part of forensic botany[9].

Histochemical analysis is essential for the study of plant secretory structures whose classification is based, at least partially, on the composition of their secretion. As each gland, may produce one or more types of substances, a correct analysis of its secretion should be done using various histochemical tests to detect metabolites of different chemical classes. Histochemical studies are used to confirm identification of cellular and tissue chemical components (secondary metabolites). Histochemical methods are employed in the identification, density of accumulation and distribution of chemical compounds within biological cells and tissues in different organs under microscopes using the colour stain reaction technique and photographic recording. These include the preparation of fixed variably stained specimens and then the examination under the microscopic devices. It is successfully applied in detection and localization of cellular components of active cell constituents such as proteins,

carbohydrates, lipids, nucleic acids, and a range of ionic elements occurring in the cell solutions, in addition to identifying the characterization of secretory structures and the chemical nature of the secreted compounds. Histochemical analysis reveals the presence of alkaloids, phenols and proteins in epidermis, cortex and vascular bundles of root, stem and leaf [6]. This work intended to full the 'Anatomical and histochemical characterization of three endangered species in the Westernghats of family Fabaceae– *Prioria pinnatum (*Roxb. Ex DC.) Breteler, *Humboltia bourdilloni* Prain *and Humboltia vahiliana Wight*'.

2. Materials and methods

Present study aims to compare anatomical and histochemical features of three plants belongs to the family fabaceae, *Prioria pinnatum (*Roxb. Ex DC.) Breteler, *Humboltia bourdilloni* Prain *and Humboltia vahiliana Wight*' collected respectively from Nadugani & Kulamavu- Idukki district and Vazhachal from its natural habitat. These plants are critically endangered plant species of Western ghats in Kerala. Stem, leaves, rachis & pulvinus leaf base were characterized using anatomical and histochemical analysis. The materials and methods used for the study are mentioned below;

2.1 Plant materials

1. ***Prioria pinnatum* (Roxb. Ex DC.) Breteler**

It is an endemic and endangered tree of the Southern Western ghats, belonging to the family Leguminosae and subfamily Caesalpinioideae. It carried out in the area of the Western Ghats. Seventeen populatons of *P. pinnatum* were Identified in 13 forest locatons of the Kerala area of Western Ghats. *P. pinnatum* is listed as an endangered plant species under IUCN red listed plants.Flowers very small, numerous, in panicles of racemes. *P. pinnatum* known as the 'Malabar mahogany', a vulnerable and endangered medicinal plant is used in curing sores of elephants. The oleo-gum-resin of this plant species is used in gonorrhoea and catarrhal conditions of genito-urinary and respiratory tracts. Mainly overexploitation leads to the extinction of the species.

2. ***Humboldtia bourdillonii* Prain**

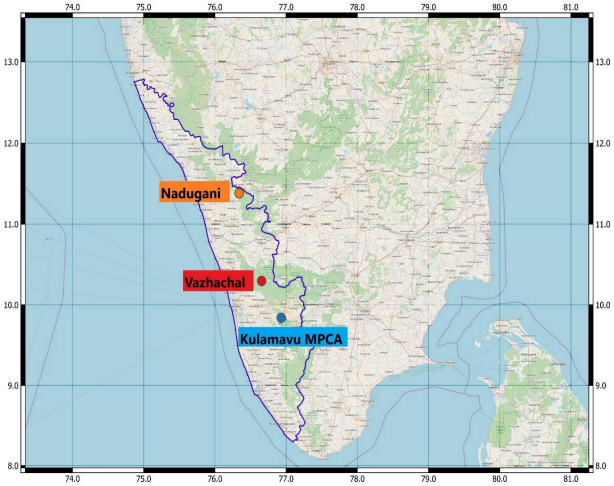
Humboldtia bourdillonii is reported from the Southern Western Ghats, India. But further information on its population and distribution is lacking and thus the species gained the 'endangered' status of IUCN. The study revealed that *H. bourdillonii* occurs in tropical wet evergreen forest from 450 – 800 m altitude as growing along and away from water courses in the

Arjunan Kotta and Poonkavanam forest of southern Western ghats. The species has a discrete distribution, found in seven patches, with an area of occupancy of 0.06 Km^2 and area of occurrence of approximately 2 Km^2.

***3. <u>Humboldtia</u> <u>vahliana</u>* Wight**

It is an endemic plant of Southern Western Ghats, Family: Fabaceae/leguminosae (subfamily: Caesalpinioideae) was carried out in wild conditions.

2.2 Study area



Nadukani is located on the Calicut- Nilambur-gudalur road 20 km north east of Nilambur town. *Prioria pinnatum* were collected from this location. Kulamavu is best known for being one of the three dams of the Idukki reservoir project. It constructed in 1961 to restrict the flow of water into Kallivally rivulet. Study material, *Humboldtia bourdillonii* were collected from this location. Vazhachal Forest stretches from Anaimalai to Athirapilli Falls (60 km) in Tamil Nadu, Kerala border. These forests are situated on the western slope of the Western Ghats and therefore receive copious amount of rainfall. The rest of the area is under various *Humboldtia Vahliana* were collected from this place.

<u>2.3 Stains used for histochemical studies</u>

<u>Lugol's reagent</u>

For examining the presence of starch grains. A solution composed of iodine and potassium iodide. Dissolve KI in about 20-30 ml of distilled water. Add iodine and heat gently with constant mixing until iodine is dissolved. Dilute to 100 ml with distilled water.

Coomassie blue

For examining the presence of proteins. To prepare this solution, dissolve 2g Coomassie Blue (Serva Blau) in 250ml water and slowly add 75ml of glacial acetic acid. Add 500ml of ethanol and makeup to 1000 ml with water.

Sudan black

To test the presence of lipids. It is made by preparing a staining solution of 500 mg Sudan Black B in 20 ml of acetone. This is added to 15 ml of acetic acid, and then added to 85 ml of water. Stir the mixture for 30 minutes and centrifuge to remove the precipitate.

NADI solution

For examining the presence of essential oils. This solution is made by dissolving 1% alpha-naphthol in 95% ethanol (solution 1). Solution 2 is made by adding 1% N, N-dimethyl-p-phenylenediamine HCl in water. Solutions 1 and 2 are mixed in equal volumes just prior to use.

Ferric chloride

For examining the presence of phenolic compounds. Dissolve 5 g of ferric chloride in 100 ml of water.

Wagner's reagent

To test the presence of alkaloids. To prepare this reagent 2 g iodine 6 g of potassium iodide (KI) in 100 ml of water to produce solution.

Glycerin gelatin

It is used as the mounting medium.

2.4 Stains used for permanent slide preparation

Safranin, Alcohol, Fast green, Clove oil , Xylene, Dibutylphthalate polystyrene Xylene (DPX)

2.5 Materials used in the maceration

Nitric acid (HNO_3), Test tube, Test tube holder, Spirit lamb

2.6 Equipments used

Blade, Microscope slides, Coverslip, Cavity block, Compound microscope (10-100x)

Steriomicroscope (Steriomicroscope (Olympus) with camera attachment is used. It helps to view sections at lowest magnification and gradually increase magnification to closely examine macroscopic details of the sample).

2.7 Methods of study

Study was conducted during the period of March – June 2022 at the Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri. Plant materials were collected from natural habitats of Idukki, Trissur (Kerala) and Nilgiris (Tamilnadu).

Microscopic or anatomical evaluation

Thin transverse sections of the fresh stems, leaves, petiole and pulvinous leaf bases were taken by hand. The microscopical evaluation of sections was carried out to confirm the structural details of the material. For effective results permanent slide preparation were also done. Chemical constituents of plants were also evaluated by applying the histochemical stains on small quantities.

Histochemical staining procedures.

In order to estimate the presence of various cell inclusions like Starch grains, proteins, lipids, essential oils, phenolic compounds and alkaloids. The following methods were followed.

Test for Starch:

To examine the presence of Starch

- Sections were Submerge in the Lugol's reagent for 10 min.
- Rinse briefly with distilled water.
- Mount the slides using distilled water or Lugol's reagent itself.
- This reaction highlights the starch grains in dark blue to black.

Test for Proteins

To examine the presence of proteins

- Stain the sections in 0.25% Coomassie blue for 15 min.
- Differentiate in 7% acetic acid.
- Rinse briefly in distilled water.

- Mount in glyceringelatin.
- It stains proteins blue.

Test for Lipids

To examine the presence of lipids

- Stain the sections with Sudan black B for 20 min.
- Rinse briefly in 70% ethanol.
- Wash in distilled water.
- Mount in glyceringelatin.
- It stains lipids dark blue to black.

Test for Essential oils

- To examine the presence of essential oils, Apply NADI reagent for 1 h in the dark.
- Wash in sodium phosphate buffer (0.1 M, pH 7.2) for 2 min.
- Mount in the same buffer.
- This reagent produces differential staining, with essential oils staining blue and resins staining red.

Test for Phenolic compounds

For examining the presence of phenolic compounds,

- Apply the sections with10% ferric chloride for 30 min.
- Wash twice in distilled water to remove surplus ferric chloride.
- Mount in glyceringelatin. This method highlights phenolic compounds through iron precipitation, producing a dark color , usually black , sometimes brown.

Test for Alkaloids

For examining the presence of Alkaloids,

- Apply Wagner's reagent for 20 min.
- Rinse briefly in distilled water. Mount in distilled water.
- This method stains alkaloids red or red brown .

Maceration

Small pieces of wood of the plant materials are heated with concentrated nitric acid in a test tube with the help of a test tube holder. The complete volume should not be larger than 1/10 of the reaction container, because a lot of gas develops very quickly at cautious heating. The surfaces of the wooden pieces are strongly attacked. The single cells can be scraped off after washing of the preparation and examined under the microscope.

3.RESULTS

In the present study, it is aimed to study the microscopic features of three members of the family, Fabaceae – *Kingiodendron pinnatum, Humboldtia bourdillonii And Humboldtia Vahliana*, collected from their natural habitat. The stem, leaves, petiole and leaf bases are selected for the anatomical and histochemical studies. This study also includes the study of stomatal index and tracheids by maceration. The results obtained can be summarised as follows.

***1.Prioria pinnatum* (Roxb. Ex DC.) Breteler**

Stem cylindrical 4 to 8 mm diameter, internodes being 4.5 to 5 cm in length, nodes are prominent, bark 5-8 mm thick, surface greyish-brown with green blotches, rough, exuding a reddish sticky resin. The outline of the transverse section is almost circular. Periderm is composed of phellem, phellogen and phelloderm. The cells of the phellem are rectangular, thickly suberized and dead. A few lenticels also occur in the phellem. There is no hairs or trichomes present in the stem. Cortex is undifferentiated and wholly parenchymatous, the cells are filled with starch. Small intercellular spaces are present. Secretory glands are present in the cortex. Vascular bundles are arranged in a ring, secondary growth is present. Vascular bundle is conjoint, collateral, endarch and open. Secondary xylem is grooved at four places. Secondary phloem present above the secondary xylem. A few phloem cells are thick walled. These are called bast or phloem fibers. A well defined parenchymatous pith present in the centre. Prismatic crystals present in the pith region. They are calcium oxalate Crystals**.** Presence of Starch grains observed in the Stem.

The transverse section of the lesf shows central bulged region and lateral wings

Lamina is dorsiventral with palisade and spongy cells.

Epidermis is bounded by thin cuticularised upper and lower epidermis. The upper epidermis is uniseriate thick walled cells with cuticle. The lower epidermis is made up of uniseriate row of rectangular cells and contain paracytic stomata. The outline of the lower epidermis is angular. Epidermis is followed 4-5

layered mesophyll differentiated into palisade and spongy tissue. Palisade cells contain a large number of chloroplasts which is meant for photosynthesis and spongy tissues are arranged below the palisade cells with large intercellular spaces. Vascular bundles are almost parallel series. Vascular bundles are surrounded by a thick layer of sclerenchymatous cells. Vascular bundles are endarch. Vascular bundles are also seen in the wings of leaf.

Petiole round, 5-10 mm, stout, grooved above, glabrous, lateral nerves 8-13, pinnate, slender, prominent.The outline of the transverse section is almost circular**.** Detailed TS shows a single layered epidermis with elongated compact parenchymatous cells. Trichomes absent. Just below the epidermis, there is 6-7 layered chlorenchymatous hypodermis and few layered parenchymatous tissues. Prismatic crystals present in the hypodermis. Vascular bundles are arranged in a concentric ring and they form additional bundles with in the central region. In the outer vascular bundles xylem is endarch,closed .Central vascular bundles is amphivasal vascular bundle . In Pulvinous leaf base Epidermis is uniseriate parenchymatous cells.Cortex is undifferentiated and wholly parenchymatous, the cells are filled with starch. Small intercellular spaces are present. Secretory glands are present in the cortex.Vascular bundles endarch, conjoint and crescent shaped with leaf traces. Vascular bundles are surrounded by a thick layer of Sclerenchymatous cells. Vascular bundles are endarch.

Table 1- Comparison between two species of *Humboldtia– Humboldtia bourdillonii and Humboldtia vahliana*

Characters		***Humboldtia bourdillonii***	***Humboldtia vahliana***
Stem	Epidermis	Diagrammatic view of the T.S. has a wavy outline with four pronged sides.	Diagrammatic view of the T.S. has a wavy outline with four pronged sides
		Trichomes present	Trichomes absent
	Hypodermis	2-3layered sclerenchymatous cells present	2-3 layered sclerenchymatous cells present
		Secretory glands absent	Secretory glands present
		Sclerenchyma fibres present	Sclerenchyma fibers present

	Vascular bundle	Conjoint,collateral and open	Conjoint**,** collateral and open
	Pith	Large pith made of thin walled parenchymatous cells	Large pith made of thin walled parenchymatous cells
Leaf	**Epidermis**	Formed of single layer of square cells	Formed of single layer of square cells
		Paracytic stomata present on the lower epidermis	Paracytic stomata present on the lower epidermis
	Hypodermis	Palisade cells 2 layered	Palisade cells 2 layered
		2-3layered sclerenchymatous cells present	3-4 layered sclerenchymatous cells present
	Vascular bundle	Crescent shaped	Crescent shaped
		Amphivasal	Amphivasal
Petiole	**Epidermis**	Uniseriate epidermis	Uniseriate epidermis
	Hypodermis	Parenchymatous 5-6 layers.	Parenchymatous and sclerenchymatous
		2- 3 layered Sclerenchymatous cells present	2- 3 layered Sclerenchymatous cells present
	Vascular bundle	Crescent shaped	Arranged in a wavy ring
		Amphivasal	Amphivasal
	Pith	Large	Large, also contains an amphivasal vascular bundle smaller than outer bundle
Leaf base	**Epidermis**	Uniseriate epidermis	Uniseriate epidermis

	Cortex	Wide & consists of parenchymatous soft tissues	Wide & consists of parenchymatous soft tissues
		Secretary glands absent	Secretary glands present
	Vascular bundle	Conjoint, collateral amphiphloic	Conjoint, collateral amphiphloic
		Crescent shaped	Crescent shaped

3.2 Histochemical analysis

Histochemical analysis like the presence of starch, proteins, lipids, essential oils, phenols and alkaloids detected in this study. Findings are concluded as following:

Table 2- Histochemical analysis of *Kingiodendron pinnatum*

	Stem	Leaf	Petiole	Leaf base
Starch	✓	✓	✓	✓
Proteins	✓	✓	✓	✓
Lipids	×	✓	✓	✓
Essential oils/resins	✓	✓	✓	✓
Phenols	✓	✓	✓	✓
Alkaloids	✓	✓	×	×

Table 3- Histochemical analysis of *Humboldtia bourdillonii*

	Stem	Leaf	Petiole	Leaf base
Starch	✓	✓	✓	✓
Proteins	✓	✓	✓	✓
Lipids	✓	✓	×	✓
Essential oils/resins	✓	✓	✓	✓
Phenols	✓	✓	✓	✓
Alkaloids	✓	✓	✓	✓

Table 4- Histochemical analysis of *Humboldtia Vahliana*

	Stem	Leaf	Petiole	Leaf base
Starch	✓	×	✓	×
Proteins	✓	✓	✓	✓
Lipids	✓	✓	✓	✓
Essential oil/resins	✓	✓	✓	✓
Phenols	✓	✓	✓	✓
Alkaloids	×	×	✓	×

STOMATAL INDEX

Stomatal index of the leaf is the ratio of the number of stomata to the total number of stomata and epidermal cells.

$$\text{Stomatal index} = \frac{\text{No. of stomata cells per unit area}}{\text{No. of epidermal cells per unit area}}$$

Prioria pinnatum

	No. of stomata	No. of epidermal cells	Stomatal Index (%)
1	7	33	17.5
2	9	36	20
3	8	35	19.04
4	7	25	21.9

$$\text{Stomatal index} = \frac{17.5+20+19.04+21.9}{4} = 19.61$$

Humboldtia bourdillonii

	No. of stomata	No. of epidermal cells	Stomatal Index (%)

1	11	62	15.1
2	12	60	16.7
3	11	65	14.4
4	14	70	16.6

Stomatal index = 15.1+16.7+14.4+16.6 = 15.7

4

Humboldtia vahliana

	No. of stomata	No. of epidermal cells	Stomatal Index (%)
1	13	51	20.32
2	9	64	12.32
3	11	60	15.4
4	13	59	18.05

Stomatal index= 20.32+12.32+15.4+18.05 = 16.5

4

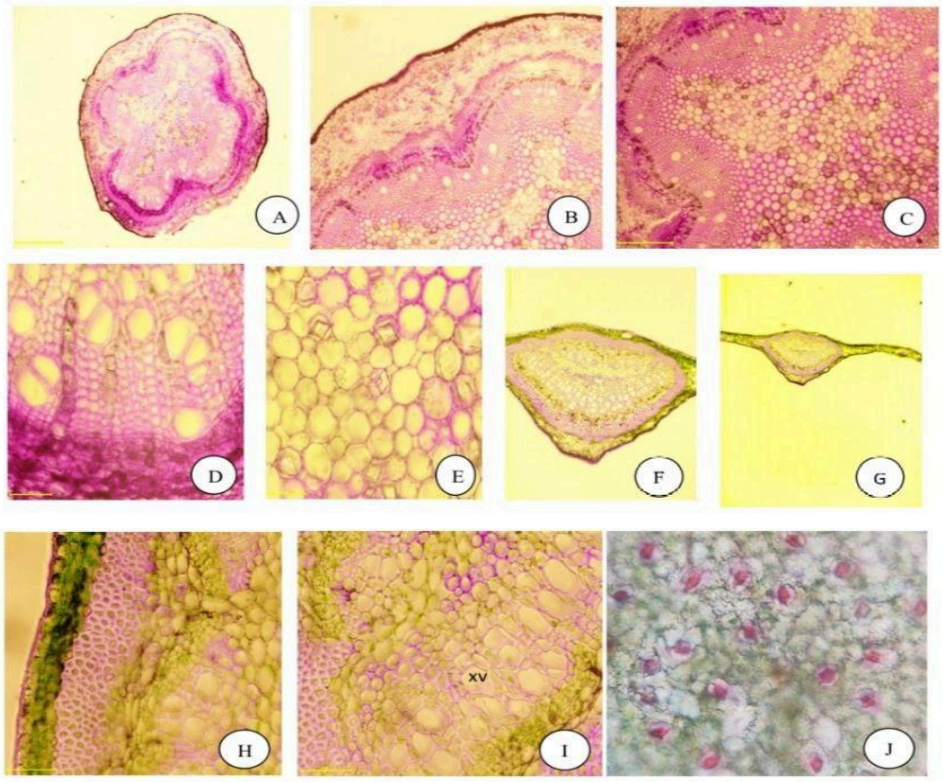


Plate – 4: Fig (A-J) Microscopy of *Prioria pinnatum (*Roxb. Ex DC.) Breteler

(A) T. S. of the entire stem, (B) Portion enlarged, composed of phellum, phellogen and phelloderm, (C) T. S. showing pith region, (D) T. S showing xylem regions. Vascular bundles are arranged in a ring, (E) Detailed T. S. showing calcium oxalate crystals in the pith, (F) T. S. of leaf in the midrib region enlarged, (G) T. S. showing midrib region, (H) T. S. showing outer portion of leaf, (I) Xylem region (xv-xylem vessels), (J) Lower epidermal peeling showing paracytic stomata.

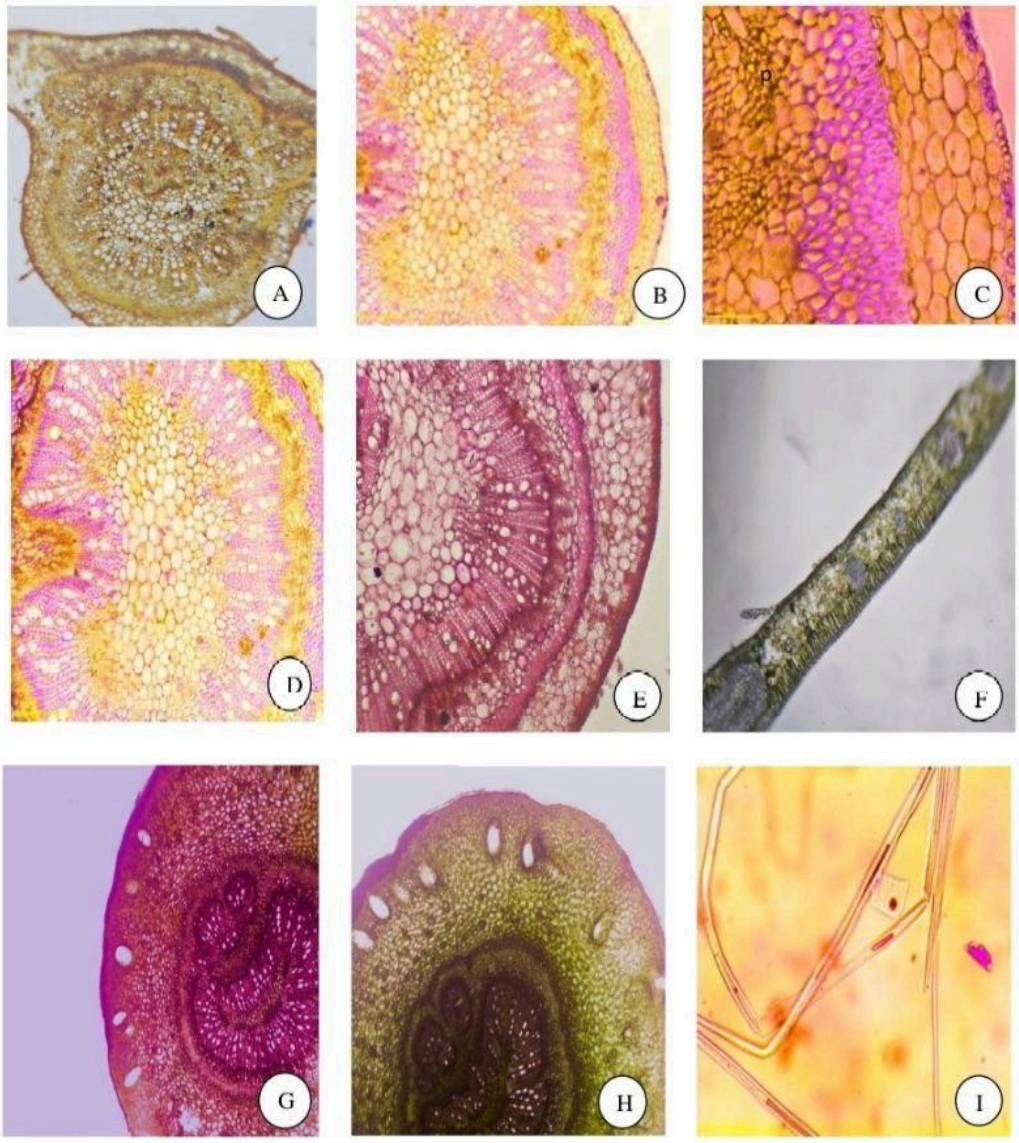


Plate – 7: Fig (A-I) Microscopy of *Humboldtia bourdillonia* prain.

(A) T. S. of entire petiole, (B& C) T. S. showing portion enlarged (P-Phloem), (D) T. S. showing pith region (E) T. S. showing enlarged outer portion, (F) T. S. showing lamina region of the petiole, (G) T. S. Showing leaf base, (H) T. S. showing leaf base outer and inner regions, (I) Tracheids.

5.Discussion

The present study provides macroscopic, microscopic and histochemical characterization of the stem, leaves, petiole and leaf base of *Prioria pinnatum, Humboldtia bourdillonii & Humboldtia Vahliana* belongs to the family fabaceae. There is no evidences for the regarding studies.The macroscopic study of the stem, leaves, people and leaf base showed similarities in their appearances. Since all the three species belong to the same family, fabaceae, are almost same. Stem Morphology is same in the two species of Humboldtia . But it is different in case of P. pinnatum, which is cylindrical in structure.In case of leaves, all species has compound leaves of paripinnate, slightly glabrous, having pulvinus leaf base.The petiole of the both humboldtia species is winged. Anatomical characterization revealed that the basic structure of stem, leaves, petiole and leaf base of the selected plants are almost. There are certain differences also seen. Diagrammatic T. S of the stem of the *P. pinnatum* showed circular outline with distinct epidermis, hypodermis, cortex,Stele and pith in the centre. In *H. bourdillonii* & *H.vahiliana* , the outline have a wavy nature with four- grooved sides. All species show uniseriate epidermis of thick walled cells. Cells are squarish to rectangular in shape. High variation is seen in trichome structure. It is absent in *P.pinnatum* and *H.vahiliana, H. bourdillonii* possess unicellular, non- glandular trichomes on its epidermis. Xylem fibers are present in the stem of Humboldtia species. Oil glands are present in the hypodermis region of stem of the H.vahiliana. Xylem region consists of radially arranged vessels of different sizes,fibres and alternating uniseriate medullary rays in all species. Microscopic structure of leaves of all Species are almost same. Comparison of leaves shows uniseriate upper and lower epidermis present in all species which are made up of thick walled cells. Trichomes are absent in all species. All three species show paracytic stomata in the lower epidermis. Upper epidermis is devoid of stomata. In *P. pinnatum* epidermis is followed by 3-4 layer of chlorenchymatous. sclerenchymatous bundle cap is present in all species. Several radial rows of xylem arranged in a crescent shape. Collenchymatous cells are present in the Midrib region. Vascular bundles are conjoint collateral and Amphivasal. Small vascular bundles are present in the leafy region. Microscopic comparison of petiole shows high variation in diagrammatic level. The petiole of both *humboldtia* species is winged, but that of *P. pinnatum* is not winged. The petiole of three species having uniseriate epidermis. *P.pinnatum* have cuticle covering. Following the epidermis, *P.pinnatum* have 7-8

layer of chlorenchymatous cells, *H. bourdillonii* have 5-6 layer of parenchymatous tissues and *H. Vahliana* have parenchymatous and chlorenchymatous cells. Sclerenchymatous bundle cap is present in all the three species. Vascular bundle of *P. pinnatum* is arranged in a ring. The pith region also contain a vascular bundle of the same pattern but small. Vascular bundle of *H. bourdilloni* is crescent shaped and Amphivasal. Pith large.*H. vahiliana* has Vascular bundle arranged in a wavy ring structure.pith also contain Amphivasal vascular smaller than surrounding bundle.In the leafy region vascular bundles are arranged at regular intervals surrounded by sclerenchymatous tissue. Microscopic comparison of leaf bases shows almost same structure. All species have pulvinus leaf base. Epidermis is uniseriate parenchymatous in all species. Cortex is undifferentiated made up of parenchymatous soft tissues. Cortex of *P.pinnatum* & *H.valiliana* contains secretary glands.it is absent in *H.bourdilloni.* Vascular bundle of *P. Pinnatum* is endarch, conjoint, surrounded by thick layer of parenchymatous cells. Vascular bundle of H. *bourdillonii* is conjoint, collateral and amphiploic and that of *H. vahiliana* is crescent shaped, it is surrounded by sclerenchymatous fibres[7] . Histochemical analysis reveals the presence of starch, proteins, lipids, essential oils, phenols and Alkaloids in the stem, leaves, petiole and leaf base. *P. pinnatum* contains starch grains in stem, Leaf, petiole and leaf base in the hypodermis and cortical regions. Presence of proteins also confirmed lipids present in the vascular region of leaf, petiole and leaf base. It is absent in the stem. Presence of essential oil is examined by NADI test. It is present in the almost all regions of the stem, leaf, petiole and leaf base. Phenols present in all sections of *P. pinnatum* [4]. Alkaloids present in the stem and leaf in cortex and vascular regions. It is absent in petiole and leaf base[4]. In *Humboldtia bourdillonii*, starch and proteins are present in stem, leaf, petiole and leaf base at random regions. Lipid is absent in petiole, but present in the vascular region of the stem, leaf and leaf base. The presence of essential oils, phenols and alkaloids are confirmed in stem, leaf, petiole and leaf base of *H. bourdillonii* [3]. In case of *Humboldtia vahliana*, Starch grains are present in the stem and petiole, absent in the leaf and leaf base. Starch granules mainly seen in the pith region. Protein is present in the all regions of *H. vahiliana* [11]. Lipids, essential oils, phenols are present in all among them in variable regions. Alkaloids are detected only in the petiole.it is absent in the other parts[8]. Stomatal index is the ratio of the number of epidermal cells to the number of stomata in a given area of a leaf. The three species have high stomatal index. High stomatal index correlate with a higher net rate of photosynthesis [10]. *P. pinnatum* shows higher stomatal index as compred to *Humboltia vahiliana* and *Humboltia bourdillonii.* Anatomical characters are conserved and stable and thus can be used as a taxonomic character for plant taxonomy. Anatomical features can be used in taxonomy for the

identification of plants, establishing genetic relationships and solving taxonomic disputes. All the three members possess paracytic stomata in their leaves. Paracytic stomata have one or more subsidiary cells parallel to the opening between the guard cells. All species have uniseriate epidermis, paracytic stomata on the abaxial surface.

5. **Conclusion**

This is the first attempt to compare the microscopy and histochemistry of *Prioria pinnatum, Humboltia bourdilloni & Humboltia vahiliana.* Macroscopic characterization of the selected species showed almost similar morphological characters and some differences also. In histochemical view, these three species show similarities. Starch granules are commonly found in all species. From the study it is clear that the basic structure is same in the stem, leaves, petiole and leafbase of *Prioria pinnatum, Humboltia bourdilloni and Humboltia vahiliana.* The minor differences observed can be taken as diagnostic characters for the identification of the selected species.

6.Acknowledgement

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Study on allelopathic effects of some selected native plant species of Kerala against the weed Ipomoea obscura using seed germination analysis and GC-MS

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Abstract

Background: Weeds are described as plants with capacity to significantly infiltrate damaged or intentionally cultivated ecosystems inhabited by humans and to suppress or replace native plant populations or plants intentionally maintained for their commercial, aesthetic or ecological value. Allelopathy is a natural and environmentally beneficial method for controlling weeds that also boosts crop yields, lessens our reliance on artificial pesticides and enhances the ecological environment. Allelochemicals selectively prevent the occurrence of competitive species, such as soil microbes or other plants in their surroundings. **Methods:** The present research has been focused to evaluate the allelopathic potential of methanolic leaf extract of plants viz., *Macaranga peltata, Garcinia cambogia, Bauhinia acuminata, Averrhoa bilimbi* and *Ficus auriculata* on the germination percentage and plant growth characters of weed *Ipomoea obscura* (L.) Ker-Gawl both in laboratory condition (*in vitro*) and in soil (*in vivo*).The germination percentage and growth parameters viz, shoot length, root length and number of leaves were tested in *Ipomoea obscura* by the methanolic leaf extract of the selected plants. To determine the allelochemicals present in *Garcinia cambogia*, using Gas chromatography Mass Spectrometry analysis of leaf extract was performed. **Findings:** The present study revealed that leaves were found to allelopathic and decreased the germination percentage and the growth. The present study revealed that L- lysine which is a phenolic allelochemical present in *Garcinia cambogia.*

Keywords: *Allelopathy, Garcinia cambogia, Weed, Ipomoea obscura, GC-MS*

1.Introduction

Weeds are described as plants with capacity to significantly infiltrate damaged or intentionally cultivated ecosystems inhabited by humans and to suppress or replace native plant populations or plants

intentionally maintained for their commercial, aesthetic or ecological value (1). The prevalence of weeds in agricultural systems lowers the production of many crops (2). Typically, mechanical means and synthetic herbicides are used to control them. Meanwhile, mechanical methods require a lot of time and effort, and the use of herbicides not only increases environmental pollution, but also has perceived negative effects on agricultural output (3). The danger of weed resistance emerging and the high cost-benefit ratio are additional drawbacks of using synthetic herbicides and insecticides (4). In order to control weeds in agricultural systems, novel strategies such plant allelopathic effects have been investigated recently (5). It is now well acknowledged that allelopathy is essential for weed control and crop productivity in nature (6).

Allelopathy is a natural and environmentally beneficial method for controlling weeds that also boosts crop yields, lessens our reliance on artificial pesticides and enhances the ecological environment. There have been attempts to use a variety of plants that are said to have allelopathic potential to suppress weeds. The Greek terms “Allelon”, which means “of each other”, and “Pathos”, which means “to endure” or “suffering”, are the source of the word allelopathy. When a plant species interferes chemically with other plants in settings other than nutritional emergencies, it denotes the harmful effects of one plant species on another (7). According to the release of chemicals (allelochemicals) from plant parts through leaching, root exudation, volatilization, residue composition and other processes in both natural and agricultural systems, allelopathy refers to the either beneficial or harmful effect of one plant upon another, both crop and weed species. It was initially thoroughly researched in forestry systems. Allelopathy also has a significant effect on a number of other areas of plant ecology, including as occurrence, plant succession, community structure, dominance, diversity, and plant productivity. Other edaphic, external factors, such as physiological and environmental stresses, pests and diseases, sun radiation, herbicides and less-than-ideal nutrition and moisture levels, etc., may have an impact on weed suppression. Rice (1984) defined allelopathy as ‘any direct or indirect harmful or helpful influence by one plant (including microbes) on another through synthesis of chemical substances that escape into the environment’. Both stimulatory and inhibitory biochemical interactions are present in this process (8).

Allelochemicals selectively prevent the occurrence of competitive species, such as soil microbes or other plants in their surroundings (9). Allelochemicals work by preventing the germination of other plants or harming their shoot and root development. They may also be harmful to cells (10). These substances, which also include natural herbicides, phytoalexins and seed germination inhibitors play a

part in the chemical conflict between plants (allelopathic interaction) (11). Allelochemicals, or inhibitory substances are released into the environment where they have an impact on the growth and development of nearby plants. In both natural and agricultural systems, the allelochemicals are located in plant parts such leaves, flowers, roots, stems, rhizomes and seeds from which they are released into the soil by volatilization, root exudation, leaching and breakdown of plant wastes (12).

Hence we aimed to evaluate allelopathic potential of methanolic leaf extract of plants viz., *Macaranga peltata, Garcinia cambogia, Bauhinia acuminata, Averrhoa bilimbi* and *Ficus auriculata* on the germination percentage and plant growth characters of weed *Ipomoea obscura* (L.) Ker-Gawl both in laboratory condition (*in vitro*) and in soil (*in vivo*) and also to determine the allelochemicals present in *Garcinia cambogia.*

2. Materials and methods

2.1. Allelopathic assay of weed

2.1.1. Selection of seeds

The viable, healthy and uniform seeds of weed *Ipomoea obscura* were acquired from KAHM Unity Women's College, Manjeri, Malappuram, Kerala (GPS data: 11° 7' 13.0728" N and 76° 7' 11.8848" E.).

2.1.2. Collection of plant materials

Five different plants were selected to test allelopathic effect against weed *Ipomoea obscura*. Plants viz. *Macaranga peltata, Garcinia cambogia, Bauhinia acuminata, Averrhoa bilimbi* and *Ficus auriculata*. Among the plants *Garcini*a *cambogia* and *Averrhoa bilimbi* leaves were collected on 2022 March 8 from area Parappanangadi, Malappuram, Kerala. The plants *Macaranga peltata, Bauhinia acuminata* and *Ficus auriculata* leaves were acquired from KAHM Unity Women's College campus, Narukara, Malappuram, Kerala. Just after procurement, fresh leaves of the plants were washed under running tap water and air dried. The dried plant material was powdered in a mechanical blender (Panasonic MX-AC 300-HI) and the powder was kept in an air-tight container for use in the study.

2.1.3. Preparation of extract

The powdered plant material weighed 20 g using weighing balance (CAS -CAY 220) and were extracted with methanol solvent (250 ml) by using Soxhlet extraction apparatus (KEMI) by continuous heat extraction. The extract was filtered and concentrated to dryness by evaporating the solvent under reduced pressure. The dry extract was kept in a refrigerator (Whirlpool) for further use. Different concentrations of leaf extract such as 5, 10, 15, and 20 % were prepared.

2.1.4. Bioassay

(i) Under Laboratory Condition *(in vitro*)

The seed germination trial was performed in petri dishes (Borosil). Uniform, healthy and viable seeds of plant under test were collected. They were surface cleaned and subjected to germination trial in response to the treatments. Sterilized petri dish was lined with a filter paper above absorbent cotton. For this purpose, this was spread evenly on the surface and saturated with the specific concentration. Ten seeds of *Ipomoea obsura* were placed in a spread over moist cotton kept in petri dishes (15 cm diameter). The treatments were replicated 3 times and 3 replicates of control treatment with distilled water were also prepared. The petri dishes were kept under natural light dark cycle. The whole set up was placed in a laboratory maintained at 27± 2° C temperature, relative humidity of 25-28 % and continuous light for day period. The seed were observed every day and numbers of germinated seeds were recorded. Distilled water was added just to moisten the seed when required. The experiment was conducted in Completely Randomized Block Design (CRBD) which includes five treatments and one control for each crop seeds. For each experiment ten seeds were sown, the experiment was replicated at three times and performed from March to April 2017.

The emergence of the radical from the seed was regarded as germinated. Every day each petri dish was carefully observed for the emergence of the radical of each seed were made with help of hand lens and observation continued for 21 days of germination, number of plants emerged were counted. Seedlings were carefully uprooted and their shoot length, root length, leaf area, number of leaves and number of roots were determined.

(ii) Under soil (*in vivo*)

Sterilized sand maintained at laboratory used as substrate for germination and growth of the target species. Ten healthy and viable seeds of weed plant were sown at in each rounded paper cups containing well sterilized sand. Extract solution was added to each cup while distilled water was used as

control. Under the laboratory conditions the experiment was maintained in Completely Randomized Block Design (CRBD) with three replicates and performed from April to May 2022. Adequately watered daily. After 21 days of germination, Number of plants emerged were counted. Seedlings were carefully uprooted and their shoot length, root length, number of leaves and number of roots were determined.

2.1.5. Determination of germination percentage

The seeds were placed in petri dishes and paper cups wetted with methanolic leaf extract of different plants used in this study in different concentrations (5 %, 10%, 15 %, 20 % and with distilled water as control to allow germination. Care was taken to avoid drying of filter paper and sterilized sand. The number of seeds germinated in each day was noted and the percentage of germination was calculated.

2.1.6. Determination of shoot length, root length and number of leaves

The length of shoot and root was measured using a graduated scale and was expressed in centimeter. And leaf area was also calculated.

2.1.7. Statistical analysis

Germination percentage were calculated by the following formula.

$$\text{Germination percentage} = \quad \times 100$$

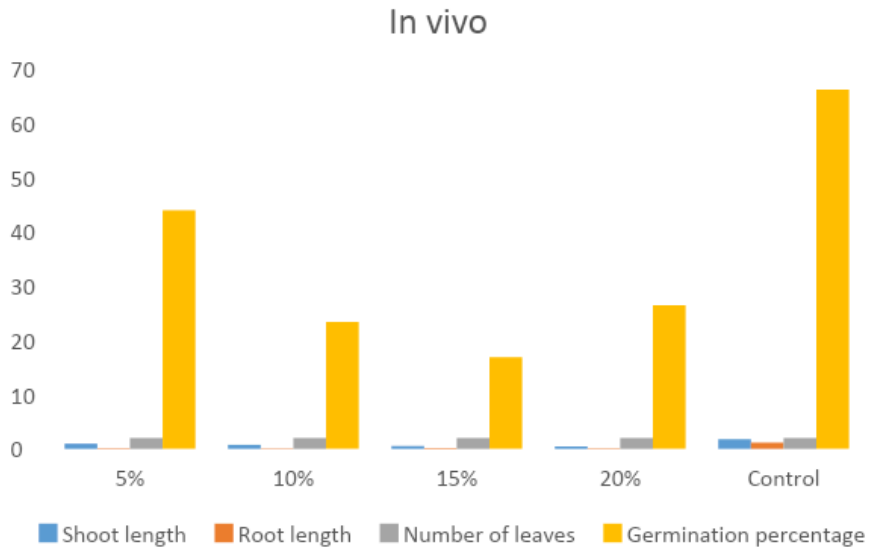
2.2. Profiling of Bioactive Components by GC-MS

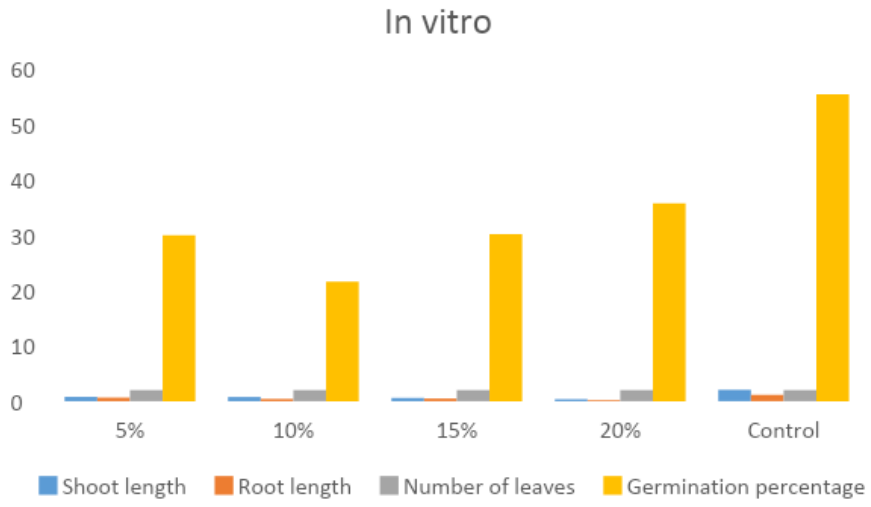
Ten grams of shade dried, powdered leaf tissues of *Garcinia cambogia* were packed in thimbles and subjected to extraction using various solvents with the help of Soxhlet apparatus. The extract obtained was used for further analysis. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of leaf extracts was performed using Thermo Scientific Trace 1300 Gas chromatograph with TG- 5MS Column (30 m x 0.25 mm ID x 0.25µM) interfaced to an ISQ-QD Mass Spectrophotometer (Perkin-Elmer GC Clarus 500 system)

3. Results

3.1. Allelopathic studies in *Macaranga peltata*

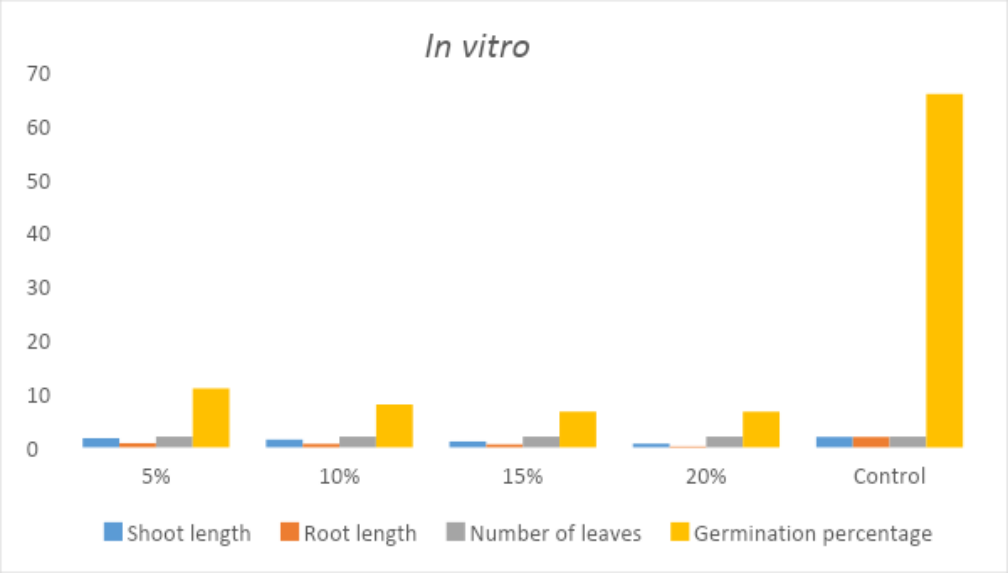
The results presented in table 1 and 2 clearly showed the effects of *Macaranga peltata* on growth parameters and germination percentage of the weed *Ipomoea obscura*. The inhibitory effects of the methanol extract of *Macaranga peltata* leaves was found to depend on the extract concentration (5%, 10%, 15%, 20%) of leaf extract of *Macaranga peltata* was generally found to be allelopathic and decreased the germination percentage of the same weed. As the increasing concentration of leaf extract, it decreased the germination percentage and growth parameters of the weed as compared to the control both in *in vitro* and *in vivo* treatments.

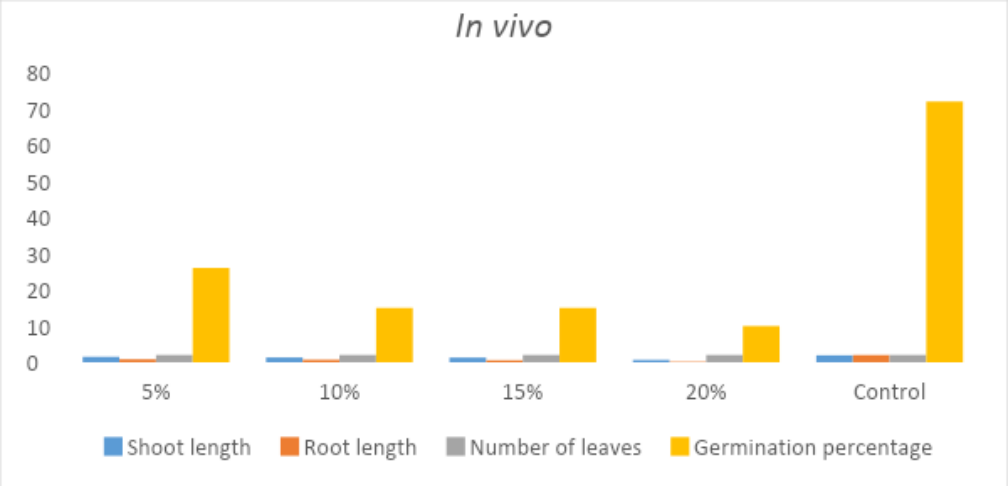




3.2. Allelopathic studies in *Garcinia cambogia*

Garcinia cambogia leaf extract in all concentrations (5%, 10%, 15% and 20%) were found to be strongly allelopathic and inhibited the germination of the weed both *in vitro* and *in vivo* treatment as compared to the control (Table 3 and 4).

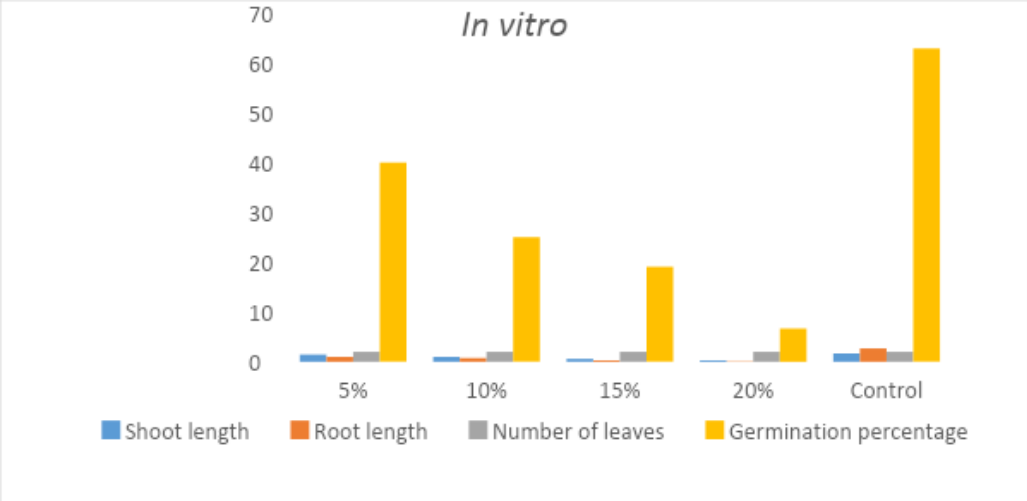


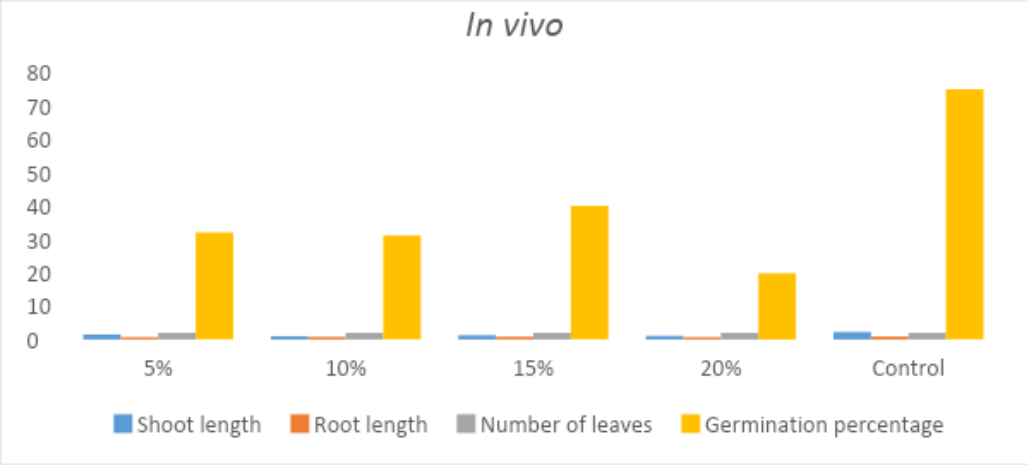


3.3. Allelopathic studies in *Bauhinia acuminata*

The result presented in the Table 5 and 6 clearly showed the effect of *Bauhinia acuminata* on growth parameters and germination percentage of the weed *Ipomoea obscura*. The inhibitory effect of the methanol extract of *Ipomoea obscura* leaves was found to depend on the extract concentration. All concentrations (5%, 10%, 15%, and 20%) of leaf extract of *Bauhinia acuminata* was generally found to be allelopathic and decreased the germination percentage of the weed. As the increasing concentration

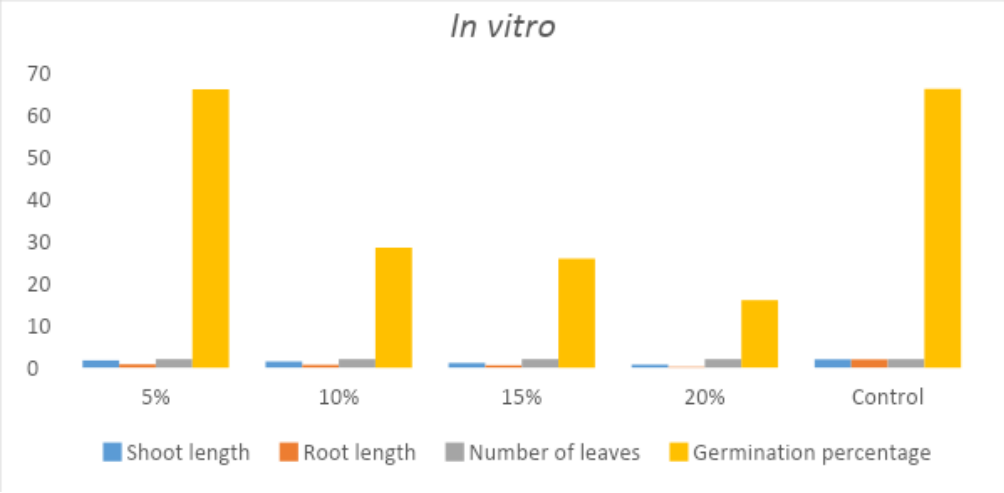
of the leaf extract, it decreased the germination percentage and growth parameters of weed as compared to the control both in *in vitro* and *in vivo* treatments.

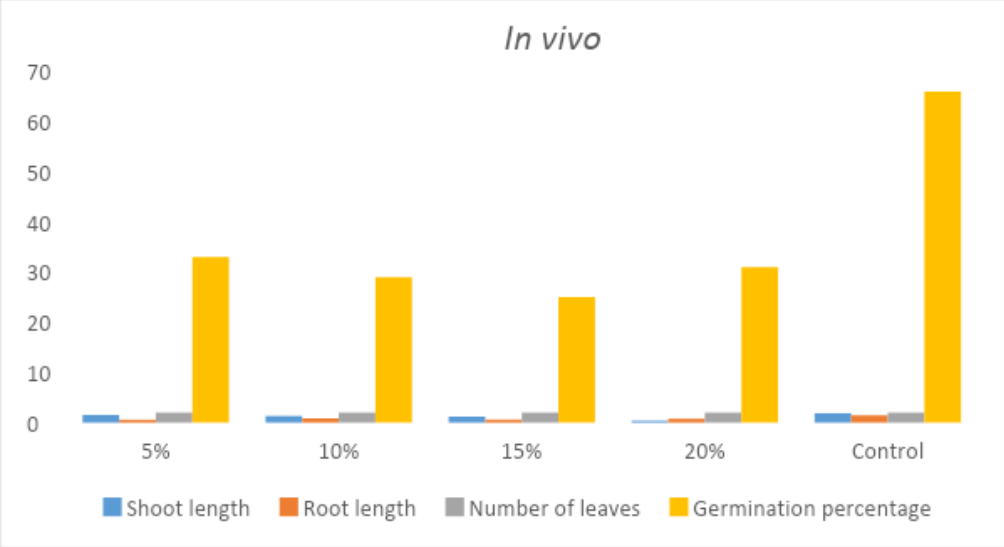




3.4. Allelopathic studies in *Averrhoa bilimbi*

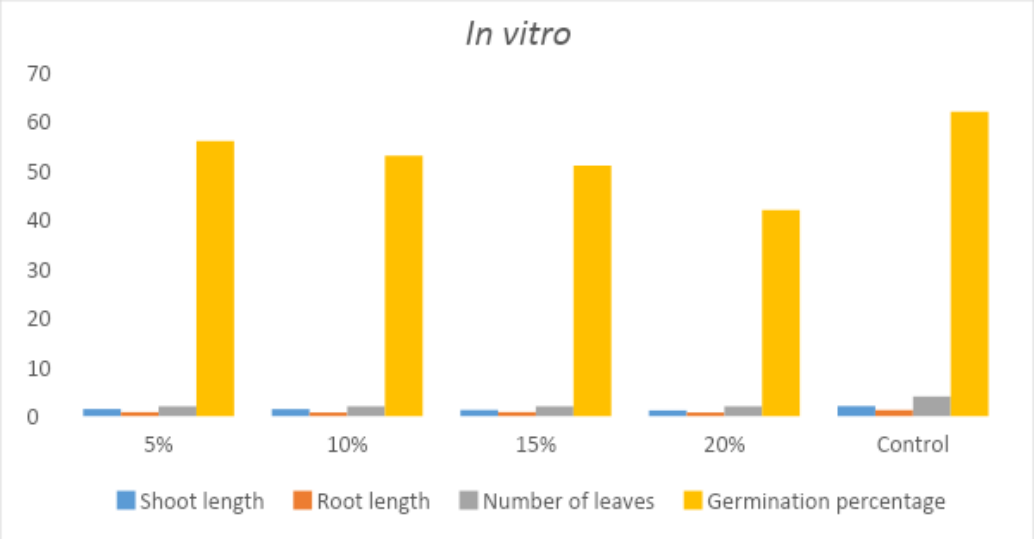
Averrhoa bilimbi leaf extract in all concentrations (5%, 10%, 15 %, and 20%) were found to be not strongly inhibited the germination of the weed both *in vitro* and *in vivo* treatment as compared to the control (Table: 7 and 8).

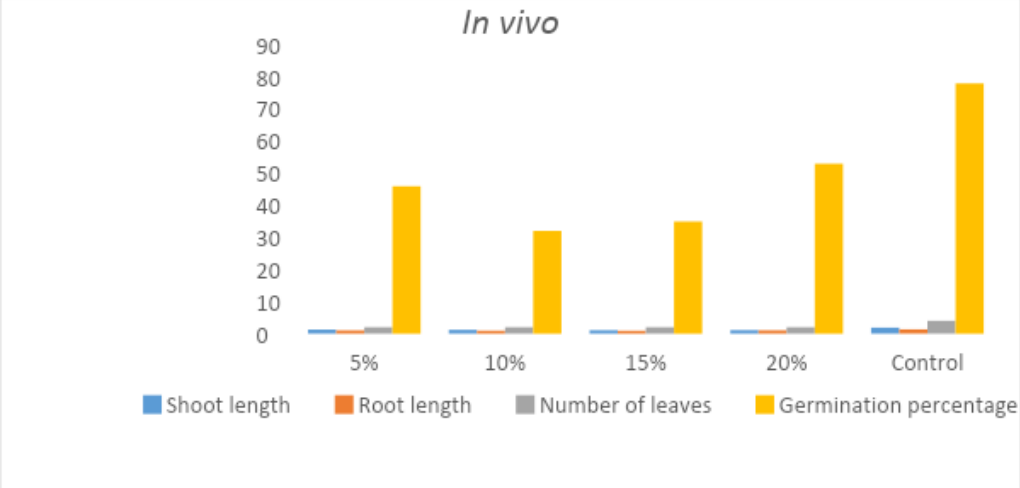




3.5. Allelopathic studies in *Ficus auriculata*

Ficus auriculata leaf extract in all concentrations (5%, 10%, 15 %, and 20%) were found to be not strongly inhibited the germination of the weed both *in vitro* treatments as compared to the control (Table: 9 and 10).





3.6. GC-MS analysis

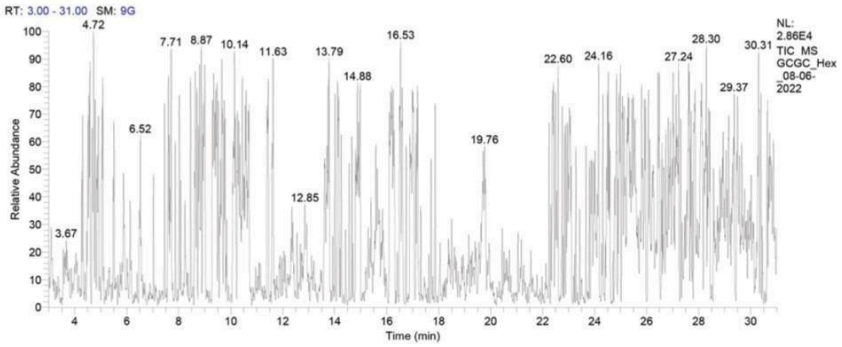


Plate 10: GC-MS Chromatogram of the Methanolic extract of *Garcinia cambogia* - Leaf

List of identified components

R.T	Name of compounds	Molecular formula	Molecular weight	Area %
4.72	L-Lysine, N6-acetyl-N2-[N-[N-[N-(N2-acetyl-N, N,N2-trimethyl-L-asparaginyl)-N-met hyl-L-phenylalanyl]-N-methyl-L-phenyl alanyl]-N,1-dimethyl-L-tryptophyl]-N 2,N6-dimethyl-, methyl ester	C53H72N8O9	964	1.04
7.71	1,1'-Diacetoxy-4,4'-dichloro-5,5',8,8'-tetr amethoxy-6,6'-dimethyl-2,2'-binapht halene	C30H28Cl2O8	586	1.35
8.87	L-Lysine, N6-acetyl-N2-[N-[N-[N-(N2-acetyl-N,N, N2-trimethyl-L-asparaginyl)-N-met	C53H72N8O9	964	1.44

	hyl-L-phenylalanyl]-N-methyl-L-phenyl alanyl]-N,1-dimethyl-L-tryptophyl]-N 2,N6-dimethyl-, methyl ester			
10.14	Hexa-2,4-dien-2,5-zirconium-3,4-molibd enum-tetra(cyclopentadienyl)	C26H26MoZr	526	1.16
11.63	Tungsten, dicarbonyl-(ü-4-pinocarvone)[1,2-bis(di methylphosphino)ethane]	C18H30O3P2W	540	1.37
16.53	Zeranol, 3TMS derivative	C27H50O5Si3	538	1.35
30.31	Pregn-4-en-18-al, 3-(methoxyimino)-20-oxo-11,21-bis[(tri methylsilyl)oxy]-, 18-(O-methyloxime), (11á,17à)-	C29H50N2O5Si2	562	0.78

4. Discussion

4.1. Allelopathic effects of plant extracts on weed

Methanol extracts of various concentrations of leaf of selected plants viz., *Macaranga peltata, Garcinia cambogia, Bauhinia acuminata, Averrhoa bilimbi* and *Ficus auriculata* had varying degrees of inhibition on the germination and growth of weed *Ipomoea obscura*, reflecting the allelopathic potential of the plants. Such inhibition on the shoot, root and leaf growth and seed germination of the test plant species may be due to the presence of allelochemicals in each methanolic leaf extract. The allelopathic effects were either inhibitory or stimulatory depending on test species. Allelopathic effect of 5, 10, 15 and 20% of methanolic extract of leaves of *Garcinia cambogia* was clearly demonstrated on germination percentage, shoot and root length of *Ipomoea obscura.* Generally, leaf extracts reduce all the measured growth parameters. In *in vivo* treatment of *Garcinia cambogia* showed lesser germination when compared with control. In the present study, responses revealed that the inhibition of growth parameters of seedlings was more pronounced than inhibition of seed germination.

Zaman et al. (2014) found that through the release of various water soluble allelochemicals from the live portions and litter into the nearby soil, it was discovered that *Mallotus philippensis* has considerable allelopathic capability against the tested plant species (13). When compared to control, *Mallotus philippensis's* growth parameters and germination percentage decreased both *in vivo* and *in vitro*. The results of *Mallotus philippensis*, allopathic effects on weed *Chromolaena odorata* clearly showed that these effects are inhibitory.

Masry et al. (2019) indicated the possibility of using the allelopathic activity of the leaf powder of *Ficus nitida* as a selective bioherbicide for controlling annual weeds accompanied *Vicia faba* plants (14). The *Boerhavia procumbens* exhibited the maximum inhibition of germination and seedling growth of *Lactuca sativa* (lettuce) among 196 species. It has numerous medicinal uses but its allopathic effects are least reported in literature. However, *Boerhavia procumbens* has been considered as a threat to sustainable agriculture due to its vast distribution and impact on crop production (15). Al-Snafi (2017) reported the presence of cardiac glycosides, amino acids, alkaloids, tannins, flavonoids, saponins, carbohydrates and phenols in aqueous and methanolic extract of Datura metel (16). Ramachandran (2017) tested the ability of Datura metel to control the noxious weeds particularly *Parthenium hysterophorus* L in a laboratory bioassay (17). The aqueous extract of Datura metel had successfully inhibited the early seedling growth and germination of *P. hysterophorus* L.

Amini et al., (2016) recorded the strong inhibitory allelopathic effect of *Berberis vulgaris* on *Lactuca sativa* (lettuce) seedling growth and germination out of 68 plant species leaf litter through sandwich method (18). Similarly, Mardani et al., (2016) reported the allelopathic effect of *Berberis vulgaris* while studying 178 Caucasian plant species impact on *Lactuca sativa* (lettuce) growth in sandwich method (19). Peterson et al., (2005) reported the rusting and damage to stems of cereal and wheat from Berberisvulgaris due to release of allelochemicals (20). However, the finding of the present study also revealed the inhibitory allelopathic effect of *Berberis vulgaris* on lettuce germination and seedling growth.

4.2. GC-MS analysis

The present study confirmed that, the L-lysine, which is a phenolic allelochemical present in *Garcinia cambogia*. The chemical composition of leaf tissue of *Garcinia cambogia* was analyzed by using GC-MS. The chromatograms show six compounds were detected in the leaf tissue of *Garcinia*

cambogia in methanol extract such as L-lysine(C53H72N8O9), zeranol, 3tms derivative(C27H50O5Si3) etc. Zhao et al. (2010) found that phenolic allelochemicals can reduce or inactivate the physiological activity of plant hormones, which may then inhibit the normal physiological process of plants and hydroxyl benzoic acid, polyphenols, and other compounds could affect the decomposition process of indoleacetic acid and gibberellin (21).

In a case of allelopathic research on silvergrass (Vulpiaspp.), a significant weed in southern Australia, GC/MS has been employed to characterize the natural toxins. Twenty-one allelochemicals were identified and quantified and their biological actives were tested and identified through a bioassay procedure, which revealed strong correlations between individual phytotoxins and levels of measured phytotoxicity (22). There were a variety of allelochemicals in root exudates and de-composed products in strawberry roots, and p-hydroxybenzoic acid, one of the allelochemicals with the highest content and strongest autotoxicity, could contribute to the incidence of strawberry wilt disease (21).

An investigation of the chemical basis for rice allelopathy to the rice weed arrowhead (*Sagittaria montevidensis*) was undertaken using GC/MS techniques. Twenty-five compounds were isolated and identified from the root exudates of both allelopathic and non-allelopathic rice varieties. Phenolics, phenylalkanoic acids, and indoles were among the chemical classes identified. Two indoles previously unreported in rice were detected in the exudates, 5-hydroxy-2-indolecarboxylic acid and 5. hydroxyindole-3-acetic acid (23).

5. Conclusion

The present study revealed that *Macaranga peltata, Bauhinia acuminata, Averrhoa bilimbi, Ficus auriculata* and *Garcinia cambogia* leaves were found to allelopathic and decreased the germination percentage and the growth parameters of the weed *Ipomoea obscura* in both *in vitro* and *in vivo.* The compound L-lysine, which is a phenolic allelochemical present in *Garcinia cambogia*.

6. Suggestions

Natural products (allelochemicals) produced by plants may help to reduce the use of synthetic herbicides for weed management and cause less pollution, safer agricultural products as well as alleviate human health concerns. Other factors regarding to supporting the allelopathic potential of these plants can be checked and suggest as a natural herbicide for the public.

7. Acknowledgments

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