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.

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

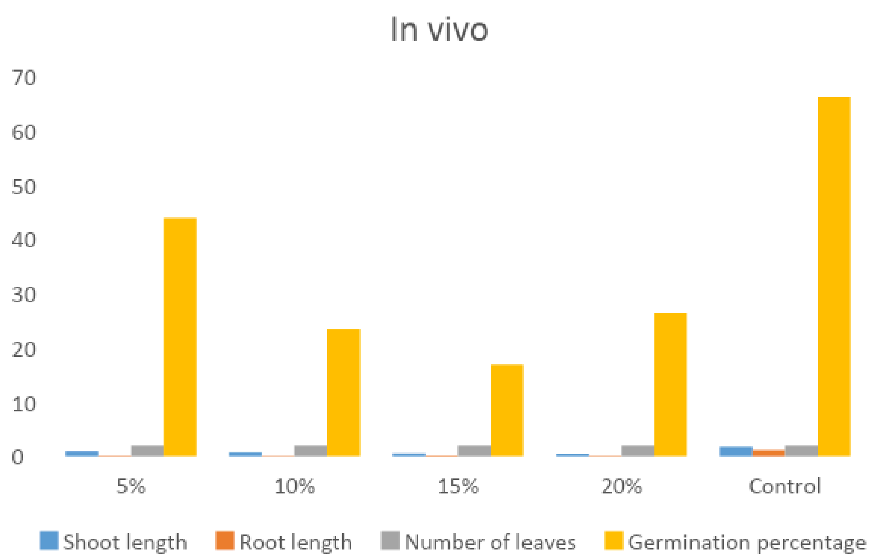
2.2. Profiling of Bioactive Components by GC-MS

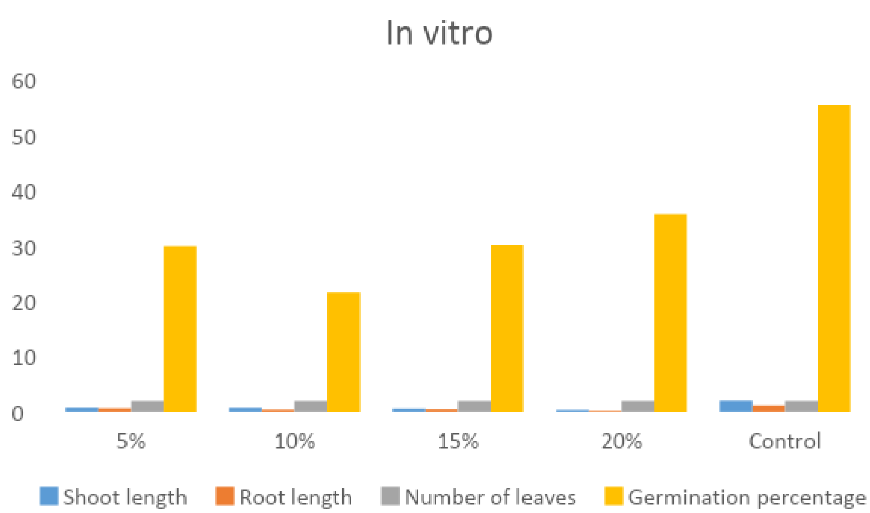
Ten grams of shade dried, powdered leaf tissues of *Garcinia cambogia* were packed in thimbles and subjected to extraction using various solvents with the help of Soxhlet apparatus. The extract obtained was used for further analysis. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of leaf extracts 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*

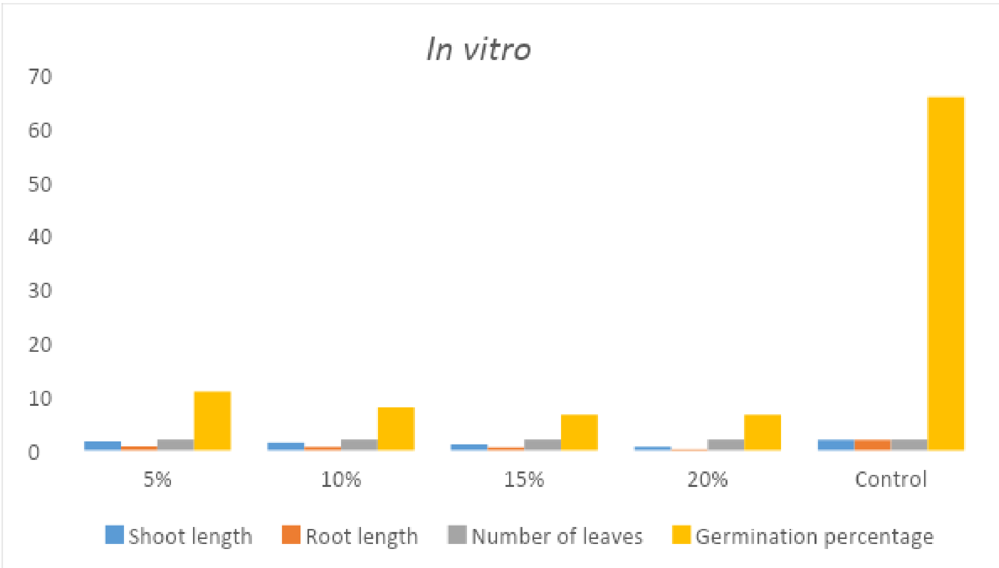
The results presented in table 1 and 2 clearly showed the effects of *Macaranga peltata* on growth parameters and germination percentage of the weed *Ipomoea obscura*. The inhibitory effects of the methanol extract of *Macaranga peltata* leaves was found to depend on the extract concentration (5%, 10%, 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.

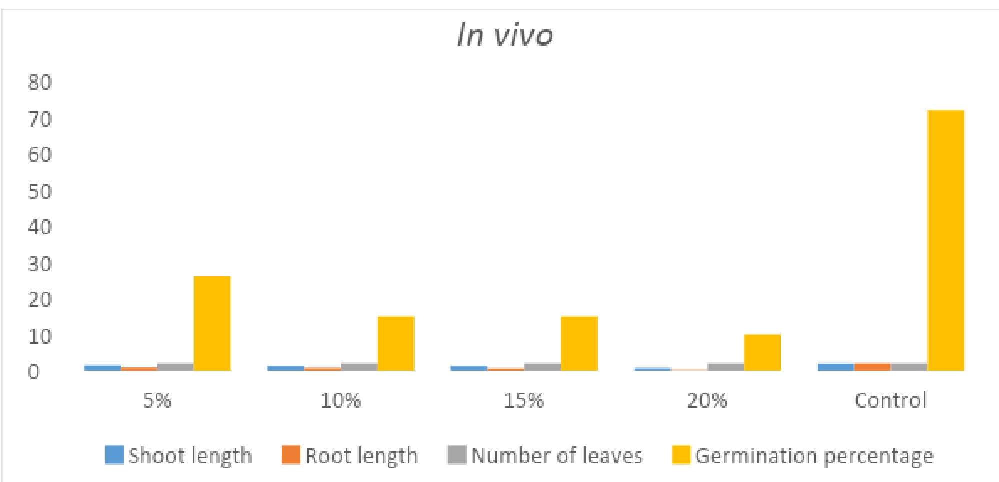




3.2. Allelopathic studies in *Garcinia cambogia*

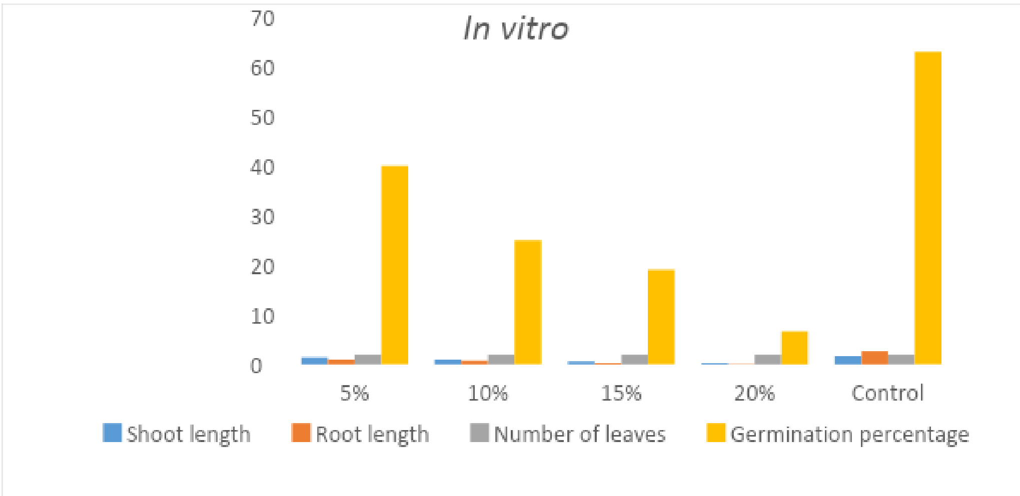
Garcinia cambogia leaf extract in all concentrations (5%, 10%, 15% and 20%) were found to be strongly allelopathic and inhibited the germination of the weed both *in vitro* and *in vivo* treatment as compared to the control (Table 3 and 4).

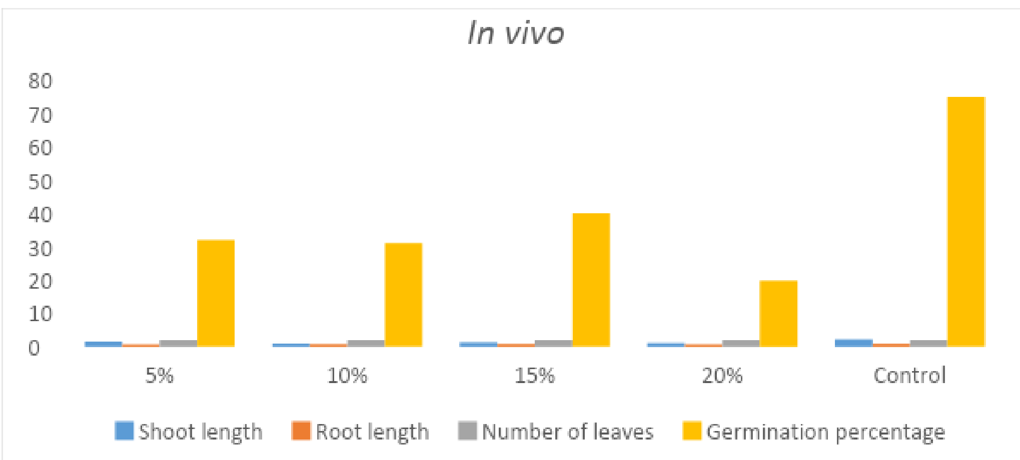




3.3. Allelopathic studies in *Bauhinia acuminata*

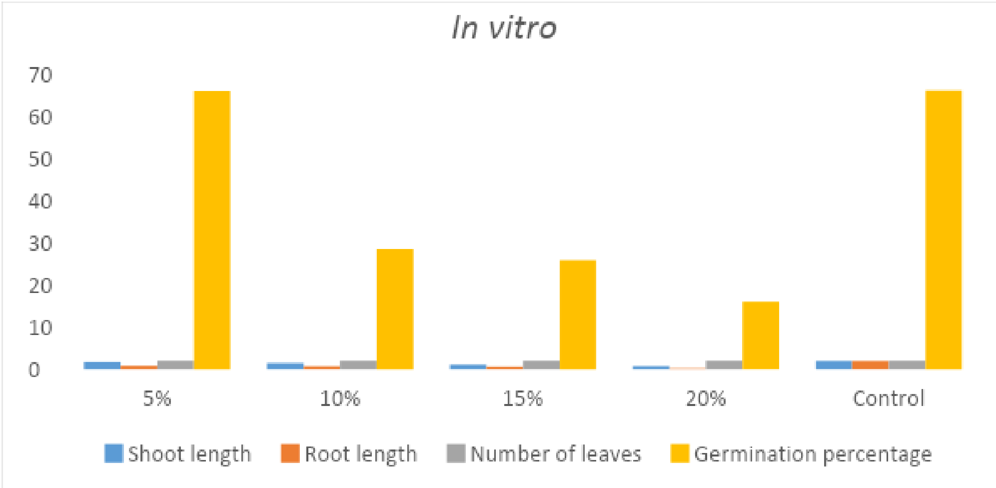
The result presented in the Table 5 and 6 clearly showed the effect of *Bauhinia acuminata* on growth parameters and germination percentage of the weed *Ipomoea obscura*. The inhibitory effect of the methanol extract of *Ipomoea obscura* leaves was found to depend on the extract concentration. All concentrations (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.

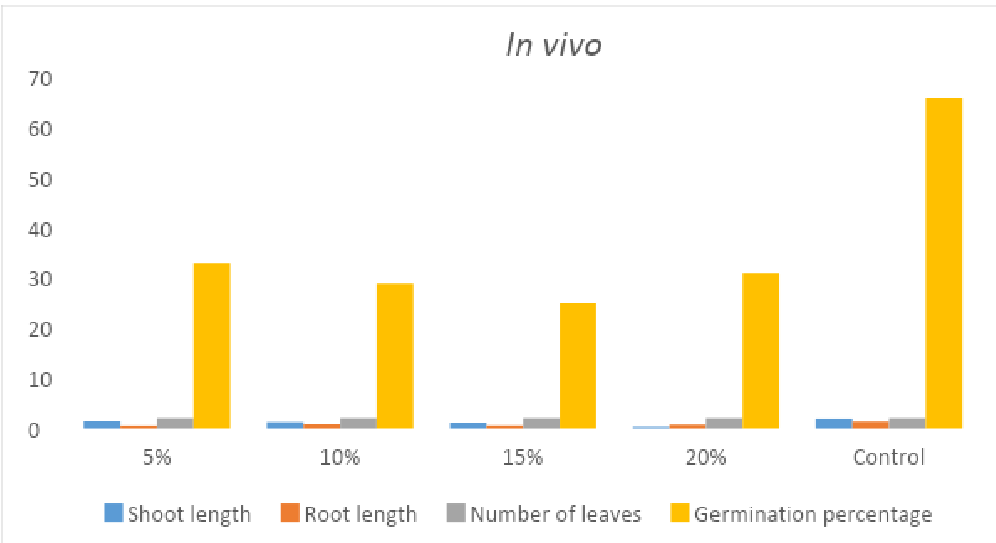




3.4. Allelopathic studies in *Averrhoa bilimbi*

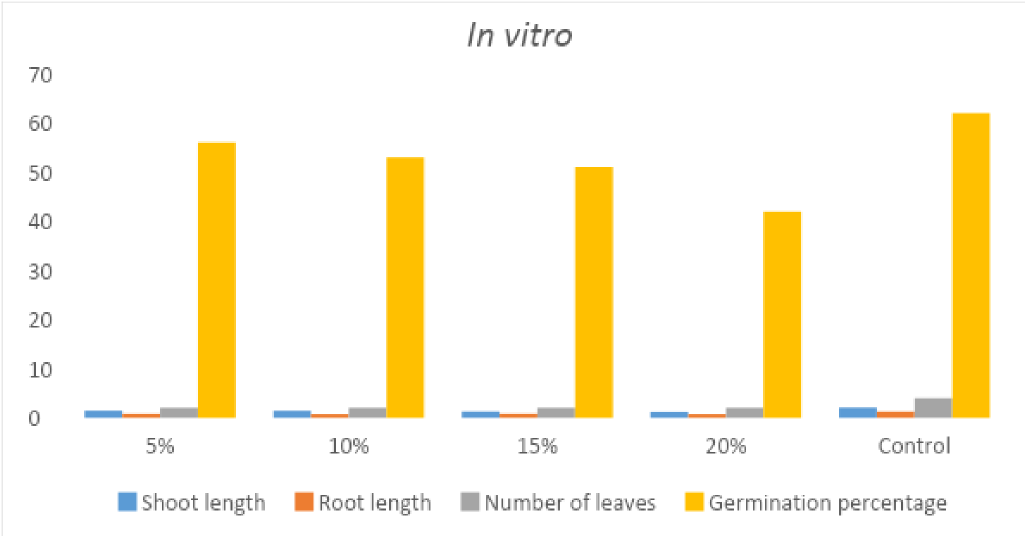
Averrhoa bilimbi leaf extract in all concentrations (5%, 10%, 15 %, and 20%) were found to be not strongly inhibited the germination of the weed both *in vitro* and *in vivo* treatment as compared to the control (Table: 7 and 8).

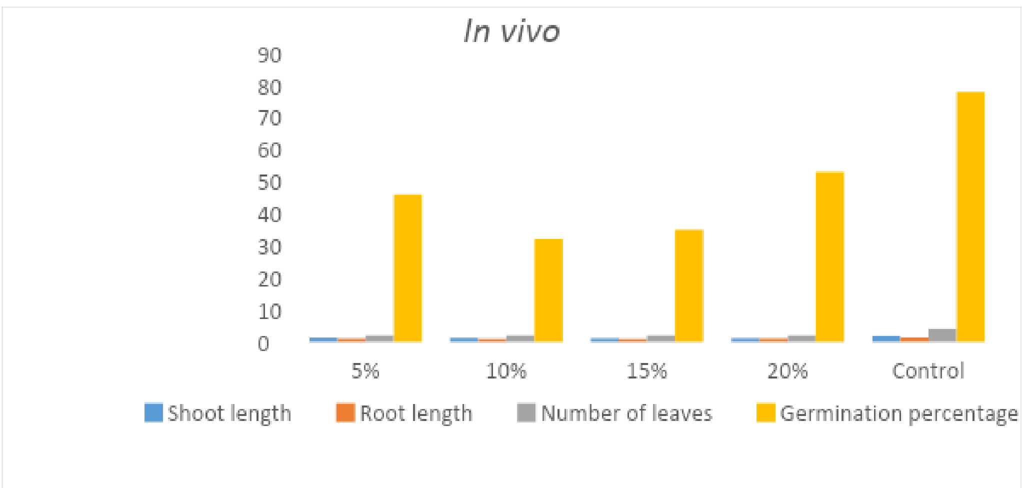




3.5. Allelopathic studies in *Ficus auriculata*

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

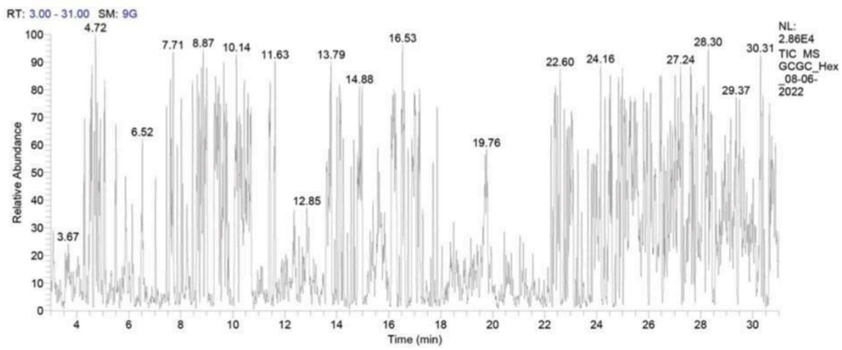


Plate 10: GC-MS Chromatogram of the Methanolic extract of *Garcinia cambogia* - Leaf

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-methyl-L-phenylalanyl]-N-methyl-L-phenylalanyl]-N,1-dimethyl-L-tryptophyl]-N2,N6-dimethyl-, methyl ester	C53H72N8O9	964	1.04
7.71	1,1'-Diacetoxy-4,4'-dichloro-5,5',8,8'-tetramethoxy-6,6'-dimethyl-2,2'-binaphthalene	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.

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