RESEARCH ARTICLE

Allelopathic Potential and HPTLC Analysis of *Ipomoea carnea*

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ABSTRACT- In the present study, sandwich method was used to study the allelopathic interactions of *Ipomoea carnea* on two test weed seeds i.e. *Amaranthus spinosus* and *Cassia fistula*. Pot experiments were also conducted where Ipomoea extracts were applied to germinated seedlings in the bags and the effect was observed after regular application of the Ipomoea extract as a weedicide. Both methods showed inhibition of the weeds with respect to growth of seedlings. However, the results were more significant in Sandwich method as compared to Spray Bioassay, indicating the allelopathic properties of *I. carnea* is more significant on un-germinated seeds compared to grown plantlets. HPTLC analysis revealed the presence of flavonoids, phenols, tannins and terpenoids in *I. carnea*. Since all the four phytochemicals were present in *I. carnea*, these could be responsible for allelopathic properties of *I. carnea* on *A. spinosus* and *C. fistula.*

Key-words- Allelopathy, *Amaranthus spinosus*, *Cassia fistula*, Carnea, HPTLC, Ipomoea, Weedicide

INTRODUCTION

Allelopathy is the inhibitory effect of one living plant upon another by the release of toxic substances. It is an interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth [1]. The study of allelopathy increased in the 1970s and has undergone rapid development since the mid-1990s, becoming a popular topic in botany, ecology, agronomy, soil science, horticulture, and other areas in recent years. Allelopathic interaction can be one of the significant factors contributing to species distribution and abundance within plant communities and can be important in the success of invasive plants [2]. Allelopathy has effects on several aspects of plant ecosystem which includes occurrence of the plants, their growth, dominance, productivity, divergence and succession. Initially, many of the forestry species evaluated had negative allelopathic effects on food and fodder crops, but in the 1980s, research was begun to identify species that had beneficial, neutral, or selective effects on companion crop plants [3]. The main purpose of research on allelopathy include the application of the observed allelopathic effects to agricultural production, reduction of the input of chemical pesticides and consequent environmental pollution, and provision of effective methods for the sustainable development of agricultural production and ecological systems [4].

In the laboratory, leachates and plant extracts are usually screened for their effects on seed germination with further isolation and identification of allelochemicals from greenhouse tests and field soil, confirming laboratory results. Interactions between host crops, allelopathic plants, and other non-target organisms must also be considered [5]. An allelopathic weed can effectively be used to control bothersome weeds near crop by planting a variety with allelopathic qualities. Alternatively, application of allelopathic compounds along with, before, or after synthetic herbicides could increase the total effect of both materials, thereby reducing application rates of synthetic herbicides. Several studies have been carried out to evaluate the allelopathic potential of weeds on other plants.

I. carnea is one common weed popularly known as Besharam [6]. It is found growing in waste waters and lands. This plant has been listed to have medicinal values. Different extracts of *I. carnea* plant possess anti-bacterial, anti-fungal, anti-oxidant, anti-cancer, anti-convulsant, immunomodulatory, anti-diabetic, hepatoprotective, anti-inflammatory, anxiolytic, sedative and wound healing activities [7]. Using Ipomoea for weed control can be an excellent method as cultivating it is easy and it does not require high maintenance.

The present study intends to study the allelopathic potential of *I. carnea* against two commonly growing weed plants i.e. *C. fistula* and *A. spinosus*. The study also suggests ways to use *I. carnea* as a biological control for

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C. fistula and *A. spinosus.*

MATERIALS AND METHODS

The current research was carried out at the Laboratory of Botany Department, at Jai Hind College, Mumbai. The leaves of *I. carnea* was collected from local areas of Mumbai, India. Mature plants were selected for this study. Leaves of *I. carnea* plants were removed from the stem. These leaves were surface sterilized and later air dried, weighed and transferred in a homogenizer where they were crushed into powder, which was stored in a dark bottle at room temperature until further use. The stored leaf powder was used for further allelopathic analysis.

Sandwich method

The allelopathic effect of *I. carnea* was tested on seeds of 2 donor weed plants (*A. spinosus* and *C. fistula*), which were used as subjects. Sandwich method was employed to determine the allelopathic effect. These are commonly grown weeds, which grow locally. The sandwich method consists of the use of a special six well multi-dishes in which dry leaf powder from the donor plant are placed above a layer of autoclaved agar cooled to 40˚C to 45˚C. In the current study a modified sandwich method was used where, instead of the special six well multi-dish well, a sterile petri-dish (90 mm in diameter) was used to prepare the media for the sandwich method and check for the germination rate.

Preparation of sandwich medium

0.75 % agar solution was prepared for the carrying out the sandwich assay [8].

Test Plate

The 0.2 gm of dried leaf powder was placed in a sterile glass petri-dish. 10 ml of 0.75% of sterile agar solution was added into each petri-dish on the leaf powder extract. After solidification of first layer of agar, another 10 ml of 0.75% agar solution was added to each petri-dish. This second layer of agar was allowed to cool and solidify. After solidification, ten seeds each of the donor weeds were placed on agar gel in each petri-dish. Each plate was then sealed with a plastic tape and incubated for 6 days at 24ºC under dark conditions. The seeds in Petri dishes were allowed to germinate for six days. Those seeds with visible radicle were considered germinated. The counting of the germination of the seed was done daily from the second day onwards. The radicle and hypocotyls lengths were recorded. Percentage of root and hypocotyl lengths of each weed seed was calculated by comparing with control. The whole experiment was repeated three times and the average values were expressed. One control was maintained with only agar gel in the petri-plate.

Spray Bioassay

50% aqueous extract of *I. carnea* leaves were prepared. The mixtures were made in sterile 125 mL Erlenmeyer flask wrapped in aluminum foil to avoid evaporation and exposure to light for 3 days at room temperature. The flasks were placed on a platform shaker at 70 rpm. After 3 days of soaking in water, the mixtures were centrifuged for 10 min at 4,000 rpm. The supernatant was collected and stored at 4°C till it was used for analysis [9]. The test weed seeds i.e. *C. fistula* and *A. spinosus* were grown in a mixture of soil and farmyard manure. The seedlings were sprayed with 10 ml of prepared weedicide using a spray bottle after 30 days of growth. The application of weedicide was done once every 3 days. The other two days, the platelets were sprayed with water. A control was set up were the test weed seeds were only treated with water. Ten bags with three seedlings each were maintained for the study with a control set. Plants were harvested after 2 weeks and data regarding root and shoot were determined.

CALCULATIONS

Percentage inhibition

Observations were made for Germination, Radical and Plumule length. Percentage inhibition (% inhibition) was calculated using the formula below to observe the magnitude of inhibition by various extracts on radical and Plumule length.

Percentage inhibition (%inhibition) = A-B/A* 100

Where, A= Effect on control, and B= Effect on test
Control and treatment means were compared using Students t-test (α- 5%) for analyzing the significance of the difference between them.

Overall Allelopathic potential (OAP)

In order to rank the data in terms of their allelopathic effects of plant, the concept of overall allelopathic potential (OAP) was applied in this study [10].

Calculations were made using the formula:

OAP= inhibition of radical growth/100

A score between 0.0 and 1.0 was obtained and the data were ranked according to this score. A maximum score of 1.0 would indicate that the test material had totally inhibited growth, while a score of 0.0 would indicate that no allelopathic inhibition had occurred.

Identification and quantification of allelopathic compounds by HPTLC

Preparation of standard and sample solutions

Standard stock solutions for Quercetin, Linalool, Gallic Acid and Tannic acid were prepared by dissolving 5mg of standard powder in 5 ml of Methanol, and sonicating it for 10 minutes. The prepared solution was the Stock solution and, 10 µl of each of these solutions was applied using sample applicator. The plant extract of 10 µl quantity (Sonicated) was used for the HPTLC chemoprofiling. HPLC grade solvents were used [11].

Chromatographic conditions

Chromatography was performed on pre-activated silica gel 60 F254 HPTLC plates and the applications of samples were done on the plates [11].

Detection and quantification of compounds

Detection of compounds were done using solvents specific for those compounds. Solvents were used as mobile phases and derivatizing agents were used for individual separation each of the phytoconstituent i.e. Phenols, Flavonoids, Tannins and Terpenoids [11-13].

Development of chromatograms

Chromatograms were developed at room temperature, in glass twin-through chambers (20 mm×20 mm, with metal lids) previously saturated with mobile phase vapor for 30 min. The development distance was 87 mm. The plate was kept in photo-documentation chamber (CAMAG Reprostar-3) and the images were captured at 254 nm and 366 nm. The compounds were investigated according to their RF values with the corresponding spot of standards. Calculations for percentage were done considering standard and sample RF and AUC [11].

RESULTS AND DISCUSSION

Percentage inhibition via Sandwich method

The results of the experiment showed that there was significant inhibition of development of radicle and plumule of treated *C. fistula* and *A. spinosus* seeds. There was 94.35% inhibition of root development and 81.99 % inhibition of shoot development of *C. fistula* seeds, treated with Ipomoea extracts, when they compared to the control seeds which were treated with water (Fig. 1). Similarly an inhibition of 81.04% was observed for root development, and 28.07% inhibition was observed for shoot development of *A. spinosus* seeds, treated with Ipomoea extracts, when they were compared with control seeds which were treated with water (Fig. 1). There was greater inhibition noted in Cassia seeds compared to *Amaranthus* seeds.

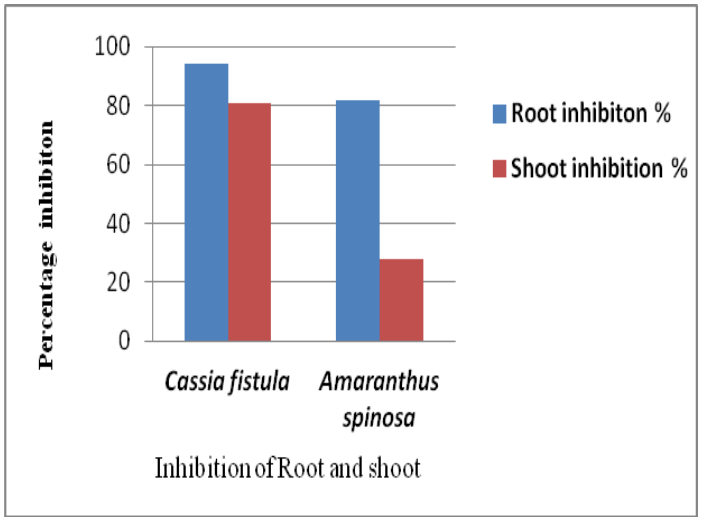

Fig. 1: Representing percentage inhibition of root and shoot development for *C. fistula* and *A. spinosus* upon treatment with *I. carnea* in sandwich method

Length of radicle

The allelopathic treatments significantly influenced root length. Treated plants showed radical length of 3.133 cm in Cassia (Table 1) and for *Amaranthus* seeds it was 14.8cm (Table 1). A more effective reduction was observed in *Cassia* seeds as the difference between root length of control and test seeds were much higher as compared to *Amaranthus* seeds. This indicated that radicles of *C. fistula* is more sensitive to Ipomoea extracts as compared to the radicles of *Amaranthus spinosus*.

Length of plumule

The plumules of test seedlings were significantly affected by Ipomoea extracts. The shoot length for Cassia was the longest compared to the shoot length of Amaranthus in the test seeds (Table 1). The test seeds were affected in an impressive manner upon treatment with *Ipomoea* extracts and there was considerable difference in the shoot size of Cassia and Amaranthus compared to the control seeds. The shoot length of test seeds of Cassia was 14.8 cm and that for Amaranthus was 31.6cm. Both the test seedlings showed a shorter shoot length compared to control seedlings (Table 1). However, seeds of *C. fistula* showed a greater difference between control and test seeds. This indicates that shoots of *C. fistula* is affected more by Ipomoea extracts as compared to shoots of *A. spinosus*.

Overall Allelopathic potential

It can be seen from Table 1, that the overall allelopathic potential of *I. carnea* was 0.94 for *C. fistula* and 0.81 for *A. spinosus*. Thus, it was observed, the overall allelopathic potential of *I. carnea* was more profound in *C. fistula* as compared to *A. spinosus*. However, Ipomoea showed a high allelopathic potential on both the test plants, with a value of more than 0.5 OAP.

Table 1: Overall allelopathic effect (OAP) and effect of *I. carnea* on root and shoot length of *C. fistula* and *A. spinosus* for sandwich method

		Cassia fistula	*Amaranthus spinosus*
Length of Radicle	Control	55.466±4.097	31.666±2.097
	Test	3.133±0.957	6±0.364
Length of Plumule	Control	82.2±2.548	43.933±1.141
	Test	14.8±5.953	31.6±0.652
OAP		0.94	0.81

Values presented are means ± SE, * = significance at P < 0.05

Spray Bioassay

Cassia fistula

The receptor plants of *C. fistula* had certain variations in them when compared to normal plants which were treated with water. There occurred variation in the root length, shoot length of the test and control seedlings. *C. fistula* roots displayed a length of 48cm for control and 47cm for the test plants. The shoot lengths for *C. fistula* were 103±19.6 cm for Control and 88±2.005 cm for the test plants (Table 2). As it can be observed, there was not a significant difference between the root and shoot lengths of control and test plants of *C. fistula.*

Amaranthus spinosus

The roots of Amaranthus plants were shown a length of 79.8cm for control and 66.2 cm for test plants. Similarly, the shoot length observed for Amaranthus was 17.8cm for control and 13.4 cm for test plants (Table 2). From the table and statistical analysis; it can be observed that there is difference between control and test for shoot and root length and the difference is due to application of *I. carnea* extract. The present observation suggests that *I. carnea* can be used as herbicide or a weedicide.

Table 2: Effect of aqueous extract of *I. carnea* on root and shoot length of *C. fistula,* and *A. spinosus* for spray bioassay

	Cassia	
	Control	**IC**
Root length	48±5.1475	47±7.01
Shoot length	103±19.6	88±2.005
	Amaranthus	
Root length	79.8±0.799	66.2±2.763*
Shoot length	17.8±30382	13.4±1.886*

Values presented are means±SE, * = significance at P<0.05

HPTLC Analysis

HPTLC is a novel technique which allows separating and quantifying components present in a given sample. HPTLC is used in several fields like medicine, chemistry, food analysis etc. The present work attempts to optimize the simultaneous HPTLC fingerprint profiles of secondary metabolites in leaf extracts of *I. carnea*. The sonicated leaf extract of *I. carnea*, was able to resolve many peaks in the developing solvent system. The bands for quercetin, gallic acid, tannic acid and linalool compounds in the methanolic extract of *I. carnea* was identified and confirmed by comparing UV-Visible absorption spectra with respective compound. The banding pattern with their RF value regarding the flavonoids in sonicated method using methanol, is given below.

Table 3: Rf values and concentrations of the estimated phytochemicals in *I. carnea* using HPTLC method

No.	Phytochemical	Rf	Concentration (µg/ml)
1	Flavonoids	0.92	5.167
2	Phenols	0.46	9.717
3	Terpenoids	0.25	0.925
4	Tannin	0.86	2.078

The estimation of the four phytochemicals was assessed. The amount of total flavonoids and total phenols estimated in *Ipomoea carnea* was 5.167 µg/ml and 9.717 µg/ml respectively. The quantity of Terpenoids was 0.925 µg/ml and that of tannic acid was 2.078 µg/ml respectively. Phenols were found in maximum quantity followed by flavonoids, tannic acid and Terpenoids. This indicates that *I. carnea* is a rich source of phenols and flavonoids as compared to tannins and Terpenoids.

Sandwich method is a unique method used to determine the allelopathic effect. A related study was conducted where 20 medicinal plants were tested for their phytotoxicity using the sandwich method. They research concluded that there was significant reduction on root and shoot length of the test seedlings [14]. A similar study was conducted where *Garcinia gummi-gutta* leaf leachates were used to test weedicial effects. Their study indicated that the plant was exerting its allelopathic effect only on the germination stage of seeds, whereas a null effect was observed with matured seedlings [9]. In another study, 239 medicinal plant species were studied in Pakistan for their allelopathic activity using the sandwich method. Their results indicate that there was a noteworthy reduction the germination percentage of the test seeds. They also suggested that allelopathic studies should be carried out in a standardized manner in order to study and identify plant species which possess allelopathic properties [15]. In Japan, a study was carried out where 251 plant species collected from the Sino-Japanese Floristic Region were screened for allelopathic plant species. Sandwich method and dish pack method were respectively used to screen plant leaf leachates. Among all the plants tested, 84 species showed inhibitory effects on the seedlings which were tested [16]. The Allelopathic effects of *Euphorbia guyoniana* and *Retama retam* was investigated on germination efficiency of two weeds (*Bromus tectorum* and *Melilotus indica*) and one crop species (*Triticum aestivum*) by a group of researchers in Egypt. Their study concluded that the test weeds had significant effect on the receptor plants [17]. All the studies above focus on the fact that allelopathy is in an important phenomenon which occurs in plants and it should be studied further towards identification and extraction of the responsible allelochemicals.

CONCLUSIONS

In the current study, *I. carnea* was subjected to sandwich method and it exhibited the allelopathic effect on the two test weed crops i.e. *C. fistula* and *A. spinosus*. Cassia seeds displayed greater inhibitory effects as compared to Amaranthus. *I. carnea* also displayed weedicidal effect against both the test weeds, when they were subjected to the spray bioassay method. When the Sandwich method and Spray method were compared, *I. carnea* was shown greater inhibition in the sandwich method as compared to the Spray method. HPTLC analysis revealed the presence of flavonoids, phenols, tannic acid and Terpenoids in the plant extract. The concentration of Phenols was found to be the highest, followed by flavonoids, tannins and terpenoids indicating that Phenols might be responsible for the allelopathic potential of *I. carnea*. Determination and isolation of the responsible allelochemicals should be carried out in order to take the study further towards the development of a weedicide. Also, several other weeds can be tested against *I. carnea* to have a broad spectrum of sensitive weeds.

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