Eco. Env. & Cons. 28 (November Suppl. Issue) : 2022; pp. (S298-S304)
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ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i07s.049

Microcosm investigation on the allelochemic potential of *Ageratum conyzoides* on selected food crop

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(Received 11 March, 2022; Accepted 17 May, 2022)

ABSTRACT

Invasive alien plants like *Ageratum conyzoides* influence the growth, yield, development, and productivity of food crops. The various allelochemicals present in *A. conyzoides* exert stimulatory or inhibitory effects on the food crops. In this microcosm investigation through pot experiments, we evaluated the allelopathic potential of *A. conyzoides* on the germination and seedling growth of a leafy vegetable of nutritional value i.e., *Lactuca sativa*. The soil samples were collected from two different sites i.e., healthy forest soil and soil invaded by *A. conyzoides*. The healthy forest soil was considered as control pot while *A. conyzoides* invaded soil was selected for the experimental pot. The inhibitory effect of the invasive plant was observed on the tested crop of dietary value in human nutrition. The results revealed that the germination of the seeds is inhibited and suppressed by *A. conyzoides*. Microcosm studies demonstrated that out of the 15 seeds planted on both pots, 13 seeds germinated and thrived on the experimental pot and 14 on the control pot. The seedling height (32.1 cm), shoot length (27.65 cm), seedling biomass (1.866 g) and vigor index (6.829) were recorded higher in the experimental pot. Whereas, the root length (4.8 cm), germination potential (0.93), germination percentage (93.33%), germination index (4.66) and germination rate index (434.91) were noted higher in the control pot. *A. conyzoides* therefore showed both the inhibitory and stimulatory effects on *L. sativa* and thereby exhibited both positive and negative allelopathic effects. Further, bioassay experiments with other bioassay crops can elucidate the chemical ecology and allelopathy potential of *A. conyzoides*.

Key words : *Allelopathy, Allelochemicals, Ageratum conyzoides, Novel Weapon Hypothesis, Lactuca sativa, Microcosm, Plant Invasion, Chemical Ecology*

Introduction

Allelopathy is an interference mechanism in which plant release secondary metabolites also known as allelochemicals into the environment that could have either inhibitory or stimulatory effect on the growth of nearby plants (Kaur, 2012; Mushtaq, 2020; Khatri, 2020). Differential inhibitory effects of various parts are attributed to variability in the amount of phytotoxic compounds in different plant tissues (Kaur, 2015). When the two plant species co-exist, the interactions between them can be either inhibitory or stimulatory through direct or indirect allelopathic interaction (Kumar, 2006). Allelochemicals in plants act like herbicides preventing the germination and growth of the seedlings of competing species (Willis, 2005, Rai, 2022). Several allelopathic interactions of agricultural importance have been reported including crop to weed (*Brassica juncea* on *Amaranthus retroflexus)*, weed to crop (*A. conyzoides* on *Lactuca sativa*), crop to crop (*Brassica nigra* on *Lactuca sativa)*, weed to weed (*A. conyzoides* on *Amaranthus spinosus*), plant to insect (*Azadirachta indica* L to *Bemisia tabaci*) and plant to pathogen (*A.

conyzoides on *Aspergillus niger)*, thereby, affecting the economical outcomes of plant production (Abbas, 2017; Ercoli, 2005; Weston, 2005; Chahal, 2021; Erida, 2019; Elshae, 2003). Many aspects of plant physiological and biochemical processes have been proved to be affected by allelochemicals (Gniazdowska, 2005). Heavy infestations of the weeds modify the soil environment through root exudation, affecting soil structure and mobilizing or chelating nutrients (Singh, 2003). They also harm other plants by restricting light from reaching them, preventing photosynthesis. (Chengxu, 2011; Rai, 2015). In agroecosystems, several weeds, crops, agroforestry trees and fruit trees have been shown to exert allelopathic influence on the crops, thus affecting their germination and growth adversely (Kohli, 1998; Rai, 2020). When compared to native plant species, invasive alien plant species proliferate and respond quickly to dominate the habitat (Sakachep, 2021a, b; Vanlalruati, 2021). *A. conyzoides* is a serious problem in cultivated lands in the hilly tracts of northwestern India, where it forms dense thickets in commonly grown crops such as chickpea, rice, maize and wheat, and adversely affects crop yields (Kohli, 2006). Novel Weapon Hypothesis provides strong evidence that chemicals released by invasive plants in a new alien environment prove toxic to the native species and other organisms such as microbes and herbivores and help the donor plants to expand their territories. (Batish, 2013; Rai, 2021; Rai, 2022). The impact of plant invasion on biodiversity patterns and distribution can be studied through chemical ecology (Rai, 2020a).

Ageratum is one of the genera in the family Asteraceae and consists of about 30 species. As a member of family Asteraceae, the plant is non-woody in habit, found throughout tropic and semi-tropical regions around the world including India (Okunade, 2002). Though the genus consists of thirty species, however only a few species are phytochemically investigated (Kamboj, 2008). In this respect, Sakachep (2021) reported that *A. conyzoids* was a dominant species among the other invasive alien species in the Hailakandi district of Assam. A large number of allelochemicals have been found and identified and some are involved in the plant defence systems (Deepmala, 2019). Depending on the plant, allelochemicals can be released from a plant's flower, leaf debris and leaf mulch, stem, bark, root or soil surrounding the roots and can persist in soils affecting other plants that present around them as well as succession plants (Kruse, 2000).

These allelochemicals are classified into various groups based on their chemical properties. Phenolics, alkaloids, terpenes, fatty acids and indoles are the most ordinarily occurring allelochemicals in plants (Noguchi, 2008). These allelochemicals are chemically diverse, being represented by phenolic compounds, terpenoids, alkaloids and nitrogen-containing chemicals, and many other chemical families (Macías, 2019). *A. conyzoides* also have various chemical compounds such as flavonoids, alkaloids, tannins, saponins, cardiac glycosides, and anthraquinones (Okunade, 2002). Upon exposure to various stress factors, *A. conyzoides* was found to release more allelochemicals, such as ageratochromene and its analogues, flavones, sesquiterpenes, and monoterpenes (Kong, 2004). The constituents identified by Ekundayo (1988); Rana (2003); and Rao (1973), include 20 monoterpenes, 20 sequiterpenes and three phenylpropanoids and benzenoids. In the *Ageratum conyzoides* intercropped citrus orchard, *A. conyzoides* released allelopathic flavones and agreatochromene into the soil to reduce the populations of soil pathogenic fungi *Phytophthora citrophthora, Pythium aphanidermatum* and *Fusarium solani* (Kong, 2006). Kohli (2004) observed a huge loss in plant diversity and density (50-64%) of native flora after invasion by *A. conyzoides* in the Shivaliks, North India. Some species such as *Sonchus oleraceus, Sonchus asper, Vernonia cinerea, Abutilon indicum, Agave americana* and *Medicago lupulina* are not found in weed-infested areas, while they were found to be growing abundantly in weed-free areas (Dogra, 2008).

Allelopathy plays a key role both in natural and managed ecosystems. Allelochemicals is also a biological control of plant pathogens and diseases. Kumar (2018(a)) stated that allelopathy is not only a detrimental factor but also has positive aspects, providing a better soil or plant internal environment to cope with environmental stress. Even though allelopathy includes both positive and negative effects of one plant on the other but most of the studies seem to focus only on its deleterious impacts alone. Proper weed management is paramount in sustaining biodiversity. Biological control is the most effective and long-term solution for controlling the spread of invasive plants (Rai, 2015). Therefore, the objectives of the present microcosm study were to collect the soil of invaded region of *A. conyzoids* and study its impact on growth and development of selected native plant (*L. sativa*) in pot experiment.

Materials and Methods

The study was done at Mizoram University located in Tanhril, Aizawl District, Mizoram, India. A soil sample along with the debris of the plant is collected from the region invaded by *Ageratum conyzoides* and kept in a pot marked as experimental pot. Another control pot experiment is set in which the pot was filled by a healthy forest soil. Fifteen number of fast growing native plant seeds of *Lactuca sativa* are grown in both the pots, since it is a fast growing food crop and commonly grown in the state. Both of the pots are provided with same amount of sunlight and water. On the 3rd day and 7th day, the number of seeds germinated was noted. The plants on the two pots were carefully examined at a different time intervals and noted the change in morphology. On day 59 when it was fully grown two replicas (Sample A and B) from each pots were taken. The plant height (root and shoot length) was measured with a ruler and fresh weight (biomass) was taken in a laboratory electronic balance. The data are then used for calculating different growth parameters. The results of both the control and experimental are analysed and compared with each other. Parameters that used to analyse the data are Seedling Height, Root length and shoot length, Seedling Biomass, Germination Percentage, Germination Potential, Germination Index, Germination Rate Index and Vigor Index.

Results and Discussion

Seeds germination

The germination of the seeds of the *L. sativa* species was higher in Control Pot and lower in Experimental Pot. On the 3rd day the control pot germinates 14 individuals while experimental pot germinates 11. On the 7th day control pot remains the same but experimental pot increased to 13 numbers of individuals (Fig 1). This shows that the germination of the seedlings has been suppressed and shows the presence of allelopathy. Several environmental factors such as, soil moisture regime, soil temperature alternate wetting, drying of the soil, soil nitrate level also affect the seed germination percentage (Reigosa, 1999).

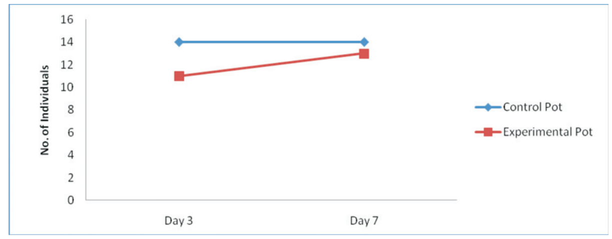

Fig. 1. Number of *Lactuca sativa* seeds germinated on the 3rd and 7th day on Control Pot and Experimental Pot.

Seedling Height, Root Length and Shoot Length

The morphological alterations of the native plant were faster in the experimental. Seedling Height was higher in the experimental than in control (Fig 2). Presence of *A. conyzoids* shows enhancement in growth morphologically. The mean seedling Height (both sample A and B) of control was 26.4 cm and 32.1 cm in Experimental. The Root length was higher in Control which is 4.8 cm and lower in Experimental of 4.45 cm. Cytological studies showed significant mitotic inhibition of root cells of *L. sativa* which was achievable only by the allelopathic effects of *A. conyzoides* (Bassey, 2018). On the other hand the Shoot length was of the opposite, which is higher in Experimental pot (27.65 cm) and lower in Control pot (1.6 cm). The higher seedling height and shoot length shows that the Experimental seedlings were healthy. And the root length shows that the nutrients were higher in the Experimental pot and lower in Controls pot in which the roots tend to penetrate down. Similar kind of results was also observed by Kumar (2018(b)), where low concentrations of plant extract of *A. conyzoids* stimulated the growth of *Pisum sativum*.

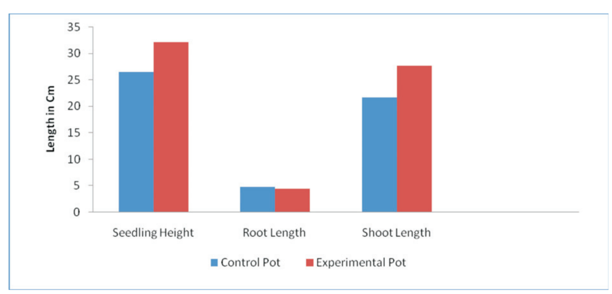

Fig. 2. Seedling Height, Root Length and Shoot Length of *Lactuca sativa* in Control pot and Experimental pot.

Seedling Biomass

Biomass was taken in fresh weight when the plant is fully grown. The Biomass of Sample A and B in

Control Pot was 0.848 g and 0.938 g and the Biomass of Sample A and B in Experimental Pot was 2.064 g and 1.669 g (Table 1). The average (mean) biomass of the species in Control Pot was 0.893 g and 1.866 g in Experimental Pot. The biomass in Experimental Pot was much higher than the control pot. The biomass of experimental pot was almost two fold the biomass of control pot. The higher biomass indicates that the soil in the experimental is fertile as compare to the control. Long root length in the control shows that the soil is less fertile and lack of nutrients in which the roots penetrate down. The presence of inhibitory chemicals or allelochemicals might be the reason for differential behavior in the growth of the seedlings. *A. conyzoides* leachate shows a positive impact on nitrogen content of leguminous crops at a lower amount (Kumar, 2018(a)). Due to higher nitrogen content the growth of the Experimental also increases. The fresh aqueous extracts of *A. conyzoids* were also found to be positive which might have practical use to increase agricultural yield and can be potential green manure (Negi, 2020).

Germination Percentage and Germination Potential

In the experiment the germination percentage was higher in Control pot than the Experimental pot. The germination percentage of control pot was 93.33% and germination potential of experimental pot was 86.66% (Fig. 3). This shows that the germination ability of seeds of *L. sativa* is much higher in Control pot than experimental pot. Also, the germination potential of *L. sativa* in control pot was 0.93 and in experimental pot was 0.73. A similar result was observed by Bassey (2018), that there is a gradual reduction in seed germination percentage of *L. sativa* and reduction of germination was concentration dependent.

Germination Index, Germination Rate Index and Vigor Index

The Germination Index of *L. sativa* was higher in the

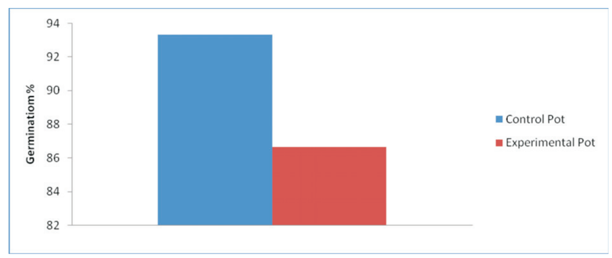

Fig. 3. Germination % of *Lactuca sativa* in Control Pot and Experimental Pot.

Table 1. List of data that shows the morphological characteristic of *Lactuca sativa* species grown in a healthy soil (control pot) and *Ageratum conyzoids* invaded soil (experimental pot)

Sl. No.	Parameters		Control Pot (Healthy Soil)	Experimental Pot (Ageratum conyzoids invaded soil)
1	Total number *Lactuca sativa* of seeds grown		15	15
2	Germination on	3^{rd} day	14	11
		7^{th} day	14	13
3	Seedling height (H)		Sample A- 26.6 cm Sample B- 26.2 cm Mean: 26.4 cm	Sample A- 32.1 cm Sample B- 32.1 cm Mean: 32.1 cm
4	Root Length (RL)		Sample A- 4.9 cm Sample B- 4.7 cm Mean: 4.8 cm	Sample A- 4.6 cm Sample B- 4.3 cm Mean: 4.45 cm
5	Shoot Length (SL)		Sample A- 21.7 cm Sample B- 21.5 cm Mean: 21.6 cm	Sample A- 27.5 cm Sample B-27.8 cm Mean: 27.65 cm
6	Seedling Biomass (B)		Sample A- 1.375 g Sample B- 0.938 g Mean: 1.156 g	Sample A- 2.064g Sample B- 1.669g Mean: 1.866g
7	Germination Percentage (GPe)		93.33%	86.66%
8	Germination Potential (GPo)		0.93	0.73
9	Germination Index (GI)		4.66	3.66
10	Germination Rate Index (GRI)		434.91	317.17
11	Vigor Index (VI)		5.391 (mean)	6.829 (mean)

control pot and lower in experimental pot. The germination index in control was 4.66 and in experimental pot was 3.66 (Table 1). The rate of germination of *L. sativa* was also higher in control pot and lower in experimental pot. Control pot with 434.91 germination rate index showed best performance as compared to the experimental pot with 317.17. The experimental pot have the higher vigor index of 6.829 which is the average vigor index of the two samples of the experimental pot, whereas the average vigor index of the two samples of control pot was 5.391 which is lower than the experimental pot. Aqueous extracts of *A. conyzoides* showed inhibitory effects on seed germination percentage, root and shoot length, fresh and dry weight (Jayaraman, 2014). Kong, 2002, also reported that the shoot extracts of *A. conyzoides* inhibited germination of *Amaranthus caudatus*, *Digitaria sanguinalis* and *Lactuca sativa*. *A. conyzoides* exhibited strong growth suppressing allelopathic effect on germination and but not the growth of *L. sativa.* Quest of exploring positive or beneficial effects of *A. conyzoides* can also facilitate its management. Invasive alien species are

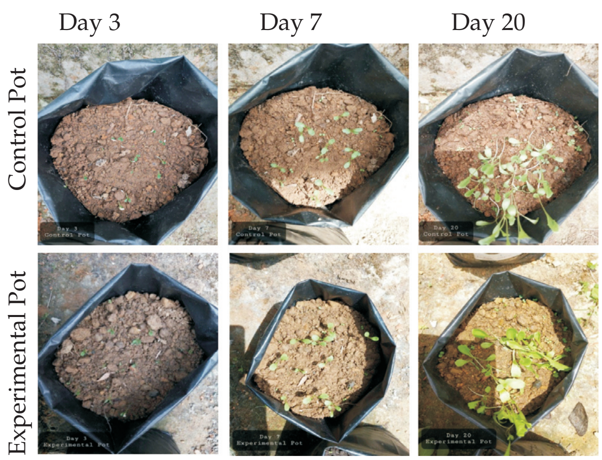

Fig. 4. *Lactuca sativa* grown on Experimental pot and Control pot at different time intervals.

seen as promising candidates for nanoparticle phytosynthesis (Rai, 2018). Also, invasive alien species can contribute to sustainability and ecological restoration by positively influencing the ecology and socioeconomic aspects of degraded ecosystems (Rai, 2020b). Some invasive alien plant species are used for medicinal purposes by indigenous Himalayan people of northeast India, and are also used in the lumber business *(Lantana camara)*, which might have socio-economic implications (Rai, 2011; Negi, 2019).

Conclusion

The study concludes that *Ageratum conyzoids* have the allelochemic potential that have both positive and negative impact on the native species. It shows that the seedling strive to grow in the beginning but thrive later. *Lactuca sativa* have better seedling height, shoot length and biomass in the experimental pot, and shows inhibition only on germination stage but on the later stage it shows positive response. Presence of small numbers *Ageratum conyzoides* with crops could be beneficial but detrimental when it is present on large amount. However, a detail study on the phytochemical profiles of *A. conyzoides* and their specific role on crop are required for understanding the mechanisms of their allelopathic potential. More research work is needed to evaluate the potential of *Ageratum conyzoides* on crops and other native species. Studies can be carried out on the effect of different plant parts based on the concentrations on native and non-native plants. Physical, chemical and biological properties like pH, organic carbon, electric conductivity, nutrients (NPK), etc. of the soil from both sites are also needed to analyse. Also, the effect of allelochemical on the soil microbes and other living organisms can be carried out for proper weed management.

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