RESEARCH ARTICLE

Microcosm Investigation on the Allelochemical Potential of *Mikania micrantha* to the Selected Food Crop

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Abstract

An experiment under microcosm was conducted to assess the allelochemical potential of *Mikania micrantha* on the growth and germination of *Lactuca sativa* through a pot culture experiment. Soil samples were collected from two different sites i.e., *M. micrantha* invaded soil (for the experimental pot) and healthy forest soil (for control pot). Various seed germination and growth parameters have been analyzed to evaluate the inhibitory and stimulatory effects of the allelochemicals released from *M. micrantha*. The results revealed that the germination and growth parameters of the crop were inhibited and suppressed by *M. micrantha*. Out of the fifteen seeds planted in both pots, thirteen seeds were able to germinate in the experimental pot, while fourteen were in the control pot. The seedling height (8.85 cm), shoot length (8.75 cm), seedling biomass (0.408 g), vigor index (0.52), root length (0.091 cm), germination potential (0.73), germination percentage (86.66%), germination index, (3.66) and germination rate index (317.17) were lower in the experimental pot and higher in the control pot. Therefore, *M. micrantha* showed an inhibitory effect on the growth and germination of *L. sativa* and induced negative allelopathic effects.

Keywords: Allelopathy, Allelochemicals, *Mikania micrantha*, Novel Weapon Hypothesis, *Lactuca sativa*, Microcosm, Plant Invasion, Chemical Ecology

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Introduction

Allelopathy is the inhibition of the growth of one type of plant by chemicals produced by other types or more widely, biochemical interactions between all kinds of plants and microorganisms (Rice, 1984; Yang *et al.,* 2011; Rai and Singh, 2020). It involves inhibitory and stimulatory effects manifested through allelochemical compounds (Willis, 2007). Chemical ecology is, therefore a subject of utmost interest in shaping terrestrial and aquatic biodiversity (Rai and Singh, 2020a). In certain studies, the allelopathic influence of a plant may result in positive outcomes. However, in general, it negatively affects the other plant (Elijarrat and Barcelo, 2001). Plant species are in a complex and multi-directional relationship with their neighboring plant species, including competition for nutrients and water, inhibition, stimulation and interdependence (Zeng, 2008; Rai and Singh, 2021). The phytochemical composition of higher plants comprises chemicals or secondary metabolites localized in different structural organs, such as leaves with potential allelopathic activity (Inderjit, 1996; Duke *et al.,* 2000).

The allelochemicals are released into the environment in adequate amounts to affect neighboring or successional plants, either as exudates from living plants or through the decomposition of plant residues (Rice, 1984; Dayan *et al.,* 2000; Einhellig *et al.,* 2004). The allelopathy can cause a significant decline in agricultural yield through the infestation of invasive weeds, thereby influencing food security (Kruse *et al.,* 2000; Rai, 2015). Allelochemicals indirectly affect plant growth by inhibiting the activity of microorganisms such as nitrogen-fixing bacteria, nitrifying bacteria, and ectomycorrhizae (Hunter and Menges, 2002). The allelopathic effect on plant growth is caused by a disruption in physiological processes such as cell division, elongation, cytology and ultrastructure in plant roots (AL-Jehaishy, 2017; Rice, 1984; Celik and Aslanturk, 2010; Gulzar *et al.,* 2016; Mohamadi and Rajaie, 2009; Mushtaq *et al.,* 2019). The chemicals released into the environment also alter nutrient dynamics, soil organisms, and soil characteristics (Koocheki *et al.,* 2013; Sakachep and Rai, 2021 a,b). Allelopathy corroborates Novel Weapon Hypothesis (NWH), wherein invasive species outcompete native species by releasing novel chemicals or allelochemicals that native species never encountered through root exudates as weapons against native species in invaded areas. Thus, root exudates that are fairly ineffective against their native neighbors due to adaptation may be highly inhibitory to newly introduced plants in invaded environments (Callaway and Ridenour, 2004). The Novel Weapon Hypothesis provides significant evidence for a plant's allelopathic nature and invasiveness; however, it must be tested in a wide range of invasive alien plants (Batish *et al.,* 2013).

Allelopathic interactions mostly depend on a plant's ability to produce secondary metabolites with biological effects on other organisms (Waller, 2004). Fungi, microbes and plants produce these secondary metabolites which can impact biological and agricultural systems (Prince and Pohnert, 2010). Allelochemicals

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and their derivatives may vary in activity and concentration throughout the growing season in different parts of the plant life cycle (Qasem and Foy 2001; Macias *et al.,* 2007; Gatti *et al.,* 2010). The amount of allelochemic compounds produced in specific habitats is regulated by long-day photoperiods, growing seasons, and plant growth stages (Al-Jobori and Ali, 2014). In this respect, the mineral deficiency increases allelochemical production, further enhanced by drought stress and relatively low temperatures (Ali, 2008). Plant with allelopathic effect releases chemicals into the environment which inhibit or suppress the growth of other plants, causing soil infertility and nutrient imbalance as well as limiting the microbial population in the soil (Singh *et al.,* 2003; Rai, 2012). Invasive alien plants also inhibit weed growth, cause stomatal closure, disturb mineral uptake, influence photosynthesis, affect respiration, induce water stress, and reduce enzyme activity (Baziramakenga *et al.,* 1995; Hussain *et al.,* 2010). Further, Zhang *et al.,* (2021) also noted that allelopathy affects remarkably influences seed germination. Allelochemicals can be categorized into the following categories based on structural distinctions and properties; water-soluble organic acids, aliphatic aldehydes, straight-chain alcohols, and ketones; polyacetylenes and long-chain fatty acids; simple unsaturated lactones; quinines; cinnamic acid; coumarins; phenolics; tannins; flavonoids; terpenoids and steroids (Li *et al.,* 2010). Various phytochemicals have been identified in *M. micrantha*, viz., phenolics, flavonoids, alkaloids, terpenes etc (Table 1).

Mikania is the largest genus in the tribe Eupatorieae in the family Asteraceae, with about 400 species (Holmes, 1982). The weed is of wide occurrence in North-East India (an Indo Burma hotspot) where it usually flowers from October to late November and senesces between December and January (Swamy and Ramakrishnan, 1987). In this respect, Yang *et al.* (2003) showed that *M. micrantha* plants grown in less fertile or dry soil had a shorter flowering period, fewer flowers, and a lower percentage seed set than those grown in more fertile soil. This mile-a-minute weed propagates locally via vegetative propagules (Rai, 2022). Also, this weed can markedly restrict the light or incoming photosynthetic active radiation to adversely influence the cellular metabolism of surrounding plant species (Huang *et al.,* 2000).

Several microcosm studies noted inhibitory effects of *M. micrantha's* on food crops (Li *et al.,* 2008; Wu *et al.,* 2009; Kaur *et al.,* 2012). The germination and growth of *L. sativa* seedlings were inhibited by three sesquiterpenoids compounds identified in *M. micrantha*, with deoxymikanolide being the most phytotoxic allelochemic molecule (Shao *et al.,* 2005). Chinese cabbage (*Brassica* spp.) seeds, radicles, hypocotyls, and biomass growth were adversely influenced by plant (i.e., leaves, stems, and roots) extracts of *M. micrantha* (Ismail and Mah, 1993; Ismail and Chong, 2002; Li and Jin, 2010). Additionally, it has been found that *M. micrantha* leachates increase soil nitrification with concomitant lowering of soil pH and soil net mineralization, thereby making soil more conducive to its infestation (Chen *et al.,* 2009). Leaching of root exudates and the secondary metabolites from *M. micrantha* during rain is eventually discharged into the environment (Zhang *et al.,* 2021). Also, Jali *et al.* (2021) found that the *M. micrantha* extracts inhibit the rate of seed germination, plant growth, and biochemical parameters of *Macrotyloma uniflorum*.

Mikania micrantha alters the environmental attributes through the release of allelochemicals which can be toxic to surrounding plants. It inhibits the growth and regeneration of some native plant species/food crops and assists itself in becoming the dominant species in novel introduced landscapes (Ni *et al.,* 2007). Therefore, the objectives of the present microcosm study were to collect the soil of invaded region of *M. micrantha* and study its positive and negative impacts on the growth and development of a selected native crop (*L. sativa*) in a pot experiment.

Table 1: Phytochemicals identified in *Mikania micrantha*

Number	*Compound*
Phenolics	
1	Vanillic acid
2	Resorcinol
3	Caffeic acid
4	p-Hydroxybenzaldehyde
5	Isobutyl acetate
6	3,5-Di-o-caffeoylquinic acid n-butyl ester
7	3,4-Di-o-caffeoylquinic acid n-butyl ester
Flavanoids	
8	Mikanin
9	Eupalitin
10	Eupafolin
11	3,4′,5,7-Tetrahydroxy-6-methoxyflavone3-o-β-D-glcopyranoside
12	Luteolin
Alkaloid	
13	2-Butanamine
Terpenes and their derivatives	
14	Dihydromikanolide
15	Deoxymikanolide
16	2,3-epoxy-1-hydroxy-4,9-germacradiene-12,8:15,6-diolide
17	Mikanolide
18	Scandenolide
19	Dihydroscandenolide
20	Anhydroscandenolide
21	Mikanokryptin
22	Limonene
23,24	α-, β-Copaene
25-27	α-, β-, γ-Terpinene
28	Longipinene
29-30	α-, β-Bergiberene
31	α-Zingiberene

Modified after Ni *et al.* (2007)

Materials And Method

The microcosm investigation was conducted in the Department of Environmental Science, Mizoram University, Aizawl, Mizoram, India (Latitude of 23.7307° N and Longitude of 92.7173° E). Mizoram is located in the North Eastern parts of India, comprising approximately 8% of the country's total land area. Northeastern biodiversity is endowed with genetically diverse endemic plants, animals, and microbes. Among 34 biodiversity hotspots, the Indo Burma is listed as a priority hotspot for biodiversity conservation, including the northeast region (Rai and Vanlalruati, 2022). The experiment was done during the months of April and May, 2021. *M. micrantha* invaded soil was collected from the rhizosphere of the plant and put in the pot marked as the Experimental pot. Similarly, the healthy forest soil was also collected from the forest where *M. micrantha* is absent, kept in a pot and marked as a Control Pot. A native plant (*Lactuca sativa)* seed was selected, as it is a fast-growing crop commonly cultivated in the state of Mizoram. Fifteen seeds were sown in each pot, watered with tap water and kept in a controlled greenhouse. At different time intervals, it was carefully examined and the changes in the morphology of the plant were noted down. On the third and seventh days, the number of seeds germinated was noted. On day 59, when the crop is fully grown two samples were taken from each pot and measured the growth parameters. Different growth parameters that were used to analyze the data are Seedling Height, Root Length and Shoot Length, Seedling Biomass, Germination Percentage, Germination Potential, Germination Index, Germination Rate Index and Vigor Index (Singh *et al.,* 2014). 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 analyzed from each pot were compared and represented in Table 2.

Results And Discussion

Seedling Height, Root Length and Shoot Length

The estimated mean seedling height, root length and shoot length of *L. sativa* (Sample A and B) sown in the Experimental pot and Control pot are shown in Fig. 1. The Seedling height was 8.85 cm in Experimental Pot and 25.85 cm in the Control pot. The Root Length was 0.091 cm in Experimental Pot and 4.55 cm in Control Pot. The Shoot Length on the Experimental Pot was 8.759 cm, and 21.3 cm on the Control Pot. All three parameters, i.e., Seedling Height, Root Length and Shoot Length are much higher on the Control Pot as compared to the Experimental Pot. It shows that *M. micrantha* inhibits the growth and development of *L. sativa*. The inhibition is more on the root than the shoot. Wu *et al.* (2015) also found similar results that *M. micrantha* leaves extract influenced seed germination percentage, initial germination time, and germination rate of *L. sativa* substantially. High concentration of allelochemicals of M. micrantha was also found to decrease the seed germination, root and shoot growth, and fresh weight of *Biden pilosa* (Ma *et al.,* 2021). The activity of recipient plant root cells was reduced, and plant bud growth was hindered when the concentration exceeded the allelochemical threshold (Ren *et al.,* 2018; Carmello and Cardoso, 2018). Allelochemicals such α-amyrin, stigmasterol, and ferulic acid inhibited the growth of the seedlings of food crops, including *R. sativus*, *B. campestris*, and *Oryza sativa* (Li *et al.,* 2008).

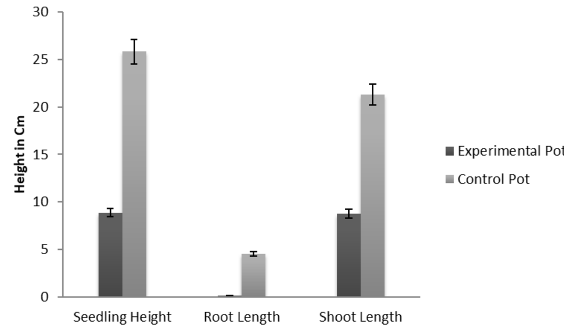

Fig. 1: Seedling height, Root Length and Shoot Length of *Lactuca sativa* in Experimental Pot and Control Pot.

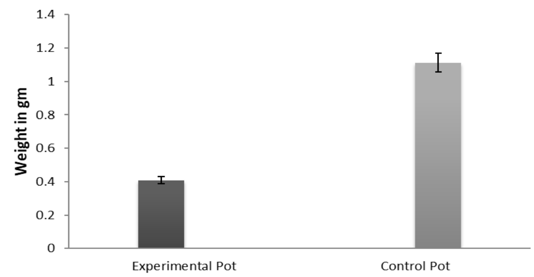

Fig. 2: Seedling biomass of *Lactuca sativa* in Experimental Pot and Control Pot.

Biomass

The seedling biomass of *L. sativa* was 0.408 g on Experimental Pot and 1.112 g on the Control Pot. It was much higher in Control Pot than in Experimental Pot (Fig. 2). The biomass or fresh weight of the plant decreases in the Experimental Pots because it shows an allelopathic effect on the length of root and shoot. Plants' monoterpenes and sesquiterpenes are believed to induce active information exchange with their surroundings (Dudareva *et al.,* 2006). Monoterpenes present in *M. micrantha*, which restrict cell proliferation and DNA synthesis in apical tissues, impact seedling growth. (Nishida *et al.,* 2005; Delory *et al.,* 2016).

Table 2: Morphological characteristic of *L. sativa* species grown in *M. micrantha* invaded soil (Experimental Pot) and Forest healthy soil (Control Pot).

Parameters	Control pot	Experimental pot
Seedling height (cm), (Mean ± SE)	25.85 ± 0.75	8.85 ± 0.35
Root length (cm), (Mean ± SE)	4.55 ± 0.35	0.091 ± 0.027
Shoot length (cm), (Mean ± SE)	21.3 ± 0.4	8.759 ± 0.377
Seedling Biomass (g), (Mean ± SE)	1.112 ± 0.1	0.408 ± 0.09
Germination percentage (%)	93.33	86.66
Germination potential	0.93	0.73
Germination index	4.66	3.66
Germination rate index	434.91	317.17
Vigor index	5.18	0.52

Germination Percentage and Germination Potential

Seed germination is a crucial stage in sapling enlistment and community dynamics for seed plants, and seed scarcity is acknowledged as one of the major challenges in restoring natural ecosystems that have been overrun by exotic species. (Xing *et al.,* 2003, Bakker & Wilson 2004). The germination potential of *L. sativa* in the Experimental Pot was 0.73 and 0.93 in the Control pot (Fig. 3A). The germination percentage in Control Pot was 86.66 % and 93.33 % in Experimental Pot (Fig. 3B). Both germination percentages and germination potential of *L. sativa* were lower on the experimental pot. In past studies also, allelochemic volatiles from the weed leaves significantly hampered seed germination of *L. sativa* (Ma *et al.,* 2021). *M. micrantha's* volatiles are considered an allelopathic stress signal, promoting tissue disorder, disrupting the cell's internal structure, interrupting mitosis and gene expression patterns, and inhibiting recipient plant growth (Ismail and Mah 1993; Oracz *et al.,* 2007).

Germination Index, Germination Rate Index and Vigor Index

The germination index in the Control Pot was 4.66 and in the Experimental Pot was 3.66. Fig. 4A shows that the Germination Index of *L. sativa* was higher in the control pot and lower in the Experimental Pot. Fig. 4B shows that the germination rate of *L. sativa* was also higher in the Control Pot and lowers in the Experimental Pot. The Control Pot with a 434.91 germination rate index showed the best performance as compared to the Experimental Pot with 317.17. The Experimental Pot has a lower Vigor Index of 0.52, the average Vigor Index of the two samples of the Experimental Pot. The average Vigor Index of the two samples of the Control Pot was 5.18, which is higher than the Experimental Pot. The seed germination rate was determined by the speed and consistency with which seeds germinated (Bao, 2019). Ismail & Chong (2002) found that allelochemicals in *M. micrantha* aqueous extracts caused a decrease in seeds germination speed and radicle lengths of *Lycopersicon esculentum* Mill and *Brassica chinensis* L. Allelopathy has a broad application prospect in increasing crop production, forest tending, plant protection, biological control, etc. (Yan *et al.,* 2000). The volatile oil of *M. micrantha* flower contains α-Pinene and β-Pinene in high concentrations and is an effective insect repellent (Hao & Ge, 1999). Alcohol extracts of *M. micrantha* significantly decreased the survival of citrus red mite (*Panonychus citri*) eggs, larvae and nymphae (Cen *et al.,* 2005). Laboratory and pot culture experiments by Wu *et al.,* (2009) and Kaur *et al.,* (2012) also showed that *M. micrantha* shoots produce allelochemicals that can alter the recruitment and growth of native species with negative influence on their diversity.

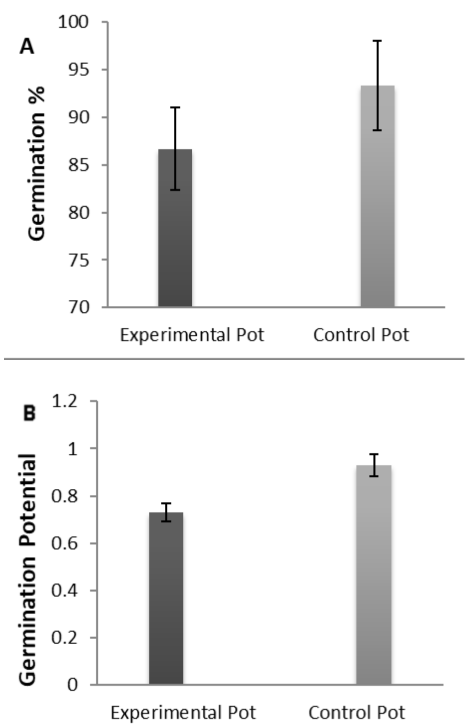

Fig. 3: (A) Germination percentage and (B) Germination potential of *L. sativa* in Experimental Pot and Control Pot.

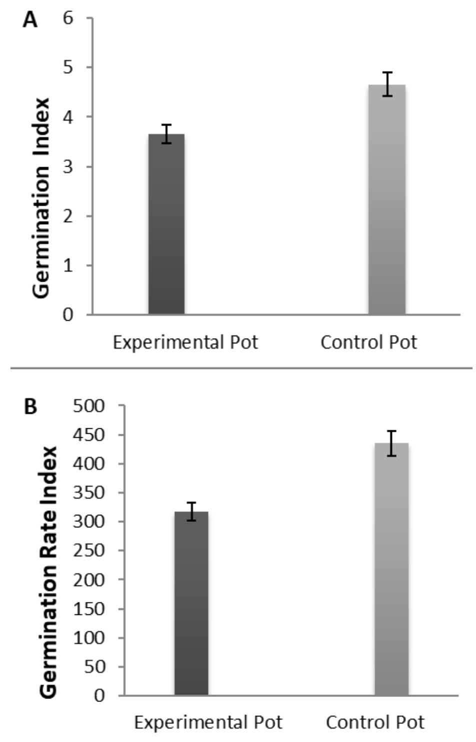

Fig. 4: (A) Germination Index and (B) Germination Rate Index of *L. sativa* on Experimental Pot and Control Pot.

Conclusion

Allelopathic effects of *M. micrantha* adversely influence the germination and seedling growth of food crop i.e., *L. sativa*. The impact of the invasive alien plant was inhibitory to the plant growth parameters like root length and shoot length. Germination potential, germination percentage, germination index, and germination rate index were also reduced due to allelopathic effects of *M. micrantha*. However, the extent of reduction was lower than the growth parameters. Vigor Index was much lower in the Experimental Pot when compared with the control. These results validate the Novel Weapon Hypothesis, as the invasive alien plant *M. micrantha* inhibits native plants or food crops. More research is needed to evaluate the potential of *M. micrantha* on edible crops and other plant species. Studies can be carried out on the effect of different crop/plant parts, based on the specific concentrations of allelochemicals and soil analysis of the invaded region. Also, the effect of allelochemical on the food crop-soil microbe interactions and other living organisms can be carried out to elucidate the chemical ecology of *M. micrantha*. Proper weed management is needed to control the abrupt growth of invasive alien plant species in agricultural lands. Further long-term mesocosm studies are warranted to elucidate the mechanisms of allelopathy, the natural release of allelochemicals in the environment, allelopathic native-invasive interactions, and the beneficial prospects for drugs and medicinal purposes.

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Author Contribution

Roger Bruce Syngkli, Lalnunhlui, Sarah Lallianpuii and P.K Rai designed the protocol and framework of the present work. Roger Bruce Syngkli and Lalnunhlui conducted the experiment and compiled the data. Sarah Lallianpuiiand P.K Rai made the necessary corrections to finalize the writing of present article.

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