ENVIRONMENTAL REPORTS

Original Research Article
ISSN: 3041-556X
Journal homepage: https://er.researchfloor.org/

Study on the impact of *Mikania Micrantha* Kunth on soil chemistry, crop yields, and forest canopy in an Indo-Burma biodiversity hotspot region

Roger Bruce Syngkli, Vanlalruati, Prabhat Kumar Rai*

Department of Environmental Science Mizoram University, (A Central University) Aizawl, Mizoram - 796004, India

Citation: Roger Bruce Syngkli, Vanlalruati, Prabhat Kumar Rai (2024). Study on the impact of *Mikania Micrantha* Kunth on soil chemistry, crop yields, and forest canopy in an Indo-Burma biodiversity hotspot region. *Environmental Reports; an International Journal*. **08 to 14. DOI: https://doi.org/10.51470/ER.2024.6.2.08**

Corresponding Author: **Prabhat Kumar Rai** | E-Mail: **(pkraimzu@gmail.com)**

Received 19 July 2024 | Revised 22 August 2024 | Accepted 17 September 2024 | Available Online October 03 2024

ABSTRACT

Mikania Micrantha Kunth is an invasive alien plant pervasive in an Indo-Burma global biodiversity hotspot, which can exert a major impact on biodiversity, agriculture, socioeconomy, and human health. The presentstudy attempts to evaluate the allelopathic effect of M. micrantha leaf aqueous extract on the germination of Pisum sativum L. (Pea). Major allelochemicals like total phenolic compounds (TPC) and tannins present in the leaves and flower extracts of M. micranthawere analyzed by spectrophotometry method. We also investigate the habitat attributes of M. micrantha infested site by analyzingsoil physicochemical characteristics, photosynthetically active radiation (PAR), leaf area index (LAI), and canopy openness. The results showed that M. micrantha aqueous extract inhibits P. sativum germination, biomass, plumule, and radicle length at all concentrations, with the highest inhibition observed at 25gL^{-1}. The presence of allelochemicals (total phenolic content and tannins) in the extracts also demonstratesallelochemicthe potential of M. micrantha, thus, validatingthe 'novel weapon hypothesis (NWH)'. Further in respect of abiotic environmental components, study area with high soil temperature, soil moisture content, water holding capacity, nitrogen, and potassium facilitated the invasive spreadof M. micrantha. This study concludes that novel disturbed habitats with increased availability of PAR, low LAI, and high canopy openness are more susceptible to invasion of M. micrantha. On the contrary, a pristine environment with low canopy openness and less anthropogenic disturbances are rather resistant tothe invasion of M. micrantha. Henceforth, an integrated study comprising site characteristics, soil chemistry, and bioassay is necessary to devise sustainable management of M. micrantha.To this end, the sustenance of dense canopy can be the most effective management strategy to control this worst global plant invader.

Keywords: *Allelochemicals, Forest Canopy Openness, Leaf Area Index, Gap Light Analyser, Mikaniamicrantha, Novel Weapon Hypothesis,Pisum sativum*

1. INTRODUCTION

Invasive plants have a significant negative impact on crop productivity, biodiversity, and the environment [1-3]. Global food safety and agricultural stability are affected by several interrelated and tightly linked factors, including population growth, poverty, environmental deterioration, and the use of synthetic herbicides [4]. Crop yields are negatively impacted by weed competition for resources including light, nutrients, space, water, and allelopathy [5-7]. The financial burden and unsustainable nature of traditional weed management practices jeopardized farmers livelihoods, which strained the general public and policymakers [8-9].

*Mikaniamicrantha*Kunth. ("mile-a-minute" weed)is an extremely fast-growing perennial vine belonging to the Asteraceae family and genus *Eupatorium* in the Compositae family [10] (Figure 1a). It is native to the sub-tropical regions of South, Central, and North America [11]. *M. micrantha* was known as the top 100 noxious invaders by the International Union for Conservation of Nature (IUCN) [12-13]. The flowers of *M. micrantha* produced crown hair seeds that can proliferate over a long distance and hence difficult to control [14] (Fig. 1a). Invasive spread of *M. micrantha* results in a notable decrease in the output of agricultural crops and hence endanger food security [15]. The harmful effects of *M. micrantha* are primarily caused by allelopathic effect (release of allelochemicals/ secondary metabolites) which modulated the soil chemistry, microbial diversity, agricultural yield, and biodiversity in a manner to sustain its invasive spread [16-18]. In terrestrial ecosystems, *M. micrantha* climbs and cover trees and rapidly causes the death of trees and shrubs, thereby severely impacting the native biodiversity [11]. This abrupt spread of *M. micrantha*can perturb the normal photosynthetic metabolism of native plants, enforcing their senescence and expanding the canopy gaps (Fig. 1b). In this respect, past studies also observed that *M. micrantha* compete with other neighboring plants/edible crops for nutrients and other abiotic resources by blocking the sunlight [45, 2119-20]. This above-canopy spread of *M. micrantha*significantly lowers the growth and productivity of a number of crops and has the ability to choke, suffocate, penetrate crowns, and pull over plants [21].

In this study, we investigate the allelopathic effects of *M. micrantha* on a selected food crop (i.e., *Pisum sativum* L.) of wide dietary intake and nutritional value.*P. sativum*(locally known as green pea) isanutritious legume crop that is high in protein, vitamins, minerals, and health-promoting beneficial compounds [22-23]. Pea has been used for experiments for decades pertaining to a plethora of cytogenetics, and ecological studies as it is susceptible to broad leaf herbicides, can be used year-round, and performs well in bioassays. In this respect, the analysis of major allelochemicals present on the plant parts, allelopathy, physicochemical characterization of soil, and their linkage with the habitat attributes of *M. micrantha* (such as light intensity, canopy gap analysis, and leaf area index (LAI)) was studied which is a rather novel integrated approach in its

invasion ecology. Henceforth, this article aimed to evaluate the allelopathic potential of *M. micrantha* by the (i) Phytotoxic effects of the leafextract on the growth of*P. sativum* (ii) Total phenolic content (TPC) and tannins present ininvasive plant parts,and(iii) Physicochemical characterization of soil

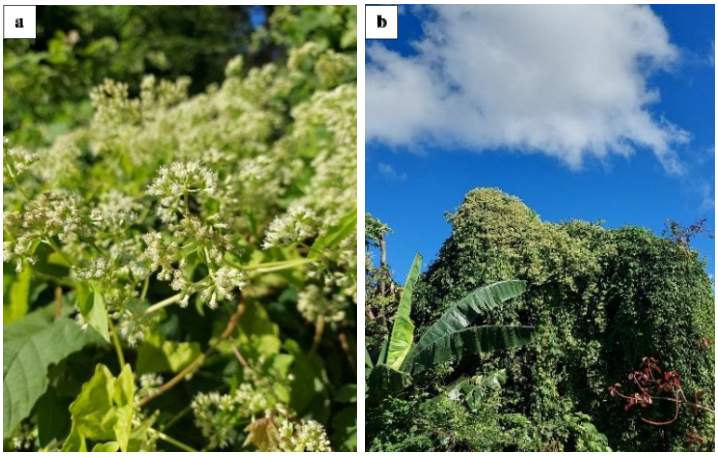

Figure 1a-b. Description of invasive alien plan and study area(a)*Mikaniamicrantha* plant during flowering stage with abrupt spread covering the shrubs/tree vegetation (b). *M. micrantha* penetrates the tree crown and covers the canopy which reduces the sunlight reaching the forest floor which may ultimately lead to their senescence and hence perturb the dense canopy (photo courtesy R. B. Syngkli)

2. MATERIALS AND METHODS

2.1 Study Site

The research was carried out in Aizawl district, Mizoram, North East India ('23.87 ºN 92.89 ºE')at an altitude of '800 meters to 1200 meters' which is underlying in an Indo-Burma global biodiversity hotspot (Figure 1c). The area receives 2350 mm of rain falls annually. Summer temperatures range from 20 to 35 °C, while winter temperatures range from 9 to 21 °C. The research was conducted during May, 2023toApril, 2024.Laboratory work was done at Mizoram University, Tanhril, located 846 meters above sea level in Mizoram, India (23.73784167ºN 92.66741944ºE). The ambient light of the site ranged 2248.33-3152.33 LUX during the study period.

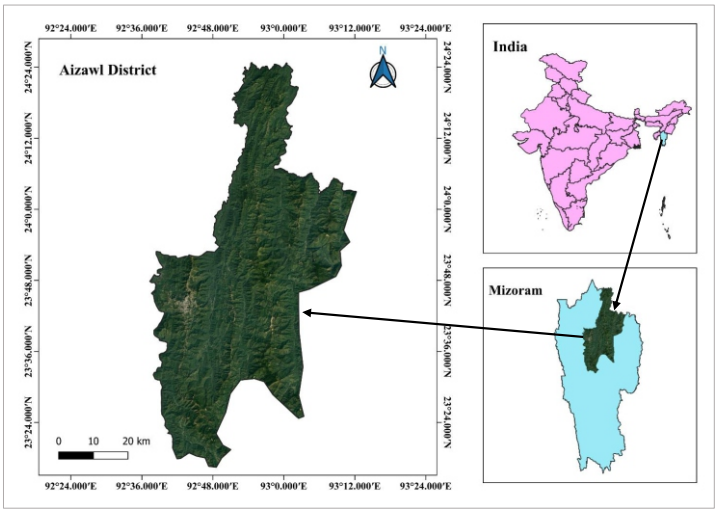

Figure 1 (c). Map of Aizawl district, Mizoram, North East India, undelying in an Indo Burma global biodiversity hotspot region.

2.2 Preparation of leaves aqueous extracts of *M. micrantha* and its effects on the growth of test crops

Aqueous leaf extract of *M. micrantha* was prepared by following the method of Ismail and Chong [24]. Fresh leaves of *M. micrantha* were collected from the invaded region, and faced with several anthropogenic disturbances. The leaves were washed thoroughly with clean water to remove dust and dirt and chopped into small pieces to keep them in a conical flask. Further, 100 ml distilled water was added into the flask and agitated in a rotary shaker (Rotary Flask Shaker, Scientech, Science Enterprises, Delhi, India) at 140-150 RPM for 8 h. The aqueous extract was then filtered through 2 layers of filter papers (Whatman No. 41) and maintained it eventually into 4 concentrations (i.e., 50 gL^{-1}, 37.5 gL^{-1}, 25 gL^{-1}, and 12.5 gL^{-1}). Besides, these concentrations of aqueous extracts, a control (distilled water) was used to compare the results. The petri dishes (90 x15 mm) were covered with filter paper which acted as medium to absorb the leaf extracts. Ten seeds of *P. sativum* were kept in petri dishes. Further, 5ml of different extract concentrations and control were added in petri dishes and covered with filter papers. All experiments along with the control were done in duplicate for better comparative analysis. The petri dishes were covered and incubated Biochemical Oxygen Demand (BOD) Incubator, at 30º C for 4 days. Finally, the seed germination percentage (%), radicle, and plumule length of the test crops were measured to compare the effects of *M. micrantha*-based leaf extracts in comparison to the control.

2.3 Sample extractions

Allelochemicals of *M. micrantha*were extracted from the plant parts(i.e., leaves and flowers) using a traditional cold maceration solvent extraction method, which was slightly modified as per Handa et al. [25]. An extraction was conducted using a sample-to-solvent ratio of 10:100 (w/v). A 250 mL stoppered Erlenmeyer flask with 100 mL of 95% methanol was used to macerate 10 grams (10 g) of the air-dried soil samples. The mixtures were let to stand at room temperature while being stirred frequently which lasted for 72 hours. Following filtration using Whatman No. 1 filter paper (125 mm), the solvent was evaporated using a rotary evaporator (BUCHI Rotavapour R-300). After evaporation, a crude extract of leaves and flowerswas obtained.

2.4 Quantification of allelochemicals

Total phenolic content (TPC)

The Folin-Ciocalteu colorimetric method by Ainsworth and Gillespie [26] was applied to determine the total phenolic content. 0.5 mL of aliquot of 10, 20, 40, 80, and 100 µg/mL gallic acid solutions were mixed with 5 mL of 10% Folin-Ciocalteu and kept for 5 mins. 4 mL of 7% Sodium Carbonate (Na_2CO_3)wasadded, and the mixture was allowed to incubate at dark room temperature for 60 mins. The optical density was measured using a spectrophotometer at 765 nm. The same procedure was used for all plant parts extract. The stock solution for plant extract was prepared using 20 ml of methanol and 20 mg of the crude extract. TPC was calculated using gallic acid's standard calibration curve and represented as µg/mL gallic acid equivalent (GAE) of the dry extract.

Tannins

The Folin-Ciocalteu method by Lah are et al. [27] was used to determine the total tannin content of the crude extract. The stock solution was prepared using 20 ml of methanol and 20 mg of the crude extract.7.5 ml of distilled water was used to dissolve

0.1 ml of crude extract. The mixture was mixed with 0.5 ml of Folin reagent, 1 ml of 35% (Na_2CO_3), and diluted with 10 ml of distilled water. The mixture is allowed to stand at room temperature for half an hour after being thoroughly mixed. Using a spectrophotometer, the optical densities of the sample and standard solutions were determined at 725 nm. The tannin concentration was measured in terms of gallic acid equivalents (GAE) per gram of extracts, with gallic acid concentrations (10, 20, 40, 80, and 100 µg/ml) being selected as standard.

2.5 Physicochemical characterizationof soil on *M. micrantha* invaded site

Soil temperature were measured onsite with a portable soil analysis kit. For other parameters, the soil was collected from the rhizosphere (15cm depth)and surrounding of *M. micrantha*using a shovel and kept in a clean plastic tray. Unwanted debris such as roots, stones, leaves, and plant materials were removed. Soil pH was analyzed by 'potentiometric method' with a 5:1 ratio of water to the soil using a pH glass electrode (µ pH system 361, Systronics). The moisture content was determined by the 'gravimetric method' [28].The soil was air-dried and sieved by 2 mm sieve. Water holding capacity was analyzed by standard methods. Soil organic carbon (SOC) was estimated by following 'Walkley and Black's'[29]'rapid dichromate oxidation method'.The estimation of available soil Nitrogen was done by 'Kjeldahl's Digestion Method[28]. Available Phosphorus was estimated by using the 'Bray method'. The available potassium will be determined by a standard method using a flame photometer.

2.6 Canopy openness, leaf area index, and light intensity of invaded site

The canopy openness of *M. micrantha* infested site was analyzed by hemispherical photography and gap light analyzer (GLA) software method, as devised by Sooraj et al. [30]. The hemispherical photographs were taken in a skyward direction perpendicular to the ground using a fish eye lens (Skyvik Sign One 10mm Fisheye Lens) mounted on a smartphone (Samsung Galaxy M51). Hemispherical photos were taken in triplicates from 16 random areas (named asG1, G2, G3, ……., and G10) on a cloudy day and select 1 best photograph to act as an input to GLA software for canopy gap analysis (Figure 2). Coordinates and elevation of the site were taken using GPS (Garmin GPSMAP 64s). The magnetic declination for the configuration settings of GLA was taken from the National Oceanic and Atmospheric Administration (NOAA) website[31]. The light intensity and LAI of the area were measured using a plant canopy analyzer (Integration of handheld and Software from Kaizen Imperial and Quantum Sensors from Apogee Instruments, USA) from 16 random spots under the forest canopy. The data of the plant canopy analyzer (PCA) were recorded and stored in the handheld device attached to the sensors which were later extracted using PCA software. Herein, plant canopy analyzer data and hemispherical photographs were collected to facilitate the study on the habitat ecology and further elucidate the role of canopy gaps in influencing the invasive spread of *M. micrantha*, which might be tightly linked with its allelochemical attributes.

3. RESULTS AND DISCUSSION

3.1 Impacts on the growth of *Pisum sativum*

The aqueous leaf extract of *M. micrantha* has negative inhibitory effects on the growth of *P. sativum.* In this respect,50gL^{-1}the extract showed the lowest inhibition, whereas, 25gL^{-1} showed the highest inhibition (26.7% reduction) in terms of seeds germination (Fig. 3a). The biomass of the seedlings was reduced by 33.4% at 25gL^{-1}but the reduction was recorded only up-to the extent of 1.01% at higher concentration of50gL^{-1} (Fig. 3b). The radicle length also showed inhibitory effect at all concentration with the highest inhibition being recorded at 47.51% at 25gL^{-1} (Fig. 3c). Similarly, the length of plumule were also reduced at all concentrations with the highest reduction observed at 25gL^{-1} (31.97% reduction).The aqueous leafextract of *M. micrantha*has therefore exhibited a significant effect on Pea plant growth parameters at 25gL^{-1}and 37.5gL^{-1}. Past studies such as those of Sahu and Devkota[32] also corroborated that *M. micrantha* aqueous leafextract inhibits the germination and seedling growth of *Oryza sativa*L. and *Raphanussativus*L. Similarly, Wu et al. [33] also found that *M. micrantha* inhibits the germination and seedling growth of 26 species which comprised native, naturalized exotic species, legumes, and non-legumes species.Further, it has also been observed that volatile organics of the leaves and flowers of *M. micrantha* also inhibit the germination rates of *Lactucasativa*L., *Chrysanthemum coronarium*L., *Bidenspilosa*L., and *Abutilon theophrasti*Medik.[34].

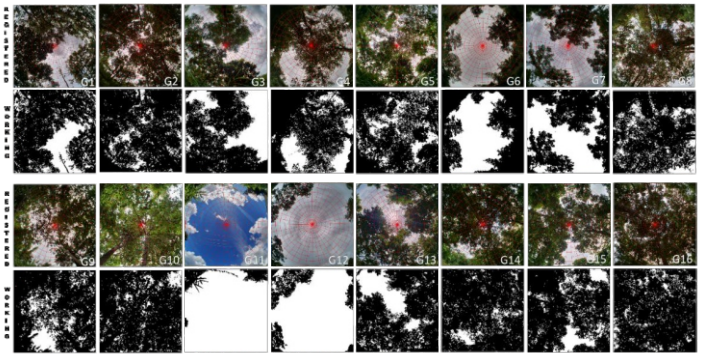

Figure 2. Hemispherical photographs used for forest canopy openness estimation. The monochromatic images are processed (working image) whereas the colour images are the original images (registered image) used to analyze canopy openness on Gap light analyzer software.

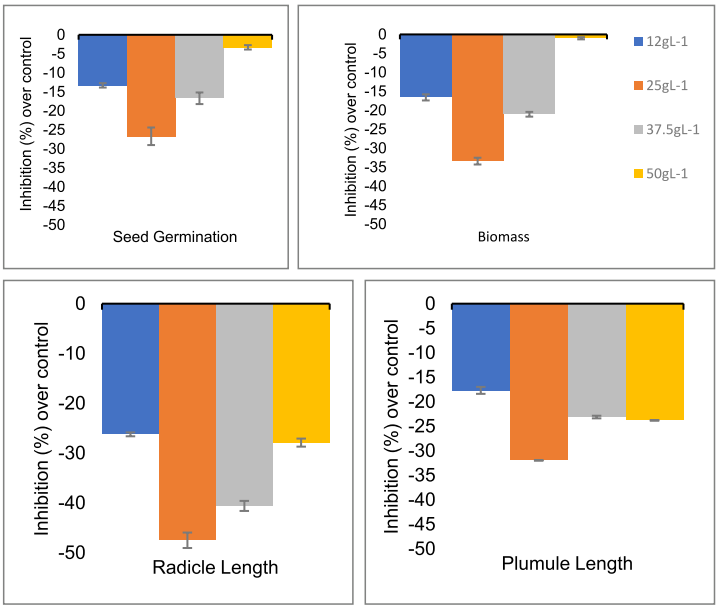

Figure 3. Allelopathic effect of *M. micrantha* on (a) Germination, (b) Biomass, (c) Radicle length, and (d) Plumule length of *P. sativum.*

3.2 Allelochemicals analysis

The TPC values recorded in the leaves (15.13 µg) and flowers (29.97 µg) ascertained the presence of phenolics as the most ubiquitous allelochemic compound. Similarly, tannins were also estimated in the leaves (15.84 µg) and flowers (35.67 µg), demonstrating the abundance of secondary metabolites in plant parts of *M. micrantha*. Interestingly, TPC and tannins were recorded higher concentrations in floral extract when compared with the leaves. The presence of these allelochemic compounds confirms the inhibitory effect of *M. micrantha* aqueous extract on *P. sativum,* thereby, validating the NWH**.** Past studies havealso reported that *M. micrantha* contains structurally diverse allelochemicals that have a significant bioactivity (Ríos et al., 2014; Dong et al., 2017; Ishak et al., 2018; Sumantri et al., 2020)[35-38]. Thus, present reports on allelochemic potential of *M. Micrantha* was well corroborated by past studies and pave the way for further global studies in sustainable management of invasive alien plants.

Table 1. Total phenolic content and tannins present on the leaves and flowers of M. micrantha.

Allelochemical	Sample	Concentration* (µg GAE)	Wavelength (nm)
TPC	Leaf	15.13±0.41	765
	Flower	29.97±0.92	
Tannins	Leaf	15.84±0.43	725
	Flower	35.67±1.57	

**Each value represents the mean (± standard deviation) of three replicates.*

3.3 Physicochemical characterization of soil

The *M. micrantha* invaded site remarkably altered the soil physicochemical parameters when compared with the control (Table 2). The soil temperature at the highly invaded site was recorded 25.3°C, which reflects the ability of *M. micrantha* to withstand high temperatures, ascribed to its phenotypic plasticity [39-40]. Similarly, soil moisture content and water holding capacity (17.88%, 87.47%) were also estimated higher at the invaded site. Therefore, it is quite evident that environmental variables can influence the invasion success of plant invaders in concert with their biological traits[41]. In this respect, abiotic environmental factors such as water is one of the key determinants in the spread of invasive alien plants [42-43].Especially, *Mikania* prefers habitats with high water content in the soil such as wetlands and other aquatic ecosystems [19].Likewise, Yue et al. [41]also found that *M. micrantha* has higher growth characteristics and population in wet habitats than dry habitats. Similarly, available nitrogen (229.97 kg/ha) was also higher at the invaded site when compared with the control. However, pH (5.18), SOC (1.74%), available phosphorus (16.85 kg/ha), and available potassium (339.73 kg/ha) were lower at the invaded site as compared to the control site. Importantly, past studies also elucidated that *M. micrantha* alters the nutrients in the soil by mobilizing the activities of microbial enzymes which inturn affect the functional metabolism of microbial nitrogen and phosphorus [18]. Previous studies demonstrated that other invasive alien species from Asteraceaelike *Chromolaena odorata*(L.) R.M. King & H. Robprefer to grow on soil with low phosphorus content [44-45]. Agricultural fieldsrich in nutrient content are therefore prone to infestation of invasive alien plants, which reduce agricultural yield to threaten food security and socioeconomic or farmer's livelihood[46-47].

Table 2. Physicochemical parameters of soil on M. micranthainvaded site

Sl. No	Parameters	Control	Invaded site
1	Soil temperature (°C)	23.7±0.71	25.3±0.71
2	Soil moisture content (%)	12.66±0.32	17.88±0.58
3	Water holding capacity (%)	81±1.92	87.47±3.68
4	pH	5.77±0.06	5.18±0.11
5	Soil organic carbon(%)	2.01±0.12	1.74±0.13
6	AvailablePhosphorus(kg/ha)	21.95±1.43	16.85±2.17
7	Available Nitrogen (kg/ha)	209.07±7.24	229.97±14.48
8	Available Potassium (kg/ha)	545.07±17.11	339.73±23.31

3.4 Forest canopy openness, leaf area index, and photosynthetically active radiation (Incoming and diffused)

The forest canopy openness analysis was to determine the overall canopy/forest cover of the study site invaded by *M. micrantha*. Sites where *M. micrantha* was absent were denoted by '0'while'1'was assigned for sites that marked their presence (Table 3). The site comprises of forest area, human settlements, and other anthropogenic activities such as quarry, agricultural activities, small industries, and rock grinding mills. The canopy openness of the site ranged from 10.71 % (lowest) to 98.73 % (highest) with the average value being 35.32 % (Table 3).In this respect, the average LAI was estimated 1.55 at the invaded site wherein 5.3 was the highest and 0.05 was the lowest recorded value(Table 3).The incoming average PAR was 396.48 µ mol m^{-2} s^{-1} and diffused PAR was 183.40 µ mol m^{-2} s^{-1}. Diffused PAR is the sunlight that reach the forest floor which is already absorbed and reflected by the forest canopy. The canopy openness was observed higher in the area where *M. micrantha* was present as the dominant invasive plant. Past studies in this Indo-Burma biodiversity hotspot region also noted that high canopy openness, high light availability, and low LAI indicate the high disturbance status of the novel introduced site [48]. Huang et al. [44] found that light intensity escalates the growth of invasive alien plants like *C. odorata,* therefore, an open canopy area with abundant light is prevalent to *M. micrantha*. When there is less availability of under story light, *M. micrantha* climbs the forest canopy for sunlight (Fig. 1b).

Table 3: The forest canopy openness, leaf area index, and photosynthetically active radiation (Incoming and diffused) of the site invaded by M. micrantha.

Gaps	GPS Coordinates	Elevation	Mikania-status (0-Absent 1-Present)	Photosynthetically Active Radiation (PAR)		Leaf Area Index (LAI)	Canopy Openness (%)
				Incoming PAR *µ mol m^{-2} s^{-1}*	Diffused PAR *µ mol m^{-2} s^{-1}*		
G1	N23°45'27.97" E092°40'01.19"	692	1	195.33	131	0.67	29.45
G2	N23°47'43.06" E092°43'57.92"	1147	0	29.33	20	0.71	16.4
G3	N23°45'31.12" E092°39'57.14"	665	1	748.33	477.33	0.87	33.2
G4	N23°45'32.88" E092°40'07.28"	703	1	65.00	35.67	1.07	28.71
G5	N23°47'55.13" E092°44'11.42"	951	1	150.67	90.33	0.88	27.12
G6	N23°45'33.61" E092°40'09.13"	708	1	80	77.67	0.05	59.24
G7	N23°45'26.14" E092°39'50.52"	649	1	307.33	141	1.30	48.97
G8	N23°47'33.97" E092°44'05.72"	1057	0	210	41	2.72	19.6
G9	N 23°47'56.24" E092°44'02.77"	1075	0	61	29.33	1.24	25.85
G10	N23°47'54.73" E092°44'12.44"	938	0	604.33	33.33	5.32	10.71

G11	N23°45'18.98" E092°40'16.34"	768	1	1620	821	1.63	98.73
G12	N23°45'19.39" E092°40'16.97"	784	1	342.67	239	0.60	90.14
G13	N23°45'17.90" E092°40'21.20"	799	0	1172.67	649.67	1.44	29.8
G14	N23°45'17.57" E092°40'19.95"	789	0	582.00	45.33	4.12	13.73
G15	N23°47'54.21" E092°44'09.10"	994	1	150.67	90.33	0.88	22.03
G16	N23°47'41.04" E092°43'59.74"	1138	0	24.33	12.33	1.35	11.38
Mean				**396.48**	**183.40**	**1.55**	**35.32**

4. CONCLUSION

This study revealed that *M. micrantha* aqueous extracts have negative inhibitory effects on the germination and growth of *P. sativum*at different concentrations. The results were supported by the presence of TPC and tannins on the leaves and flower extracts of *M. micrantha* which were tightly regulated by invasive plants induced modulation of soil physico-chemical attributes. The high canopy openness, light availability (diffused PAR), and low LAI indicated that *M. micrantha* tends to grow and spread abruptly at sites faced with high anthropogenic disturbance. These disturbances facilitated the spread of *M. micrantha* which was inextricably linked with its allelopathic potential and soil chemistry, as clearly demonstrated in results of present bioassay and ecological investigation. To this end, areas with less canopy cover have low LAI which allows solar radiation to reach the forest floor. Therefore, it further facilitates the spread of *M. micrantha* and other invasive alien species which have allelopathic impacts on surrounding plants and crops. In

present bioassay study, *P. sativum* growth parameters were affected by *M. micrantha*due to imposed competition forthe availability of PAR, soil nutrients, and allelopathy. Thus, establishment of explicit interrelated framework of abiotic parameters such as PAR, LAI, and canopy gaps need to be studies to elucidate the allelochemic potential of *M. micrantha* on edible crops like pea. Sustenance of pristine or undisturbed sites with low canopy openness and less anthropogenic disturbances can be more resistant to the invasion of *M. micrantha*.

ACKNOWLEDGEMENT

The authors are also thankful to the Ministry of Tribal Affairs, Government of India for financial assistance in the form of a National Fellowship for ST, Department of Biotechnology (DBT-BT/PR24917/NER/95/907/2017), and department of Science and Technology (DST- Nexus Project) vide research project no. DST/TMD/EWO/WTI/2K19/EWFH/2019 (C), for financial assistance. Authors are grateful to the Department of Environmental Science, Mizoram University, Aizawl, Mizoram, India, for providing the laboratory facilities.

DECLARATION

The authors announce that they have no conflict of interest and follow the ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCE

1. Ali, H.H., Peerzada, A.M., Hanif, Z., Hashim, S. and Chauhan, B.S. (2017). Weed management using crop competition in Pakistan: A review. Crop Protection, 95, pp.22-30.

2. Sarić-Krsmanović, M., GajićUmiljendić, J., Radivojević, L., Šantrić, L., Potočnik, I. and Đurović-Pejčev, R. (2019). Bio-herbicidal effects of five essential oils on germination and early seedling growth of velvetleaf (*Abutilon theophrasti*Medik.). Journal of Environmental Science and Health, Part B, 54(4), 247-251.

3. Hussain, M.I., Danish, S., Sánchez-Moreiras, A.M., Vicente, Ó., Jabran, K., Chaudhry, U.K., Branca, F. and Reigosa, M.J. (2021). Unraveling sorghum allelopathy in agriculture: Concepts and implications. Plants, 10(9), 1795.

4. Ain, Q., Mushtaq, W., Shadab, M. and Siddiqui, M.B. (2023). Allelopathy: an alternative tool for sustainable agriculture. Physiology and Molecular Biology of Plants, 29(4), 495-511.

5. Callaway, R. M. and Ridenour, W. M. (2004). Novel weapons: invasive success and the evolution of increased competitive ability. Frontiers in Ecology and the Environment, 2(8), 436-443.

6. Macías, F.A., Mejías, F.J. and Molinillo, J.M. (2019). Recent advances in allelopathy for weed control: from knowledge to applications. Pest management science, 75(9), 2413-2436

7. Farooq, N., Abbas, T., Tanveer, A. and Jabran, K. (2020). Allelopathy for weed management. Co-evolution of secondary metabolites. 505-519.

8. Rai, P. K. and Singh, J. S. (2020). Invasive alien plant species: Their impact on environment, ecosystem services and human health. Ecological indicators, 111, 106020.

9. Rai, P. K. and Singh, J. S. (2024). Ecological insights and environmental threats of invasive alien plant *Chromolaena odorata*: Prospects for sustainable management. Weed Biology and Management, 24(1), 15-37.

10. Chen, L., Cai, M., Zhang, Q., Pan, Y., Chen, M., Zhang, X., Wu, J., Luo, H. and Peng, C. (2024). Why can *Mikaniamicrantha* cover trees quickly during invasion?. BMC Plant Biology, 24(1),511.

11. Ali Khan, M., El-Kersh, D.M., Islam, M.S., Ara Khan, S., Kamli, H., Sarkar, C., Bhuia, M.S., Islam, T., Chandra Shill, M., Gobe, G.C. and SönmezGürer, E. (2023). *Mikaniamicrantha*Kunth: An ethnopharmacological treasure trove of therapeutic potential. Chemistry & Biodiversity, 20(11), p.e202300392.

12. Lowe, S., Browne, M., Boudjelas, S. and De Poorter, M. (2000). *100 of the world's worst invasive alien species: a selection from the global invasive species database* (Vol. 12, p. 12). Auckland: Invasive Species Specialist Group.

13. Liu, B.O., Yan, J., Li, W., Yin, L., Li, P., Yu, H., Xing, L., Cai, M., Wang, H., Zhao, M. and Zheng, J., 2020. *Mikaniamicrantha* genome provides insights into the molecular mechanism of rapid growth. Nature communications, 11(1), 340.

14. Zhao, N., Ze, S., Liu, N., Hu, L., Ji, M., Li, Q. and Yang, B. (2021). Exogenous phytohormone application and transcriptome analysis of *Mikaniamicrantha* provides insights for a potential control strategy. Genomics, 113(3), 964-975.

15. Kaur, R., Malhotra, S. and Inderjit.(2012). Effects of invasion of *Mikaniamicrantha* on germination of rice seedlings, plant richness, chemical properties and respiration of soil. Biology and Fertility of Soils, 48, 481-488.

16. Chen, B.M., Peng, S.L. and Ni, G.Y. (2009). Effects of the invasive plant *Mikaniamicrantha* HBK on soil nitrogen availability through allelopathy in South China. Biological Invasions, 11, 1291-1299.

17. Sun, F., Ou, Q., Yu, H., Li, N. and Peng, C. (2019). The invasive plant *Mikaniamicrantha* affects the soil foodweb and plant-soil nutrient contents in orchards. Soil Biology and Biochemistry, 139, p.107630.

18. Zhao, P., Liu, B., Zhao, H., Lei, Z. and Zhou, T. (2023). Significant changes in soil microbial community structure and metabolic function after *Mikaniamicrantha* invasion. Scientific reports, 13(1), p.1141.

19. Zhang, L. Y., Ye, W. H., Cao, H. L., and Feng, H. L. (2004). *Mikaniamicrantha* H. B. K. in China – an overview. Weed Res. 44, 42–49.

20. Jia, P., Wang, J., Liang, H., Wu, Z.H., Li, F. and Li, W. (2022). Replacement control of *Mikaniamicrantha* in orchards and its eco-physiological mechanism. Frontiers in Ecology and Evolution, 10, p.1095946.

21. Poudel, M., Adhikari, P. and Thapa, K. (2019). Biology and control methods of the alien invasive weed *Mikaniamicrantha*: a review. Environmental Contaminants Reviews, 2(2), 06-12.

22. Fahmi, R., Ryland, D., Sopiwnyk, E., and Aliani, M. (2019). Sensory and physical characteristics of pan bread fortified with thermally treated split yellow pea (*Pisum sativum* L.) flour. Journal of food science, 84(12), 3735-3745.

23. Han, X., Akhov, L., Ashe, P., Lewis, C., Deibert, L., Zaharia, L.I., Forseille, L., Xiang, D., Datla, R., Nosworthy, M. and Patterson, N. (2023). Comprehensive compositional assessment of bioactive compounds in diverse pea accessions. Food Research International, 165, 112455.

24. Ismail, B.S. and Chong, T.V. (2002). Effects of aqueous extracts and decomposition of *Mikaniamicrantha* HBK debris on selected agronomic crops. Weed Biology and Management, 2(1), 31-38.

25. Handa, S.S., Khanuja, S.P.S., Longo, G. and Rakesh, D.D. (2008). Extraction technologies for medicinal and aromatic Plants, no. 66. *Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology.* Trieste, 21-25.

26. Ainsworth, E.A. and Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature protocols, *2*(4), 875-877.

27. Lahare, R.P., Yadav, H.S., Bisen, Y.K. and Dashahre, A.K. (2021). Estimation of total phenol, flavonoid, tannin and alkaloid content in different extracts of *Catharanthusroseus* from Durg district, Chhattisgarh, India. Scholars Bulletin, 7(1), 1-6.

28. Anderson, J.M. and Ingram, J.S. (1994) Tropical soil biology and fertility: a handbook of methods. Soil Science, 157(4), 265.

29. Walkley, A., Black, I.A. (1934) An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37, 29–37.

30. Sooraj, N.P., Jaishanker, R., Sajeev, C.R., Kumar, V.S., Lijimol, D. and Ammini, J. (2020). Influence of forest canopy gaps on establishment of *Mikaniamicrantha*kunth, an invasive plant, in a tropical forest in Southern Western Ghats, India. Applied Ecology and Environmental Sciences 8(5): 199-206

31. National Oceanic and Atmospheric Administration (NOAA). https://www.ngdc.noaa.gov/geomag/calculators/magcalc.shtml#declination (accessed 12-11-2023)

32. Sahu, A. and Devkota, A. (2013). Allelopathic effects of aqueous extract of leaves of *Mikaniamicrantha* HBK on seed germination and seedling growth of *Oryza sativa* L. and *Raphanussativus* L. Scientific World, 11(11), 90-93.

33. Wu, A. P., Li, Z. L., He, F. F., Wang, Y. H., and Dong, M. (2015). Screening allelochemical-resistant species of the alien invasive *Mikaniamicrantha* for restoration in South China. PLoS One, 10(7), e0132967.

34. Ma, H., Chen, Y., Chen, J., Ji, J. and He, H. (2021). Identification and comparison of allelopathic effects from leaf and flower volatiles of the invasive plants *Mikaniamicrantha*. Chemoecology, 31(6), 355-365.

35. Ríos, E., León, A., Chávez, M. I., Torres, Y., Ramírez-Apan, M. T., Toscano, R.A., Bravo-Monzón, Á.E., Espinosa-García, F.J. and Delgado, G. (2014). Sesquiterpene lactones from *Mikania micrantha* and *Mikania cordifolia* and their cytotoxic and anti-inflammatory evaluation. Fitoterapia, 94, 155-163.

36. Dong, L. M., Jia, X. C., Luo, Q. W., Zhang, Q., Luo, B., Liu, W.B., Zhang, X., Xu, Q.L. and Tan, J. W. (2017). Phenolics from *Mikania micrantha* and their antioxidant activity. Molecules, 22(7), 1140.

37. Ishak, A. H., Shafie, N. H., Esa, N. M., Bahari, H. and Ismail, A. (2018). From weed to medicinal plant: Antioxidant capacities and phytochemicals of various extracts of *Mikania micrantha*. International Journal of Agriculture and Biology, 20(3), 561-568.

38. Sumantri, I. B., Wahyuni, H. S. andMustanti, L. F. (2020). Total phenolic, total flavonoid and phytochemical screening by FTIR spectroscopic of standardized extract of *Mikania micrantha* leaf. Pharmacognosy Journal, 12(6).

39. Deng, X. (2010). Morphological and physiological plasticity responding to different light environments of the invasive plant, *Mikaniamicrantha*H. B. Kunth. Ecology and Environment, 19(5), 1170.

40. Ahmed, N.U., Park, J.I., Jung, H.J., Yang, T.J., Hur, Y. and Nou, I.S. (2014). Characterization of dihydroflavonol 4-reductase (DFR) genes and their association with cold and freezing stress in *Brassica rapa*. Gene, 550(1), 46-55.

41. Yue, M., Yu, H., Li, W., Yin, A., Cui, Y. and Tian, X. (2019). Flooding with shallow water promotes the invasiveness of *Mikaniamicrantha*. Ecology and Evolution, 9(16), 9177-9184.

42. McElrone, A.J., Choat, B., Gambetta, G.A. and Brodersen, C.R. (2013). Water uptake and transport in vascular plants. Nature Education Knowledge, 4(5), 6.

43. Gavrilescu, M. (2021). Water, soil, and plants interactions in a threatened environment. Water, 13(19), 2746.

44. Huang, L., Liao, M., Liao, H., Liu, Z., Cai, H., Zhou, W., Xu, Z., Ouyang, K., Yang, W. and Jian, S. (2024). High phosphorus availability and low light intensity reduce the competitive ability of the invasive plant *Chromolaena odorata* in tropical coral islands. Biological Invasions, 26(2), 471-487.

45. Rai, P. K., Lee, S. S., Bhardwaj, N. and Kim, K. H. (2023). The environmental, socio-economic, and health effects of invasive alien plants: Review on *Tithoniadiversifolia* (Hemsl.) A. Gray in Asteraceae. South African journal of botany, 162, 461-480.

46. Seebens, H., Blackburn, T.M., Dyer, E.E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M. and Bacher, S. (2017). No saturation in the accumulation of alien species worldwide. Nature communications, 8(1), 14435.

47. Kariyawasam, C.S., Kumar, L. and Ratnayake, S.S. (2021). Potential risks of invasive alien plant species on agriculture under climate change scenarios in Sri Lanka. Current Research in Environmental Sustainability, 3, p.100051.

48. Syngkli, R.B. and Rai, P.K., (2024). Allelopathic effects of *Ageratum conyzoides* L. on the germination and growth of *Zea mays* L., *Lactucasativa* L. and *Solanumlycopersicum* L. Allelopathy Journal, 62(2), 193-204.

