ALLELOPATHIC EFFECTS OF AQUEOUS EXTRACT OF LEAVES OF *MIKANIA MICRANTHA* H.B.K. ON SEED GERMINATION AND SEEDLING GROWTH OF *ORYZA SATIVA* L. AND *RAPHANUS SATIVUS* L.

Anita Sahu* and Anjana Devkota*
*Central Department of Botany, Tribhuvan University, Kathmandu.

Abstract: Allelopathic effects of aqueous extract of leaves of *Mikania micrantha* were studied on seed germination and seedling growth of *Oryza sativa* L and *Raphanus sativus* L. Seed germination and seedling growth were inhibited by concentrated aqueous extract of *M. micrantha*. *R. sativus* was more sensitive to inhibitory effects of leaf aqueous extract of *M. micrantha*. The extract had strong inhibitory effect on root elongation of seedling than in shoot elongation.

Keywords: Alien invasive; Seed germination; Seedling growth.

INTRODUCTION

Allelopathy is the effects of one plant on another due to chemicals released by them, or the breakdown products of their metabolites (Willis 1994). Allelopathy has been suggested as a mechanism for the impressive success of invasive plants by establishing virtual monoculture and may contribute to the ability of particular exotic species to become dominants in invaded plant communities (Kanchan & Jayachandra 1979). Allelopathy is expected to be an important mechanism in the plant invasion process because the lack of co-evolved tolerance of resistant vegetation to new chemicals produced by the invader could allow these newly arrived species to dominant natural plant communities . In fact, allelopathic interference is one of the important mechanisms for the successful establishment of invasive exotic weeds (Ridenour & Callaway,2001).

Mikania micrantha H.B.K. (Asteraceae) is originated in Central and South America. This genus is found in the tropics of America, Asia, and is widely known as Guaco. *Mikania micrantha* damages or kills other plants by cutting out the light and smoothering them. In this respect it is especially damaging young plantations and nurseries. Although *Mikania micrantha* has suppressed growth of the plant species grown next to it which is a serious problem in Nepal and no remedy yet is found. No effort has been made to control *Mikania micrantha,* neither there is any study in Nepal to examine the effect of its invasion to native ecosystem. To explore allelopathic potential of *Mikania micrantha* we examined effect of aqueous extract of leaves of this plant on seed germination and seedling growth of test plants (*Raphanus sativus* L. and *Oryza sativa* L.).

MATERIALS AND METHODS

Species Characters

Mikania micrantha is one of the worst and most aggressive invasive weeds in Nepal. It spreads at an alarming rate, readily climbing and twining on any vertical support, including crops, bushes, trees, walls and fences. Its shoots have been reported to grow up to 27 mm a day (www.issg.org/database). Vegetative reproduction is also efficient and vigorous. Although intolerant of heavy shade it readily colonizes gaps. *Mikania micrantha* damages or kills other plants by cutting out the light and smoothering them. In this respect it is especially damaging in young plantations and nurseries. It also competes for water and nutrients, but perhaps even more importantly, it is believed that the plant releases substances that inhibit the growth of other plants (Tiwari *et al.* 2005).

The successful invasion of *M. micrantha* is not only due to its high reproduction and wide eco-physiological tolerance but also due to its allelopathic effects on

Author for Correspondence: Anjana Devkota Central Department of Botany, Tribhuvan University, Kathmandu. E-mail: devkotaa@gmail.com.

neighbouring native plants. Allelochemicals of *M. micrantha* are released by decomposition of plant debris or volatilization. Volatile oil of this plant significantly inhibited the growth of various plants and pathogenic fungi. The volatile oil from flowers of *M. micrantha* contains high concentrations of α-pinene and β-pinene, both of which are effective insect repellents. Sesquiterpene lactones, essential oil, diterpenes, flavanoids including mikanin,alpinetin and -3-O-sulfate had been reported (Cuenca *et al*. 1988, Herz *et al*. 1975, Boeker *et al*. 1987, Nicollier & Thompson 1981). These sesquiterpenoids and flavanoids inhibited both germination and seedling growth of tested species with deoxymikanolide possessing the strongest phytotoxicity.

Collection of Plant Materials

Fresh leaves of *Mikania micrantha* were collected at vegetative stage in Chitwan National Park, Sauraha (27° 30'N, 84° 20' E) and air dried in shade for a week. The fully dried sample was ground into fine powder with the help of grinder and then was stored in air tight plastic bag at room temperature for further experiment.

Test plants

Seeds of *Oryza sativa* and *Raphanus sativus* were used as test plants for initial screening of species to check allelopathic potentialities. Seeds were taken from Botany Division, NARC (National Agriculture Research Council), Khumaltar, Lalitpur. The seeds of these two species germinate easily, easy to handle, showed pronounced effects after the application of aqueous extracts.

Filter paper was used in Petri dishes. For sterilization of the medium from dust particles or fungal attack on Petri plates of 9 cm, cleaned ethanol dipped cotton was used, and then filter paper was placed. Different concentration of aqueous extracts of *Mikania* was applied on the test plant.

Aqueous extract preparation: Twenty six gram of ground leaves was soaked in 380 ml of distilled water for 24 h (method given by Zobel *et al.* 1987) at room temperature and filtered using Whatmann No.1 filter paper. The volume of collected filtrate was made 400 ml by adding distilled water. Thus solution of the 10% extract was formed and used as stock solution. The stock solution was then diluted with distilled water to prepare the different concentrations of extract (viz. 2%, 4%, 6%, 8% and 10%). Control taken at 0% of distilled water.

Seeds of *Oryza sativa* L. and *Raphanus sativus* L. were soaked in 2 ml of each concentration. Each concentration had five replicates with 10 seeds in petridishes. Total seed germination and length of root and shoot were measured on the 7th day of incubation.

RESULT

Germination was inhibited with increasing concentration (Table 1). The inhibition of germination was found strong in *Raphanus sativus*. At 2% concentration, *Oryza sativa* exhibited 100% germination,while for *R. sativus* it was 90%. At 10% concentration, *O. sativa* showed 68% germination; while it was only 2% for *R. sativus* (Table 1). The leaf extract of *Mikania micrantha* inhibited the germination of *R. sativus* more than *O. sativa* (Table 1). The germination percentage was decreased with increase in concentrations,and average germination percentage was 56.34% of *R. sativus* and 86.34% of *O. sativa*.

There was significant difference ($p<0.001$) between treatments in root and shoot length of both test seedlings (Table 2). The homogeneity test showed that root length of both *Raphanus sativus* and *Oryza sativa* at 2 − 10% was significantly different from that of control. The shoot length at 4-6% were significantly different from that of control in *Oryza sativa* (Table 2). Increasing concentration of leaf extract of M*ikania micrantha* decreased growth of root and shoot length in *R. sativus* and *O. sativa.* (Table 2, Figs. 1 & 2). Root length growth was inhibited more than shoot length in both the tested plants. Maximum shoot length was in 2% and minimum in 8% in both the tested plants (Table 2). No or least growth of root and shoot was observed at 10% concentration.

Table 1: Effect of leaf extract of *M. micrantha* on germination percentage in *R.sativus* and *O.sativa.(n=50).*

Plant species	Concentration (%)	Total germination (%)	Mean (%)	Germination
Raphanus sativus				
	0	100		
	2	90		
	4	88		
	6	42	56.34	
	8	16		
	10	2		
Oryza sativa	0	100		
	2	100		
	4	96	83.34	
	6	72		
	8	68		
	10	62		

Table 2. Effect of aqueous extract of ***Mikania micrantha*** on root and shoot length of different plant species measured after one week. Different letters in superscript of the values in horizontal rows indicate that the values are significantly different ($\alpha = 0.05$).(n=50).

Total Average length of root length and shoot length of *R. sativus* (cm)

	0%	2%	4%	6%	8%	10%	Fvalue	P value
Root length	5.91±2.01^{d}	4.42±1.05^{c}	4.17±1.25^{c}	0.83±0.21^{b}	0.00±0.02^{a}	0	122.39	0.000
Shoot length	8.44±2.01^{d}	5.91± 2.01^{c}	3.17± 1.90^{b}	0.82±0.02^{a}	0.23±0.12^{a}	0.00±0.02^{a}	147.21	0.000

Total Average length of root length and shoot length of *Oryza sativa* (cm)

Root length	4.44±1.44^{d}	2.74±0.84^{c}	0.94±0.43^{b}	0.11±0.09^{a}	0.06±0.12^{a}	017	8.857	0.000
Shoot length	3.25±0.92^{d}	2.88±0.90^{c}	2.026±1.14^{b}	0.35±0.21^{a}	0.27±0.13^{a}	0.12±0.21^{a}	355.767	0.000

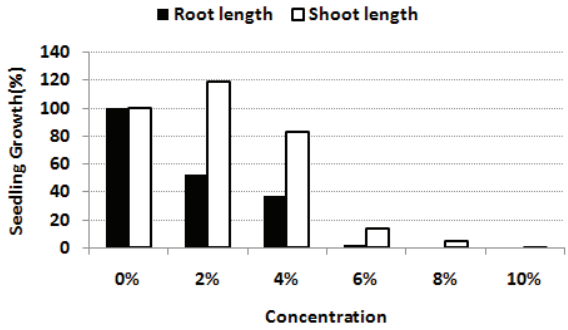

Fig. 1: Effect of leaf extract of *Mikania micrantha* on the root length and shoot length growth of *Raphanus sativus*.

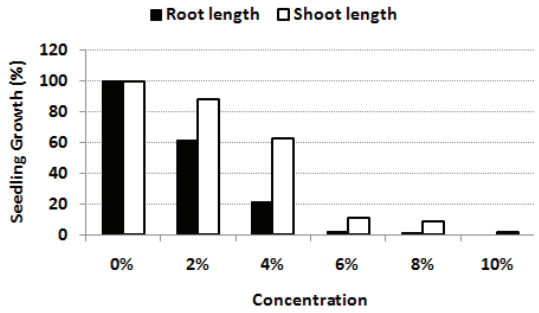

Fig. 2: Effect of leaf extract of *Mikania micrantha* on growth of root length and shoot length of *Oryza sativa.*

DISCUSSION

From preliminary screening it was found that leaf extract had the strongest allelopathic effect on seed germination. Higher the concentration of the extract, lower the germination of the seeds and vice-versa. Tefera (2002) also found that the inhibitory allelopathic impact of leaf extract was more powerful than of other vegetative parts. The study demonstrated that leaf aqueous extracts of *Mikania micrantha* exhibited significant inhibitory effects on seed germination and seedling growth of both tested species (Figs. 1-2, Table 2). The leaf extract of *Mikania micrantha* inhibited the germination of *Raphanus sativus* more than *Oryza sativa* (Table 1). That could be due to species of crucifers like *R. sativus* be the most sensitive than *O. sativa* to inhibitory effect of leaf aqueous extract of *Mikania micrantha*. Earlier works have also reported that foliar leachates of *Mikania micrantha* reduced root and shoot elongation of *Oryza sativa* , *Zea mays, Triticum aestivum* (Baral *et al.* 2011). The inhibition of the aqueous extract of *M. micrantha* on the root growth of two tested plant species was greater than that on shoot growth. These results are in agreement with the results of Stachon and Zimdahl (1980), which reported that the extracts of allelopathic plants had more inhibitory effect on root growth than on hypocotyl growth because root is the first organ to absorb allelochemical from the environment. Similar kinds of results were reported by Chon *et al*. (2000). Root length was the best indicator of allelopathic effects of plant extracts because root growth has been reported to be more sensitive to phytotoxic compounds than hypocotyl growth in alfalfa. Furthermore, the permeability of allelochemicals to root tissue was reported to be greater than that to shoot tissue (Nishida *et al*. 2005). Results of this study suggested that *Mikania* had an allelopathic influence on other plants in addition to its competitive ability, as reported previously by Wong (1964). The results have provided an evidence of the existence of inhibitory factors in *Mikania* which can influence germination and growth of certain weed species. The inhibitory effect of *Mikania micrantha* on seed germination and seedling growth of different plant species was due to presence of growth inhibitors (allelochemicals) in the extracts.

Phenolics (Wei *et al.* 2004) and terpenes are the major constituents isolated from *Mikania micrantha* and its essential oil compounds have large applications in pharmaceutical and cosmetic industries (Silva-Santos *et al*. 2004). Flavonoids and dicaffeoylquinic acid butyl esters have been recently described as bioactive for *M. micrantha* (Wei *et al*. 2004). Phenolics found in leaves also have inhibitory effects on growth of nitrogen fixing and nitrifying bacteria (Kanchan and Jayachandra 1981). According to Rice (1984) phenolics are the most common and widely distributed water soluble allelochemicals. The escape of these chemicals into the environment occurs through various mechanisms such as leachation, volatilization and microbial decay of dead and fallen parts, as well as root exudation (Rice 1984). These chemicals were reported to have

had allelopathic potential on various agronomic crops and weeds (Stephen and Sowerby, 1996) and vegetable crops (Mersie and Singh 1988). Allelopathy is expected to be an important mechanism in the plant invasion process. Lack of co-evolved tolerance of resident vegetation to new chemicals produced by the invader could allow these newly arrived species to dominate natural plant communities (Hierro and Callaway 2003). *Mikania micrantha* because of its invasive capacity and allelopathic properties has the potential to disrupt natural ecosystems (Evans 1997). Present result showed that concentrated aqueous extracts of leaf inhibited germination of tested seeds.

CONCLUSION

Raphanus sativus was more sensitive to inhibitory effects of leaf aqueous extract of *Mikania micrantha*. The extract had strong inhibitory effect on germination and root elongation than to shoot elongation. Thus, sensitivity to allelochemicals and extent of inhibition varied with species and organs of the test species. Allelopathic effect of *Mikania micrantha* may be an important mechanism involved in invasive success of this plant.

REFERENCES

1. Willis, R.J. 1994. Terminology and trends in allelopathy. *Allelopathy Journal.* **1**(1): 6-28.
2. Kanchan, S.D. and Jayachandra. 1979. Allelopathic effects of *Parthenium hysterophorus* L. I. Exudation of inhibitors through roots. *Plant and Soil.* **53**: 27–35.
3. Ridenour, W.M. and Callaway, R.M.. 2001. The relative importance of allelopathy in interference: the effects of invasive weed on native bunchgrass. *Oecologica.* **126**: 444-450.
4. www.issg.org/database/welcome/content.asp.
5. Tiwari, S., Siwakoti, M., Adhikari, B. and Subedi, K. 2005. *An inventory assessment of invasive alien plant species of Nepal*, IUCN-The World Conservation Union, Nepal.
6. **Cuenca, M.D.R.**, **Bardon, A. and Catalan, C.A.N. 1988**. Sesquiterpene lactones from *Mikania micrantha*. ***Journal of Natural Product.*** **51**:**625**-626.
7. **Herz, W.**, **Srinivasan,** A. and **Kalyanaraman, P. S. 1975.** Mikanocryptin, a new guianolide from *Mikania*. ***Phytochemistry.*** **14**: 233-237.
8. **Boeker, R.**, **Jakupovic,J., Bohlmann, F.**, **Schmeda-Hirschmann and** Germacra, **G**. **1987.** 1,10Z, 4E-dien-12,8 -olides from *Mikania micrantha*. ***Planta Medica***. **53**: **105**-106.
9. **Nicollier, G.** and **Thompson, A.C.1981**. Essential oil and terpenoids of *Mikania micrantha*. ***Phytochemistry.*** **20**:2587-2588.
10. Tefera, T. 2002. Allelopathic effects of *Parthenium hysterophorus* extracts on seed germination and seedling growth of *Eragrostis tef* (Zucc.) Trotter, *Journal of Agronomy and Crop Science.* **188**(5): 306-310.
11. Baral, B. and Maharjan, B.L. 2011. Antagonistic characteristics and phytochemical screening of invasive alien species of Nepal Himalaya. *International Journal of Pharmaceutical & Biological Archives.* **2**(5):1444-1450.
12. Stachon, W.J. and Zimdel, R.L. 1980. Allelopathic activity of Canada thistle *Cirsium arvense* in Colorado. *Weed Science.* **28**: 83-86.
13. Chon, S.U., Jang, H.G., Kim, D.K., Kim, Y.M., Boo, H.O. and Kim, Y.J. 2005. Allelopathic potential in lettuce (*Lactuca sativa* L.) plants. *Scientia Horticulturae.* **106**: 309-317.
14. Nishida, N., Tamotsu, S., Nagata, N., Saito C. and Sakai, A.2005. Allelopathic effects of volatile monoterpenoids produced by *Salvia leucophylla*: Inhibition of cell proliferation and DNA synthesis in the root apical meristem of *Brassica campestris* seedlings. *Journal of Chemical Ecology.* **31**: 1187-1203.
15. Wong, R. 1964. Evidence for the presence of growth inhibitory substances in *Mikania cordata* (Burn. F.) B. L. Robinson. *J.Rubber Research Institute of Malaya.* **18**: 231-242.
16. Wei, X.Y., Huang H.J., Wu, P., Cao, H.L. and Ye, W.H. 2004. Phenolic constituents from *Mikania micrantha. Biochemical Systematics and Ecology.* **32**: 1091-1096.
17. Silva-Santos A., Antunes, M.A.S., Bizzo, H.R., D'Avila, C.A. and Souza-Santos, L.C. 2004. The application of essential oils and terpenics/terpenoids compounds in the fields of pharmaceutic and cosmetic through the knowledge registered in patents. *Revista Brasileira de Farmacognosia.* **14**: 48-50.
18. Kanchan, S.D. and Jayachandra. 1981. Effects of *Partheniumhysterophorus* on nitrogen-fixing and nitrifying bacteria.*Canadian Journal of Botany.* **59**: 199-202.
19. Rice, E.L. 1984. *Allelopathy*. 2nd edition. Academic Press, Inc, Florida (USA).
20. Stephen, W.A. and Sowerby M.S. 1996. Allelopathic potential of the weed, *Parthenium hysterophorus* L. in Australia. *Plant Protection Quarterly.* **11**: 20-23.
21. Mersie, W. and Singh. M. 1988. Effects of phenolic acids and rageweed *Parthenium hysterophorus* L. extracts on tomato (*Lycopersicum esculentum)* growth and nutrient and chlorophyll content. *Weed Science.* **36**: 278-281.
22. Hierro, J.L. and Callaway, R.M. 2003. Allelopathy and exoticplant invasion. *Plant and Soil.* **256**: 29-39.
23. Evans, H.C. 1997. *Parthenium hysterophorus*: A review of its weed status and the possibilities for biological control. *Biocontrol: News and Information.* **18**(3): 89-98.