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Allelopathic Effect of Extract of *Mikania micrantha* on Seed Germination and Seedling Growth of *Melia azedarach*

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ABSTRACT

***Mikania micrantha* is a high threat to the natural food of mega fauna in conservation areas so an appropriate management option is essential. This research aimed to find allelochemicals effects of *M. micrantha* on seed germination and seedling growth of *Melia azedarach*. The leaves of *M. micrantha* and seeds of *M. azedarach* were obtained from Chitwan, Nepal. The chemical of *M. micrantha* was extracted in laboratory. Altogether 120 poly pots were used to assess germination percentage and growth of *M. azedarach*. The solutions of chemical and water in different proportion specifically control, 75:25, 50:50 and 25:75 were prepared and applied to treat the seeds. After sowing treated seeds in poly pots, germination percentage was estimated and seedling growth of *M. azedarach* was measured. The results showed that, germination percentage was high nearly 43.34%, the highest cumulative base diameter of *M. azedarach* was recorded about 1.55 ±0.03 cm cm and** cumulative height was about 14.44±0.40 cm in use of 75:25 solution but it was the highest number of leaves of seedling in application of 25:75. The r**epeated measure ANOVA showed there was significant difference in growth of base diameter at 95% confidence level. The use of chemical extract of *M. micrantha* can be a good management option.**

Key words: Invasive species, Allelochemicals, Germination percentage and Growth.
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INTRODUCTION

The biological phenomenon by which an organism creates biochemical which influences the germination, growth, survival, and reproduction of other living thing is called Allelopathy (Cheng and Cheng, 2005). The chemicals travel from one plant to another through root exudation, volatilization, decomposition of leaves residues and leaching. This phenomenon is carried out by participation of various secondary metabolites or chemicals compounds present in the roots, leaves, flowers and fruits of the species (Langenheim, 1994). Allelopathy is a basic biological defense and offense mechanism in plants invasion. Lack of co-evolved tolerance of resident vegetation to new chemicals provided by the invader could allow this new species to dominant the plant community. Nonetheless, plants interactions have often been controversial because of the difficulty in unambiguously demonstrate interference by chemical inhibition rather than through resource competition or other mechanisms (Cheng and Cheng, 2015). *Mikania micrantha* is an invasive species which alters the growth and reproduction of indigenous valuable species of forest ecosystem (Bijay 2015, Matawali et al., 2016). This species colonize in such a way that other species cannot grow well even in their suitable habitat. The species smother and retard or kill young trees, crops, native plants and fodder grasses.

The species has high reproductive rate (both sexual and asexual reproduction) and also high fire resistant (Azam et al., 2013; Murphy et al., 2013). Asexual reproduction occurs from root that develops from node on small

sections of stem. This natural biological characteristic provides plant to spread fast. In addition, the favorable physiological and anthropogenic factors play key role to spread this invasive species (Murphy, 2001). Burning of vegetation and manual weed control of crops support to promote the spread of *M. micrantha*. Various reports showed that the slash and burn operation increases the vigor of *M. micrantha*. Plant biodiversity of tropical countries in Asia like India, Pakistan, China and Bangladesh has been facing the problem of the infestation of *M. micrantha* every year (Ferguson et al., 2003). Most valuable species like *Shorea rosbusta* (Jackson, 1994) and associate plant species are the main victim of the invasion of *M. micrantha*. Nepal is also facing the serious problem of *M. micrantha* especially in Chitwan National Park. Chitwan National Park is famous eco-tourism avenue particularly for mega fauna like Rhino ceros. The elephant grass is the most palatable food for this animal. These days, grasses are seriously disturbed because of infestation of *M. micrantha*. The conservationists are worrying how to get rid of the problem of this mile a minute weed. Every year government and local agencies have been spending huge money to control this obnoxious weed. However, proper solutions are still unknown.

The local farmers living around the national park and conservation areas have been challenging the problem of this weed. Their crops are damaged and the tree plants like *Melia azedarach* are affected by the invasive *M. micrantha*. The *M. azedarach* is fast growing species of local community in Chitwan. The local people plant *M. azedarach* in their private land. It serves multipurpose usage particularly fuelwood, forage and fodder to local communities. Recently, research conducted using household survey in Madi valley, Chitwan Nepal showed that the *M. azedarach* was the top preference for fodder and forage whereas, second preference as fuel wood (Bishokarma et al., 2005). The species holds a special piscicidal character, which is used to catch fish in the river by the local ethnic communities (Joshi and Joshi, 2005). Recent study conducted on the effects of *M. azedarach* on Aphids showed that the concentration of crude plant material of *M. azedarach* slowed the development and growth process of sucking insects such as aphid (Neycee et al., 2012; Jiregna and Tena, 2014). Some studies show that, *M. micrantha* can be utilized as manure which can be the good option to manage this species (Swamy and Ramakrishnan, 1987). The study done in three species in Nepal, *M. micrantha*, *Lantana camara* and *Chromolaena odorata* showed that *M. micrantha* were used as fodder for goats during dry season, L. camara was used for fuelwood and medicinal purposes on the other hand *C. odorata* was commoditized as compost through human intervention with external support (Rai et al., 2012). However, no assessment of the allelopathic effects of *M. micrantha* on germination and growth of *M. azedarach* was carried out before. Thus, this research aimed to evaluate the allelopathic effects of *M. micrantha* on seed germination and seedling growth of *M. azedarach*.

MATERIALS AND METHODS

Description of the Site

The “Jal Devi Community forest” is located in Chitwan, Nepal. The forest is highly dominated by the economically valuable timber species *Shorea robusta*. The average precipitation is 2150 mm annually (CNP, 2010) and the annual mean temperature is around 25°C. The Chitwan lies in tropical zone with an altitude of 212 meters (Nelson, 2012). The nursery was established to grow *M. azedarach* in Kathmandu which lies in sub-tropical zone of Nepal having altitude around 1,400 meters. June is the hottest month in Kathmandu with an average temperature of 23°C and the coldest is January at 9°C with the most daily sunshine hours at 12 in April. The wettest month is July with an average of 325.3 mm of rain.

Materials Collection

The leaves of *M. micrantha* were collected on 25th January, 2017. Total 120 seeds of *M. azedarach* were gathered and used as host plant for the initial screening to assess the effect of aqueous extract of *M. micrantha*. Then, 120 poly bags were kept in the tunnel nursery filled with mixture of sand and forest soil.

Leaf Aqueous Extract Preparation

The collected leaves were dried in sun for 3 days and grinded into fine powder using a grinder machine. These powders were stored in a plastic bottle for laboratory analysis. The process started with the preparation of aqueous extract from the leaves of *M. micrantha* in the laboratory of Kathmandu Forestry College. To this, 400 grams of dried powder of *M. micrantha* were mixed with 1l of distilled water and was submerged for 48 h in a Jar. The mixture was strained through 4 layers of cheese cloth. The residue extracted was separated and collected in a container. The extract was passed through two layers of Whitman No.1 filter paper and was gathered in 500 ml of beaker and named as stock solution (Ferguson et al., 2003). The stock solution was then mixed with water (160 ml total) in three different concentrations (25:75, 50:50 and 75:25) plus a control solution (160 ml water only).

Pre-Germination Treatment and Nursery Establishment

The pre-germination treatments of seeds of *M. azedarach* were done for 24 h applying different solutions of the

Table 1. Germination percentage of *M. azedarach* under distinct concentrations of the extract of *M. micrantha*.

Treatments (Solutions)	Sprouts Formation in Seeds Percentage		Seeds Fully Grown into Seedlings After Sprout Stage Percentage	
	Number of Seed Germinated	Percentage	Number of Seeds Reaching To Its Seedling Stage	Percentage
75:25	13	43.34%	8	26.67%
50:50	12	40%	10	33.34%
25:75	5	16.67%	14	46.67%
Null	7	23.34%	10	33.34%

extract. In each solution of different proportion of extracts and water, 30 seeds were submerged for treatment. Then the seeds were sown into poly bags. Next, 120 poly bags were raised in outdoor tunnel nursery for exposure to sunlight.

Seed Germination

Then calculation of seed germination percentage of *M. azedarach* and measurement of seedling were done. First the total number of seeds sprouted after breaking of dormancy was counted. After 10 days, total number of sprouted seeds reaching to seedling stage was counted. Then, the germination percentage was calculated using following formula (Bhatt and Santo, 2016).

$$Germination\ \% = No.\ of\ seeds\ germinated \times 100 / Total\ no\ of\ seeds$$

Measurement

Three basic parameters specifically base diameter, height of the seedlings and number of leaves were recorded. The measurements of the seedlings were taken on 28th March, 30th March, 1st April, 3rd April and 6th April, 2017.

The normality of the data associated with the plant growth like base diameter, height of plant and number of leaves were statistically examined using Kolmogorov-Smirnov and Shapiro-Wilk test (Kothari, 2004). Next, the repeated measure ANOVA (Mcginn, 2010) was applied to evaluate the effect of different proportion of chemical constituents of *M. micrentha* on growth performance base diameter, height and number leaves of seedlings, of *M. azedarach*.

RESULTS

Allelopathic Effect of Extract of *M. micrantha* on Seed Germination of *M. azedarach*

Germination percentage of *M. azedarach* was affected by the different proportion of extracts. The number of sprouted seeds after breaking the dormancy was higher (43.34%) in high concentration extract (that is, 75:25 solution), whereas it was lower (16.67%) in low concentration of extract (that is, 25:75 solution; Table 1). On the other hand, the number of sprouted seeds reaching its final seedling stage was the higher (46.67%) in low concentration solution and it was the lowest (26.67 %) in high concentration solution (Table 1).

Effect of Mixture of Extract on Base Diameter on *M. azedarach*

The effect of different proportion of aqueous extract of *M. micrantha* on the base diameter of *M. Azedarach* was variable among treatments (Figure 1). The highest cumulative base diameter of *M. azedarach* was recorded in the extract 75:25 solution (1.55 ± 0.03 cm) and it was the lowest about in the 25:75 solution (1.08 ± 0.02 cm). The cumulative base diameter was also very low in control pot (1.23±0.03 cm).

Effect of Aqueous Extract on Height of Seedling of *M. azedarach*

The cumulative height of seedling of *M. azedarach* differed among distinct extract concentrations (Figure 2). It was found that the highest cumulative height of the seedling of *M. Azedarach* was about 14.44±0.40 cm in 75:25 solution which was the least nearly 13.03±0.30 cm in control (null). This different was 1.41 cm between these concentrations.

Effect of Solution on Number of Leaves Aqueous Extract of *M. micrantha*

The effect of solution of *M. micrantha* was also recorded on the number of leaves of the seedling of *M. azedarach*. There were the highest numbers of leaves in 50:50 solutions (18.95±0.89) while it was the lowest in case of control (16.43±0.98) pots. The effect of solution on number of leaves was about similar in case of 25:75 solutions. It was the highest number of leaves of seedling using the solution 25:75 but the lowest number of leaves was in control solution (Figure 3).

Statistical analysis

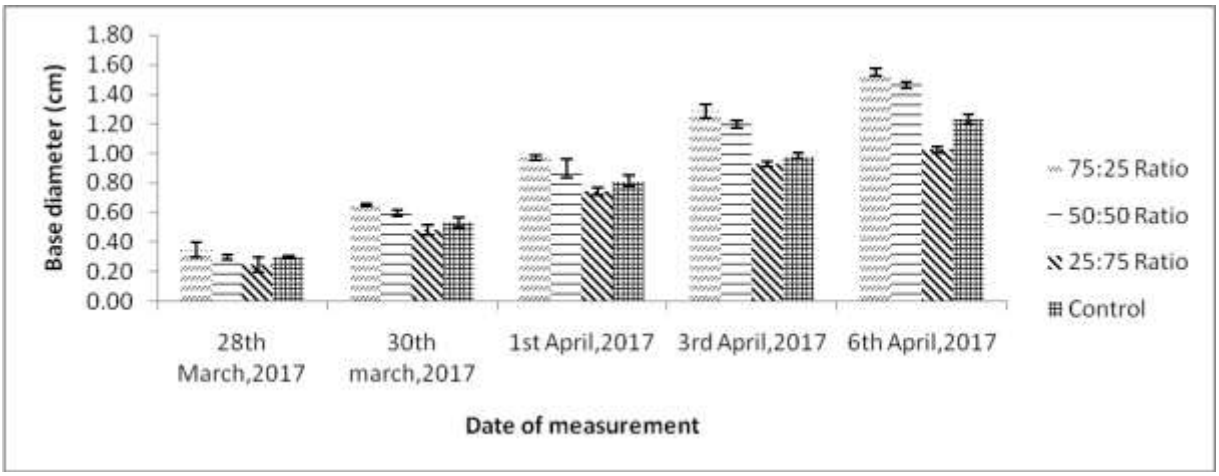

Figure 1. Cumulative base diameter of *M. azedarach* treated in different proportion of extracts of *M. micrantha.*

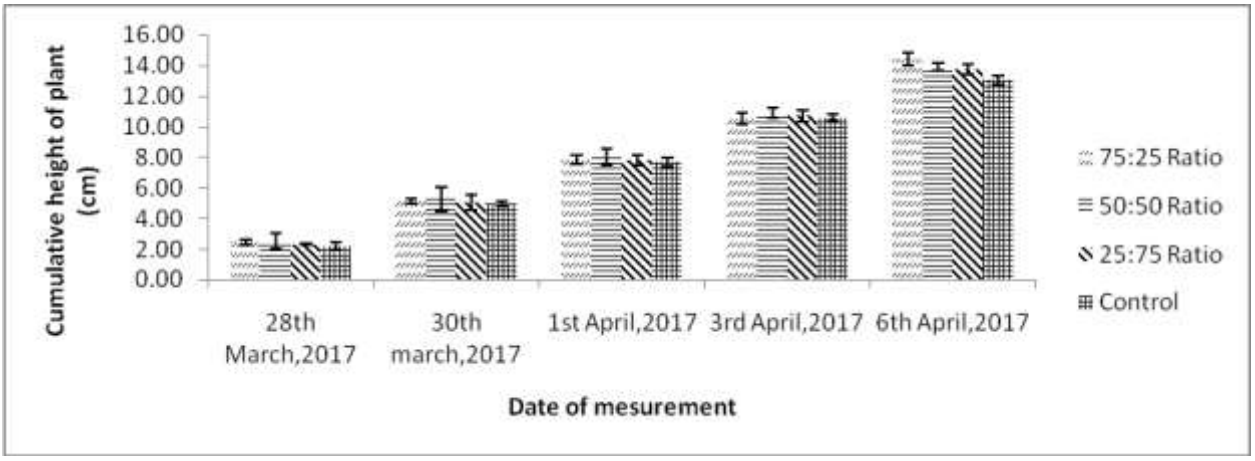

Figure 2. Effect of distinct solutions of *M. micrantha* on the height of *M. azedarach* seedlings.

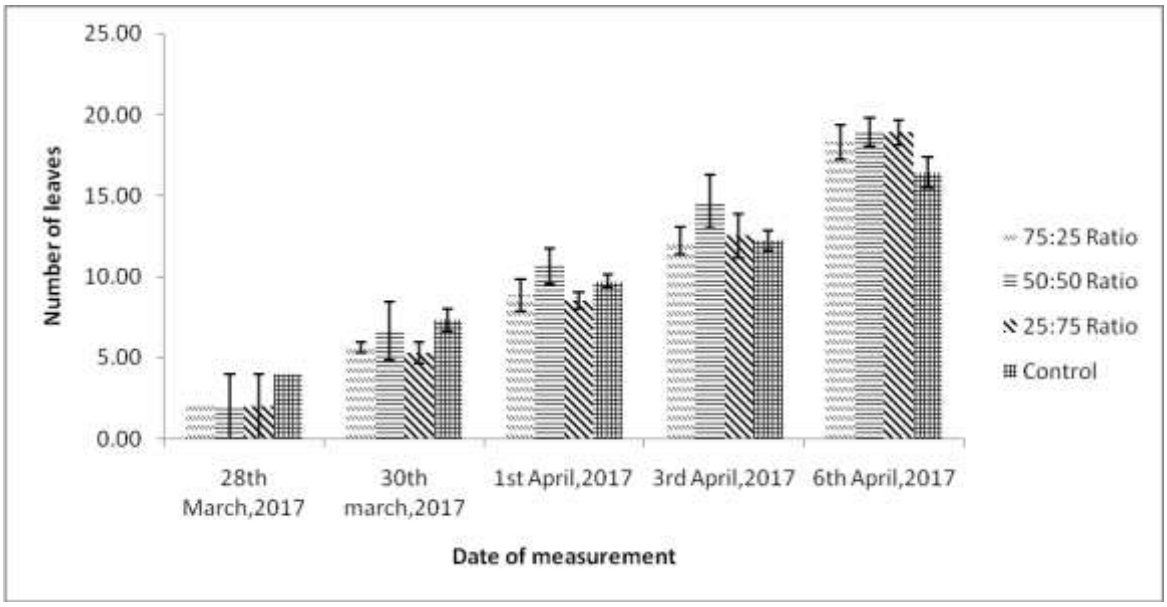

Figure 3. Cumulative number of leaves.

Table 2. Repeated measures ANOVA.

Dimensions	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Height of seedling	Intercept	367.72	1.00	367.72	303	0.00
	Error	15.76	13.00	1.21	.25	
Base diameter	Intercept	4.40	1.00	4.40	493	0.00
	Error	0.12	13.00	0.01	.74	
No of leaves	Intercept	553.14	1.00	553.14	145	0.00
	Error	49.36	13.00	3.80	.69	

The repeated measure ANOVA showed that there were significant difference in mean height, base diameter and number of leaves of the seedling *M. azedarach* using the differences concentration of extract of *M. macrantha* at 95% confidence level since the p-value were less than 0.05 ($p<0.05$) (Table 2). It indicated that the growth performance is affected due to application of difference proportion of extract of *M. macrantha*.

DISCUSSION

Thousands of national and international tourists visit Chitwan National park because this park is the most suitable habitat for mega fauna specifically rhino, elephant, beer, tigers, leopards and sloth bears (DNPWC, 2016). The *Saccharum* and *Imperata spp*. are the important food for one-horned rhinoceros. However, the obnoxious weed particularly *M. micrantha* is a creating high threat to the grass land. One of the major consequences is food insufficiency for one-horned rhinoceros (Ortolani, 2017). The mechanical removal of *M.micrantha* is very costly so proper utilization is the best way (Rai, 2012; CPN, 2016). The utilization of high concentration solution of extract of *M.micrantha* and water showed positive effect on breaking the dormancy of seeds and speeding the rate of initial germination or increasing the sprouting rate of *M. azedarach*. The treatment of seed before germination helps to expose the seed and make the favorable condition for germination. The pre- treatment of seeds prevent radicle protrusion but stimulate physiological and biochemical activities (Di Girolamo and Barbanti, 2012).

Thus the utilization of extract solution of *M. micrantha* and water showed the significant effect on germination percentage of *M. azedarach*. The study done by Sahu and Devkota (2013) showed the germination percentage was high about 83.34% in applying the extract of *M. micrantha* and on *Raphanus Sativus*. This result is not similar to the germination percentage of *M. azedarach* but the indicated that there is effect of extract on germination percentage.

There is effect of aqueous extract of *M. micrantha* on seed germination and seedling growth of different plant species was due to presence of allelochemicals (growth chemicals) in the extract (Nishida et al., 2005). Our finding also supports this view showing the higher growth of seedling (Manonmani et al. 2014).

Another study showed that the application of extract of *M. micrantha* affects the germination percentage, shoot length, root length and biomass of *Zea mays* (Lalmuanpuii and Sahoo, 2011). Another study done by Shao et al. (2003) showed that there is the allelopathic effect of *M. micrantha* on seedling growth of *Acacia mangium*, *Pinus massoniana* and *Eucalyptus robusta*.

The germination percentage of *M. azedarach* was the highest in high concentration of solution 75:25 and second highest was recorded in 50:50 solutions. The growth performance of *M. azedarach* was affected due to different concentration of solution of *M. micrantha*. The highest cumulative base diameter was recorded in 75:25 solutions which were the lowest in 25:75 solutions. The same base diameter was found in 50:50 solutions as well infant, the base diameter of seedling was not so significantly affected because the growth of base diameter of seedling is very slow in early stage of the plant (Hossain, 2012).

Similarly, the height of the seedling was also affected due to application of different concentration of solution. The cumulative height of seedling of *M. azedarach* was the highest in 75:25 solutions which was the least nearly in control. This difference was 1.41 cm between them. Utilization of solution of *M. micrantha* may be one of the potential management option (Huang et al., 2000). The alternative use of *M. micrantha* will be good options to manage the weed in Chitwan national park (Ram, 2008; Koirala, 2011).

CONCLUSION

The seed germination and sprouting rate of *M. azedarach* increased with exposure to high concentration extracts of *M. micrantha*, an invasive species in Chitwan National Park. On the other hand, the growth rate of seedlings of *M. azedarach* was affected by different proportions of the aqueous extract of *M. micrantha*. The utilization of extract of *M. micrantha* will be one of the options to manage the obnoxious weed in Chitwan national park, Nepal. However, more research works related to management of the weed is task of academic society.

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