

Allelopathic effects of *Ageratum houstonianum* Mill. on germination and growth of *Raphanus sativus* L. and *Vigna mungo* (L.) Hepper

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ABSTRACT

This study investigated the allelopathic effects of leaf leachates of invasive weed *Ageratum houstonianum* Mill. on two crop plants viz. *Raphanus sativus* L. and *Vigna mungo* (L.) Hepper in both petri-plates and pots culture. In petri-plates, the leachate had no effect on seed germination of *R. sativus*. However, in *V. mungo*, germination was reduced by higher concentrations of leachates. The leachates also reduced root and shoot length and biomass. In pot experiment, shoot and root growth, leaf number and biomass were reduced in both species. The phytotoxic effects of *A. houstonianum* on both crops suggested that the weed has potential threats to agro-ecosystems where these plants are cultivated. As the allelochemicals responsible for these effects were not identified due to resource limitations, further research should concentrate on identifying the key allelochemicals responsible for the effects on specific plants and on exploring management strategies to mitigate the impact of this invasive species.

Key words: Agro-ecosystem, crop plants, germination, pot culture, seedlings growth

INTRODUCTION

Invasive alien plant species (IAPS) have rapid growth rate, prolific seed production, efficient dispersal mechanisms and strong colonizing capacity (17). Besides such capabilities, they release allelochemicals through volatilization, root exudation, leaching or decomposition of plant residues, which cause inhibitory effects on native plants (22). Allelopathy in plant communities has been recognized in agriculture since ancient times (36). The IAPS out compete the crop plants in agro-ecosystem by releasing allelochemicals, which impacts negatively on germination, growth and development (9,19,27). Therefore, studies have increasingly highlighted the allelopathic effects of IAPS on crop plants.

Ageratum houstonianum Mill. (family Asteraceae), is one of the worst IAPS called “Nilo Gandhe” in Nepal (Figure 1). The weed, native to Central America, Mexico and Caribbean, is annual herb, which propagates by seeds and vegetatively through its stem (5). It has been spreading worldwide, posing a great threat to different ecosystems, including agriculture fields (20). It is distributed from low land Tarai to Shiwalik and Mid-Hills in degraded or marginal lands and roadsides throughout Nepal (26).

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Figure 1. *Ageratum houstonianum* Mill.

This weed produces several allelochemicals such as Sabinene, β -pinene, β - phellandrene, cineole and limonene, terpinen-4-ol, eugenol and methyl eugenol (11). Intense colonization of this weed in crop fields over the past decades has become a challenging issue. Farmers have observed that the weed has severe negative impacts on soil and several crop plants, including winter or summer cereals, legumes and vegetables. One of the causes of negative impacts of weeds on crops such as inhibiting germination, growth, development and productivity is allelopathy (7); however, crop species-specific impacts of allelopathy are yet to be documented. Such understanding would help us to know the outcompeting capabilities of both weed and crop species.

Raphanus sativus L. is a popular and nutritious edible vegetable (23). Likewise, *Vigna mungo* (L.) Hepper is the most used legume, which is grown aside from the paddy field. This legume not only contributes to the food and nutritional security but also aids in the production of other crops like paddy, facilitating them with biologically fixed nitrogen (1). Both *R. sativus* and *V. mungo* are cultivated throughout Nepal from Tarai to Mid hills. Since decades, *A. houstonianum* is invasive in the fields of these crops (2,6). Weeding is laborious and if not done, the crop fields are intensively covered by the weeds suppressing the growth of crop plants (20). Therefore, this study investigated the effects of leachates from *A. houstonianum* on seed germination, seedling growth and development of *R. sativus* and *V. mungo*, aimed to understand the impact of the weed.

MATERIALS AND METHODS

R. sativus and *V. mungo* were used in this study as the test species against the allelopathic effects of *A. houstonianum*. Fresh leaves of *A. houstonianum* were collected near Bhaktapur, Nepal (27°46'21"N, 85°50'20"E and 1286 m asl.). The leaves were collected from mature flowering plants. The seeds of *R. sativus* were purchased from local market in Kathmandu and the seeds of *V. mungo* were collected from Nepal Agricultural Research Council (NARC), Lalitpur, Nepal.

Ten g fresh leaves of *A. houstonianum* were washed and then soaked in 100 mL distilled water for 24 h. After soaking, the leachate was filtered through muslin cloth, which was used for the stock solution considering 100 % concentration (10 g/100 mL). It mimics the natural conditions such as rainwater leaching and foliar wash-off. The stock solutions were diluted into 25 % and 50 % by adding distilled water. Both the seeds and stock solutions were stored at 4 °C, until use.

Petri-plate experiment

Seeds of test species were surface sterilized using 70 % ethanol and washed by distilled water. Ten seeds of each test crop were placed uniformly on double-lined moist filter paper in sterilized petri-plates. Twenty mL distilled water for control and respective concentrations of *A. houstonianum* leaf leachate (25 %, 50 % and 100 %) were added in respective plates. Each treatment had 5 replicated petri-plates, which were incubated at 28 ± 2 °C for 10-days. The germinated seeds were counted and germination (%) was calculated. The shoot and root lengths were measured. The seedlings were harvested after 10-days and dried in the oven at 120 °C for 24 h for the measurement of dry weight.

Pot experiment

The pot culture experiment for these test crop plants was done simultaneously with the petri-plate experiment. The pots (10 cm height and 10 cm diameter) were filled with garden soil. The seeds of test plants were soaked in the moist filter paper to allow germination. The seedlings of homogeneous sized (2 cm length) were then transferred to the prepared pots. Ten seedlings were planted in each pot. The pots were irrigated with 20 mL normal water in control pots and in leachates treated pots, respective plant leachates of different concentration (25 %, 50 % and 100 %) were added. Each treatment for each test species had 5 replicated pots. Watering was done at interval of 2 days. The test plants were harvested 26 days after sowing. The shoot and root growth parameters were measured. The number of leaves were also counted. Root and shoot were dried and their biomasses were recorded. Both the petri-plate and pot experiments were conducted during October 2021.

Statistical analysis

The differences in seed germination and seedling growth parameters of each test plant among the treatments were compared using One-way ANOVA. Significant differences between the concentrations within each parameter was observed, using Tukey's HSD test with 95 % confidence interval and a significance level of $p < 0.05$ as the threshold for statistical significance. Data of shoot biomass, leaf number and leaf length of *R. sativus* did not show normal distribution and therefore, they were analyzed using the Kruskal-Wallis test. The analyses were done using the software R version 4.3.1 (25).

RESULTS AND DISCUSSION

PETRI-PLATE BIOASSAY

(i). Seed germination

There was no effect of *A. houstonianum* leachates on the seed germination (%) of *R. sativus* (Table 1) in all treatments. However, the germination of *V. mungo* was significantly reduced at 50 and 100 % concentrations of *A. houstonianum* leachates ($p < 0.001$). In total, 91.43 % seeds germinated in control plates in *V. mungo*, followed by the leachate concentrations 25 % (83.71 %), 50 % (65.71 %) and 100 % (60 %) (Table 1).

The leachates of *A. houstonianum* had selective inhibitory effects, especially on *V. mungo*, while, their impact was less significant or minimal in *R. sativus*. The germination (%) decreased with the increasing concentrations of leachates in *V. mungo* indicating that the leachates suppressed the seed germination. Such effects might be due to change in seed physiology like enzyme activity and hormonal imbalance during germination (34). Nevertheless, the germination and growth parameters were strongly inhibited by *A. houstonianum* on *R. sativus* (32). They have used powdered shoot of *A. conyzoides* and conducted in field experiment. These findings showed that the leachates of *A. houstonianum* had less phytotoxic effects than the crushed or powdered form of the weeds.

Table 1. Effects of *A. houstonianum* aqueous leachates on germination (%) of *R. sativus* and *V. mungo* in Petri plate bioassay at 10 days after sowing.

Leachate conc. (%)	<i>R. sativus</i>	<i>V. mungo</i>
Control	100	91.43 ± 3.19a
25 %	100	83.71 ± 2.38ab
50 %	100	65.71 ± 10.77bc
100 %	100	60.00 ± 11.15c

Mean ± Standard deviation. The similar alphabet in each variable for different concentrations represent no any significant difference ($p > 0.05$) between them and different alphabets for different concentrations represent significant critical difference (CD) ($p < 0.05$) between them.

Jayaraman and Ramalingam (15) had shown the inhibitory effects of *A. conyzoides* on seed germination of *V. mungo*, *Ageratum houstonianum* had also inhibitory effects on other crops such as *Sesamun indicum*, *Brassica nigra*, *Brassica juncea* and *Raphanus sativus* (24). The weeds exhibited strong inhibitory effect, which may be attributed to the bioactive compounds present in the leachate. The leaves of *A. houstonianum* contains several secondary metabolites (flavonoids, alkaloids, tannins and phenols), which can contribute in inhibition of seed germination and growth by suppressing the enzymes involved (3,35).

Moreover, the compounds isolated from *A. houstonianum* and its congeneric have potential inhibitory effect (11). Some crucial components such as precocene I, precocene II, β -caryophyllene, β -bisabolene etc. released from the plant's leaves may influence the growth and physiology of neighboring plants when growing in close proximity (10,18). In addition, through natural processes such as leaching, rainfall or decomposition some of these compounds may enter the soil and can interact with crop plants, potentially influencing soil

microenvironment (31,33). However, the rate of inhibition may depend upon the nature of test and invasive species, concentration of leachate and the plant tissue from which the chemicals were leached (15).

(ii). Seedling growth

Ageratum houstonianum aqueous leachate had no effects on shoot length of *R. sativus* and *V. mungo* (Table 2). Unlike *R. sativus*, the root length of *V. mungo* significantly decreased. Significant reduction was observed at 50 % and 100 % concentrations compared to the control (Table 2). In the root and shoot length of both test species (petri-plate experiment), only the root length of *V. mungo* was significantly affected by the leachate of *A. houstonianum*. This indicated that the root growth is more susceptible to allelochemicals, which may be due to direct contact of phytotoxins (29). Moreover, the results suggest the stimulatory effects at lower concentrations and the inhibitory effects at higher concentrations due to hormetic effects (8). This means lower concentration of allelochemicals might act as stimulants, which was previously observed in the allelopathic studies (13).

Table 2. Effects of aqueous leachates of *A. houstonianum* on root and shoot length of *R. sativus* and *V. mungo* in Petri plate bioassay at 10 days after sowing.

Leachate conc. (%)	Shoot length		Root length	
	<i>R. sativus</i>	<i>V. mungo</i>	<i>R. sativus</i>	<i>V. mungo</i>
Control	6.43 ± 0.84a	8.19 ± 1.49a	4.12 ± 1.41a	7.57 ± 0.74a
25 %	7.66 ± 0.71a	9.93 ± 4.64a	5.07 ± 0.84a	6.69 ± 0.72ab
50 %	7.09 ± 0.76a	8.46 ± 2.56a	4.03 ± 0.87a	5.99 ± 0.50c
100 %	6.20 ± 1.77a	5.96 ± 0.74a	3.80 ± 1.62a	6.06 ± 0.55c

Mean ± Standard deviation. The similar alphabet in each variable for different concentrations represent no any significant difference ($p > 0.05$) between them and different alphabets for different concentrations represent significant difference (CD $p < 0.05$) between them.

Shoot biomass of *R. sativus* and *V. mungo* was not significantly affected by *A. houstonianum* leachate (Table 3). Similarly, no significant effect was observed on the root biomass of *R. sativus* ($p > 0.05$). In contrast, root biomass of *V. mungo* was low in control than at 50 % and 100 % concentrations (Table 3).

Table 3. Effects of aqueous leachates of *A. houstonianum* on on root and shoot biomass of *R. sativus* and *V. mungo* in Petri plate bioassay at 10 days after sowing.

Leachate conc. (%)	Shoot biomass		Root biomass	
	<i>R. sativus</i>	<i>V. mungo</i>	<i>R. sativus</i>	<i>V. mungo</i>
Control	0.08 ± 0.01a	0.196 ± 0.02a	0.01 ± 0.001a	0.027 ± 0.008b
25 %	0.07 ± 0.02a	0.16 ± 0.02a	0.007 ± 0.002a	0.040 ± 0.004ab
50 %	0.06 ± 0.02a	0.10 ± 0.06a	0.005 ± 0.003a	0.045 ± 0.013a
100 %	0.08 ± 0.02a	0.15 ± 0.01a	0.006 ± 0.004a	0.045 ± 0.004a

Mean ± Standard deviation. The similar alphabet in each variable for different concentrations represent no any significant difference ($p > 0.05$) between them and different alphabets for different concentrations represent significant difference (CD $p < 0.05$) between them.

POT CULTURE

(i). Seedling growth

(a). Shoot length: *Ageratum houstonianum* leachate significantly affected both test species. In *R. sativus*, shoot length decreased from 13.99 cm (control) to 12.48 cm (50 %) and 12 cm (100 %) ($p = 0.010$) (Table 4). A similar significant reduction was observed in *V. mungo* ($p = 0.011$), with shoot length decreasing from 8.46 cm (control) to 6.55 cm (50 %). Root length of *V. mungo* was also significantly reduced, decreasing from 6.75 cm (control) to 4.61 cm (100 %), however there was no effect on root length of *R. sativus* (Table 4). *A. houstonianum* leachate significantly reduced shoot biomass in both species (*R. sativus* $p = 0.009$; *V. mungo* $p = 0.001$) (Table 5).

Table 4. Effects of aqueous leachates of *A. houstonianum* on root and shoot length of *R. sativus* and *V. mungo* in Pot culture at 26 days after sowing.

Leachate conc. (%)	Shoot length		Root length	
	<i>R. sativus</i>	<i>V. mungo</i>	<i>R. sativus</i>	<i>V. mungo</i>
Control	13.99 ± 0.75a	8.46 ± 0.29a	6.08 ± 0.95a	6.75 ± 0.80a
25 %	12.93 ± 0.70ab	7.76 ± 1.11ab	5.38 ± 0.70a	5.57 ± 0.82ab
50 %	12.48 ± 1.16ab	6.55 ± 0.62ab	5.15 ± 0.25a	4.62 ± 0.76b
100 %	12.00 ± 0.73b	7.39 ± 0.87b	5.14 ± 0.87a	4.61 ± 1.07b

Mean ± Standard deviation. The similar alphabet in each variable for different concentrations represent no any significant difference ($p > 0.05$) between them and different alphabets for different concentrations represent significant difference (CD $p < 0.05$) between them.

Table 5. Effects of aqueous leachates of *A. houstonianum* on on root and shoot biomass of *R. sativus* and *V. mungo* in Pot culture at 26 days after sowing.

Leachate conc. (%)	Shoot		Root	
	<i>R. sativus</i>	<i>V. mungo</i>	<i>R. sativus</i>	<i>V. mungo</i>
Control	0.35 ± 0.03c	0.13 ± 0.03a	0.036 ± 0.010a	0.043 ± 0.02a
25 %	0.21 ± 0.02b	0.07 ± 0.02b	0.016 ± 0.003b	0.019 ± 0.01b
50 %	0.16 ± 0.02a	0.07 ± 0.01b	0.010 ± 0.002b	0.016 ± 0.02b
100 %	0.18 ± 0.03a	0.07 ± 0.03b	0.010 ± 0.001b	0.012 ± 0.01b

Mean ± Standard deviation. The similar alphabet in each variable for different concentrations represent no any significant difference ($p > 0.05$) between them and different alphabets for different concentrations represent significant difference (CD $p < 0.05$) between them.

(b). Shoot biomass: Shoot biomass was decreased across all concentrations. *R. sativus* shoot biomass declined from 0.35 g (control) to 0.18 g (100 %), while *V. mungo* decreased from 0.138 g to 0.075 g (Table 5). Similarly, *A. houstonianum* leachate also significantly reduced root biomass in both species ($p < 0.001$). *R. sativus* root biomass declined from 0.360 g (control) to 0.010 g (100 %), while *V. mungo* decreased from 0.043 g to 0.012 g (Table 6). *V. mungo* shoot length was not significantly affected in the petri-plate experiment but showed a significant effect in the pot experiment.

Shoot biomass in both species was similar in petri-plate experiment. However, significant reduction was observed in the shoot biomass in pot experiment. This suppression may be likely due to metabolic disruptions by allelochemicals, such as in photosynthetic

efficiency and hormonal imbalance (37). The root biomass of *V. mungo* was more susceptible to allelochemicals than *R. sativus*, which reinforced that certain species and parameters exhibit higher sensitivity against certain allelochemicals (4). However, roots of *V. mungo* growing in petri-plate were stimulated (biomass) by leaf leachate (Table 3).

It can be expected that the plants growing only with water in petri plate might be in low-stress, where the leaf leachates stimulated biomass accumulation in the roots. In case of pots, roots might have faced multiple factors like nutrients competition, pH changes including soil microbial interactions or microbial transformations due to allelochemicals (29). Previous studies highlighted that the invasive plants can alter soil chemistry both directly through allelochemicals released from aerial or underground parts and indirectly by modifying microbial communities, which in turn affects the activity of the allelochemicals in soil (21,30).

(c). Leaf number and size: *A. houstonianum* leachate significantly reduced leaf number in both species. *R. sativus* (4.32 leaves in control, decreasing to 3.88 at 100 % concentration) ($p = 0.018$). Similarly, *V. mungo* leaves declined from 2.4 (control) to 1.57 (100 %) ($p = 0.010$, Table 6). Reduction in the length of leaves by *A. houstonianum* leachates was not observed (Table 6). Similar result was obtained in leaf breadth of *V. mungo*. However, leaf breadth of *R. sativus* was significantly affected by the different concentrations of *A. houstonianum* ($p < 0.001$). The leaf breadth in control, 25 %, 50 % and 100 % concentration treatment was 1.39 cm, 1.26 cm, 1.06 cm and 1.12 cm, respectively (Table 6). It highlighted the great differences in 50 % and 100 % concentration than control treatment. A significant reduction was observed in the leaf number of both test species, suggesting the allelopathic effects, particularly on *V. mungo*. Moreover, the decrease in leaf size of *R. sativus* further supported the impact of allelochemicals on leaf development. This may be attributed to the disturbance in cell division and expansion due to phytotoxin induced oxidative stress (14).

Table 6. Effects of aqueous leachates of *A. houstonianum* on leaf number, length and breadth of *R. sativus* and *V. mungo* in Pot culture at 26 days after sowing.

Leachate conc. (%)	Leaf Number		Leaf length		Leaf width	
	<i>R. sativus</i>	<i>V. mungo</i>	<i>R. sativus</i>	<i>V. mungo</i>	<i>R. sativus</i>	<i>V. mungo</i>
Control	4.32 ± 0.23a	2.40 ± 0.5 a	5.98 ± 0.62a	2.99 ± 0.26a	1.39 ± 0.12a	0.94 ± 0.07a
25 %	4.00 ± 0.01a	1.68 ± 0.33b	5.20 ± 0.37a	2.75 ± 0.23a	1.26 ± 0.10ab	0.83 ± 0.08a
50 %	3.76 ± 0.33b	1.52 ± 0.3 b	4.26 ± 0.51a	2.58 ± 0.33a	1.06 ± 0.09b	0.91 ± 0.07a
100 %	3.88 ± 0.11b	1.57 ± 0.29b	4.80 ± 0.71a	2.53 ± 0.36a	1.12 ± 0.13b	0.89 ± 0.07a

Mean ± Standard deviation. The similar alphabet in each variable for different concentrations represent no any significant difference ($p > 0.05$) between them and different alphabets for different concentrations represent significant difference ($CD p < 0.05$) between them.

Vigna mungo proved more sensitive to leachate treatments in both the experiments, particularly in root-related parameters like root length and biomass. In contrast, *R. sativus* exhibited strong response in pot experiment, with significant reductions in shoot biomass and leaf breadth, while there were minimal effects in the petri plate experiment. These differences suggest that the results from petri plate experiment reflect the direct phytotoxic effects of the leachate under controlled conditions (28), whereas the pot experiment

incorporates ecologically relevant variables, including soil chemistry and microbial interactions (16). The observed variation also underscores the complex nature of allelopathic interactions, which are influenced also by hormesis, where low concentrations of allelochemicals may stimulate growth, while higher concentrations may be inhibitory. Response of growth in *R. sativus* and *V. mungo* in this study showed the variations in sensitivity of underground and aboveground parts of seedlings, suggesting that species-specific responses and target tissue susceptibility (12).

Several conventional allelopathic studies were done previously. Unlike such studies, which rely on organic solvent leachates or powdered plant material, our experiment employs crude leachate from freshly collected leaves from *A. houstonianum*. It mimics natural processes like rain water induced leaching. Moreover, we compared petri-plate and soil-based pot experiments, which gave insights into how direct phytotoxic effects differs from those mediated by soil interactions.

CONCLUSIONS

This study showed that the invasive *A. houstonianum* leachate inhibits seed germination, root and shoot growth, root and shoot biomass and the size and number of leaves of both species, particularly in *V. mungo*. Presence of this invasive species in crop field can cause a potential damage in crop production, regarding quality or quantity. However, the varied responses of *A. houstonianum* on test species and parameters insight the species-specific interactions and selective inhibitory effects. It is important to acknowledge that the scope of this study was limited to assessing the impact of the leachates and we did not perform a chemical analysis to confirm the presence of specific allelochemicals. Future studies should focus on the identification of specifically responsible phytochemicals for crop inhibition and their mechanism of actions that could be helpful to manage the invasion of *A. hasutonianum*.

DECLARATION

We declare that all authors of this manuscript have made substantial contributions. We have not excluded any author that substantially contributed to this manuscript. We have followed our ethical norms of respective institutions.

DECLARATION OF COMPETING INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration with all authors. All authors finally approved and drafted the manuscript.

ETHICAL CONSIDERATIONS

This study adhered to ethical guidelines for research. All experiments were conducted following standard protocols and no endangered species were involved. The research did not require any human or animal subjects, thus not necessitating additional ethical approval. The use of plant materials was performed in accordance with institutional guidelines and local regulations.

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