Effects of *Ageratum houstonianum* Leaf Extract on Seed Germination and Seedling Growth of Wheat (*Triticum aestivum* L.)

Sumitra Budhathoki[1], Bimala Shakya[2], Sanu Raja Maharjan[3]*, Lal B. Thapa[4]

[1]Department of Environmental Science, Khwopa College, Bhaktapur, Nepal

[2]Natural History Museum (NHM), Tribhuvan University, Swoyambhu, Kathmandu, Nepal

[3]Department of Botany, Tri-Chandra Campus, Tribhuvan University, Ghantaghar, Kathmandu, Nepal,

[4]Central Department of Botany, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal

*Corresponding Email: botanysanu@hotmail.com

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ABSTRACT

Ageratum houstonianum Mill. (Nepali name: Nilo Gandhe) is one of the worst invasive plants in different habitats including agroecosystems in Nepal. It is reported as the weed having detrimental impacts on crop plants. However, information regarding the specific impact of A. houstonianum on different crop species including wheat (Triticum aestivum L.) is deficient. This study tested the effect of aqueous extract of A. houstonianum leaves on seed germination and seedling growth in wheat. The seedlings were grown both in petri plates and pots to measure seed germination percentage and other growth parameters. Seed germination percent, shoot growth and root-shoot biomass of wheat seedlings were reduced by A. houstonianum leaf extract in petri plates, while the negative effects were not prominent in the seedlings grown in pots. The concentration of available potassium in the pot soil was found to be elevated by the extract. The results suggest that the weed A. houstonianum imposes toxic effects on seed germination and seedling growth of wheat when the seeds are exposed to the weed's leaf extract. But the toxicity of the extract varies depending on whether seeds are directly exposed to it or if it is amended into the soil. Such differential impacts of A. houstonianum on wheat highlight the need for targeted strategies to mitigate its negative effects on wheat growth, development and productivity.

Keywords: Weeds, Invasive plants, Leaf Extract, Allelopathy.

1. Introduction

Ageratum houstonianum Mill., native to Central America and Mexico, is one of the most noxious weeds in many regions around the world (Vélez-Gavilán, 2016). The weed belongs to the family Asteraceae and it is commonly called 'Nilo Gandhe' in Nepali. Its extensive colonization is found in disturbed habitats, fallow lands, road sides, forest margins and mainly agroecosystems in Nepal and India (Singh et al., 2011; Shrestha et al., 2018). Production of a large number of minute seeds, rapid rate of germination and growth of the weed have been key factors for its invasion success (Airi et al., 2023).

Many of the invasive weeds in agroecosystems can result in competition with crop plants for resources such as light, water and nutrients (Médiène et al., 2011). In addition, the weeds can harm crop plants through allelopathy, where they release harmful chemicals (allelochemicals) that inhibit the growth of surrounding crops (Kunz et al., 2016). Allelochemicals are responsible for inhibiting the germination and growth of crop plants as well as contributing to alterations in soil physicochemical parameters (Dogra et al., 2009; Chengxu et al., 2011; Zohaib et al., 2016). Several previous studies (e.g., Marinov-Serafimov et al., 2010; Shrestha et al., 2021; Khatri et al., 2023) have highlighted the allelopathic effects of many invasive weeds on crop plants. Studies have documented that the invasive *Ageratum conyzoides* L., which is the congeneric member of *A. houstonianum*, is also phytotoxic to several crop species (Kong et al., 2004; Kohli et al., 2006; Negi et al., 2020). Despite these documentations, there is a significant lack of research concerning the specific toxic impact of *A. houstonianum* on crop plants.

Among the crops, wheat (*Triticum aestivum* L.) is a staple crop worldwide and the major cereal crop in Nepal (Subedi et al., 2019; Filip et al., 2023). Not only as a staple food, but also the wheat contributes as livestock feed and various industrial products. Infestation of *A. houstonianum* in the wheat fields may directly threaten both plant survival and productivity which subsequently impacts food availability as well as economic losses for farmers (Shrestha et al., 2018). Understanding such a threat is crucial for assessing the responses of wheat to the leached substances (allelochemicals) from the weed (Choudhary et al., 2023). By knowing how crop plants interact with and respond to allelochemicals released by invasive weeds, researchers and managers can develop strategies to mitigate the negative impacts on crop growth and productivity. Hence, this study aims to highlight the potential impacts of *A. houstonianum* extracts (leachates) on the germination and growth of *T. aestivum*.

2. Methods

2.1. Petri plate experiment

Leaves of *A. houstonianum* were collected from the Changunarayan area of Bhaktapur district (27°42'28" N, 85°26'22" E), Nepal in order to prepare water extract. Seeds of *T. aestivum* (variety WK 1204) were collected from Nepal Agricultural Research

Council (NARC), Lalitpur, Nepal in December 2021. For extract preparation, ten grams of fresh and matured leaves of *A. houstonianum* were washed and soaked in 100 mL of distilled water for 24 hours. Afterward, the extract (10 g leaf/100 mL distilled water) was used as the stock solution considering 100%, which was then diluted with distilled water to make concentrations of 50% and 25%.

The seeds of wheat were washed with distilled water and surface sterilized by using 70% ethyl alcohol. The sterilized seeds were subsequently rinsed multiple times with distilled water, and arranged on double-lined filter paper in sterilized petri plates. A total of 10 seeds were uniformly arranged on each petri plate. The filter paper of control plates was moistened by distilled water and the filter papers of extract treatments were moistened by the respective extract *concentrations* (i.e., 25%, 50%, and 100%) of *A. houstonianum.*

There were five replicated plates for each treatment. The petri plates were then incubated at 30°C (dark) for 7 days. The filter papers in the petri plates were kept moist during the incubation period by adding respective extracts and distilled water (in control). The seeds that germinated (seeds emerging with visible radicles) were counted and the final germination percent was calculated. After germination, the seedlings were allowed to grow inside petri plates. On the 7th day of incubation, the length of seedlings (root and shoot separately) and their dry weight (biomass) were measured. The plant materials were dried in a hot air oven at 80°C for 24 hours to measure the dry weight.

2.2. Pot experiment

In addition to the petri plate experiment, seedlings of wheat were grown in pots. Polyethylene pots of size 15 cm in height and 15 cm in diameter were filled with soil collected from previously wheat-cultivated land. Each pot contained 1.3 kg of soil. Similar to the petri plate experiment, wheat seedlings were grown in pots treated with distilled water (control) and *A. houstonianum* leaf extracts (25%, 50%, and 100% concentrations). Each treatment consisted of five replicated pots, with each pot containing 10 seedlings. Pots were placed in an open area at the Department of Environmental Science, Khwopa College, Bhaktapur, Nepal. The pots were irrigated with 100 mL of water (control pots) and the same volume of respective extracts (extract treated pots) at 2-day intervals.

The experiment was terminated after 40 days of sowing seeds. The seedlings were carefully removed from the pots by moistening the soil with enough water without destroying the root system. Aerial parts and roots were washed and length (root and shoot separately) were measured. The number of leaves, length of leaf lamina, and breadth (at the midpoint of the lamina) were also measured. Then the plants were dried in hot air oven, as mentioned above in the petri plate experiment, to measure dry weight (biomass). The experiment was conducted in January 2021.

2.3. Soil analyses

Soil pH, soil organic matter (SOM), total nitrogen (TN), available potassium, and available phosphorus were measured in the pot soils treated with water (control) and *A. houstonianum* extract (100%). Soil organic matter was determined by the titrimetric method given by Walkley and Black (1934). Total soil nitrogen was estimated by Kjeldahl's distillation method (Kjeldahl, 1883). Available phosphorus was determined by modified Olsen's method, and available potassium by using a flame photometer as described by Pradhan (2005). Three replicated soil samples for each treatment were analyzed in the laboratory of the Central Department of Environmental Science, Tribhuvan University, Kathmandu, Nepal.

2.4. Statistical analysis

Differences in the germination percentage of seeds (petriplate experiment), lengths and biomass of roots and shoots (both petriplate and pot experiments), and leaf parameters (pot experiment) among the treatments (control and *A. houstonianum* leaf extracts) were compared using One-way Analysis of Variance (ANOVA). Variations in the soil parameters between control and extract (100%) was compared using independent sample t-test. Statistical analyses were carried out in the software R (version 4.2.0) (R Core Team, 2022).

3. Results

3.1. Seed germination

In the petri plate experiment, the seed germination percentage of wheat was computed. The final percentage of seed germination was reduced by the aqueous extract of *A. houstonianum*. The control plate exhibited a high percentage of seed germination i.e., 86±4.51%, while the germination in the extracts (25%, 50%, and 100%) was 76.63±9.04%, 75.67±4.18% and 76.67±5.27%, respectively (Figure 1).

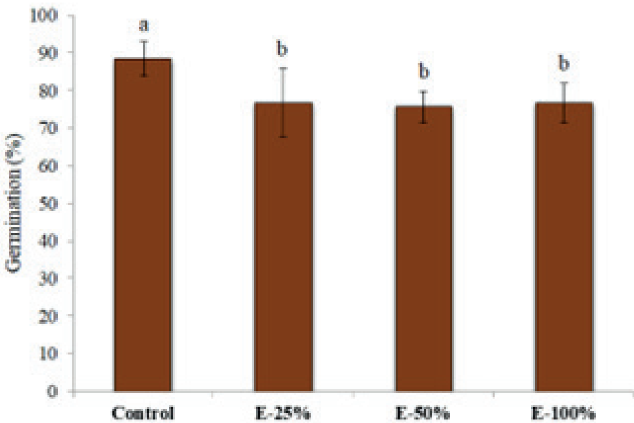

Figure 1. Effect of aqueous extract of *A. houstonianum* on seed germination percentage. Different letters above error bar indicate significant difference ($P < 0.05$) among the treatments. Extract concentrations (E-25: 25%, E-50: 50% and E-100: 100%).

3.2. Shoot and root length

With an increase in the concentrations of aqueous extract of *A. houstonianum*, the shoot length of wheat seedlings grown in petri plates decreased significantly. In the case of the seedlings grown in the pots, the differences in shoot length were not significant although the seedlings grown with extract treatments were comparatively shorter than the control pots (Table 1). Similarly, the roots of seedlings grown with aqueous extracts were shorter than the control in both petri plates and pot experiments, but the difference in the length was statistically not significant (Table 1).

Table 1. Shoot and root length of wheat seedlings in petri plate and pot experiments. The data are the average value ± standard error.

	Shoot length (cm)		Root length (cm)	
	Petri plate experiment	Pot experiment	Petri plate experiment	Pot experiment
Control	5.08±0.46 a	16.27±4.44 a	3.25±0.56 a	9.25±1.64 a
E-25	3.62±0.66 b	12.87±1.86 a	2.13±0.35 a	8.26±0.81 a
E-50	2.17±0.18 c	13.44±3.07 a	2.16±0.10 a	8.81±0.52 a
E-100	1.56±0.41 c	12.64±2.98 a	1.78±0.12 a	8.79±1.12 a

Different alphabets after mean values indicate significant difference among the concentrations in each ex-periment ($p < 0.05$). Extract concentrations (E-25: 25%, E-50: 50% and E-100: 100%).

3.3. Shoot and root biomass

Comparing the seedlings among the treatments on petri plates, the shoot biomass was significantly lower in the seedlings that were grown in the extracts (0.010 to 0.034 g) than the control seedlings (0.048 g). A similar result was found in case of root biomass in the petri plates. The root biomass was 0.042 g and 0.058 g in the extract concentrations 50% and 100% , respectively, which was significantly lower than the biomass of seedlings found in control (0.078 g) and extract concentration 25% (0.074%). The extract concentration of 25% did not inhibit the root biomass significantly compared to the control. Interestingly, the addition of *A. houstonianum* extracts did not inhibit the biomass of shoot and root in the pot experiment (Table 2).

Table 2. Shoot and root dry weight of wheat seedlings in petri plate and pot experiments. The data are the average value ± standard error.

	Shoot dry weight (g)		Root dry weight (g)	
	Petri plate experiment	Pot experiment	Petri plate experiment	Pot experiment
Control	0.048±0.01 a	0.44±0.04 a	0.078±0.008 a	0.19±0.04 a
E-25	0.034±0.03 b	0.39±0.03 a	0.074±0.005 a	0.16±0.05 a
E-50	0.014±0.01 c	0.43±0.16 a	0.058±0.008 b	0.15±0.05 a
E-100	0.010±0.01 c	0.44±0.12 a	0.042±0.004 c	0.18±0.03 a

Different alphabets after mean values indicate significant difference among the concentrations in each experiment ($p < 0.05$). Extract concentrations (E-25: 25%, E-50: 50% and E-100: 100%).

3.4. Leaf parameters

Leaf parameters (number of leaves, length and breadth of lamina) were measured in the wheat seedlings grown in pots under different treatments. The average number of leaves, length and breadth of the leaf lamina of wheat seedlings did not vary among control and different concentrations of *A. houstonianum* extracts. The average values of each parameter are given in Table 3 .

Table 3. Measurement of leaf parameters of wheat in petri plate and pot experiments. The data are the average value ± standard error.

	Leaf number	Leaf length (cm)	Leaf breadth (cm)
Control	3.82±0.28 a	8.37±1.52 a	0.29±0.01 a
E-25	3.62±0.22 a	6.70±0.57 a	0.23±0.07 a
E-50	3.56±0.17 a	6.69±0.61 a	0.25±0.02 a
E-100	3.54±0.38 a	6.80±1.01 a	0.25±0.04 a

Different alphabets after mean values indicate significant difference among the concentrations in each experiment ($p < 0.05$). Extract concentrations (E-25: 25%, E-50: 50% and E-100: 100%).

3.5. Soil parameters

Comparing the soil parameters between control and *A. houstonianum* extract (100%) in pots, available potassium was found to be significantly higher, approximately double in the extract than in the control. There was no change in other parameters of soil (pH, SOM, TN and available phosphorus) due to the addition of the extract (Table 4).

Table 4. Soil parameters. The data are the average value ± standard error.

Soil sample type	pH	SOM (%)	AK (kg/ha)	TN (%)	AP(kg/ha)
Control	6.90± 0.33	2.19±0.083 a	179.33±18.77 b	0.019±0.023 a	78.92±9.00 a
E-100	7.10± 0.24	2.30±0.052 a	299.33±13.87 a	0.022±0.005 a	74.57±4.00 a

SOM: soil organic matter, AK: available potassium, TN: total nitrogen, AP: available phosphorus. Different alpha-bets after mean values indicate significant difference among the concentrations in each experiment ($p < 0.05$). (Extract concentration, E-100: 100%).

4. Discussion

This study conducted a comparative analysis of the effect of various concentrations of *A. houstonianum* leaf extracts on seed germination and seedling growth of wheat by growing in controlled (petri plates) and in open (pots) environments. The results suggest that the toxicity observed in wheat seedlings likely resulted in an alteration in the germinability of seeds (Figure 1) and biomass accumulation (Table 2), when the seeds were exposed to *A. houstonianum* leaf extract.

Leaves of *A. houstonianum* are known to produce a large number of secondary metabolites such as saponins, flavonoids, alkaloids, tannins and phenols (Zeeshan et al., 2012; Anyanele et al., 2022). These chemicals can interfere seed germination and seedling growth by inhibiting the enzymes involved. Radwan et al. (2019) also found similar chemicals in *Calotropis procera* L. and had tested the effect of aqueous extract from this plant on the growth of wheat and barley. Higher concentrations extracts from *C. procera* inhibited germination percentage and growth of radicle and plumule of wheat significantly (Radwan et al., 2019). From these information, it is concluded that wheat exhibits sensitivity to such chemicals present in the surroundings extracted from the leaves of other plants.

Seed germination and seedling growth depend on various physiological mechanisms. Allelochemicals are responsible to reduce chlorophyll content, relative water content as well as expression and activity of the enzymes required for germination and growth (Madhan Shankar et al., 2014). Allelochemicals suppress cell division and elongation, change membrane permeability, lower nutrients uptake and hinders synthesis of endogenous hormones (John & Sarada, 2012). Allelochemical-induced such effects impact on seed germination and overall growth and development of plants.

The aforementioned explanations are based on the results of the petri plate experiment. Interestingly, the pot experiment did not reveal significant changes in the seedling growth (shoot and root length, biomass and leaf parameters) of wheat (Tables 1 to 4). This suggests that when allelochemicals are amended into soil at lower concentrations, the detrimental effects on seedlings may not be readily apparent or pronounced. In petriplate experiment, the seedlings perceived direct exposure to concentrated allelochemicals, whereas the conditions in the pot were unlike those in the petri plate environment. Actually, pot experiments simulate more realistic field conditions, where allelochemicals are diluted in a larger soil volume. In such scenarios, the availability of allelochemicals to plant roots may be reduced, potentially mitigating their negative effects on seed germination and seedling growth.

Additionally, the soil environment is complex and allelopathic effects may be influenced by various factors such as soil microbial activity and their interaction with plant itself and allelochemical input from surroundings (Scavo et al., 2019). Therefore, petriplate experiment provides insights into the direct impacts of allelochemicals on seed germination and early growth stages of wheat, while the pot experiment deals with a more realistic perspective considering a wider ecological interactions and environmental conditions.

Analyzing soil parameters, a significant increase in potassium concentration was found in the soils treated with A. *houstonianum* extract (Table 4). The elevated potassium content in the soil can have significant role in altering seedling growth dynamics as it is an essential macronutrient influencing various metabolic activities in wheat (Rao, 1986). Mojid et al. (2012) quantified the optimum doses of nutrients (nitrogen, potassium and phosphorus) for wheat. They concluded that a considerable negative effects on wheat growth were not observed due to increased concentrations of potassium, while exceeding concentrations of nitrogen (>100 kg ha-1) and phosphorus (>20 kg ha-1) showed negative impacts. This study further confirms that doubling the concentration of available potassium, as observed in pots treated with 100% A. *houstonianum* extract, does not significantly alter wheat seedling growth. However, high salt concentrations in soil limit the availability of soil water to the plant roots and create osmotic stresses on plants (Beaton & Sekhon, 1985). Therefore, understanding the impact of invasive plants' extracts on soil components is also crucial for comprehending knowledge on invasions and soil interactions in crop fields.

5. Conclusion

In conclusion, *Ageratum houstonianum* shows toxic effects on seed germination and seedling growth of wheat (*Triticum aestivum*) when the seeds are exposed to *A. houstonianum* leaf extract but the toxicity was not prominent when seeds are grown in soil. The results indicate that the degree of extract toxicity depends on the type of substrate (petri plate vs. soil) and extract concentrations. Toxicity is prominent when the seeds are directly exposed to extract but may not occur when the same concentration of extract is incorporated into the soil. Therefore, it is important to consider a real field conditions when assessing the allelopathic effects of donor to recipient plant species.

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