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**RESEARCH ARTICLE**

# Phytochemical Screening and Allelopathic Effect of *Ageratum houstonianum* on Seed Germination and Growth of *Raphanus sativus* and *Eleusine coracana*

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## Abstract

Invasive and alien plant species are increasingly reported to affect agricultural landscapes and ecosystems in Nepal. Their effects on crop production and native biodiversity have rarely been investigated. Different phytochemicals in the invasive plants enter the soil and are transported to the farmlands and cause negative impacts on the germination and development of staple plants. This research examined the allelopathic impacts of one of the most widespread invasive plants, *Ageratum houstonianum* on the germination and early growth of *Eleusine coracana* and *Raphanus sativus*. The methanolic leaf extract of *A. houstonianum* was found to contain several classes of secondary metabolites including alkaloids, flavonoids, phenolics, and reducing sugars on phytochemical screening. The leaf extract exhibited no significant antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aruginosa.* Treatment with 10% extract reduced plumule growth by 22.79% and radicle growth by 27.86% in *R. sativus*, and germination rates were significantly decreased in both crop species. Although the extract inhibited seedling growth in *R. sativus*, *E. coracana* showed a significant stimulatory response, with growth increasing by 190.48% at the 10% extract concentration. The results of this study will support the management of invasive alien species for the sustainable crop production and preservation of biodiversity.

*Keywords: Ageratum houstonianum*, allelochemicals, invasive plants, Parbat district, seedlings

## Introduction

Allelopathy refers to the phenomenon where one plant positively or negatively affects another by releasing specific biochemicals. These compounds are synthesized by the secondary metabolism as well as through decomposing plant matter, leachates, root secretions, and volatile compounds which are referred to as allelochemicals (Trezzi et al., 2016). Weeds are undesirable plants that obstruct the growth of crop by competing for space, nutrients, moisture, light, and air. They even impede the plants by releasing secondary metabolites into the environment (Lalbiakdika et al., 2022). Unwanted plants and cultivars continually compete by synthesizing various chemicals that inhibit each other's seed germination, growth, and reproduction (Marinov-Serafimov, 2010). Invasive and alien plants are more adaptive for seed germination and growth in and elevated level of carbon dioxide, greenhouse gas, and global warming. These factors excessively benefit invasive plants, leading to a loss of native biodiversity, species extinction, and distractions to hydrology and ecosystem function (Nagabhushan Arun et al., 2023). These non-native species can have severe adverse effects, worsening climate change and biodiversity loss. By outcompeting native species, altering hydrological systems, and disrupting gene pools, invasive aliens degrade ecosystems and lead to biological homogenization (Hui et al., 2011).

Nepal is a small country located in the Hindu Kush Himalayan Region (HKHR) and features a unique ecosystem and diverse landscapes. Its diverse climate, topography, glaciers and geography support a wide range of plant and animal life (Shrestha et al., 2012). Researchers regard this region as highly sensitive and vulnerable to climate change because its temperature is rising by 0.03±0.07°C annually (Thapa et al., 2018). According to Shrestha et al. (2017), 219 alien flowering plant species have become naturalized in Nepal. Among them, 26 species invaded from tropical America are reported to negatively impact agricultural production and the environment.

*Ageratum houstonianum* (Asteraceae) commonly known as Blue-mink is an annual herb that is identified by its thread-like florets and blue inflorescence (Figure 1). In tropical and subtropical areas, it grows as a weed (Chandraker et al., 2020). It is native to Central America to Mexico and has been spreading in Tarai, Mid Hills and Siwalik of Nepal from east to west ( Shrestha et al., 2017). The plants of this species are short-lived annual or biennial herbs growing up to 20-100 cm tall in height. *A. houstonianum* is one of the major invasive alien plant species found in the Parbat district of Nepal (Adhikari et al., 2022) which is widely spread across the mid-hills and lowland regions, particularly along roadsides, within protected national parks, and across agricultural and fallow lands (Lamsal et al., 2019). In Nepal, comprehensive data on alien plant species and their impacts on native species, water resources and soil physical, chemical, and microbial properties are limited.

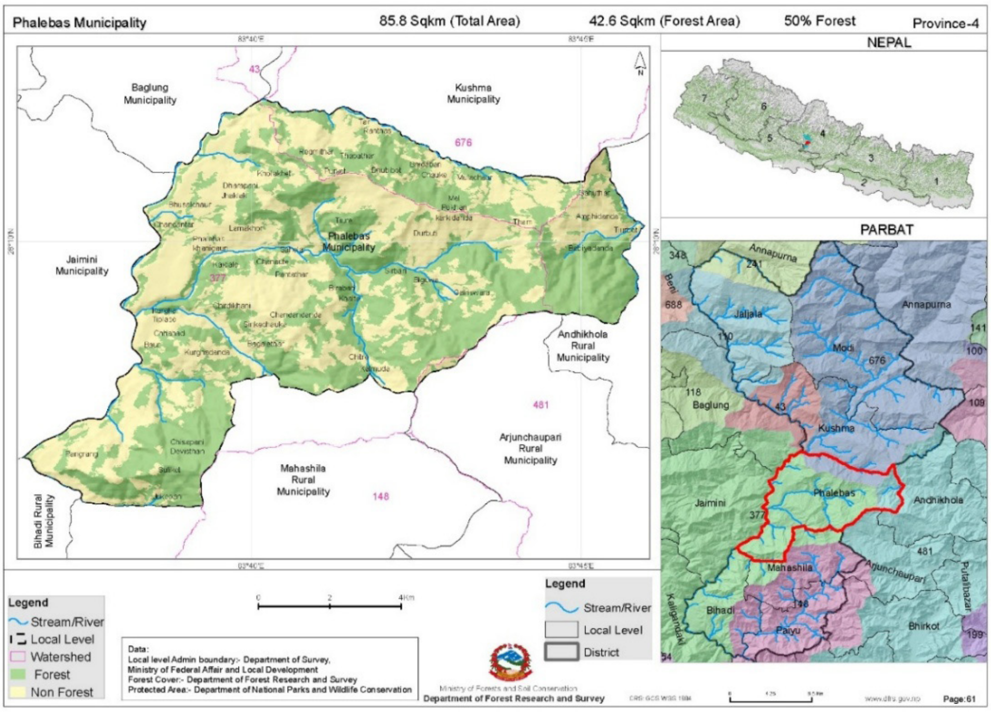
**Figure 1**

*A Flowering Structure and the Bush of Ageratum houstonianum*

This study aims to evaluate the ecological and agronomic impacts of alien plant species in agricultural regions, with a particular focus on their dual allelopathic effects and soil-mediated interactions to identify both harmful and potentially beneficial roles in crop production. In this study, we assessed the phytochemical screening, antibacterial capability and effects of *Ageratum houstonianum* on the germination and growth of *Eleusine coracana* and *Raphanus sativus* in the Parbat district of Nepal.

**Figure 2**

*Map of Phalebas Municipality*




(**Source**: Phalebas Municipality, Parbat)

## Materials and Methods

### Study Area

Whole plant samples were collected from Phalebas Municipality of Parbat district (Figure 2), whose total area is 86 km$^2$. Parbat is a mountainous district in Western Nepal's Dhaulagiri region. It spans an area of 536.86 km², with coordinates between 27° 58' to 28° 39' North and 83° 34' to 83° 59' East, and has an elevation range of 520 to 3,309 meters. The study area is chiefly a plain area and lies near the Kaligandaki River.

### Chemicals and Apparatus

The chemicals used in this study were of the high analytical purity. Distilled water (DW) was used for preparation of solutions. Different solvents of analytical grade like hexane, methanol, chloroform, and ethyl acetate (Fisher-Scientific) were used in the extraction. The chemicals of Thermo-Fisher Scientific India Pvt. Ltd., sodium hypochlorite, dimethyl sulfoxide (DMSO), concentrated sulphuric acid, Muller Hinton Reagent (MHA), vancomycin and amikacin are the other chemicals that were used. Similarly, different apparatus including electronic balance, grinder, rotary evaporator, autoclaves, hot air oven, refrigerator, incubator, sonicator, separating funnel, Petri discs, Soxhlet apparatus, and micropipettes were used.

### Sample Collection and Extraction

About 3 kg fresh leaves from healthy

matured *A. houstonianum* were collected from Phalebas Municipality, Parbat, Nepal in January, 2024. A herbarium specimen was submitted to Dr. Hom Nath Pathak of the Department of Botany, Prithvi Narayan Campus, Pokhara, for botanical identification. The samples were washed with distilled water, dried in the shed for about three weeks. It was ground into a fine powder using a mechanical mill. The extraction was performed by the Soxhlet apparatus using 80% methanol. The methanolic extract was subjected to fractionation using n-hexane, chloroform and ethyl acetate using a separating funnel and stored at 4ºC in refrigerator (Kyada et al., 2023).

**Phytochemical Screening**

The methanolic extract and its different solvent fractions were examined for the presence of different phytochemicals by using standard methods. The tests were performed for the presence of alkaloids, flavonoids, tannins, reducing sugars, phenolics, terpenoids, anthraquinones, and saponins (Shaikh & Patil, 2020).

**Antibacterial Activity**

The antibacterial susceptibility of the plant extract was evaluated by the agar well diffusion method against gram positive *Staphylococcus aureus*; and gram-negative *Escherichia coli* and *Pseudomonas aruginosa.* The strains were cultured in freshly prepared Muller Hinton Broth (MHB) at 37ºC to make the concentration equivalent to 0.5 McFarland's standard. The solution was carefully streaked onto the surface of sterile Petri discs filled with Muller Hinton Agar Media uniformly. The test samples were loaded onto the sterile bore wells using a micropipette and allowed to incubate for 24 hours at 37ºC. In the next day, the discs were taken out and the clear zones around the sample that signifies the bacterial activity was measured as zones of inhibition (ZOI) (Dosoky et al., 2016; Murray et al., 2007).

**Allelopathic Effect**

The allelopathic impact of *A. houstonianum* on two common crops, radish and millet, was assessed using standard methods (Li et al., 2021; Namkeleja et al., 2013). The tests were carried out by measuring the percentage of germination, growth of radicle and plumule in presence of the plant extract at various concentrations. The mature leaves were collected and dried in a shed, ground into a fine powder. About 500 g of this powder was soaked in 500 mL of distilled water. The mixture was then sealed in conical flasks and shaken on a horizontal shaker at 140 rpm for about 6 hours. The extracts were collected and filtered through Whatman No. 1 filter paper. This filtrate was regarded as the 100% stock solution, and specific volumes of distilled water were added to prepare 10%, 30%, 50%, and 70% dilutions. Distilled water served as the control. All filtrates were stored in a refrigerator at 4°C until further use in the allelopathic assay. Healthy seed samples were purchased from Pokhara city and surface sterilized with 0.2% sodium hypochlorite solution and rinsed three times with DW. Sterile Petri dishes were lined with two layers of Whatman No. 1 filter paper. For each treatment, 10 mL of the extract at the corresponding concentrations were applied to the filter paper using a micropipette, after which 10 seeds were placed in each dish. The discs were covered and put into an incubator at 24ºC for 7 days. Each of the set was observed at the intervals of 24 hours and 2 mL of the particular extracts were added to maintain the humidity. Each of the treatments were set in triplicates and the number of seeds germinated were recorded in 48 hours. The length of plumule and radicle each of the sample were measured and recorded on the $7^{th}$ day. The fresh and dry weights of the 10-day old seedlings of both of *R. sativum* and *E. caracana* were determined by drying the sample in an oven at 45°C for 72 hours until it reached a constant weight. The percentage allelopathic effects of each of the tests were calculated by using the formula:

% Allelopathic effect

$$= \frac{(Effect\ with\ control - Effect\ with\ treatments)}{Effect\ with\ control} X100$$

Statistical Analysis: All experiments were conducted in triplicate, and data were analyzed using Microsoft Excel. Results are presented as mean ± standard deviation (SD). Statistical differences among treatments were assessed by one-way analysis of variance (ANOVA), with significance determined at $p < 0.05$.

## Results and Discussion

### Extraction Yield

The crude methanol extract (14.8 g) of *A. houstonianum* (AHM) was subjected to fractionation. Each fraction was carefully dried and the weights were recorded and shown in Table 1. The extraction yield is significantly affected by various factors such as the type of solvent used, the extraction technique, and the maturity of stage of the plant material.

Water, being a polar and a green solvent, is particularly effective for extracting polar compounds such as phenolics, flavonoids, terpenoids, and various nitrogen-containing substances (Dhanani et al., 2017). The aim of this study was to evaluate the allelopathic influence of *A. houstonianum* on selected staple crops under natural conditions using an aqueous extract, as the aforementioned phytochemicals are known to possess such activity (Xu et al., 2023).

**Table 1**

*Weights of Methanol Extract and Different Fraction of Ageratum houstonian*

| S. No | Plant extract/fraction | Mass (g) |
|---|---|---|
| 1 | Methanol (AHM) | 14.8 |
| 2 | Hexane fraction (AHH) | 2.7 |
| 3 | Chloroform fraction (AHC) | 1.24 |
| 4 | Ethyl acetate fraction (AHE) | 0.42 |

### Phytochemical Screening

Phytochemicals are secondary metabolites synthesized within plant cells that do not participate in primary metabolic processes. Instead, they play a vital role in the plant's defense mechanisms against hostile weather, pests, herbivores, and insects, while also aiding in pollinator attraction and interactions with soil minerals (Anjali et al., 2023). The crude extract and all fractions were subjected to phytochemical screening and the results are shown in Table 2. The methanolic extract contained alkaloids, reducing sugars, glycosides and flavonoids, while hexane extract contained glycosides and tannins only. The ethyl acetate fraction contained glycosides, flavonoids, tannins and phenolics.

**Table 2**

*Results of Phytochemicals Screening of the Extract and Fractions of A. houstonianum*

| S.N. | Phytochemicals | AHM | AHH | AHC | AHE |
|---|---|---|---|---|---|
| 1 | Alkaloids (Dragedorff's test, Meyer's test) | + | - | + | + |
| 2 | Reducing sugars (Fehling's test) | + | - | - | - |
| 3 | Glycosides (Keller-Killani test) | + | + | - | + |
| 4 | Tannins ($FeCl_3$ test) | - | + | + | + |
| 5 | Flavonoids (Alkali test, Shinoda test and lead acetate test) | + | - | - | + |
| 6 | Phenolics (Lead acetate test, $FeCl_3$ test) | - | - | + | + |
| 7 | Terpenoids (Salkowski's Test) | - | - | - | - |
| 8 | Anthraquinones (Borntrager's Test) | - | - | - | - |

Similarly, chloroform extract contained alkaloids, tannins and phenolic compounds. The ethyl acetate fraction of the plant contained alkaloids, glycosides, tannins, phenolic compounds and flavonoids. The results indicated the presence of polar compounds in polar solvents in comparison to the non-polar solvents like hexane and chloroform. Similar to our results Anyanele et al. (2022) also observed the presence of saponins, flavonoids, tannins, phenols and alkaloids in the leaf extract of the plant collected from Nigeria. Similarly, methanol and DW extracts of the plant collected from Sauraha forest area of Chitwan were used to prepare methanol and DW extract. The extracts were subjected to phytochemical screening tests against several secondary metabolites. The aqueous extract contained tannins, saponins, cardiac glycosides, alkaloids and flavonoids whereas methanol extract of the same species was found to contain terpenoids, cardiac glycosides, steroids, flavonoids and alkaloids which are similar to our results (Devkota & Sahu, 2019). Among the several bioactive compounds in plants, the volatile phenolics, terpenoids, and alkaloids as well as other volatile organic compounds (VOCs) which easily leach out or evolve from roots, flowers, seeds, rhizomes, barks, buds, leaves etc. over time and exhibit allelopathy to their neighboring plants species (Kong et al., 2024; Latif et al., 2017).

**Antibacterial Activity**

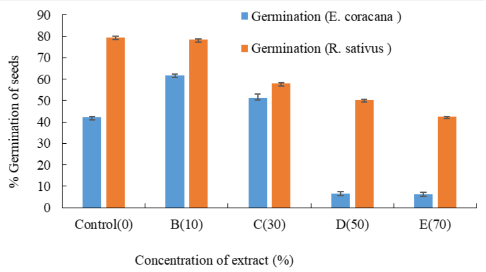
The methanolic extract of the plant did not show any antibacterial activity against the tested strains. The tests were performed using solutions of 50 mg/mL, 250 mg/mL and 500 mg/mL but none of these solutions showed significant activity against gram-positive *Staphylococcus aureus*; and gram-negative *Escherichia coli* and *Pseudomonas aruginosa.* The extract may show antibacterial action against other bacterial strains. Germination and early seedling growth are complex and highly sensitive processes influenced by various biochemical interactions. Neighboring plants produce allelochemicals such as, isoflavonoids, metacarpin, 4-methoxymedicarpin, sativan, and 5-methoxysativan etc. In addition, seedlings also release flavonoids like hyperoside, luteolin, luteolin-7-glucoside, and chrysoeriol. These compounds can control the surrounding microbiome by suppressing harmful microorganisms while promoting the growth of beneficial microbial communities that support seedling development (Abouzeid et al., 2023; Laouane et al., 2024).

**Allelopathic Activity**

The allelopathy of *A. houstonianum* on the seed sprouting of *R. sativus* and *E. coracana* was evaluated by germination method and the results are shown in the bar diagram (**Figure** 3). The application of a 10% extract increased the germination of *E. coracana* seeds from 42.08% to 61.9%, while slightly inhibiting the germination of *R. sativus* from 79.58% to 78.35%. However, higher concentrations of the extract reduced germination in both plant species.

**Figure 3**

*Percentage of Seed Germination at Different Concentrations of A. houstonianum*

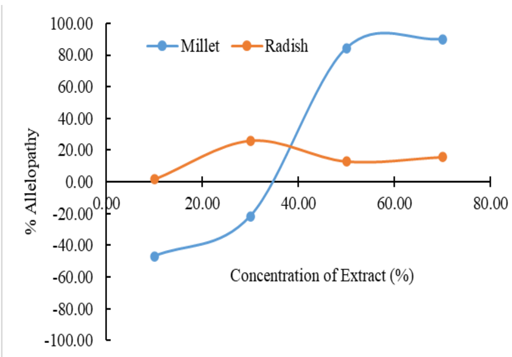



Note: Values are mean ± SD (n=3)

The percentage allelopathic effect of germination indicated both positive and negative impacts on germination. In case of *E. coracana*, a low concentration (10%) enhanced germination by 47.10%, and a moderate concentration (30%) led to a 21.79% increase. On the other hand, higher concentrations of 50% and 70% markedly inhibited germination. Similarly, the germination of *R. sativus* seeds was affected in a dose-dependent manner. A low level of inhibition (1.5%) was observed initially, which rose to 26% at a higher concentration. At the highest concentrations tested (50% and 70%), the inhibition was 12.95% and 15.71%, respectively in figure 4. The study concludes that the alien plant species exerts both positive and negative influences on seed germination. Most allelopathic effects are associated with competition for essential resources such as nutrients, light, water, and minerals. Invasive plant species often release specific chemicals that suppress the growth and development of native plants. However, soil conditions can modify or sometimes reduce these allelopathic interactions. In certain cases, allelochemicals may also interfere with germination inhibitors, thereby enhancing seed germination (Bakacsy et al., 2024; Lenda et al., 2023).

**Figure 4**

*Allelopathic Effect of A. houstonianum on the Germination*




The growth of seedlings was measured by assessing the relative growth of plumule and radicle length at specific time intervals (Figure 5). Here, we measured the percentage reduction in the growth of radicle and plumule within the 10-day period. Figure 6 shows the allelopathic effect of *A. houstonianum* extract on the growth of *E. coracana.* It shows that at concentrations of 10% and 30% the growth of plumule was enhanced but at higher concentrations the development rate was greatly inhibited by the extract.

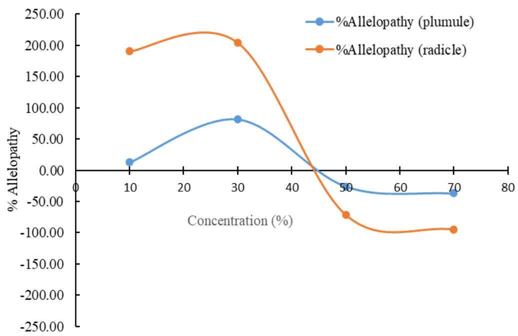
**Figure 5**

*Germination and Growth of Seedling*

Similarly, radicle growth was also improved by the extract, increasing by 190.48% at a 10% concentration and 203.94% at 30%. However, further increases in concentration resulted in significant inhibition of growth. The early growth of seedlings may have been enhanced by the action of these chemicals, and their physio-biological interactions with the soil provided additional benefits similar to a mulching effect (Basar et al., 2023).

**Figure 6**

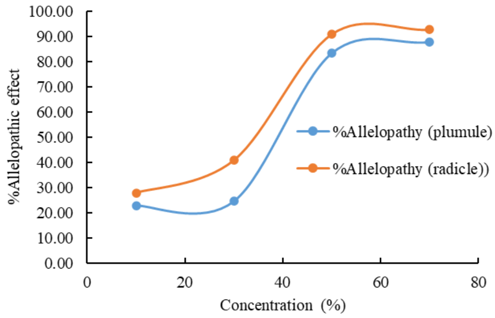
*Allelopathic Effect of A. houstonianum on the Growth of E. coracana*




For *R. sativus*, as shown in Figure 7, the application of the extract significantly inhibited overall seedling development. However, measurements of the plumule and radicle showed a reduction in growth at a 10% concentration, with the plumule decreasing by 22.79% and the radicle by 27.86%. Higher concentrations led to a more substantial inhibition of growth indicating the adverse effects of invasive plant species on crops.

**Figure 7**

*Allelopathic Effect of A. houstonianum on the Growth of R. sativus*




The growth of seedlings is greatly influenced by different types of chemicals in the plant extract. The growth inhibition was assessed by the comparative reduction of the weights of the seedling biomass at different concentrations of A. houstonianum extracts and the results are shown in Table 3. In this study, the reduction of seedling weight of E. caracana was lower than the control at 10%, 30%, and 50% treatments, but higher at 70%. The higher percentage of weight reduction (96.28±0.91) was due to strongly inhibited growth of the plumule and radicle. In case of R. sativus slightly different results are observed. The weight reduction was larger than the control at 10%, 50% and 70% but was higher in 30% (94.51±0.39), which is the effect on the growth of seedlings. It indicates that the growth of seedlings can have both positive and negative allelopathic effect at various concentration. This effect may arise from the neutralizing action of allelochemicals produced by native species against those of invasive plants. Although such outcomes are relatively rare, compounds released by one species can, in some cases, promote seed germination and stimulate early growth at certain concentration. However, these interactions require thorough investigation, particularly with respect to soil chemistry and the degradation dynamics of allelochemicals (Hierro & Callaway, 2021).

**Table 3**

*Reduction of Weight of Seedling (%) by the A. houstonianum Extracts at Different Concentrations*

| Sample | Control | 10% | 30% | 50% | 70% |
|---|---|---|---|---|---|
| E. caracana | 82.68±5.46 | 74.97±7.26 | 64.74±5.73 | 77.54±4.06 | 96.28±0.91 |
| R. sativus | 93.64±1.21 | 83.62±1.29 | 94.51±0.39 | 89.97±1.90 | 86.54±6.94 |

Note: Values are the Mean ± SD, (n = 3)

The allelopathic effect on two indigenous Chinese species, *Fallopia multiflora* and *Arthraxon lanceolatus*, was assessed using plant growth-promoting (PGP) properties of the bacterial strains obtained from the rhizosphere of *Ageratina adenophora* The findings indicated that while the invasive plant did not affect germination rates, it significantly inhibited seedling growth. Conversely, bacteria growing in the roots of *A. adenophora* demonstrated host-specific growth promotion, with no adverse effects on either native species (Fang et al., 2019). Similar to our study, a group of researchers evaluated the phytotoxic effects of *Amorpha fruticosa* L. on several native crop varieties by measuring parameters such as germination percentage, germination time, germination index, and overall seedling biomass. Their results showed that leaf extracts of this invasive species markedly suppressed the growth of agricultural plants (Krstin et al., 2021). The allelopathic effect of aqueous extracts of various parts of the invasive species *Acmella radicans* (Jacquin) R. K. Jansen on two vegetable crops, *Brassica rapa* and *Chrysanthemum coronarium*, was examined. Results indicated that the inhibitory strength of *A. radicans* extracts followed the order: flower and fruit heads > leaves > stems > roots (Shen et al., 2025).

Similar to our study, *A. houstonianum* exhibited significant reduction in the germination and development of seedlings of *Abelmoschus esculentus* (Bhindi) and *Zea mays* (maize) under controlled conditions. Measurement of seed germination, root and plumule lengths revealed better impact on okra in comparison to maize. The water soluble allelochemicals in the plant were responsible for the inhibitory effects on the growth of the crops (Inala & Amaechi, 2025). The toxic effect was stronger against the seeds of wheat (*Triticum aestivum*) in presence of the leaf extract in comparison to the nearby soil. The plant secondary metabolites including saponins, flavonoids, alkaloids, tannins, and phenols inhibited the actions of the enzymes responsible for seed germination and growth (Budhathoki et al., 2024).

In recent years, there is a growing concern over the invasive alien plant species (IAPS) in Nepal. The rapid spread of these plants and their impact on agro production, biodiversity and ecological sustainability has been assessed by several researchers. The observed allelopathic effects are likely caused by the specific bioactive compounds that interfere with the growth of neighboring plants. Comprehensive studies like GC–MS profiling, assessments of soil health, and evaluation of climate change and its impacts are needed to identify the crucial factors influencing crop output and biodiversity.

A wide range of water-soluble plant secondary metabolites existing in leaves, stems, roots, seeds, and other tissues show strong allelopathic activity. These compounds can be assimilated for their herbicidal potential to suppress weed germination and growth of cultivars. For instance, phenolic acids, sorgoleone, and aldehyde derivatives released from their aqueous extract of *Sorghum bicolor* are toxic to several weed species, including *Rumex dentatus* L. (toothed dock), canary grass, lamb's quarters, and *Convolvulus arvensis* L. (bindweed) (Khamare et al., 2022). Although complete replacement of chemical weed control is challenging, the application of an integrated weed management methods offer promising scenarios. Allelochemicals derived from alien invasive and other plants can be incorporated with existing weed management practices to curtail herbicide use, lower production costs, and support the formulation of operational strategies for controlling invasive species. This study opens up the avenues for further comprehensive research to connect allelopathy as a sustainable means for weed control in organic farming systems in Nepal.

## Conclusion

This study highlights the impact of the aqueous extract of one of the most common invasive alien plant on two crop species. The leaves of the plant contained different phytochemicals including alkaloids, flavonoids, reducing sugars and phenolics but exhibited no significant antibacterial activity. The extracts showed negative impacts on the germination of both of the plants on a dose dependent manner. For *E. caracana*, the extract promoted seedling growth at lower concentrations but inhibited it at higher doses. In contrast, *R. sativus* exhibited a significant reduction in growth across the treatments. Upcoming research should emphasize more comprehensive investigations incorporating additional physiological and biochemical parameters, along with detailed analyses of soil chemistry. Greater attention should also be given to assessing the specific impacts of invasive species on particular crop systems. Such efforts will support the formulation of effective management strategies for the conservation of biodiversity, refining agricultural productivity, and sustaining ecological balance.

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**Availability of Data and Materials**

Data are safely stored. They will be made available in special request.

**Conflict of Interest**

The authors declare that there is no conflict of interest in relation to this manuscript.

**Ethical Compliance**

“Not applicable.”

**Consent for Publication**

"Not applicable"

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