EFFECTS OF INVASIVE *Parthenium hysterophorus* LEACHATES ON SEED GERMINATION AND SEEDLING GROWTH OF WHEAT (*Triticum aestivum*)

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ABSTRACT

Parthenium hysterophorus is a noxious invasive alien weed distributed in agroecosystems. This weed shows phytotoxic properties on other plants in the invaded areas. This study aims to evaluate the phytotoxic effects of this weed on seed germination, seedling growth and development of wheat (*Triticum aestivum*) with petri plate and pot experiments. Seeds and seedlings of wheat were treated with *P. hysterophorus* leaf leachates extracted using more natural way i.e., by soaking the leaves in water rather crushing or powdering. Results show that the leachates of *P. hysterophorus* exhibits negative effects on seed germination and growth parameters of wheat in petri plate experiment. The phytotoxic effects of leachates on root-shoot length and shoot biomass was not were not significant in the pot experiment. Therefore, actual concentration of leachates that start hindrance to the recipient plants varies between the petri plate and pot bioassays. Further studies to understand the leachate interaction with soil and soil microbes are recommended.

Keywords: invasion, allelopathic effects, leachates, growth parameters

INTRODUCTION

Parthenium hysterophorus L. (Asteraceae), natively found in north to south Americas, Mexico and West Indies (Adkins & Shabbir, 2014; Bajwa *et al.,* 2016), is an annual herbaceous plant. It has become a noxious invasive weed worldwide. The weed is threatening natural and agroecosystems as it brings changes in soil quality and affects biodiversity of natural habitats and crops negatively in the invaded regions (Adkins & Shabbir, 2014; Lalita, 2018; Mao *et al.,* 2021).

In Nepal, *P. hysterophorus* was introduced from India during the 1960s and it has been spreading aggressively throughout the country (Tarai, Siwalik and Middle mountains) for two or two and half decades (Tiwari *et al.,* 2005; Shrestha *et al.,* 2019). Its distribution is found along road sides, fallow, agricultural, shrub and forest lands (Tiwari *et al.,* 2005; Shrestha *et al.,* 2015; Thapa *et al.* 2015). According to Shrestha *et al.* (2019), the occurrence of this weed is the highest in the fallow-grazing lands followed by the agriculture lands. Its introduction in crop fields and its negative impacts on soil and crop plants are the serious issues. Several studies have reported that the weed has reduced crop yield severely (Safdar *et al.,* 2015; Bajwa *et al.*, 2019, 2020).

The weed competes with other plants in the invaded regions. One of the major ways of competition is that this weed can produce a number of allelochemicals from underground and aerial parts which are phytotoxic effects to neighboring plants (Batish *et al.,* 2005; Singh *et al.,* 2005; Khaliq *et al.,* 2015). The phytotoxic effects include inhibition to seed germination and growth including retardation in morphological/ physiological activities of recipient plants by the allelochemicals (Tefera, 2002; Pandey, 2009; Hassan *et al.*, 2018).

Many of the studies on phytotoxicity tests use extracts obtained either from crushed fresh leaves or from powder of dried leaves of *P. hysterophorus* (Hassan *et al.,* 2018; Kapoor *et al.,* 2019; Thapa *et al.* 2020; Darji *et al.* 2021) which may be disparate to the natural conditions. There are following potential mechanisms to release allelochemicals in natural condition (de Albuquerque *et al.,* 2011) i.e. (i) rainwater washes the allelochemicals from aerial parts (ii) roots releases allelochemicals as exudates (iii) litters in soil adds allelochemicals either through leaching or decomposition. Therefore, just soaking the parts of donor plants in water to extract water soluble components could be the reliable method to know the effects on test plants. Hence, this study aims to analyze the effects of leachates obtained through soaking the leaves of *P. hysterophorus* in water on one of the most important cereal crops - wheat (*Triticum aestivum* L.) using both the petri-plate and pot bioassays.

MATERIALS AND METHODS

Collection of *P. hysterophorus* leaves and seeds of *T. aestivum*

The leaves of *Parthenium hysterophorous* were collected from the surroundings of invaded Sallaghari area, Bhaktapur, Nepal (27.6733° N, 85.4113° E; elevation 1318masl). The seeds of wheat (*T. aestivum*) were collected from Nepal Agricultural Research Council (NARC), Lalitpur, Nepal. The seeds were stored at 4°C in refrigerator until use. The seeds were collected in December and leaves were collected in January 2021.

Leachate preparation

Fresh and matured leaves of *P. hysterophorous* were collected and washed by tap water and distilled water (DW) to remove the dust particles attached on surface. The washed leaves (100 g) were soaked in 1 liter of DW for 24 hours and the leachate obtained was filtered through muslin cloth which was the stock leachate. This concentration of the leachate was considered as 100%. Then, the leachate was diluted to make the concentrations of 25% and 50%.

Petri plate experiment

The seeds of wheat were washed with DW and surface sterilized by using 70% ethyl alcohol. The sterilized seeds after washing again with DW several times were arranged on double lined moist filter paper in sterilized petri plates. A total of 10 seeds of wheat were uniformly scattered in each petri plate. There were following treatment plates (i) control (ii) leachates of *P. hysterophorus* (25%, 50% and 100%). The filter paper of control plates was moistened by DW and the filter papers of leachate treatments were moistened by respective concentrations of *P. hysterophorus* leachates.

There were 5 replicated plates for each treatment (5 plates × 4 treatments = 20 plates). The petri plates were then incubated at 30°C (dark) for 7 days. The filter paper in the petri plates were kept moist during the incubation period by adding respective leachates and DW (in control) at an interval of 2 days. The seeds germinated (seeds emerging visible radical) 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, lengths of seedlings (root and shoot separately) were measured. After that, the roots and shoots were wrapped in paper and dried in hot air oven at 80°C for 24 hours and then dry weight (biomass) was measured.

Pot experiment

In addition to the petri plate experiment, pot experiment was also designed. The clay pots of size 15 cm height and 15 cm diameter were taken and filled with soil collected from previously wheat cultivated land. Each pot contained ca. 1.3 Kg soil. Open-air experiment was conducted in the Department of Environmental Science, Khwopa College, Bhaktapur, Nepal in January 2021.

A total of 20 seeds of wheat were sown (1 cm below soil surface at equidistance) in the pots containing soil. Distilled water in control pots and respective leachates (25%, 50% and 100%) in leachate treatment pots were poured (equal volume of water and leachate i.e., 100 mL) in each pot in 2 days interval. After seedling emergence, the seedlings were thinned to maintain 10 plants per pot. There were 5 replicated pots for each treatment as mentioned in the petri plate experiment. The seedlings of wheat were grown for 40 days, thereafter plants were harvested.

During harvest, the plants were removed carefully from the pots by moistening the soil with enough water without destroying root system. The aerial parts and roots were washed and length (root and shoot separately) were measured using centimeter scale. Number of leaves and leaf size (length and breadth) was also measured. The harvested plants were dried in hot air oven and then dry weight (biomass) was taken as discussed in the petri plate experiment.

Statistical analysis

Differences in the percentage of seed germination, root and shoot heights (both petri plate and pot experiments) and the leaf size and number (pot experiment) among the treatments (control and three concentrations of *P. hysterophorus* leachate) were compared using One-Way Analysis of Variance (ANOVA). The shoot length data of pot experiment was analyzed using the Kruskal-Wallis test as the data was not normal. The software SPSS (Statistical Package for Social Sciences, Version 26) was used for data analysis.

RESULTS AND DISCUSSION

Seed germination

Seed germination percent of wheat was calculated in the petri plate experiment. The percentage of seed germination was high in the control plates (L-0) i.e., 88.33±4.56% followed by the 25% leachate (L-25; 87.33±3.65%) and 50% leachate (L-50; 80.33±4.47%) of *P. hysterophorus* (Fig. 1). The differences in the percent of seed germination were not significant among the control and leachate concentrations of 25% and 50% but the percent was significantly reduced by the leachate concentration of 100% (L-100) ($p = 0.010$, Fig. 1).

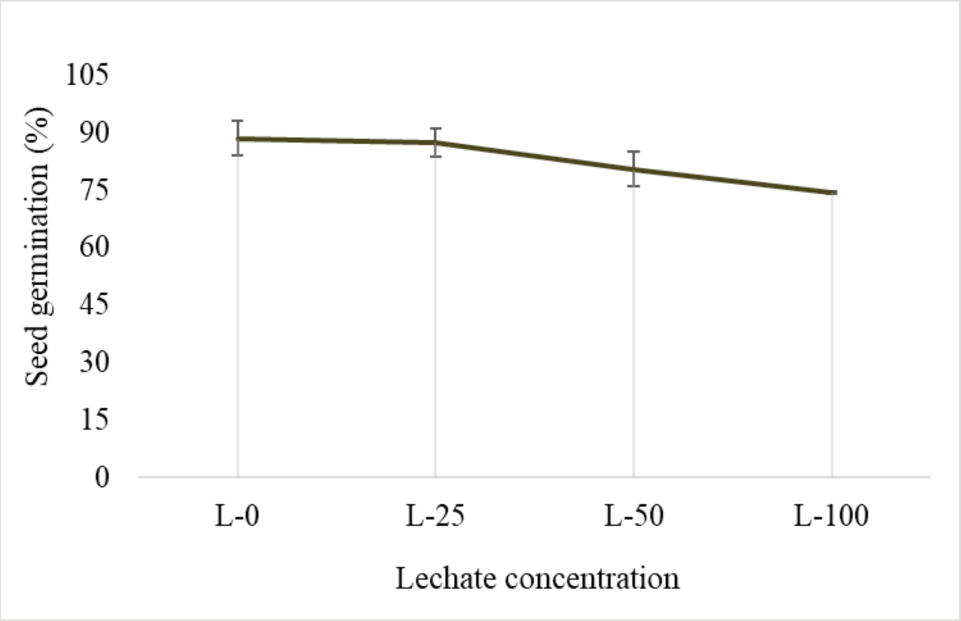

Fig. 1. Percentage of seed germination in wheat under treatments of *P. hysterophorus* leachates. Different letters above the error bars indicate significant differences ($p < 0.05$). Control (L-0), leachate 25% (L-25), leachate 50% (L-50) and leachate 100% (L-100).

Shoot and root lengths

Average shoot and root lengths of the seedlings of wheat grown in petri plates showed significant variation among the treatments. The shoot length was inhibited significantly by the leachates 50% (L-50) and 100% (L-100) ($p = 0.001$). The length of shoot was 5±0.46 cm and 4.5±0.59 cm in the control and leachate 25% (L-25), respectively whereas the length in L-50 and L-100 was < 2 cm (Fig. 2A). Similarly, the roots were shorter in L-100 (< 2 cm) comparing to other treatments (2.5 cm to 3.2 cm) ($p < 0.001$, Fig. 2A).

In case of pot experiment, significant inhibition in shoot and root by the leachates of *P. hysterophorus* was not found ($p > 0.05$) although both the roots and shoots were comparatively shorter than the control treatment (Fig. 2B). The length of shoot in the leachate treatments were 12 cm to 14 cm (per plant) whereas the length of shoots in the control pot was 16.27 cm. Likewise, the root in the control pot was about 9 cm long which gradually decreased with increasing concentrations of leachates (Fig. 2B).

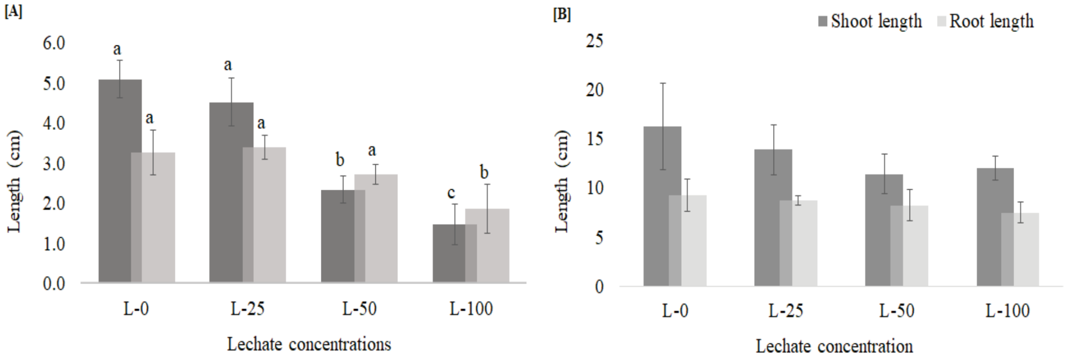

Fig. 2. Shoot and root lengths of wheat seedlings under treatments of *P. hysterophorus* leachates [A] petri plate experiment [B] pot experiment. Different letters above the error bars indicate significant differences ($p < 0.05$). Control (L-0), leachate 25% (L-25), leachate 50% (L-50) and leachate 100% (L-100).

Shoot and root biomass

In the petri plates, the biomass of both shoots and roots was reduced by high concentrations of *P. hysterophorus* leachates (L-50 and L-100) ($p = 0.007$ in shoot and $p < 0.001$ in root, Fig. 3A). The biomass accumulation in the roots of control plants (L-0) was 50% greater than the roots of L-50 plants and 4 times greater than the L-100 plants (Fig. 3A). Similarly, the difference in shoot biomass between control and L-50 plants was 22 g and the difference with L-100 plants was 38 g (Fig. 3A).

In the pot experiment, there was no significant variation in shoot biomass of wheat seedlings among different treatments, although higher concentrations of leachates (L-50 and L-100) showed inhibitory effects on the biomass (*p* = 196, Fig. 3B). Interestingly, roots of the seedlings were found more sensitive than shoots because the root biomass was significantly reduced by L-100 (*p* = 0.04, Fig. 3B). The difference in root biomass between control and L-100 plants was 8 g (Fig. 3B).

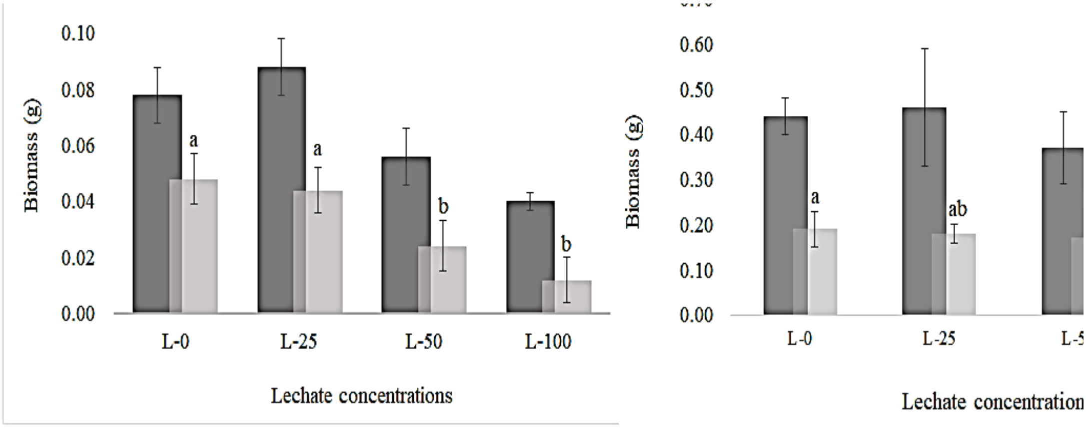

Fig. 3. Shoot and root biomass of wheat seedlings under treatments of *P. hysterophorus* leachates [A] petri plate experiment [B] pot experiment. Different letters above the error bars indicate significant differences (*p* < 0.05). The shoot biomass data of petri plate experiment was analyzed using Kruskal-Wallis test [A]. Control (L-0), leachate 25% (L-25), leachate 50% (L-50) and leachate 100% (L-100).

Leaf number and size

The number of leaves were counted and leaf length and breadth were measured in the plants grown in the pots under different treatments. There were no significant variations among different concentrations of *P. hysterophorus* leachates and control treatments in these leaf parameters (data not shown). The number of leaves per plant was 3 to 4. Average leaf length in the control plants (8.37±1.51 cm) was greater than the leaves in plants grown under treatment of leachates. The leaf length ranges from 6.10±0.87 cm to 6.54±1.07 cm in the plants of leachate treatment showing inhibitory effects on leaves also.

P. hysterophorus leaf leachate is phytotoxic to wheat

In this study, *P. hysterophorus* leaves were soaked in water to extract the leachate which is more natural than crushing fresh leaves or powdering dry leaves to obtain the extract. This study has not screened allelochemicals present in *P. hysterophorus* leachates as some of the previous studies (Pandey, 2009). The study demonstrated that the leachates of *P. hysterophorus* exhibit negative effects on seed germination and growth parameters of wheat. As shown in Fig. 1, the percentage of seed germination was high in the control and gradually reduced on increasing the concentration of leachates but significant reduction in germination was found in the leachate 100%.

Also, growth parameters (length and biomass) of the wheat seedlings in petri plates showed significant variation among the treatments. High concentrations of leachates were found inhibitory to both lengths and biomass of shoot and root of wheat seedlings (Fig. 2A, 3A). Greater negative impacts are shown in the biomass than the seedling length as the biomass accumulation in the roots of control plants was 50% to 4 times greater than the biomass in the roots of high concentrations of *P. leachates* (Fig. 3A). The results from the present study are consistent with previous studies such as Maharjan *et al.* (2007) and Afridi & Khan (2015). In general, negative effects of *P. hysterophorus* on seed germination and seedling growth of wheat are evident. Therefore, wheat plants are affected negatively when cultivation is done in *P. hysterophorus* infested fields or by infestation of *P. hysterophorus* in wheat cultivated lands.

Leachate interaction with soil minimizes phytotoxicity

The case of pot experiment was different. Significant reduction in seedling length and shoot biomass of wheat by the *P. hysterophorus* leachates was not found although the seedlings were comparatively shorter in the leachate treatments than the control plants (Fig. 2B, 3B). It is noticeable that the negative impacts of the *P. hysterophorus* leachates disappeared in the pot experiment while the effect was evident in the petri plate experiment. From this, it can be expected that the leachates poured in soil may be diluted and the efficacy of leachate toxicity is minimized. Hence, the actual concentration of leachates that start interference to the recipient plants vary between the petri plate and pot bioassays. However, continuous addition of leachates increases the allelochemicals in soil and may alter soil quality which could be harmful for recipient plants (Batish *et al.,* 2002; Belz *et al.,* 2009). Specially, addition of phenolics from *Parthenium* have adverse impacts on soil chemistry which is then responsible to decrease the growth of crop plants (Batish *et al.,* 2002; Li *et al.,* 2010).

In the petri plates, the seedlings were in contact with pure leachates. In the pot experiment, the leachates were poured in soil, the pots were air exposed and natural soil was used. Soil harbors numerous microorganisms as the soil was not sterilized. The microbes may transform allelochemicals with modified biological properties which affect the overall allelopathic competence of the donor plant (Jilani *et al.*, 2008). Therefore, studies on allelochemical interactions with soil and soil microbes to evaluate the level of toxicity between crude form and in soil could be interesting for enhancing knowledge of allelopathy. Such studies will have significance on developing strategies of invasive plant infested soil management with application of microbes.

This study tested allelopathic effect *P. hysterophorus* on a common cereal crop wheat (*T. aestivum*). Soaking the leaves of *P. hysterophorus* in water was a simple method to obtain leachate and performed both petri plate and pot assays to know the toxicity of the leachate on seed germination and seedling growth of wheat. The results show that the *P. hysterophorus* leaf leachate is phytotoxic to wheat. Therefore, actual concentration of leachates that start hindrance to the recipient plants varies between the petri plate and pot bioassays. Further studies to understand the leachate interaction with soil and soil microbes are recommended.

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