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Research Article

EFFECT OF INVASIVE *AGERATINA ADENOPHORA* ON SEED GERMINATION, SEEDLING GROWTH AND DEVELOPMENT OF RICE (*Oryza sativa* L.)

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ABSTRACT. *Ageratina adenophora* (Crofton weed), commonly called the forest killer plant, is the worst invasive weed. This plant is known to harm other plants including native wild species and crops in the invaded range by releasing allelochemicals. In this study, the effects of fresh leaf leachate and dry leaf extract of *A. adenophora* have been tested on the growth and development of rice in Nepal. Fresh leaf leachate was obtained by soaking fresh leaves in water and dry leaves were soaked in water and the filtrate was used as a dry leaf extract. Seeds of rice (*Khumal-11*) were grown in petri dish under treatment of the fresh leaf leachate and dry leaf extract. It was found that both the fresh leaves and dried leaves of *A. adenophora* were toxic to seed germination and seedling growth of rice. Comparing the fresh leaf leachate, the dry leaf extract was more toxic for root shoot growth and root number of rice. These results have questioned the practice of local people who use *A. adenophora* as fertilizer in the rice field. However further studies and field-level trials/tests are required to confirm our findings.

Keywords: *Crofton weed, leaf leachate, litter, allelochemicals, toxicity.*

INTRODUCTION

Introduction and spread of alien plant species have negatively impacted the soil nutrient dynamics, native biodiversity, growth, and development of native and crop plants in natural and agroecosystems [1, 2, 3, 4]. *Ageratina adenophora* (Crofton weed), native to Mexico, is one of the worst invasive weeds worldwide including South Asian countries [5, 6, 7]. This plant is commonly called ‘Forest Killer’ and ‘Banmara’ in Nepal. It has been observed that this weed is one of the densely colonizing invaders around the crop fields, near roadsides, fallow lands, and forest margins in Nepal. This species can produce a large number of small seeds with a high germination rate which has enabled this species to spread as a serious weed throughout the world and established in various types of ecosystems [8, 9, 10].

Rapid and severe colonization of *A. adenophora* throughout Nepal has caused several ecological problems such as changing plant community composition, degrading soil quality, replacing native species [7, 11, 12], thereby threatening the agroecosystems and staple crops. This plant can produce several allelochemicals which are harmful to the growth and development of native species and crop plants [13, 14, 15]. It grows luxuriantly in the rainy season and therefore, its allelochemicals are washed in a huge amount by rain and mix into soil which may have negative impacts on other plants growing in the invaded soils [15, 16, 17].

As the *A. adenophora* grows near crop fields it may release leachates through rainwater which can be mixed into the paddy field. Previous studies have shown that there are toxic effects of *A. adenophora* on the growth and development of native species [15, 16, 17, 18] and crop plants [19], however, it has been observed that some rural farmers in Nepal have applied *A. adenophora* in the crop field as green manure. People's perception is that *A. adenophora* can improve soil quality and crop productivity. Therefore, scientific studies are needed to validate such practices. In this regard, this study has been carried out to know the effects of *A. adenophora* leaves on the growth and development of rice in Nepal.

MATERIALS AND METHODS

Sample collection

Fresh leaves of *A. adenophora* were collected from the Swyambhu area, Kathmandu, Nepal in July 2016. The leaves were shade dried at room temperature for four days. Seeds of rice variety *Khumal-11*, which is very common to Nepalese farmers, were collected from Nepal Agriculture Research Council (NARC), Khumaltar, Lalitpur, Nepal.

Preparation of Ageratina fresh leaf leachate and dry leaf extract

Ten grams of each fresh leaves and dry leaves were soaked separately in distilled water (DW) for 72 hours at room temperature. The leaves were soaked in 100 ml DW in a jar. Then, the leaves were removed out from the jar and the water that remained in the jar was used as the leaf leachate. Similarly, dry leaves were soaked in 500 ml DW, the extract was filtered with the muslin cloth and the filtrate was used as the dry leaf extract. The stock solution of both fresh and dry leaves was considered as 100% (fresh leaves = 10 g/100 ml. DW and dry leaves = 10 g/500 ml DW). The filtrate was diluted into 50% and 25% using distilled water.

Treatment of rice seeds and measuring parameters

The experiment was performed in the laboratory of Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu, Nepal. During the experiment, three different treatments were executed (i) *A. adenophora* fresh leaf leachate, (ii) *A. adenophora* dry leaf extract, and (iii) distilled water as the control. Within each leaf type, there were three sub-treatments viz. 100%, 50%, and 25 % concentrations.

The rice seeds were washed severally with distilled water and double-layered filter paper soaked in distilled water was set into petri dish. Twenty milliliters of *A. adenophora* each fresh leaf leachate, dry leaf extract, and distilled water were poured on the respective petri dishes. Ten seeds were arranged over the moist filter paper in each petri dish and incubated at room temperature (25±5°C day temperature) for seedling development. Each treatment had five replications.

The rate and percent of seed germination were calculated. Length of root and shoot of the seedlings were measured separately after a week of incubation. Both the roots and shoots were dried in a hot air oven at 70°C for 72 hours and the weight of dry biomass was taken.

Statistical analysis

Data were subjected to one-way ANOVA to test treatment effects on root and shoot length, plant biomass, and root number. All statistical analyses were performed using software R version 3.6.3 (R Core Team, 2019) [20] with a significant level $p < 0.05$.

RESULTS AND DISCUSSION

Effect of fresh leaf leachates and dry leaf extract on seed germination

The study revealed that the high concentration of *A. adenophora* fresh leaf leachate (i.e. 100%) inhibited rice seed germination. However, other concentrations (25%and 50%) did not inhibit the germination of seeds significantly (Fig. 1). The percentage of seed germination was significantly lower in all the concentrations of *A. adenophora* dry leaf extract (25%, 50%, and 100%) comparing to the control treatment in Day-1 but the seeds were germinated later in dry leaf extracts and the percentage was not affected (differ) significantly (Fig. 1).

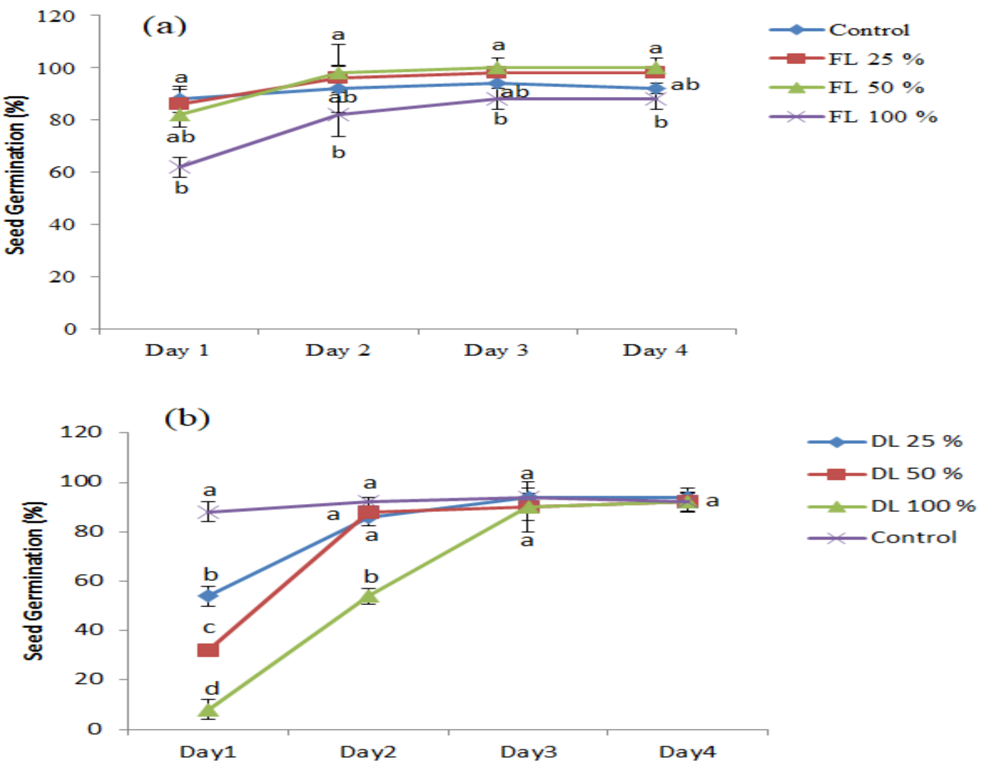

Fig. 1. *Effect of A. adenophora fresh leaf leachate (a) and dry leaf extract (b) on rice seed germination (The letters above error bar show significant difference among the treatment)*

Effect of fresh leaf leachate and dry leaf extract on shoot length

A. adenophora fresh leaf leachate did not show the inhibitory effect on the shoot length of rice seedlings ($P = 0.665$). The shoot length in control was the highest i.e. 5.24±47 cm followed by fresh leaf leachates 25% (4.67±0.26 cm), 100% (4.61±0.40), and 50% (4.49±0.47 cm) (Fig. 2).

Shoot length was reduced by 100% dry leaf extract (3.38±0.40 cm) while other concentrations (25% and 50%) did not show inhibitory effect (4.71±0.25 cm in 25% and 4.02±0.37 in 50%, P = 009) (Fig. 2).

Effect of fresh leaf leachate and dry leaf extract on root length

A. adenophora fresh leaf extracts did not show the inhibitory effect on root length of rice (Fig. 3, P = 0.441). The root was the longest in control i.e. 6.84±0.70 cm and shortest in the treatment of 100% leaf leachate i.e. 5.51±0.39 (Fig. 3). All the concentrations of dry leaf extracts (25%, 50%, and 100%) showed an inhibitory effect on the root length (Fig. 3, P < 0.001). Comparing to the control the root length was more than 2 cm shorter in all the concentrations of dry leaf extracts. The roots were the shortest under the treatment of dry leaf extract i.e. 3.38±0.37 cm. The root length in treatment of 25% and 50 % dry leaf extract were 4.71±0.55 cm and 4.02±0.25 cm, respectively (Fig. 3).

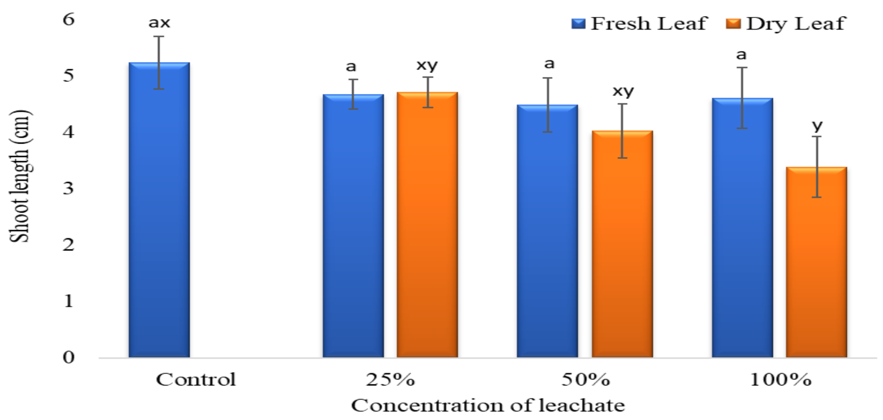

Fig. 2. *Effect of A. adenophora fresh leaf extract on shoot length of rice seedlings (The letters above error bar show significant difference among the treatment)*

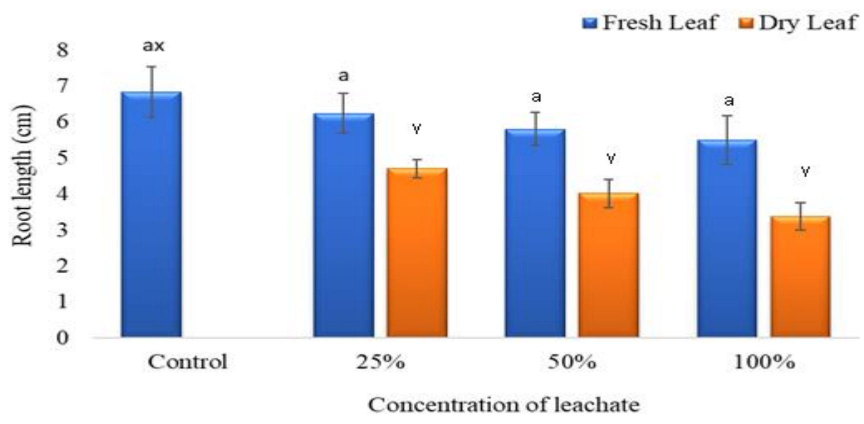

Fig. 3. *Effect of A. adenophora fresh and dry leaf extracts on root length of rice seedlings (The letters above error bar show significant difference among the treatment)*

Effect of fresh leaf leachate and dry leaf extract on seedling biomass

Similar to the effect of fresh leaf leachate of *A. adenophora* on rice shoot and root length there was no inhibitory effect on seedling biomass also (P = 0.691) although the biomass was the least under the treatment of fresh leaf leachate 100% (0.374±0.022 mg). The biomass of the seedlings was 0.380±0.051 mg under treatment of the leaf extract 25% and 0.404±0.014 mg in 50% whereas in the control the biomass was 0.428±0.021 (Fig. 4).

The highest concentration of the *A. adenophora* dry leaf extract had a significant reduction in the rice seedling biomass while the lower concentrations (25% and 50%) did not reduce the biomass (P = 0.006). The biomass in 25% *A. adenophora* dry leaf extract was 0.342±0.031 mg followed by 0.332±0.025 mg in 50% and 0.284±0.020 mg in the extract 100% (Fig. 4).

Effect of fresh leaf leachate and dry leaf extract on root numbers

Similar to the shoot length the treatment of *A. adenophora* fresh leaf extract did not show an inhibitory effect on numbers of roots in rice (P = 0.205) although the highest number of roots per plant was 6 in control and 25% fresh leaf leachate while the number was 5 in average in other concentrations of fresh leaf leachate (Fig 5). High concentration of dry leaf extract (100%) reduced root number but the lower concentrations (25% and 50%) had no effect (P = 0.008). The number of roots in dry leaf extract varies from 4 to 5 per plant (Fig. 5).

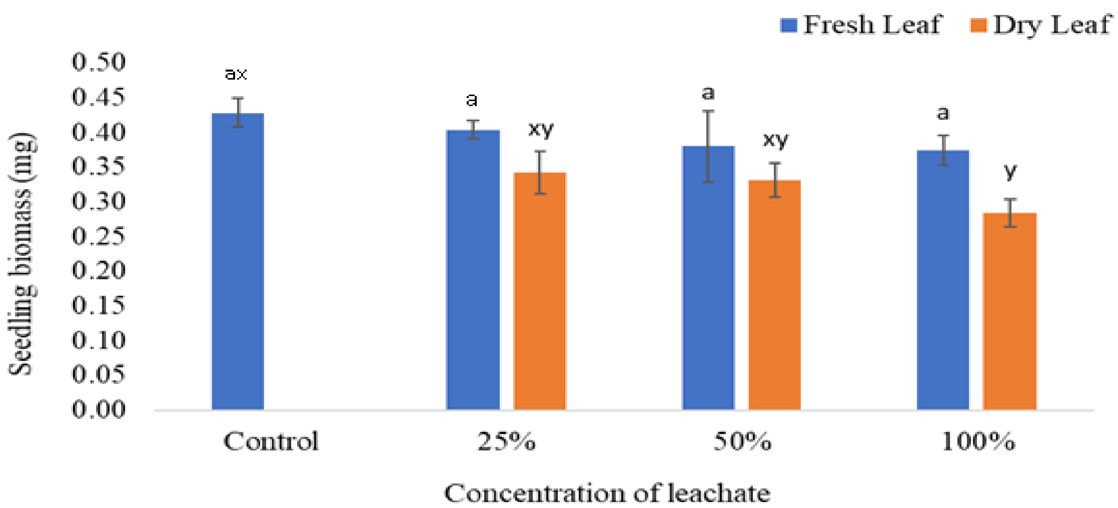

Fig. 4. *Effect of A. adenophora fresh and dry leaf extracts on seedlings biomass (The letters above error bar show significant difference among the treatment)*

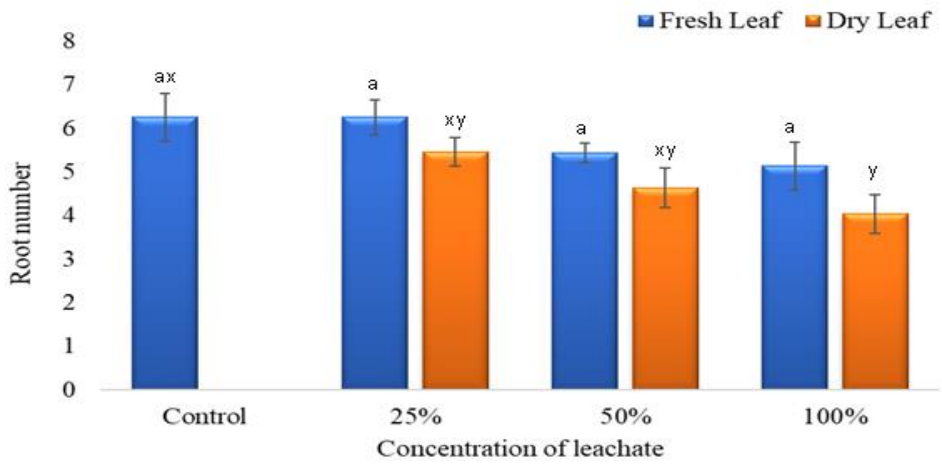

Fig. 5. *Effect of A. adenophora fresh and dry leaf extracts on root numbers (The letters above error bar show significant difference among the treatment)*

The results of this study show that the high concentration of *A. adenophora* fresh leaf leachate inhibits rice seed germination. Comparing to the fresh leaf leachate dry leaf extracts of *A. adenophora* were more toxic to the rice. Our study also showed that the roots of rice seedlings are more sensitive to the *A. adenophora* leaf extracts as their effect is more pronounced in roots than in shoots. As the leaf leachate was obtained by just soaking the fresh leaves of *A. adenophora* not all the allelochemicals might have released out in dissolved form with water and therefore, the toxic effect of fresh leaf leachate on rice seedlings was lesser than the dry leaf extracts. The allelochemicals released from *A. adenophora* leaves in the extract might have inhibited rice seed germination and seedling growth and development.

Similar to our experiment, an experiment was conducted by Khatri et al. (2020) and found the suppressive effect of *A. adenophora* on the germination, fresh weight, dry weight, and other growth parameters of rice such as the relative water content, hypocotyl length, radicle length, total seedling length, and seed vigor. They concluded that the inhibitory effects are high in the rice seedlings on the increasing concentration of the extracts and the leaf extract is more inhibitory than root and dry plant extracts. The results of our study confirmed their findings. The results of Khatri et al. (2020) and this study clearly show that the dry leaves are more phytotoxic to the rice seedlings than the fresh leaves. It might be due to either release of a high amount of allelochemicals from the dry leaves than the fresh leaves or the allelochemicals might be different than the fresh leaves.

Similarly, Yan et al. (2009) tested the effects of *A. adenophora* invaded soil and its methanol extract on the seed germination and seedling growth of upland rice [21]. They observed that both the invaded soil and its extract were toxic to the seed germination and seedling growth of the upland rice and concluded that the allelochemicals released by *A. adenophora* could accumulate in the soil, which might have affected the growth of rice.

Zhang et al. (2012) identified and characterized several allelochemicals from fresh leaf tissues of *A. adenophora* such as α-phellandrene, camphene, ρ-cymene, 2-carene, α-pinene, limonene, and (z)-3-hexen-1-ol as the foliar volatile components [19]. They have found that these components are inhibitory to lateral root formation in rice. The allelochemicals such as limonene, 2-carene, α-pinene, phellandrene and camphene had no phytotoxic effect on shoot growth while (z)-3-hexene-1-ol and ρ-cymene were toxic for both shoot and root elongation. Zhou et al. (2013) had identified other allelochemicals in *A. adenophora* such as 3-(2-hydroxyphenyl)propyl methyl malonate, 3-(2-hydroxyphenyl)-1-propanol, and *o*-coumaric acid which were toxic to *Arabidopsis* seed germination [22].

Fresh leaf leachate of *A. adenophora* had no inhibitory effect on the shoot length of rice while the higher concentration of dry leaf extract (100%) have an inhibitory effect (Fig. 2). Similarly, the fresh leaf extracts had no inhibitory effect on root length while the dry leaf extract had inhibited the root length (Fig. 3). The number of roots was decreased by the dry leaf extract (Fig. 5). Comparing to the shoots, roots were found sensitive to the extracts. This result was in support of Thapa et al. (2017) who tested the effect of *A. adenophora* on native trees *Alnus nepalensis* (Nepali name - Uttis) and *Schima wallichii* (Nepali name - Chilaune) and found that the roots were more sensitive to the *A. adenophora* leaf litters [16].

Zhang et al. (2012) have concluded that the effects of allelochemicals on root growth are severer than on the shoot growth [18] which is supported by our findings. Yang et al. (2011) reported sesquiterpene-derivative compounds as the major putative allelochemicals in aqueous leachates of *A. adenophora* [23]. These compounds were responsible to alter the normal shape and arrangement of root tip cells and intracellular activities (e.g. protein translocation) in upland rice seedlings. The allelochemicals are known to have negative effects on seed germination by altering cell membrane permeability, cell division, and differentiation [24, 25, 26]. Cellular respiration, protein synthesis, gene expression, and hormone synthesis are also impaired by allelochemicals [27, 28].

CONCLUSION

In conclusion, both the fresh leaves and dried leaves of *A. adenophora* are toxic to seed germination and seedling growth of rice. Utilization of *A. adenophora* in rice fields as fertilizer can harm rice growth and development. Rainwater may wash allelochemicals from this invasive plant and rice fields may be contaminated if there is an invasion in and nearby agricultural fields. Hence, rice fields should be protected from the contamination of *A. adenophora* leachates/extracts. Further studies and field level trials /tests are required to confirm our findings.

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