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Research Article

Impact of Invasive *Ageratina adenophora* on Soil Fungi in Native Plant-Grown Soils in Nepal

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Abstract

Invasion of alien plants impacts both the above- and below-ground communities. The specific response of soil microbes to alien plants may depend upon the presence of native inhabitants and the origin of the byproducts released from different parts of invasive plants. This study evaluated the response of common soil fungi against *Ageratina adenophora* byproducts (extracts) in the presence of two native shrubs, *Elsholtzia blanda* and *Osbeckia stellata*, in Nepal. A total of eight fungal species were isolated from soils where these two native species were grown separately, and the occurrence of the fungi was evaluated. The occurrence and frequency of fungus species varied with extracts of *A. adenophora* leaves, litter and roots as well as with the presence of specific native plants. Particularly, *A. adenophora* further inhibits the fungi that are naturally less frequent in soil, like *Hormodendrum* sp. and fresh leaves and litter of *A. adenophora* were responsible for inhibiting antagonistic fungi like *Trichoderma harzianum.* Influence in below-ground fungal communities by *A. adenophora* is one of the reasons for poor growth and development of native seedlings and the mechanism could be a strategy of invasion of *A. adenohora* in novel areas.

Keywords: Antagonists, Frequency, Invasive plant, Native shrubs, Soil fungi

Introduction

Ageratina adenophora (Spreng.) R.King. & H.Rob., one of the invasive alien species native to Mexico and Central America, has been extensively spread all over the world, including Nepal (Wang & Wang, 2006; Cronk & Fuller, 2014; Shrestha, 2016). Its priority areas of invasion are roadsides, fallow and degraded lands, disturbed forests and agro-ecosystems from tropical to sub-tropical regions (Tiwari et al., 2005; Thapa et al., 2016a). Most of the studies on *A. adenophora* are confined to its ecology, distribution and impacts on native vegetation (Tererai & Wood, 2014; Fu et al., 2018; Wu et al., 2020). Negative impacts of the weed, such as altering native plants' species composition and inhibition of seed germination and growth of native plants as well as crops, have been reported by several previous studies (Thapa et al., 2020b; Darji et al., 2021; Khatri et al., 2023). Besides the impacts

of *A. adenophora* on above-ground vegetation, some of the work has been concentrated to impact on the below-ground communities. For example, Niu et al. (2007) found that the weed is responsible for increasing the abundance of soil vesicular-arbuscular mycorrhizal fungi. Similarly, the modification of soil microbial communities and functions by *A. adenophora* is expected as one of the mechanisms for successful invasion in novel areas (Bo et al., 2014; Balami & Thapa, 2017; Xia et al., 2021).

Soil microbes, especially pathogenic and symbiotic microbes, will have a strong influence on above-ground plant communities and ecosystem functioning, and alien plant invasion can have major effects on these microbial activities in soil (Van der Putten et al., 2007). Invasive plant, *A. adenophora* can have a range of impacts on microbial communities, as explored previously (Niu et al., 2007; Kong et al., 2017), but specific interactions between invasive species and particular microbes could be a complex phenomenon. Moreover, the interaction mechanisms between soil microbes and invasive species might depend on the presence of particular native species.

Ageratina adenophora has been invading degraded forests where *Elsholtzia blanda* (Benth.) Benth. and *Osbeckia stellata* Buch.-Ham. ex D.Don. are the frequent native shrub species in the Chitlang area of Makwanpur district, Nepal. Darji et al. (2021) found that these two native species were negatively affected by *A. adenphora.* Allelopathic inhibition of *A. adenophora* has been observed as one of the causes of the diminished growth and development of natives and on the other hand, the soil microbes might have been impacted by *A. adenophora* as well. Hence, this study aims to evaluate the response of common soil fungi to invasive *A. adenophora* in the presence of native shrubs *E. blanda* and *O. stellata*.

Materials and Methods

Pot experiment

Seeds of the native plant species, *Elsholtzia blanda* and *Osbeckia stellata,* growing in Takhtar Community Forest (27°24′59.99″N and 85°01′60.00″E) nearby Chitlang village, Makwanpur district, Nepal were collected. The seeds were spread on moist filter paper to allow germination in the dark at a temperature of 25±5°C. Polyethene pots were filled with homogeneously mixed garden soil, then soil in each pot was saturated by distilled water. The native plant seedlings of homogeneous size were transplanted into the pots. Each pot had six seedlings of each native plant.

Extraction of plant materials and treatment of plants

Extracts from the leaves and roots of *Ageratina adenophora* were prepared by soaking 10 g (leaves and roots separately) per 100 ml of distilled water. The seedlings transplanted in the pots were irrigated with respective extracts. In the litter treatment, irrigation was done over the litter spread on the surface of the soil in the litter pots (Darji et al., 2021). The pots were exposed to the following treatments for each of the native seedlings: (i) irrigation by distilled water – control, (ii) irrigation by leaf extract, (iii) irrigation by root extract, and (iv) irrigation with litter extract.

Fungi culture condition

The plants were grown in pots in the glasshouse of the Central Department of Botany, Tribhuvan University, Kathmandu, Nepal. The temperature of the glasshouse varied from 20-38 °C and moisture ranged from 50-88%. After 48 days of seedling transplantation, the plants were harvested and soil from each pot was sampled for fungal assay. A potato dextrose agar (PDA) medium was used for fungal culture. Each soil sample (0.05 g) was mixed thoroughly with 20 ml molten PDA medium in sterile petriplates (Warcup, 1950). Altogether, 72 petriplates were used for each native species (3 plates per pot × 6 pots × 4 treatments). The plates were incubated at 25°C for 7 days. The fungi grown on PDA plates were then isolated and recultured as a pure colony. The fungi from pure culture were observed under a digital microscope and photographs were taken. They were identified based on macro- and micro-morphological characters following Watanabe (2002) and with the help of experts. The frequency of isolated fungi was calculated using the formula:

Frequency = Number of plates where the fungal species present × 100/Total number of plates incubated.

Results and Discussion

Occurrence of fungal species

The most commonly occurring 8 fungal species were isolated from pot soil where the native species *Elsholtzia blanda* and *Osbeckia stellata* were grown. They were *Rhizopus stolonifer* (Ehrenb.) Vuill, *Mucor hiemalis* Wehmer, *Trichoderma harzianum* Rifai, *Penicillium chrysogenum* Thom, *Geotrichum candidum* Link, *Trichophyton* sp., *Hormodendrum* sp. and *Aspergillus flavus* Link. Among them, *M. hiemalis* and *R. stolonifer* belong to the class Mucoromycetes; and the species *Aspergillus flavus, Penicillium chrysogenum* and *Trichophyton* sp. belong to Eurotiomycetes. *Hormodendrum* sp. and *T. harzianum* represented the class Dothiodeomycetes and Sordariomycetes, respectively. Two species *Aspergillus flavus* and *Hormodendrum* sp. were present and *Trichophyton* sp. was absent in the soil where *O. stellata* seedlings were grown (Table 1).

Interestingly, *A. flavus* has appeared and *T. harzianum* disappeared in *O. stellata* soil treated with *Ageratina adenophora* root extract. The species *G. candidum* disappeared in *E. blanda* soil treated with *Ageratina adenophora* litter extract. *Hormodendrum* sp. and *Trichophyton* sp. were found only in the control soil of *O. stellata* and in the *E. blanda* soil treated with root extract, respectively. Another species, *M. hiemalis* was present in both *E. blanda* and *O. stellata* soils except the soils treated with leaf extract. The species present in all the treatments in both the native plant species were *P. chrysogenum* and *R. stolonifer* (Table 2).

Table 1: List of fungal species isolated from soils of native species grown.

S.N.	Name of species	Class	*Elsholtzia blanda*	*Osbeckia stellata*
1	*Aspergillus flavus* Link	Eurotiomycetes	-	+
2	*Geotrichum candidum* Link	Saccharomycetes	+	+
3	*Hormodendrum* sp.	Dothiodeomycetes	-	+
4	*Mucor hiemalis* Wehmer	Mucoromycetes	+	+
5	*Penicillium chrysogenum* Thom	Eurotiomycetes	+	+
6	*Rhizopus stolonifer* (Ehrenb.) Vuill	Mucoromycetes	+	+
7	*Trichoderma harzianum* Rifai	Sordariomycetes	+	+
8	*Trichophyton* sp.	Eurotiomycetes	+	-

'+' means presence; '-' means absence

Table 2: Occurrence of fungal species in soils treated with *Ageratina adenophora* extracts.

S.N.	Name of species	*E. blanda*				*O. stellata*			
		Control	ALE	ALiE	ARE	Control	ALE	ALiE	ARE
1	*A. flavus*	-	-	-	-	-	-	-	+
2	*G. candidum*	+	+	-	+	+	+	+	+
3	*Hormodendrum* sp.	-	-	-	-	+	-	-	-
4	*M. hiemalis*	+	-	+	+	+	-	+	+
5	*P. chrysogenum*	+	+	+	+	+	+	+	+
6	*R. stolonifer*	+	+	+	+	+	+	+	+
7	*T. harzianum*	+	+	+	+	+	+	+	-
8	*Trichophyton* sp.	-	-	-	+	-	-	-	-

ALE: *Ageratina* leaf extrat, AliE: *Ageratina* litter extract, ARE: *Ageratina* root extract; '+' means presence; '-' means absence

Fungal frequency

The frequency of the soil fungi associated with *E. blanda* differed with treatments of *A. adenophora* leaves, litter and root extracts. In the control soil of *E. blanda*, the most frequent fungi were *T. harzianum* and *R. stolonifer* (100%) followed by *M. hiemalis* (60%) and *P. chrysogenum* (53%). The fungus *G. candidum* had the lowest frequency i.e., 13% in the control soil of *E. blanda*. The frequency of all of these fungi found in the control soil of *E. blanda* was reduced in the control soil of *O. stellata* (Figure 1a).

In the treatment of *A. adenophora* leaf litter, the fungus *T. harzianum* and *R. stolonifera* were 15% more frequent while the frequency of *H. hiemalis* was significantly lower in *E. blanda* than *O. stellata*. Two of the fungi *G. candidum* which was not found in *E. blanda* but its frequency was 7% in *O. stellata*. Similarly, *O. stellata* increased the frequency of *P. chrysogenum* (33%) than *E. blanda* (13%) (Figure 1b).

The fungi isolated from the soil of both *E. blanda* and *O. stellata* treated with *A. adenophora* leaf extract were common with variation in occurrence frequency (Table 2, Figure 1c). Comparing the frequencies, *G. candidum*, *T. harzianum* and *P. chrysogenum* showed high and *R. stolonifera* showed low frequencies towards the soil of *E. blanda* than *O. stellata* (Table 2, Figure 1c).

Among the seven fungal species, *Trichophyton* sp. and *T. harzianum* showed 13% and 20% occurrence towards the soil of *E. blanda* which were absent in *O. stellata* treated with *A. adenophora* root extract. The fungus *A. flavus* was nearly 50% frequent in the soil treated with root extract towards *O. stellata* which was absent in *E. blanda*. Two fungal species *P. chrysogenum* and *H. hiemalis* were more frequent in the soil of *O. stellata* than *E. blanda* while the frequency of *R. stolonifera* was just reverse (Figure 1d).

Most of the fungi isolated from the soils untreated and treated with *A. adenophora* leaf, litter and root extracts were common saprophytes. The fungal species *A. flavus* (Figure 2), *G. candidum*, *M. hiemalis* (Figure 2), *P. chrysogenum*, *R. stolonifera* (Figure 2), *T. harzianum* and *Hormodendrum* sp. are among the species associated with decomposing soil nutrients and contributors in the nutrient cycling and facilitate plant growth and development (Robinson, 2014; Nayak et al., 2020).

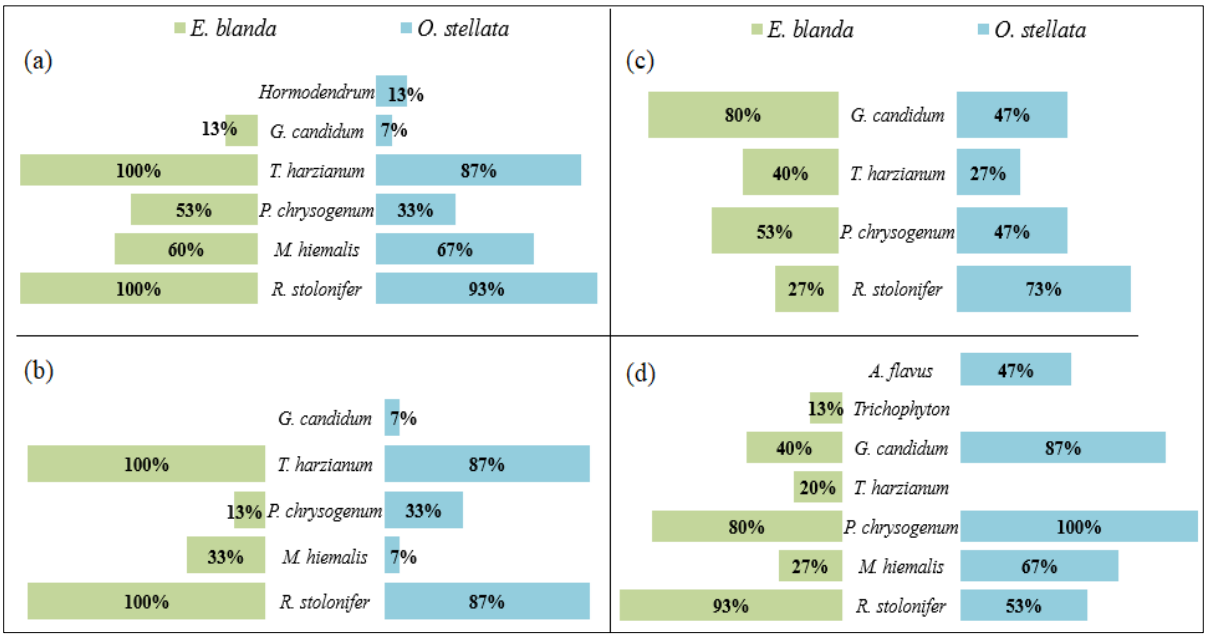

Figure 1: Frequency of fungi in soils of native species *E. blanda* and *O. stellata* treated with (a) normal water-control (b) *A. adenophora* litter extract (c) *A. adenophora* leaf extract (d) *A. adenophora* root extract.

One of the fungi *Trichophyton* sp. isolated from soil treated with *A. adenophora* root extract in *E. blanda* soil is uncommon in soil as many of the species of *Trichophyton* are explained as dermatophytes (de Hoog et al., 2021). Several species of this genus such as *T. terrestre*, *T. verrucosum*, *T. mentagrophytes*

have also been isolated from soils (Papini et al., 1998; Mahmoudabadi & Zarrin, 2008). As the presence of *Trichophyton* sp. only in the potting soil treated with *A. adenophora* root extract in *E. blanda*, it cannot be said that the fungus is prevalent in our soil samples and there was an effect of native plants and root extract of *A. adenophora* particularly on this fungus. Pontes et al. (2013) conclude that dermatophytes like *Trichophyton* may be prevalent in soils of urban areas which are influenced by several non-biological factors including soil organic matter and pH.

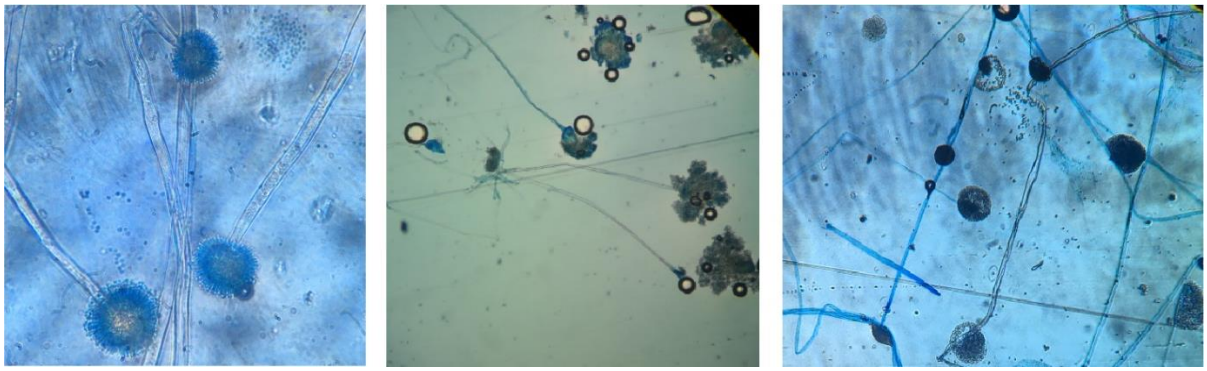

Figure 2: Isolated fungi from native plant species grown pot soil (*A. flavus* (left), *R. stolonifera* (middle) and *M. hiemalis* (right)).

Similar to *Trychophyton*, *A. flavus* appeared only in the soil of *O. stellata* treated with *A. adenophora* root extract with a frequency of 47% (Figure 1). *A. flavus* is a common saprophyte found in soil and sometimes behaves as an opportunistic pathogen of certain plants, mainly the crop plants (Klich, 2007). The fungus did not show its presence in control and all treatments with *A. adenophora* in the soils of *E. blanda* and *O. stellata* (except root extract). Specific interaction of the growing seedlings of native plants and the addition of extracts of invasive *A. adenophora* with the occurrence of this fungus can be expected from the result. Also, the particular components of *A. adenophora* root extract which influenced *A. flavus* to grow with *O. stellata* could be an issue of further studies.

The presence of *Hormodendrum* sp. only in the control soil of *O. stellata* shows its specific association with this native plant. The fungus grows on organically rich soil and is involved in decomposing plant products showing a high rate of cellulolytic activity (Đukić et al., 2018). Even in the control soil, its frequency was relatively lower than other fungi (13%) (Figure 1). The results indicate that *A. adenophora* can have toxic substances in its extract that may be harmful to the less frequent fungi like *Hormodendrum* sp. Also, the less frequent fungi may be influenced by plant-plant and soil interactions. Two of the fungi, *P. chrysogenum* and *R. stolonifer* were the species representing all treatments in soils of both native plants (Table 2). This indicates that these fungi are less sensitive to the extracts from *A. adenophora* but variation in their frequencies in different treatments with the native plants was evident. Figure 1 explains that the frequency of *P. chrysogenum* was high (80 to 100%) in the soils of both native plants which were treated with root extract while the frequency was low (<33%) in the soils treated with litter extract of *A. adenophora*. This indicates that the litter extract of *A. adenophora* inhibited and the root extract promoted the growth of *P. chrysogenum*. Similarly, *R. stolonifer* is less frequent towards *O. stellata* compared to *E. blanda* but its frequency was significantly low (27%) in the *A. adenophora* leaf extract with *E. blanda* (Figure 1). These results indicate that these two fungi show specific response patterns towards extract type from *A. adenophora* with particular native seedlings. A notable result is that *A. adenophora* litter extract is harmful to *P. chrysogenum* and leaf extract is toxic to *R. stolonifer*.

Three of the fungi, *G. candidum*, *T. harzianum* and *M. hiemalis* are extremely common in soil with a worldwide distribution and play a significant role as organic matter decomposers in natural ecosystems (Singh et al., 2016; Botha, 2006; Khalid et al., 2006). The leaf and root extracts of *A. adenophora* also increased the frequency of *G. candidum* (40-80% in *E. blanda* and 47-87% in *O. stellata*, respectively) while it was negligible in control and litter extract-treated soils (Figure 1). In addition, the fungus

disappeared in *E. blanda* soil treated with litter extract (Figure 1). Hence, it can be highlighted that leachates from *A. adenophora* leaves and roots may colonize *G. candidum* in the soils infested by *A. adenophora.*

Just contrary to *G. candidum*, *T. harzianum* showed extensive occurrence in control soils with both native plants (Figure 1a). The addition of extract from *A. adenophora* litter did not reduce the frequency of this fungus with both native species (Figure 1b). However, the occurrence percentage of *T. harzianum* reduced significantly with the addition of extracts from leaves and roots. Moreover, the fungus could not grow in soil with *O. stellata* treated with root extract of *A. adenophora* (Figure 1c, d). Similarly, *M. hiemalis* occurrence was nil in soils treated with *A. adenophora* leaf extract with both of the native plants and the frequency was decreased by root and litter extracts (Figure 1c). This signifies that *T. harzianum* and *M. hiemalis* are highly sensitive to allelochemicals present in live plant materials (leaves and roots) of *A. adenophora.*

Trichoderma harzianum is considered a biological control agent against a wide range of economically important plant pathogens as it may fight pathogens through antibiosis and competition (Mukhopadhyay & Kumar, 2020). Similarly, *M. hiemalis* also suppress the growth of pathogenic fungi like *Thielaviopsis paradoxa* and acts as an entomopathogen for plant pests of Dipteran members (Zhu et al., 2022; Hammia & Bouatrous, 2023). The frequency occurrence of these two fungi in the presence of *A. adenophroa* extracts clearly illustrates that these antagonistic fungi can be diminished in *A. adenophora*-infested soils. It can be anticipated that *A. adenophora* may compete with native species by inhibiting the growth of such antagonistic fungi in natural ecosystems. Fungal antagonists play a crucial role in controlling plant pathogens and diseases (Thambugala et al., 2020). If the population antagonists get diminished, a pathogenic fungal population may increase with an increasing rate of disease incidence of native plant species.

Darji et al. (2022) have shown the presence of hydroxyl compounds, alkynes, amines and C-H stretching (aromatic) or C-O-C stretching (ethers) in extracts of *A. adenophora* leaves, roots and litters which were responsible for reducing growth and development of seedlings of native *O. stellata* and *E. blanda*. The current study has clearly shown that there is a serious alteration in fungal community composition and their frequency occurrence by *A. adenophora*. These changes in fungal communities are another reason for the poor growth and development of native seedlings in *A. adenohora*-invaded soils.

Conclusion

The fungal species *A. flavus, G. candidum, M. hiemalis, P. chrysogenum, R. stolonifer, T. harzianum* and *Hormodendrum* sp. isolated from the soils untreated and treated with *A. adenophora* leaf, litter and root extracts were common saprophytes. *A. flavus* has appeared only in the soil of *O. stellata* treated with *A. adenophora* root extract and therefore, the components of *A. adenophora* root extract influencing *A. flavus*. *A. adenophora* is harmful for the less frequent fungi like *Hormodendrum* sp. indicating they are influenced by plant-plant and soil interactions. The fungi *P. chrysogenum* and *R. stolonifer* show their presence in all types of *A. adenophora* extracts but are less frequent in the litter and leaf extracts indicating they have specific response patterns towards alien invasion with particular native seedlings. Based on the responses of *G. candidum*, *T. harzianum* and *M. hiemalis*, the antagonistic fungi can be diminished in *A. adenophora*-infested soils and it is predicted that *A. adenophora* may compete with native species by inhibiting the growth of such antagonistic fungi in natural ecosystems. Hence, changes in fungal communities are another reason for the poor growth and development of native seedlings in *A. adenohora*-invaded soils. It would be extremely advantageous to employ molecular techniques for identifying fungi and conducting tests on specific allelochemicals for confirming the interactions between alien plants and soil fungi.

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