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COMPARATIVE ANALYSIS OF RHIZOSPHERE FUNGI IN AGERATINA ADENOPHORA AND ASSOCIATED NATIVE SPECIES IN FAR-WESTERN NEPAL

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ABSTRACT

Root exudates play a significant role in influencing the rhizosphere microbes. Fungi are an important group of microbes that are influenced by plant root exudates. As invasive alien plant species secretes several allelochemicals and impact soil microbes, this study compared the fungal community in one of the invasive weeds *Ageratina adenophora* with native species (*Rubus ellipticus, Shorea robusta* and *Imperata cylindrica*) in far-western Nepal. The rhizosphere soil was sampled by uprooting respective plants, and the soils were cultured using Czapek Dox Agar and PDA media. A total of 49 fungal species were identified from the plant rhizospheres. *A. adenophora* altered the species richness, occurrence and frequency of fungi in soil. The pathogenic fungi *Aspergillus niger, Myrothecium* sp., *Phoma* sp., *Rhizoctonia* sp., *Pythium oligandrum, Verticillium* spp. were the most frequently occurring rhizosphere fungi in all plants and they showed their host specificity in the rhzosphere. The fungi species such as *Paecilomyces lilacinus, Aspergillus flavus*, *Myrothecium* sp., *Penicillium citrinum, P. chrysogenum, Rhizoctonia* sp., *Mucor circinelloides, Hypocrea* sp., *Trichoderma hypoxylon, T. sparsum, Gliocladium* sp., *Hypomyces* sp., *Aspergillus niger* and *M. circinelloides* were common in all native plants. Even minor variations in the physicochemical properties of soil can change the presence of fungal species in the root zone, as indicated by the analysis of soil chemicals.

Keywords: soil fungi - culture - plant-soil feedback - pathogenic fungi - allelochemicals

INTRODUCTION

The rhizosphere is that part of the soil ecosystem where plant roots, soil and the soil biota interact with each other benefiting plants by increasing soil fertility and speeding up the breakdown of harmful chemical compounds (Hartmann *et al.* 2008). Exudates from plant roots have a critical role in determining the rhizosphere functions (Toal *et al.* 2000). Sugars, mucilage, organic acids, and amino acids are some of the substances secreted by plant roots and leachates from aerial parts have influence on the rhizosphere communities in the soil (Gupta & Mukerji 2002, Lombardi *et al.* 2018).

Among rhizosphere microbes, rhizosphere-dwelling fungi are biotic inducers that benefit their host plants in a variety of ways. Plants receive either negative or positive feedback from the fungal community (Bonanomi *et al.* 2005). The positive feedback encompasses root fungal mutualism and the secretion of molecules that promote plant growth. The negative feedback, for instance, may involve pathogenic effect, synthesis of bioactive compounds, and formation of allelochemicals (Bias *et al.* 2006). Additionally, the plant-soil feedback system is responsible for the regulation of the nutrient cycle (Johnson *et al.* 1997). The diversity and relative abundance of the above- and below-ground organisms are maintained by such a plant-soil feedback system (Van der Putten *et al.* 1993).

Plant-soil feedback systems are influenced by factors such as competition, stress, and disturbance, and plant traits can impact these systems by altering soil microbes in response to these factors (Beals *et al.* 2020). In this regard, one of the interesting aspects of study is impacts of invasive alien plant species (IAPs) on the plant-soil feedback systems.

There are several studies highliting the changes in above ground community by invasion and the understood fact is that such changes are linked with plant-soil feedback systems (Bohlen 2006, Van der Putten *et al.* 2013, Gioria & Pyšek 2016). Broz *et al.* (2007) concluded that IAPs can have significant effects of IAPs not only on above ground biodiversity but also on the native soil microbial community. Similarly, Zhang *et al.* (2019) showed that invasive plants can increase bacterial biomass through litter pathway and Arbuscular mycorrhizal fungal biomass in rhizosphere region and concluded that litter- and root-based loops might be linked to

generate positive feedback of IAPs on soil systems. Additionally, Wei *et al.* (2021) concludes that the IAPs have only a weak impact on soil fungi and native fungi may adapt to invasive species. Therefore, before generalizing the role of soil microbes such as fungi and their feedback mechanism, it is crucial to identify and analyze their relationship and impact on specific host plant. Hence, this study compares the rhizosphere fungal communities of the invasive species *Ageratina adenophora* with those of selected native species from the far-western region of Nepal.

METHODS AND MATERIALS

Plant species and rhizosphere soil sampling

The invasive plant species selected for the study of rhizosphere fungi was *A. adenophora* (Spreng.) RM, King and H. Robinson (Asteraceae). Three plant species (i) *Imperata cylindrica* (L.) P. Beauv (Poaceae) (ii) *Rubus ellipticus* Sm. (Rosaceae) and (iii) *Shorea robusta* C.F. Gaertn. (Dipterocarpaceae) were the selected native species which are commonly found associated in the *A. adenophora* invaded sites.

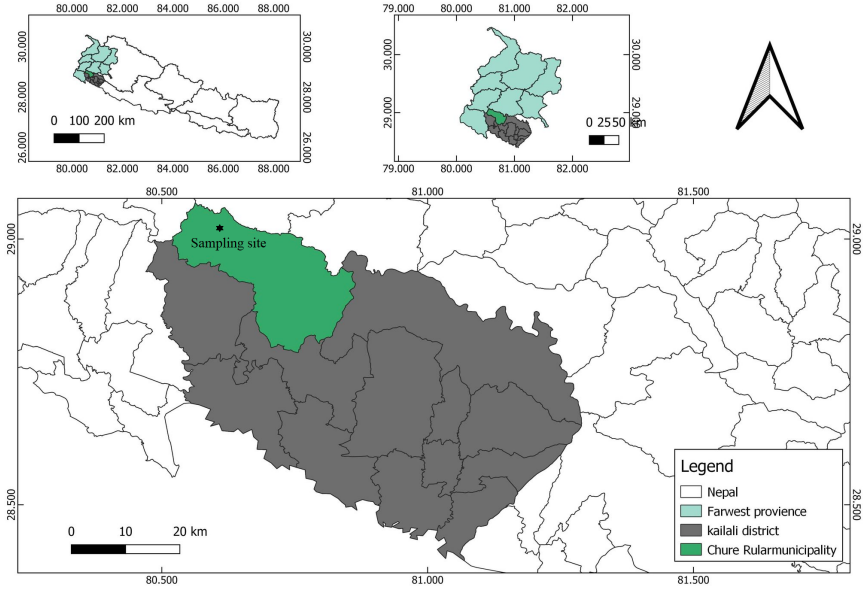

Figure 1: Soil sampling site

The plants were sampled from two community forests of Chure Rural Municipality of Kailali District, Sudurpaschim Province, Nepal named Saalghari Community Forest (SCF) and Jangagran Community

Forest (JCF) located at 28° 55′ to 29°01' N and 80° 34′ to 80° 50′ E (elevation ranges from 1150 to 1450m asl) (Figure 1). The area is characterized by subtropical climate. The mean yearly maximum and minimum temperature of the area is 31.04°C and 19.94°C, respectively. The average annual precipitation is 188.07mm. The associated common species with the native species were *Pinus roxbughii, Myrica esculenta, Phyllanthus emblica, Woodfordia fruticose, Osbeckia stellata* etc.

Rhizospheric soil samples were collected at the month of November 2020. Altogether 10 quadrats of size 1×1 m. square were sampled randomly in each community forest. Three individuals of each native species (seedlings in case of *S. robusta*) and *A. adenophora* from each quadrat were uprooted and the soil around the roots was shaken off for the collection of rhizospheric soil. The soils from 3 individual plants from each quadrat were mixed together to make a composite sample (Balami *et al.* 2017). The soil was collected in a sterile plastic bag and sealed. They were transported in ice box to the laboratory and stored at 4°C in refrigerator until use.

Soil culture, isolation and identification rhizospheric soil fungi

For growing and isolating fungi from the composite soil samples, the serial dilution method Aneja (2003) was used followed by the pour plate technique (Benson 2002). Potato dextrose agar (PDA) and Czapek-Dox agar medium (sterile) was used for culture of rhizosphere fungi. As the antibacterial agent, 30 mg/L Amoxicillin was added in the medium. There were altogether 240 plates (120 PDA and 120 Czapek medium). Each soil sample had three replicates (10 soil samples/plant sp. × 3 replicates = 30 × 2 media = 60 × 4 plants = 240 plates).

The plates were incubated at 25±5°C for 10 days. When colony of suitable size appeared in the culture medium, hyphae were picked and transferred into new medium for pure culture. The fungi grown in the plates were photographed. Identification of the fungi was done on the basis of diagnostic morphological characters (colony morphology) and microscopic characters (vegetative and reproductive characters) with the help of expert consultation and using relevant literatures (Humber 1997; Navi *et al.* 1999; Watanabe 2010). Fungi classification follows *Index Fungorum (http://www.indexfungorum.org/)*.

Measuring parameters

After identification of fungi, following parameters were measured (i) fungal species richness (ii) fungal species composition (iii) fungal

frequency. Fungal species richness is the total number of species of fungi reported in the rhizospheric soil of each plant species. The fungal frequency was calculated as the percentage of species occurrence in total number of plates incubated for each plant rhizosphere soil.

Soil chemical analysis

Soil pH, Soil organic carbon (SOC), soil organic matter (SOM) and total nitrogen (N) were estimated in the composite soil samples. The pH was measured using digital pH meter in 1:2 soil-distilled water mixture. Soil organic carbon (SOC), soil organic matter (SOM) and total nitrogen (N) were estimated following Walkley & Black (1934) and the Kjeldhal method (Pradhan, 1996). The analyses were carried out at the Central Department of Botany and the Central Department of Environment Science, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal.

Statistical analysis

Soil chemical parameters were analyzed using One-way analysis of variance (ANOVA) using the software R (version 4.1.3).

RESULTS AND DISCUSSION

Fungal species richness

Altogether 49 species of rhizospheric soil fungi were isolated from selected native and invasive plant species of two community forests namely JCF and SCF (Annex I). They belong to 9 classes, 14 orders and 28 genera (Table 1). Five fungal species were unidentified.

Table 1: Number of fungal species, genera, order and classes

SN	Class	Order	Genus	Species
1	Agaricomycetes	1	1	1
2	Blastocladiomycetes	1	1	1
3	Dothideomycetes	1	1	1
4	Eurotiomycetes	1	3	8
5	Leotiomycetes	1	1	1
6	Mucoromycetes	1	5	8
7	Peronosporea	2	3	4
8	Saccharomycetes	1	2	2
9	Sordariomycetes	5	11	18
10	Unknown	-	-	5
	Total	14	28	49

Maximum number of fungal species were isolated from rhizospheric soil of invasive *A. adenophora* and native *I. cylindrica* (37 species each) followed by *S. robusta* (32 species) and *R. ellipticus* (28 species) (Table 2). In JCF, fungal species richness was high in *A. adenophora* followed by *I. cylindrica, S. robusta* and *R. ellipticus*. Similarly, in SCF, fungal species richness was high in *A. adenophora* followed by *S. robusta* and *R. ellipticus* and *I. cylindrica* (Table 2).

Table. 2: Fungal species richness in invasive *A. adenophora* and native plant species

Class	*A. adenophora*		*I. cylindrica*		*R. ellipticus*		*S. robusta*	
	JCF	**SCF**	**JCF**	**JCF**	**SCF**	**SCF**	**SCF**	**SCF**
Agaricomycetes	0	1	1	0	1	0	1	1
Blastocladiomycetes	0	1	0	1	0	1	0	1
Dothideomycetes	1	1	1	1	1	1	1	1
Eurotiomycetes	6	5	5	5	4	6	6	6
Leotiomycetes	1	0	0	0	0	0	0	0
Mucoromycetes	4	2	3	3	3	3	5	2
Peronosporea	3	2	3	1	1	2	2	2
Sordariomycetes	15	12	2	0	7	9	9	8
Saccharomycetes	0	0	11	7	0	0	0	0
Unknown	1	0	3	0	0	0	3	1
Total	31	24	29	18	17	22	27	22

The class Sordariomycetes was found to be the richest class in terms of number of species, genera and order in both the forests. In this class, there were 4 orders, 11 genera and 17 species. The second rich class was Eurotiomycetes (2 orders, 4 genera and 8 species) followed by Peronosporea, Mucoromycetes, Dothideomycetes, Agaricomycetes and Blastocladiomycetes (Table 2).

Similarly, the class Sordariomycetes was found to be the richest class in terms of number of species, genera and order in both the forests in *I. cylindrica*. In this class, there were 4 orders, 10 genera and 14 species. The second rich class was Peronosporea (2 orders, 2 genera and 3 species) followed by Eurotiomycetes, Mucoromycetes, Dothideomycetes, Agaricomycetes and Blastocladiomycetes. One species is recognized as an unknown fungal species (Table 2).

Likewise, in *R. ellipticus* the class Sordariomycetes was also found to be the richest class in terms of number of species, genera and order in both the forests. There were 3 orders, 8 genera and 12 species in this class. The second rich class was Eurotiomycetes (2 orders, 4 genera and 6 species) followed by Peronosporea Mucoromycetes, Dothideomycetes, Agaricomycetes and Blastocladiomycetes. Sordariomycetes was the richest class in terms of number of species, genera and order in both the forests in *S. robusta* having 3 orders, 8 genera and 11 species (Table 2). The second richest class was Peronosporea (2 orders, 2 genera and 2 species) followed by Eurotiomycetes, Mucoromycetes, Dothideomycetes, Agaricomycetes and Blastocladiomycetes. One species is recognized as an unknown fungal species (Table 2).

Fungal frequency

Frequency of fungi was classified into <25%, 25-50%, 50-70% and >70%. Table 3 shows the frequency class and the list of fungal species. Based on the frequency, the distribution and occurrence of fungal species vary with forest type even within same plant species. The fungus *A. niger* in JCF and *Myrothecium* sp. in SCF showed the frequency >70% in *A. adenophora* whereas *Phoma* sp. had this level of frequency in *R. ellipticus* only in SCF (Table 3). Frequency of the *Aspergillus niger* and *Myrothecium* (the most frequent species in *A. adenophora* rhizosphere) were lesser in other plant species. Likewise, the *Phoma* sp. was less frequent in the rhizosphere of invasive *A. adenophora* and native *S. robusta* and *I. cylindrica* (Table 3).

Two different fungal species *Rhizoctonia* sp. and *Chaetomium* sp. had the highest frequency (>70%) in JCF and SCF in *S. robusta*, respectively while its frequency was lower in other plants. Similarly, *Pythium oligandrum* and *Verticillium dahlia* were the most frequent (>70%) in *I. cylindrica* in the forests JCF and SCF, respectively and less frequent in other native plants and the invasive *A. adenophora* (Table 3).

Soil chemical parameters

Except soil pH in SCF there were no significant differences in the values of chemical parameters among the rhizospheric soils of invasive *A. adenophora* and native plant species. The soil pH in *I. cylindrica* was high in SCF in comparision to other species (Table 4). Nonetheless, there were variations in the content of SOM, SOC and N among the plant species

within each forest and within same plant between two forests. The values of the parameters are given in Table 4.

There is a close relationship between plant-soil and microbial interactions since both plants and microorganisms depend on soil, and both organisms depend on one another either directly or indirectly. The root of the plant mainly exudes carbon-based chemicals, inorganic acids, oxygen, water etc. and these substances play a role in attracting helpful organisms and establishing mutualistic relationships in the rhizosphere (Bais *et al.* 2006). Rhizosphere is an important region for fungi colonization and soil fungus are the most crucial elements in the underground communities which help in the absorption and uptake of minerals, nutrients, and ions as well as offer essential chemical compounds that promote plant growth (Fazeli-Nasab *et al.* 2022).

Most commonly, the IAPs exudates allelochemicals which are harmful to the soil quality, native plants and microbial communities (Zheng *et al.* 2012, Timilsina *et al.* 2011, Thapa *et al.* 2017, Darji *et al.* 2021). The interactions between IAPs and soil microbes remain a subject of unresolved debate. Some of the studies show that the IAPs accumulates saprophytic fungi, bacteria and mycorrhizal symbionts (Ehrenfeld 2003; Reinhart & Callaway 2006; Zhang *et al.* 2019) whereas others have highlighted that the IAPS are able to accumulate parasitic microbes such as *Alternaria, Fusarium* etc (Balami *et al.* 2017). On the other hand, Wei et al. (2021) concluded that the IAPs have only a weak impact on soil fungi and native fungi may adapt to invasive species. These debates motivate the researcher to explore more on the relationship of various invasive plants and soil microbes to find out exact and specific mechanisms of their interaction.

As the invasive weed *A. adenophora* is highly problematic IAPs (Thapa *et al.* 2020a, 2020b) and its mutual relationship with the soil fungi is debatable, this study compares the fungal component in its rhizosphere with selected native species. These forests were Saal (*S. robusta*) dominant and the native shrub *R. ellipticus* and grass *I. cylindrica* were the common plants across the invaded sites. The study documented only the fungi that appeared in PDA and Czapek Dox Agar media. The results indicated that the fungal species richness and composition are altered by *A. adenophora* in the native range of *S. robusta, R. ellipticus* and *I. cylindrica*.

Table 3: Frequency of invasive A. adenophora and selected native plant species

Fungal Freuency (%)	*A. denophora*		*R. ellipticus*		*S. robusta*		*I. cylindrica*	
	JCF	**SCF**	**JCF**	**SCF**	**JCF**	**SCF**	**JCF**	**SCF**
>70	*Aspergillus niger*	*Myrothecium* sp.	--	*Phoma sp.*	*Rhizoctonia* sp.	*Chaetomium* sp.	*Pythium oligandrum*	*Verticillium dahliae*
50-70	*Fusarium oxysporum* *Gliocladium* sp. *A. kiliense* *A. fumigatus* *Chaetomium* sp. *Cunninghamella* sp. *Myrothecium* sp. *Acremonium byssoides* *Phoma* sp. *P. oligandrum* *Scedosporium* sp. *Trichoderma koningii*	*Phoma* sp. *Trichoderma viride* *Aspergillus flavus* *A. niger* *F. oxysporum* *Fusarium* sp. *Paecilomyces lilacinus* *P. oligandrum* *Verticillium* sp.	*Rhizoctonia sp.* *F. oxysporum* *P. oligandrum*	*P. oligandrum* *Aspergillus fumigatus* *Aspergillus niger* *Myrothecium* sp. *Acremonium byssoides* *Pythium* sp.	*P. oligandrum* *Trichoderma viride* *Verticillium* sp. *Chaetomium* sp.	*P. oligandrum* *Fusarium* sp. *Phoma* sp. *Physoderma* sp. *V. dahliae*	*Scedosporium* sp. *V. dahlia* *Chaetomium* sp. *Penicillium citrinum* *Phoma* sp.	
25-50	*Actinomucor* sp. *Trichoderma viride* *Verticillium* sp. *Unknown 3* *Achyla sp.* *A. flavus* *Hypomyces* sp. *Mucor mucedo* *Trichoderma* sp. *Trichoderma hypoxylon* *Trichoderma sparsum*	*Actinomucor* sp. *Chaetomium* sp. *Penicillium expansum* *Physoderma* sp. *T. koningii*	*Chaetomium* sp. *Mucor indicus* *Phoma* sp. *T. hypoxylon* *A. flavus* *Cunninghamella sp.* *Scedosporium* sp. *T. koningii*	*Fusarium* sp. *P. lilacinus* *Acremonium kiliense* *Chaetomium* sp. *F. oxysporum* *M. mucedo* *P. expansum* *T. koningii* *Trichoderma viride*	*A. flavus* *A. niger* *Cunninghamella* sp. *Phoma* sp. *T. koningii* *T. hypoxylon* *A. kiliense* *A. fumigatus* *Gliocladium* sp. *Lichtheimia corymbifera* *Lichtheimia ramosa* *M. indicus* *P. lilacinus* *V. dahliae*	*M. indicus* *Myrothecium* sp. *P. lilacinus* *P. expansum* *Achyla* sp. *A. niger* *Rhizoctonia* sp. *Scedosporium* sp. *Trichoderma viride* *Unknown 3*	*A. niger* *M. circinelloides* *M. indicus* *P. chrysogenum* *Pythium sp.* *Trichoderma viride* *Unknown 3*	*A. fumigatus* *Chaetomium* sp. *Cunninghamella* sp. *Myrothecium* sp. *T. koningii* *T. viride* *A. flavus* *F. oxysporum* *Phoma* sp. *P. oligandrum*

< 25	*Hypocrea sp.* *P. chrysogenum* *Phytophthora* sp. *V. dahliae* *A. alliaceus* *Botrytis* sp. *M. indicus*	*Achyla sp.* *Acremonium byssoides* *Pestalotiopsis* sp. *T. sparsum* *V. dahliae* *Gliocladium* sp. *Hypomyces* sp. *M. mucedo* *Rhizoctonia* sp.	*Mucor circinelloides* *P. lilacinus* *Trichoderma viride* *V. dahliae* *A. fumigatus*	*A. flavus* *Gliocladium* sp. *Physoderma* sp. *Rhizopus* sp. *M. indicus* *T. sparsum*	*Achyla* sp. *Myrothecium* sp. *Unknown 2* *A. alliaceus* *M. circinelloides* *P. citrinum* *Unknown 3* *Unknown 5*	*Cunninghamella* sp. *P. chrysogenum* *P. citrinum* *T. koningii* *Gliocladium* sp.	*Achyla* sp. *Actinomucor* sp. *A. flavus* *Gliocladium* sp. *Hypocrea* sp. *Hypomyces* sp. *Saccharomyces. cerevisiae* *T. hypoxylon* *T. sparsum* *Verticillium* sp. *Unknown 1* *Unknown 4* *Aspergillus alliaceus* *Geotrichum candidum* *Pestalotiopsis* sp.	*Actinomucor* sp. *A. niger* *P. lilacinus* *P. expansum* *Physoderma* sp. *Gliocladium* sp. *M. indicus*

Table 4: Chemical parameters of rhizospheric soil

	pH		SOM		SOC		N	
	JCF	SCF	JCF	SCF	JCF	SCF	JCF	SCF
S. robusta	6.27±0.03	5.90±0.06b	1.87±0.25	3.38±0.77	0.83±0.11	1.51±0.34	0.04±0.02	0.14±0.02
I. cylindrica	6.13±0.06	6.23±0.03a	1.52±0.15	4.06±0.25	0.68±0.06	1.81±0.11	0.14±0.11	0.28±0.12
R. ellipticus	6.13±0.08	5.87±0.33b	1.88±0.43	3.36±0.62	0.84±0.19	1.51±0.28	0.49±0.09	0.16±0.04
A. adenophora	6.10±0.02	5.83±0.67b	2.12±0.51	4.42±0.26	0.95±0.23	1.97±0.12	0.04±0.02	0.09±0.04

The alphabets 'a' and 'b' indicates significant differences among the plant species ($p<0.05$)

The fungal species richness was high in Ascomycota followed by Mucoromycota and Oomycota (Annex I). The least richness was reported in Blastocladiomycota and Basidiomycota. It indicates that the Ascomycetous fungi are most common in the soil rhizosphere. It might be because, they obtain nutrition from dead and decaying matter easily and also found associated with mycorrhiza (Egidi *et al.* 2019). The fungal species richness was high in invasive *A. adenophora* and native *I. cylindrica* than other native species (Table 2). This suggests that these species may accumulate diverse group of soil fungi. In case of *A. adenophora,* the result supports the finding of Mangla & Callaway (2008). Mangla & Callaway (2008) investigated on the rhizosphere soils of an invasive *Chromolaena odorata* (close relative of *Ageratina adenophora*) and found that there was high accumulation of generalist soil borne fungi. Moreover, Li et al. (2015) concluded that the soil microbes may gradually adapt to the allelochemicals of invasive *A. adenophora* and alleviate its allelopathic effects.

Balami *et al.* (2017) accumulates pathogenic fungi (*Alternaria alternate, Colletotrichum gloeosporioides, Rhizoctonia solani, Fusarium oxysporum,* etc.) in soil. This study has also reported the fungi like *Fusarium oxysporum, Rhizoctonia solani* in the rhizosphere of *A. adenophora*. The identified fungi include both parasites and saprophytes, but those with a high frequency such as *A. niger, Myrothecium* sp., *Phoma* sp., *Rhizoctonia* sp., *P. oligandrum,* and *Verticillium* spp. (Table 3) are parasitic in nature. The results indicate that these parasitic fungi are host specific as *A. niger* and *Myriothecium* were found in *A. adenophora, Phoma* sp. was in *R. ellipticus, Rhizoctonia* sp. in *S. robusta* and *P. oligandrum* and *Verticillium* spp. were found in *I. cylindrica* (Table 3). Other fungi belonging to the genera *Penicillium, Aspergillus*, and *Chaetomium* were common decomposers (Fu-qiang *et al.* 2004). The fungi species such as *Scedosporium* sp.*, P. lilacinus, A. flavus*, *Myrothecium* sp., *P. citrinum, P. chrysogenum, Rhizoctonia* sp., *M. circinelloides, Hypocrea* sp., *T. hypoxylon, T. sparsum, Gliocladium* sp., *Hypomyces* sp., *A. niger* and *M. circinelloides* were occurred in the rhizospheric soil of all native plants (Table 3). It might be because of having some similar types of root exudates in all native plant species.

This study did not find significant variations in soil chemical contents among the plant species, as shown in Table 4. It can be expected that even slight variations in soil physicochemical properties can alter the occurrence of fungal species in the root zone. However, the chemical compounds in soil and plant root exudates were not analyzed in this study

but some of the previous studies have analyzed the chemical compounds in the plant species selected for this study. For example, chemical compounds like flavonoid aglycones, quercetin 3-glucoside, quercetin 3-rutinoside, tannins and phenolic compounds are released by *S. robusta* (Joshi 2003, Poornima 2009). The compounds 1-octacosanol, flavonoids and phenolic compounds are common in *R. ellipticus* (Vadivelan *et al.* 2009) and iso-eugenol, iso-ferulic acid, linoleic acid, ferulic acid, vanillin, 4-acetyl-2-methoxyphenol, 2,4-di-tert-butylphenol etc. are the chemicals found in the root zone of *I. cylindrica*. The sesquiterpenes like amorpha-4, eupatorenone, 9-oxo-ageraphorone and esters like dibutyl phthalate, bis(2-ethylhexyl) phthalate and other several compounds are found in *A. adenophora* root exudate (Yang *et al.* 2013, Zhou *et al.* 2013).

CONCLUSION

In conclusion, present study reported 49 fungal species from rhizospheric soil of invasive *A. adenophora* and native *R. ellipticus, S. robusta* and *I. cylindrica* using Czapek Dox Agar and PDA media. *A. adenophora* alters species richness, occurrence and frequency of fungi in soil. The pathogenic fungi *A. niger, Myrothecium* sp., *Phoma* sp., *Rhizoctonia* sp., *P. oligandrum, Verticillium* spp. are the most frequently occurring fungi in rhizosphere of all plants showing their host specificity. The fungi species such as *P. lilacinus, A. flavus*, *Myrothecium* sp., *P. citrinum, P. chrysogenum, Rhizoctonia* sp., *M. circinelloides, Hypocrea* sp., *T. hypoxylon, T. sparsum, Gliocladium* sp., *Hypomyces* sp., *A. niger* and *M. circinelloides* are commonly occurring fungi in all native plants indicating similarity in nature of root exudates. Even slight variations in soil physicochemical properties can alter the occurrence of fungal species in the root zone. Further research exploring the effects of specific chemical compounds found in root exudates on the fungal community could yield novel insights into the complex mechanisms that govern plant-soil microbial interactions, particularly those related to biological invasion and feedback mechanisms.

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Annex I: List of fungal species with phylum, class and order

S.N.	Fungi species	Phylum	Class	Order
1	*Achlya* sp.	Oomycota	Peronosporea	Saprolegniales
2	*Acremonium byssoides* W. Gams & T.M. Lim	Ascomycota	Sordariomycetes	Hypocreales
3	*Acremonium kiliense* Grütz	Ascomycota	Sordariomycetes	Hypocreales
4	*Actinomucor* sp.	Mucoromycota	Mucoromycetes	Mucorales
5	*Aspergillus alliaceus* Thom & Church	Ascomycota	Eurotiomycetes	Eurotiales
6	*Aspergillus flavus* Link	Ascomycota	Eurotiomycetes	Eurotiales
7	*Aspergillus fumigatus* Fresen.	Ascomycota	Eurotiomycetes	Eurotiales
8	*Aspergillus niger* Tiegh.	Ascomycota	Eurotiomycetes	Eurotiales
9	*Botrytis* sp.	Ascomycota	Leotiomycetes	Helotiales
10	*Chaetomium* sp.	Ascomycota	Sordariomycetes	Sordariales
11	*Cunninghamella* sp.	Mucoromycota	Mucoromycetes	Mucorales
12	*Fusarium oxysporum* sensu Smith & Swingle	Ascomycota	Sordariomycetes	Hypocreales
13	*Fusarium* sp.	Ascomycota	Sordariomycetes	Hypocreales
14	*Geotrichum candidum* Link	Ascomycota	Saccharomycetes	Saccharomycetales
15	*Gliocladium* sp.	Ascomycota	Sordariomycetes	Hypocreales
16	*Hypocrea* sp.	Ascomycota	Sordariomycetes	Hypocreales
17	*Hypomyces* sp.	Ascomycota	Sordariomycetes	Hypocreales
18	*Lichtheimia corymbifera* (Cohn) Vuill.	Mucoromycota	Mucoromycetes	Mucorales
19	*Lichtheimia ramosa* (Zopf) Vuill.	Mucoromycota	Mucoromycetes	Mucorales
20	*Mucor mucedo* L.	Mucoromycota	Mucoromycetes	Mucorales
21	*Mucor circinelloides* Tiegh.	Mucoromycota	Mucoromycetes	Mucorales
22	*Mucor indicus* Lendn.	Mucoromycota	Mucoromycetes	Mucorales
23	*Myrothecium* sp.	Ascomycota	Sordariomycetes	Hypocreales

24	*Paecilomyces lilacinus* (Thom) Samson	Ascomycota	Eurotiomycetes	Eurotiales
25	*Penicillium chrysogenum* Thom	Ascomycota	Eurotiomycetes	Eurotiales
26	*Penicillium citrinum* Thom	Ascomycota	Eurotiomycetes	Eurotiales
27	*Penicillium expansum* Link	Ascomycota	Eurotiomycetes	Eurotiales
28	*Pestalotiopsis* sp.	Ascomycota	Sordariomycetes	Amphisphaeriales
29	*Phoma* sp.	Ascomycota	Dothideomycetes	Pleosporales
30	*Physoderma* sp.	Blastocladiomycota	Blastocladiomycetes	Physodermatales
31	*Phytophthora* sp.	Oomycota	Peronosporea	Peronosporales
32	*Pythium oligandrum* Drechsler	Oomycota	Peronosporea	Peronosporales
33	*Pythium* sp.	Oomycota	Peronosporea	Peronosporales
34	*Rhizoctonia* sp.	Basidiomycota	Agaricomycetes	Cantharellales
35	*Rhizopus* sp.	Mucoromycota	Mucoromycetes	Mucorales
36	*Saccharomyces cerevisiae* (Desm.) Meyen	Ascomycota	Saccharomycetes	Saccharomycetales
37	*Scedosporium* sp.	Ascomycota	Sordariomycetes	Microascales
38	*Trichoderma koningii* Oudem.	Ascomycota	Sordariomycetes	Hypocreales
39	*Trichoderma hypoxylon*	Ascomycota	Sordariomycetes	Hypocreales
40	*Trichoderma sparsum* W.T. Qin & W.Y. Zhuang	Ascomycota	Sordariomycetes	Hypocreales
41	*Trichoderma viride* viride Pers.	Ascomycota	Sordariomycetes	Hypocreales
42	*Trichoderma* sp.	Ascomycota	Sordariomycetes	Hypocreales
43	*Verticillium dahliae* Kleb.	Ascomycota	Sordariomycetes	Glomerellales
44	*Verticillium* sp.	Ascomycota	Sordariomycetes	Glomerellales
45	*Unknown 1*			
46	*Unknown 2*			
47	*Unknown 3*			
48	*Unknown 4*			
49	*Unknown 5*			