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ANTIFUNGAL ACTIVITY OF INVASIVE ALIEN PLANT SPECIES (IAPS) – IMPLICATION FOR MANAGEMENT

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Abstract–The antifungal activity of aqueous and methanolic extract of six invasive alien plants species (IAPs) *Ageratina adenophora, Ageratum houstonianum, Parthenium hysterophorus, Xanthium strumarium, Ipomoea carnea* and *Mikania micrantha* was investigated against *Alternaria brassicae, Botrytis cinerea, Fusarium oxysporum, Phytophthora capsici* and *Sclerotium rolfsii* at different concentrations (5, 10, 15 , 20 and 25%). Methanolic extract of plant exhibited strong inhibitory effect than aqueous extract. At 25% maximum antifungal potential was observed with the Methanolic extracts of *Ageratum houstonianum* and *Xanthium strumarium* against *F. oxysporum* (100%), *P. capsici* (100%), *S. rolfsii* (100%), followed by *A . adenophora*, which recorded excellent inhibitory activity against *F. oxysporum*(100%) *P. capsici* (100 %) and *S. rolfsii* (73%). Likewise methanolic extract of *I. carnea* at 25%, recorded maximum inhibitory activity against *F. oxysporum* (100%) which was followed by *A. brassicae* (64.0%), extract of *Parthenium hysterophorus* had shown excellent inhibitory activity against *Fusarium oxysporum* (100%) and *S. rolfsii* (100%). Extract of *Mikania micrantha* exhibited inhibitory effect on *B. cinerea* (61.50%), followed by *F. oxysporum* (61.3%). Test revealed the presence of tannins, cardiac. glycosides, alkaloids, and flavanoids in all the tested extracts. This study demonstrated that all studied plant species could be used as a potential fungicides in plant disease management. Utilization of IAPs as antifungal agent, could be helpful for its management.

INTRODUCTION

Invasive Species is the term that refers to a subset of plant and animals that spread beyond their native range, not necessarily harmful, or species introduced to a new range that establish themselves and spread (Jeschke and Strayer, 2005). Invasive alien species reduce biodiversity, replace economically important native plant species and decrease the investment in agriculture and silviculture, disrupt prevailing vegetation dynamics, alter nutrient cycling and cause changes in the pattern of plant succession. Several exotic plants have invaded the high-value biodiversity areas and have adversely affected the natural and semi-natural vegetation/ecosystems (Tripathi, 2009). Farmers, taxonomists and ecologist are now well aware of the invasion of alien species into natural areas and associated negative effects on Global patterns of native biodiversity. Once established some alien species have the ability to displace or replace native plant species the problem will likely worsen with time because of climatic changes that promote species migration worldwide. Plant pathogenic fungi attack most crops in the field and also post harvest thereby decreasing production and shelf life of many agricultural crops (Agrios, 1997). The most important method of protecting plants against fungal attack is the use of fungicides.

Invasive alien plant species (IAPS) has become one of the major threats of biodiversity loss in Nepal so their proper management is essential. Many invasive alien plant species found in Nepal, have become a problem and a topic of real concern for the country (Tiwari *et al.,* 2005). The country is facing problem in managing these weeds, and on the other hand use of these weeds in making useful products to mankind is very limited or in many cases not known. Extensive efforts for integrated management of harmful invasive species have been conducted including cataloging alien species and ecological risk assessment. One of the main activities for

management of invasive plant species has been the physical eradication with little success due to their prolific nature. On the other hand, the successful habitation of the invasive species has prompted intense interest in the mechanisms for the success (Pimentel *et al.,* 2005). Many invasive plant species release chemical compounds into the environment, which are not generally harmful to them, but those chemicals suppress the growth of other plant species growing in close proximity of such invasive species. Prime importance can be given for the bioprospecting of novel active compound which can be utilized for the management of several plant diseases.

The use of invasive plants in curing human and plant pathogenic disease may decrease the dependency on many medicinal plants which are rare, threatened or at the edge of extension from the country. This will also on the other hand manages the spread of such invasive plants. Management of IAS can also save huge loss of money in the agriculture field each year due many pathogenic fungi for the agriculture based country like Nepal and similarly, this can also reduce the dependency and import of the drugs against microorganisms from other countries.,

Besides, invasive plants are easily available throughout the year and are found growing around the crop lands. This will enable the farmers to use these plants in crop protection against various phytopathogenic diseases. Hence, the present work is aimed to investigate invasive plant species for antifungal activity against plant pathogens in order to develop a useful product from them fulfilling the concept of environment as well as health protection.

MATERIALS AND METHODS

Sample collection sites: Leaf samples of *Parthenuim hysterophrous, Ageratina adenophora,* and *Xanthium strumarium* were collected from different areas of Kirtipur, TU ($27^0$40′55"N and $85^0$17′21"E.)., while *Mikania micrantha* and *Ageratum houstonianum* were collected from different areas of Sauraha, Chitwan ($27^0$34′29"N and $84^0$29′37"E) and the leaf sample of *Ipomoea carnea* ssp. *fistulosa* was collected from river side of Jhojhi Kataiya village, Dhanusa district (26°412N 85°592E/ 26.68°N 85.98°E). in the month of June and August 2012.

Sample collection and Preservation: Fresh and healthy leaves were collected and washed properly with tap water. Plant samples were identified by the expert of Central Department of Botany, Tribhuvan University. Herbaria of the samples were prepared and deposited in the herbarium of Central Department of Botany, Tribhuvan University (Ref No. AH No.13 and AH No. 14). The leaves were cut into small pieces and were shade dried. The dried leaves were ground into fine powder with the help of electric grinder. The ground plant samples were preserved into zipper bag for further analysis.

The ground plant leaf sample of 25 g was soaked in 250 ml of distilled water and methanol (99 %) separately in a conical flask for 72 h. Each mixture was stirred at 24 h interval using a sterilized glass rod. The samples were filtered using three layers of muslin cloth. Distilled water extract was evaporated on heating mantle using water bath till the thick residue was formed (Mahida and Mohan, 2007) and methanol was evaporated using rotary evaporator at 60 ^{o}C. It was made into semisolid form by evaporation to water bath. After the solvent evaporation, each of the solvent extract was weighted and preserved in air tight bottles until further use in the refrigerator at temperature 4-10 ^{0}C.

Phytochemical screening: The phytochemical screening of crude extracts from the test plant species were carried out to determine the presence of active secondary plant metabolites. The plant extracts were screened for the presence of tannins, saponin, cardio-glycosides, terpenoids, steroids, flavonoids and alkaloids according to the established procedures. Preliminary qualitative phytochemical screening was carried out on the powdered samples applying the following standard procedures described by several researchers (Harborne, 1973; Sofowara, 1993) and for the result sharp change in color was noted.

Collection of Test organism*:* The pure fungal strains were collected from Nepal Agriculture and Research Council (NARC), Khumaltar, Kathmandu. The five strains used for the test were *Sclerotium rolfsii, Phytopthora capsici, Alternaria brassicae, Fusarium oxysporum* and *Botrytis cinerea.* All fungi were cultured on PDA (Potato Dextrose Agar) (Difco, USA) plates, and incubated at 27 °C for one week. Purification of the resulting isolates was done using the hyphal tip or single spore techniques to obtain pure cultures. The de-tected isolates were then transferred into a slant of PDA and kept at 4°C for further studies. Pure cultures of the isolated fungi were identified according to the cultural properties, morphological, and microscopic characteristics of

each fungus (Watanabe, 2002). The antifungal activity of plant extracts was evaluated against food-associated fungi by using Poisoned Food Technique applying the method of (Khalil and Dababneh, 2007; Kushwaha and Maurya, 2012), with some modifications considering the access and availability of equipments and chemicals.

Preparation of different concentrations (extract): Distilled water and methanol semisolid leaf extract of plant specieswere used for the preparation of concentrations viz. 5%, 10%, 15%, 20% and 25%. These concentrations were diluted in distilled water and methanol separately hence distilled water and methanol were used as negative control.

The test fungi were inoculated for 7 days at 27 °C. Control petriplates were run following the same process. The fungal colony was measured on the 7th day of incubaton for the data analysis. Minimum and maximum readings of the colony diameter were taken using millimetre ruler. For each treatment seven replicates were used and mean value was taken. The result was compared with control.

Data Analysis: The values were expressed as mean ± standard deviation (SD). Each value was a mean of seven replicates. The one – way Analysis of Variance (ANOVA) was used to determine the significant differences between the parameters and the Duncan test was done to compare the differences at $p < 0.01$ using statistical package SPSS version 20.The percentage of linear growth reduction of pathogenic fungi compared with control was calculated using the formula as given by Khalil and Dababneh (2007).

$$\text{Linear growth reduction (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}}$$

RESULTS

In – vitro antifungal activities of invasive plant extracted with distilled water and methanol were compared againsts five fungal phytopathogens. The rate of growth inhibition was corroborated with its concentration. In case of all tested plant extracts, there was significant different in effect to mycelial growth reduction among different concentration (Table 2 and 3). It was observed that at 25 % concentration of leaf extract (aqueous) of *Ageratina adenophora*, 80% growth inhibition was recorded against *Fusarium oxysporum*, it was followed by *Alternaria brassicae* (70.80%), *Phytopthora capcisii* (66.67%), *Sclerotium rolfsii* (65.67%) and minimum for *Botrytis cinerea* (48.10%) (Table 2). Likewise methanolic extract of plant at 25%, 100% reduction in growth was recorded in *F. oxysporum* and *P. capsici*, which was followed by *A. brassicae* (74%), *S. rolfsii* (73.30%) and minimum for *B. cinerea* (59.09%) (Table 3).

Leaf extract (aqueous) of *Ageratum houstonianum* at 25% exhibited 77.92% reduction in growth of *Fursarium oxysporum* which was followed by *B. cinerea* (51.85%) and *A. brassicae* (50%), and least effect was exhibited by *Phytophthora capsici* at all concentration. Effect of extract was more pronounced in methanolic, than aqueous as, methanolic extract at 25% concentration, completely inhibited growth of *Fusarium oxysporum*, *Phytophthora capsici* and *Sclerotium rolfsii* which were followd by *A brassicae*, (68.60%), and least effective against growth of *B. cinerea* (54.50%).

Aqueous extract of *Ipomoea carnea* at all concentration did not inhibit the growth of *P. capsici*

Table 1. Preliminary phytochemical screening of tested plant species

Plants	Phytochemical Constituents							
	Solvent	Tannin	Saponin	C.glycoside	Terpenoides	Steroids	Flavonoid	Alkaloid
Ageratina adenophora	Dist. water	+++	++	+	+++	_	_	+++
	Methanol	+++	+++	_	_	+	+++	+++
Ageratum houstonianum	Dist. water	++	+	-	-	++	+	_
	Methanol	_	+++	+++	+++	++	+++	_
Ipomoea carnea ssp. fistulosa	Dist. water	+	++	+	+	_	_	+++
	Methanol	_	+++	+	+	_	+++	+++
Mikania micrantha	Dist. water	+	++	-	-	-	+	+
	Methanol	+ +	+	++	+++	+	+++	++
Parthenium hysterophorus	Dist. water	+++	+++	-	-	-	++	+
	Methanol	_ -	+	++	+++	+	+++	++
Xanthium strumarium	Dist. water	_	++	+++	+++	_	_	++
	Methanol	+	+++	_	+++	+++	+++	+++

Responses to various tests were denoted by (+), (++) and (+++) signs indicating weak, moderate and strong reactions respectively while (-) for no reaction; c. glycoside- cardiac glycoside, Dist. water- distilled water

and *S. rolfsii* while it was more effective for the growth of *F. oxysporum* (85.71%) which was followed by *A. brassicae* (62.50%), *B. cinerea* (37.03%) at 25% concentration . Likewise methanolic extract of *I. carnea* inhibited 100% growth for *F. oxysporum* at 25% concentration and which was followed by *A. brassicae* (64.0%), *B. cinerea* (40.90%), *S.rolfsii* (22.22%) and minimum growth was exhibited for *P. capsici* (7.78%). There was significant differences in mycilial growth at different concentration (Table 2 and 3).

Extracts (both aqueous and methanolic) of *P. hysterophorus* at all concentration inhibited growth of all tested organisms to some extent. Maximum growth was inhibited by aqueous extract at 25% to *A. brassicae* (55.56%) which was followed by *Sclerotium rolfsii* (54%), *B. cinerea* (51.85%) and *F. oxysporum* (46.75%) and growth was not effected for the case of *P. capsici*. Methanolic extract of *P. hysterophorus* completely inhibited growth (100%) of *F. oxysporum* and *S. rolfsii* at 25% concentration, which was followed by *B. cinerea* (50%), *A. brassicae* (45.1%), *P. capsici* (34%). Likewise leaf extract (both aqueous and methanolic) of *Mikania micrantha* inhibited growth of all tested organisms. Maximum inhibition was exhibited by *B. cinerea* (61.50%) at 25% concentration, growth was not effected by aqueous extract even at high concentration (25%) for *P. capsici*.

Likewise, aqueous extract of *Xanthium strumarium* at *25%* concentration inhibited growth of *F. oxysporum* (78.94%) which was followed by *A. brassicae* (61.11%), *B. cinerea* (51.85%), *S. rolfsii* (6.66%) and growth was not effected for the *P. capsici* (0%), while in methanolic extract at 25% completely inhibited (i.e 100%) the growth of *P. capsici, Fusarium oxysporum* and *S.rolfsii* which was followed by *A. brassicae* (60%), and *B. cinerea* (54.54%) (Table 2 and 3).

Methanolic extract of plant exhibited strong inhibitory effect than aqueous extract It was observed that at 25% concentration of methanolic leaf extract *A houstonianum* and *Xanthium*

Table 2. *In vitro* growth inhibition (%) of phytopathogens by the leaf extract of different plants. (Distilled water) (n=7). Values within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.01$)

Pathogens	Growth inhibition (%) at different concentration of leaf extract						
	Concentrations of phyto-extract	*Ageratina adenophora*	*Ageratum houstoniaum*	*Ipomoea carnea*	*Parthenium hysterophorus*	*Mikania. micrantha*	*Xanthium strumarium*
Alternari abrassicae	5%	22.22a	9.72a	34.72a	12.5a	22.22a	15.30a
	10%	36.10b	16.67b	40.3b	15.28a	26.39ab	20.83a
	15%	48.60c	36.11c	47.22c	25b	31.96b	44.44b
	20%	54.20d	41.67d	52.78c	29.17b	43.06c	45.83b
	25%	70.80e	50.0e	62.50d	55.56c	47.22cd	61.11c
Botrytis cinerea	5%	14.80a	14.81a	7.4a	14.81a	10.81a	11.11a
	10%	22.20b	22.22b	18.51b	22.22b	18.92b	37.03b
	15%	25.90b	25.93b	22.22c	33.0c	29.73c	44.44c
	20%	44.40c	33.33c	29.63c	44.44d	48.65d	48.14c
	25%	48.10c	51.85d	37.03d	51.85d	59.46e	51.85c
Fusarium oxysporum	5%	43.40a	14.29a	33.76a	25.97a	28.57a	5.26a
	10%	44.73a	29.87b	48.10b	38.96b	33.77b	21.05b
	15%	46.05b	71.43c	62.30c	41.56c	36.36b	57.89c
	20%	52.63c	75.32c	72.72d	44.0cd	40.26c	64.47d
	25%	80.26d	77.92cd	85.71e	46.75d	51.0d	78.94e
Phytopthora capsici	5%	5.0a	0a	0a	0a	0a	0a
	10%	6.8a	0a	0a	0a	0a	0a
	15%	7.5a	0a	0a	0a	0a	0a
	20%	20.0b	0a	0a	0a	0a	0a
	25%	66.67c	0a	0a	0a	0a	0a
Sclerotium rolfsii	5%	7.0a	0a	0a	0a	0a	0a
	10%	8.12a	0a	0a	0a	0a	0a
	15%	8.23a	0a	0a	0a	0a	0a
	20%	18.90b	0a	0a	21b	0a	4.44b
	25%	65.67c	43b	0a	54c	20b	6.66c

strumarium, was most effective to inhibit the mycelial growth, followed by, *Ageratina adenophora, Parthenium hysterophorus*, *Ipomoea carnea* and *M. micrantha.*

DISCUSSION

In – vitro antifungal activities of invasive plant extracted with distilled water and methanol were compared against five fungal phytopathogens. The rate of growth inhibition was corroborated with its concentration. In case of all tested plant extracts, there was significant different in effect to mycelial growth reduction among different concentration (Table 2 and 3). The result of present study indicated that, antifungal activity showed by the tested plant extracts had inhibitory effects on the growth of *A. brassicae, B.cinerea, Fusarium oxysporum, Phytophthora capsici* and *Sclerotium rolfsii.* These results further revealed that antifungal activities of the extracts were enhanced by increasing the concentration from 5 to 25 %; hence the inhibition activities of the extracts were concentration dependent. This is in agreement with the report of previous researchers (Chiejina and Ukeh, 2013, Ilondu 2012) who indicated that increase in the antifungal activities had corresponding increase in concentration of plant extracts. The *in vitro* efficacy of *Ageratina adenophora*, *Ageratum houstonianum, Parthenium hysterophorus, Xanthium strumarium, Ipomoea carnea* and *M. micrantha* against different pathogens has been investigated by various research-ers (Baral *et al*., 2011; Kushwaha and Maurya, 2012; Ginesta-Peris *et al.,* 1994). The antifungal activity attained by these plant extracts is attributed to their chemical composition.

A. houstonianum and *Xathium strumarium* exhibited high fungitoxic effect in inhibiting mycelial growth reduction against the tested fungi. These remarkable antifungal and fungicidal results are in agreement with those reported in previous studies (Amerjothy *et al*., 2007; Sharifi-Rad *et al*.,

Table 3. *In vitro* growth inhibition (%) of phytopathogens by the leaf extract of different plants (Methanol extract). Values within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($p \leq 0.01$)

Pathogens	Concentrations of phyto-extract	Growth inhibition (%) at different concentration of leaf extract (methanolic extract)					
		Ageratina adenophora	*Ageratum haustoniaum*	*Ipomoea carnea*	*Parthenium hysterophorus*	*Mikania micrantha*	*Xanthium strumarium*
Alternaria. brassicae	5%	34a	29.40a	38a	15.70a	31.37a	26a
	10%	46b	51.00b	46b	29.40b	33.34a	30a
	15%	64c	54.90b	52b	33.34b	37.25ab	48b
	20%	64c	58.82b	54b	37.30cd	47.06b	50b
	25%	74d	68.60c	64c	45.10d	54.90c	60c
Botrytis cinerea	5%	27.27a	13.63a	9.10a	27.30a	18.18a	27.27a
	10%	45.45b	22.70b	18.18b	31.80b	27.27b	45.45b
	15%	45.45b	36.40c	22.70c	36.40b	40.90c	50b
	20%	50b	45.50cd	31.81d	40.90c	50.00d	50b
	25%	59.09c	54.50d	40.90e	50.00c	61.50e	54.54c
Fusarium oxysporum	5%	22.58a	38.70a	35.48a	3.22a	35.50a	9.67a
	10%	48.38b	41.90b	51.61b	9.67a	41.90b	29.03b
	15%	58.1c	45.20b	64.5c	25.80b	48.40b	100c
	20%	61.29d	100.0c	100d	48.40c	54.80c	100c
	25%	100e	100.0c	100d	100.0d	61.30d	100c
Phytophthora capsici	5%	21.11a	25.0a	0a	5.0a	0a	0a
	10%	70b	56.0b	0a	6.0a	15.00b	65.56b
	15%	100c	66.00c	0a	17.00b	17.00b	70.0b
	20%	100c	74.00d	0a	22.00b	19.00b	100c
	25%	100c	100.0e	7.78b	34.00c	28.00c	100c
Sclerotium rolfsii	5%	8.0a	36.00a	0a	4.0a	0a	3.34a
	10%	17.78b	44.00b	0a	5.0a	0a	11.11b
	15%	28.90c	100.0c	0a	19.00b	24.00b	100c
	20%	58.90d	100.0c	5.60b	40.00c	35.00c	100c
	25%	73.30e	100.0c	22.22c	100.0d	39.00c	100c

2015). Fungitoxic properties of the studied plant species could be attributed to the presence of saponin, terpenoids, steroids, and alkaloid, chemical components (Table 1) which has been identified as antifungal agents in the plant (Kumar *et al*., 2008).

All the tested IAS showed varied degree of antifungal activity which might be correlated to the various phytochemicals present in their respective extract (Sule *et al.,* 2011) and also this may be due to the reason that the agrochemicals present in the plants are the supply of natural fungicides, insecticides and pesticides (Shu, 1998, Cordell, 1995). Similarly, Bajpai *et al.,* (2012) also reported antifungal activity of invasive alien plants species against *Magnaporthe oryzae, Rhizoctonia solani, Botrytis cinerea, Phytophthora infestans, Puccinia recondita, Blumeriagraminis f. sp. hordei, Colletotrichum coccodes.*

In this study the plant extracts by methanol provided more consistent antifungal activity compared to those extracted by distilled water. Similar result was found by Parekh and Chanda (2007) on five microorganisms by methanolic and distilled water extract of twelve species of Indian medicinal plants. This tends to show that the active ingredients of the plant parts are better extracted with methanol than other solvents. The methanol extracts contain alkaloids, coumarins and tannins (Okemo, 1996). Methanol has a high polarity index (Cowan, 1999) than the water and thus is able to extract more phenolic and flavonoid compounds (Table 1)**.** The inhibitory effect may be as a result of the change in pH and chemical constituent(s) of aqueous and methanolic extracts of these plants. The mechanisms of action of these phytochemicals may be via lysing of cell, increasing permeability of cell wall and membrane, inhibition of protein and DNA synthesis and or inhibiting the transport of nutrient across the cell wall of membrane (Cowan, 1999).

A. brassicae and *B. cinerea* were found most resistant fungi against plant extract while *F. oxysporum, P. capsici* and *S. rolfsii* were found most susceptible fungi. This might be due to the presence of more complex cell wall with rigidity and also might be due to reason that the fungi differ in optimum growth conditions such as pH, production rate of manganese and lignin peroxidases and their resistance to toxic chemicals (Rucksdeschel and Renner, 1986).

There was significant difference ($p< 0.01$) between the linear mycelium growth and the different concentrations for all the tested plants and the tested fungus. Different species were effective against different fungal stains. So all tested plant species could be used as potential fungicide for disease management.

CONCLUSION

This study demonstrated that *Ageratina adenophora, A haustoniaum, Ipomoea carnea, P. hysterophorus, M. micrantha* and *X. strumarium* could be used as a potential biocide in plant disease management, as they showed fungicidal and fungitoxic ability. Methanolic extract of plants had more fungi toxicability than aqueous extract. The utilization of plant extracts to control disease in vegetable minimizes or eliminates the risks and hazards of toxic fungicides, especially on freshly consumed vegetables. This work proves that some invasive plants have potential and could be useful in combating plant fungal pathogens. The use of plant extracts could enable the development of inexpensive and environmentally acceptable fungicides based on locally available natural products. That helps to manage invasive weed as well.

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