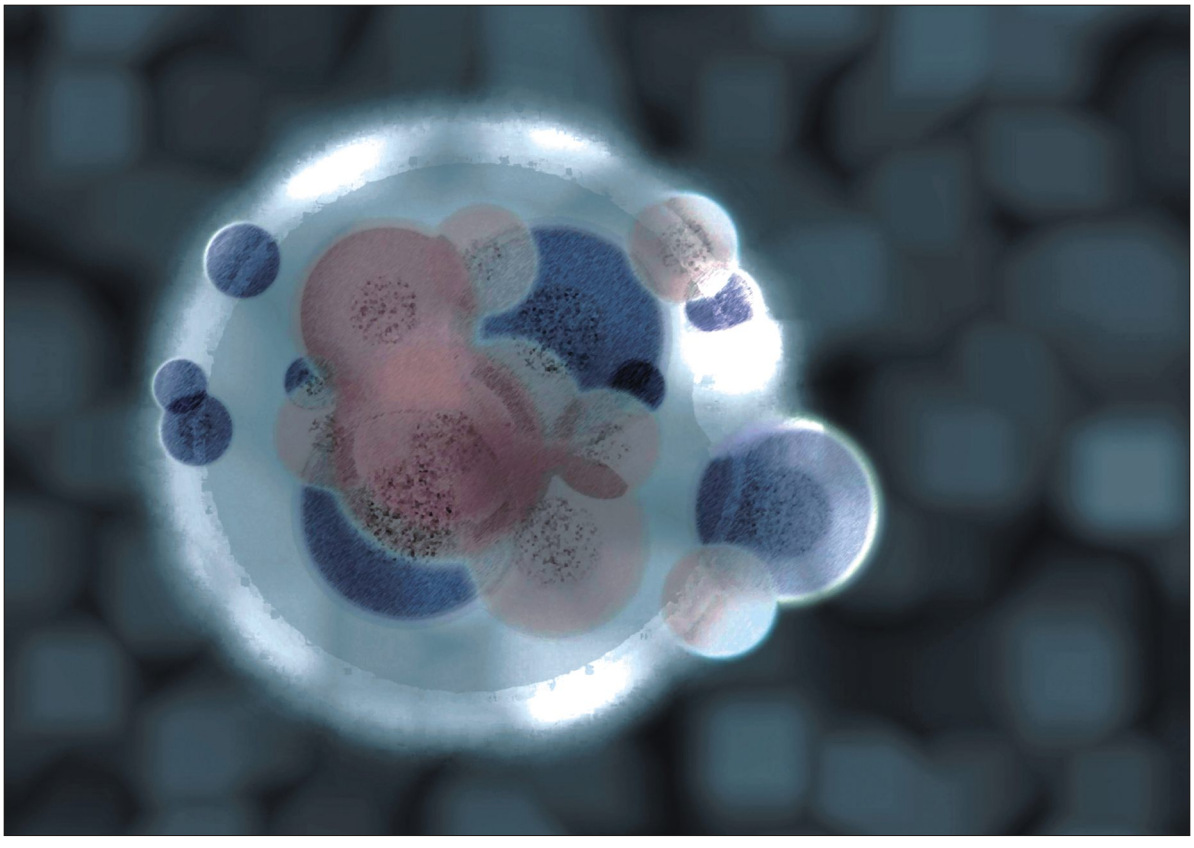

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Isolation of Fungi from Roots of *Parthenium hysterophorus* and *Desmostachya bipinnata* and Antibacterial Activity of Their Root Extracts

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Abstract: The present study was conducted to quantify the effects of soil pollutants on soil inhabiting root-associated fungi. Roots of *Parthenium hysterophorus* (L.) subjected to fungal isolation revealed more diversity in fungal association compared to *Desmostachya bipinnata* (L.). *Aspergillus* was found most commonly associated fungi with the roots of both the plants. A total of seven different fungal species were isolated from the two plants. The root extracts of both the plants were found to have antibacterial agents. However, gram negative strains of bacteria were observed least sensitive. It seems that either plants or fungi, on polluted sites have co-adapted themselves in order to survive. Their interdependence is a manifestation of an association that cannot be strictly regarded as symbiotic but leads to an endorsing reciprocity.

Key words: Antibacterial activity, fungal isolations, root extract, soil pollution

Introduction

One of the unique features among soil inhabiting fungal population is their diversity in habitat. The occurrence of fungal species in soils of varying edaphic as well as pedaphic characteristics, is a major attribute of fungal adaptation. Even under severe stress conditions, for instance acidic soils, or soils with heavy metal toxicity or polluted due to industrial effluents, doesn't restrain the survival of fungi (Steinberg *et al*., 1999). There are numerous reports on occurrence of soil and root inhabiting fungi from polluted sites. Heterogeneity in the rhizospheric soil is another compelling factor determining the diversity of soil-borne root associated fungi (Grose *et al*., 1996). Keeping these elements in account, the present study was planned to find out the diversity of root-associated fungi among plants growing on polluted soil.

Materials and Methods

Rhizospheric soil of *Parthenium hysterophorus* (L.) and *Desmostachya bipinnata* (L.) along with their roots was collected form heavily polluted area near Kala-Shah Kaku (Gujranwala–Lahore road). The site form where sampling was carried out, receiving enormous amount of industrial effluents from surrounding industries. Soil samples were subjected to texture and pH determinations while plant root samples were used for fungal isolation and antibacterial test.

Fungal Isolation: Small root pieces (1 cm) of *Parthenium hysterophorus* and *Desmostachya bipinnata* were washed with tap water for 24 hours. After that these were placed on petri plates containing sabrouad (65 g of Sabrouad "nutrient medium") was dissolved, boiled and autoclaved in 1 liter distilled water. After cooling the media, it was poured into petri plates within laminar flow chamber. After that the culture plates were incubated for 3 to 4 days at 30°C

Antibacterial Activity: Root pieces were washed with water to remove debris, cut in small pieces and then these were soaked in 60% ethanol for 72 hours. Suspension of root material was then filtered through Whatman Filter Paper No.1 and stored in sterilized glass bottles. Nutrient agar plates were (7.5 cm diameter) prepared having 0.5 cm thick layer of medium. Each plate was inoculated with one bacterial strain by the help of sterile swab. Pure cultures of *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus*, were used as inoculum. One ceftriaxone (antibiotic) disc was placed in the center of each inoculated plate using sterile forceps. In each plate, two wells were bored (same diameter as of antibiotic disc). One well was filled with ethanol root extract while in the other extracting solvent (60% ethanol) was poured. Plates were left in a cooled incubator at 4°C and these were incubated at 37°C for 18-20 hours. The zone of inhibition was measured in diameter (Hili *et al*., 1997).

Results

Results of the fungal isolates have indicated the occurrence of fungi at acidic conditions (Table 1). More fungi were isolated with *Parthenium hysterophorus* roots as compared to the *Desmostachya bipinnata* as four fungal species were isolated from *Parthenium hysterophorus* while three from *Desmostachya bipinnata*. *Aspergillus* was found the most common as three different species were isolated and it is the only genus, which is isolated from the roots of both the plant species.

The root extract of *Parthenium hysterophorus* revealed antibacterial activity. The gram positive bacteria were observed more sensitive than gram negative against the root extracts. Same trend was observed in case of antibiotic ceftriaxone (Table 2). The ethanol root extract obtained from *Desmostachya bipinnata* was tested against three bacterial strains (Table 3). *Staphylococcus aureus* showed maximum resistance while *Klebsiella pneumoniae* was found the most sensitive with zone of inhibition 6 mm (Table 3). Against cerftriaxone, again *Staphylococcus aureus* exhibited the maximum resistance with only 19 mm diameter zone of inhibition while *Escherichia coli* was divulged as the most sensitive.

Discussion

The occurrence of fungi in association with the roots of both the plant species displays a mechanism of adaptation by these plants. The soil in which these plants were growing, was under severe stress in terms of acidity and industrial pollutants. It may be speculated that plants were able to grow on such sites due to the presence of diverse fungal species in their roots particularly *Fusarium* species, which mainly invade in the vascular tissues of root and stem. Thus acting as a plug to check the sap movement in plants as well as a filter that baffles the excess entry of salts and pollutants in xylem tissues. As *Desmostachya bipinnata* was collected from much more acidic conditions than *Parthenium hysterophorus* and

Table 1: Fungal isolates from the roots of *Parthenium hysterophorus* and *Desmostachya bipinnata* collected from polluted area

Plant species	Root isolated fungi	*Effluent source	**Soil pH	Soil gorup	Topography
Parthenium hysterophorus	*Aspergillus fumigatus* Fresenius	Itteehad chmicals	5.7 ± 0.4	Sandy loam	Even with gentle slope
	Aspergillus luchuensis Inui				
	Penicillium islandicum Sopp	Kohinoor chemicals			
	Rhizoctonia solani				
Desmostachya bipinnata	*Fusarium dimerum* Penizg	Itteehad chmicals	4.8 ± 0.7	Silt loam	Steep slope
	Aspergillus niger van Tieghem	Kohinoor chemicals			
	Mucor geophilus Oudemans				

*Industries located within the radius of 200 m with reference to sampling point

**Average pH value of 3 sample ± standard deviation ($p < 0.05$)

Table 2: Antibacterial activity of root extract of *Parthenium hysterophorus* compared with antibiotic ceftriaxone

Bacterial strain	Type	Ceftriaxone (Zone of inhibition)	Disc potency	♣ Root extract (Zone of inhibition)
Escherichia coli	Gram positive	24 mm	30 µg	5 mm
Staphylococcus aureus	Gram negative	19 mm	30 µg	3 mm

♣Values were adjusted after subtracting from those of solvent (60% ethanol)

Table 3: Antibacterial activity of root extract of *Desmostachya bipinnata* compared with antibiotic ceftriaxone

Bacterial strain	Type	Ceftriaxone (Zone of inhibition)	Disc potency	♣ Root extract (Zone of inhibition)
Klebsiella pneumoniae	Gram positive	22 mm	30 µg	6 mm
Escherichia coli	Gram positive	24 mm	30 µg	5 mm
Staphylococcus aureus	Gram negative	19 mm	30 µg	3 mm

♣Values were adjusted after subtracting from those of solvent (60% ethanol)

Fusarium was isolated from *Desmostachya bipinnata* only so that a correlation between stressed habitat of *Desmostachya bipinnata* and occurrence of *Fusarium* can be established on the basis of above speculation. Moreover, several workers have also reported Fusaria at such conditions (Benhamou and Belanger, 1998).

The isolation of only one species of *Penicillium* and *Rhizoctonia* from the roots of *Parthenium hysterophorus*, and three species of *Aspergillus* is an indication of possible role of soil borne root-associated fungi in the survival of prevailing vegetation type under a particular environmental conditions (Delgado *et al*., 1998) because in many plants, induced systemic resistance is contributed by fungal root colonization (Meera *et al*., 1995). During the sampling, it was also observed that *Parthenium hysterophorus* was more frequently distributed than *Desmostachya bipinnata* and our result have shown greater diversity of root-associated fungi in *Parthenium hysterophorus* than *Desmostachya bipinnata*. This trend strengthens the view of burgeoning of plants in stressed conditions, like the one that is observed in the present study, entails a facilitated role of fungi in plant establishment (Redman *et al*., 1999).

Many soilborne fungal plant pathogens remain as resting propagules until the appearance of a potential host stimulates their germination. Several workers (Ruan *et al*., 1995) have investigated the nature of stimulus. The plant-derived stimulus for germination has generally been assumed to be nutrients exuded from roots (Oyarzun *et al*., 1998). As the present study was conducted on the site where industrial waste and salt accumulation in soil was on rise, a condition that fosters the exudation of root sap out from halophytic plants. Probably that situation has enabled fungal propagules to perpetuate their viability in the soil. The *Parthenium hysterophorus* was more commonly observed at these sites so its role in providing potential stimulus is quite obvious. That is why, comparatively more fungi were isolated from the roots of *Parthenium hysterophorus* as compared to *Desmostachya bipinnata*.

The root extracts of both the plants have shown considerable antibacterial activity. Both the plants were tested against gram positive and gram negative strains but extent of sensitivity of root extracts was almost same. However, maximum resistance against the root extracts shown by *Staphylococcus aureus* (gram negative) may be an attribute of the wall characteristics as gram negative bacteria possesses a complex wall nature (Rashid and Hashmi, 1999). This might be the most plausible explanation that zone of inhibition was observed less among gram negative bacteria.

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