PREVALENCE OF BENEFICIAL FUNGAL SPECIES IN AGRICULTURAL AND BARREN LAND SOIL AND THEIR ROLE IN PLANTS GROWTH PROMOTION

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ABSTRACT

In current study, soil samples were analyzed for the diversity of fungal species from agricultural and barren lands. The total fungal load was up to 2.57× 10^4 CFU/g dwt in agricultural and 2.11× 10^4 CFU/g dwt in barren land soils. The isolated fungal species from both types of the soils were successfully identified and found to belong from the division: ascomycota, deuteromycota and zygomycota. Results revealed, total seven fungal species namely Aspergillus niger, A. flavus, A. terreus, A. nidulence, Curvularia sp., Alternaria sp. and Mucor sp. were isolated from agricultural soils and total five fungal species (Aspergillus niger, A. terreus, A. nidulence, Curvularia sp. and Alternaria sp.) were detected from barren land. Obtained predominant fungal species were Aspergillus flavus (32.72%), Aspergillus niger (17.33%) from agricultural soil and only Aspergillus terreus (61.47%) from barren land soil. Further, these fungal species (A. niger, A. flavus, A. terreus, Trichoderma sp. and mixture of all four fungus) were tested for the growth and development of two plant species: Triticum aestivum L. and Vigna mungo (L.) Hepper. In all treatments, fungal spores were mixed (10^5spores/g) in the sterilized soil samples. By the pot experiments, best result was recorded in the treatment of A. terreus, it was very effective in enhancing the total fresh weight of shoot and root of Vigna mungo in comparison to positive control (agricultural land soil without sterilization) whereas in case of Triticum aestivum maximum total fresh weight of shoot and root was observed by the treatment of A. flavus in comparison to positive control.

KEYWORDS: *Fungal diversity, agricultural and barren land soil, Triticum aestivum, Vigna mungo.*

1. INTRODUCTION

Soil is a major reservoir for maintenance of fungi. The majority of beneficial and pathogenic fungi for plants and human inhabit freely in soil. The variation and occurrence of these fungi in soil depend on the environmental and nutritional conditions of areas.

Soil microorganisms play very important role in biogeochemical cycles and have been used for crop production for decades. Plant–fungal and plant-bacterial interactions in the rhizosphere are the determinants of plant growth, health and soil fertility. Free-living soil microorganisms beneficial to plant growth, usually referred to as plant growth promoting microorganisms, are capable of promoting plant growth. Plant growth promotion and development can be facilitated both directly and indirectly. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms. This can be achieved by the production of siderophores, i.e. small metal-binding molecules.

Trichoderma is a free-living fungus that is highly interactive in soil, root and foliar environments. It has been reported from many years that they produce a wide range of antibiotic substances [1] and that they parasitize other fungi. They can also compete with other microorganisms; for example, they contend for key exudates from seeds that stimulate the germination of propagules of plant-pathogenic fungi in soil [2] and, more generally, compete

with soil microorganisms for nutrients and or space [3]. Furthermore, they inhibit or degrade pectinases and other enzymes that are essential for plant-pathogenic fungi, such as *Botrytis cinerea*, to penetrate leaf surfaces [4].

Fungi are one of the most important organisms on earth, serving as decomposers of organic matter. Since they lack chlorophyll they live either on dead organic matter as saprophytes or on living organic matter as parasites. Fungi have an effect on soil fertility, suppress plant diseases and promote mushroom growth [5]. They also degrade complex polymers such as polyaromatic compounds or plastics and are being increasingly applied to bio-remediate soils contaminated with a wide range of pollutants [6-8]. Monitoring fungal diversity is essential to detect fungi hazardous to plants, humans, and animals and to optimize compost quality standards [9].

Fungi produces various beneficial secondary metabolites like flavonoids, phenolic, quinones, steroids, terpenoids, benzopyranones, tetralones, xanthones, chinones etc. which are responsible for plant growth promotion and plant self defense mechanism and some harmful metabolites like aflatoxins that may be potent toxins and carcinogens in food of birds, fishes, humans, and other animals.

Fungi are also a prominent source of pharmaceuticals and are used in many industrial fermentative processes, such as the production of enzymes, lipids, glycolipids, polysaccharides, polyhydric alcohols, vitamins and pigments,. Fungi are extremely useful in making high value products like mycoproteins and acts as plant growth promoters and diseases suppressor.

Among microbial communities, arbuscular mycorrhizal fungi are well hypothesized to alleviate the effects of root pathogens [10, 11] and improve host nutrients and stress tolerance [12, 13]. These root symbionts alter the microbial population composition that external hyphae (mycorrhizosphere) [14, 15] agricultural crop production both under field and greenhouse conductions.

Effective biological control methods are necessary to minimize the harmful effects on plants. *Trichoderma* species belongs to genus imperfect fungi, fast growing in culture and produce many green spores and are commonly associated with soil, root and plant debris [16]. *Trichoderma* species have long been recognized as popular biological agents to control plant diseases and protect crops against plant pathogens all over the world. An antibiotic, gliovirin, frome *Trichoderma virens* demonstrated strong inhibition of *Pythium ultimum* and the *Phytophthora* species [17]. The plant treated with *Trichoderma* in the root zone can produce higher levels of peroxides. Moreover, some strains may enhance plant growth and development. These phenomena was observed by several researchers who treated plants with *Trichoderma harzianum* resulting in large increases in the root area and cumulative root length, as well as a significant increase in dry weight, shoot length, and leaf area over that of the untreated control [16]. The use of *Trichoderma* as a biological agent of plant diseases has long been known.

In this study, we previously isolated the fungal species from the agricultural and barren land soil and observed the interaction between different fungi (*Aspergillus niger, Aspergillus flavus, Aspergillus terrieus*) and *Trichoderma* species with respect to their role in growth and development of two plant species: *Triticum aestivum* L. and *Vigna mungo* (L.) Hepper.

2. MATERIALS AND METHODS

2.1 Collection of soil samples: Soil samples were collected from different sites of agricultural and barren lands of Gwalior region. Samples were collected in sterile zipper polyethylene bags and stored at 4 ^{o}C before processing. Samples were used for the isolation of fungal organisms.

2.2 Reagent and chemicals: All media components and chemicals used in the study were of analytical grade and purchased from Hi-media laboratory Pvt. Ltd and Sigma–Aldrich.

2.3 Preparation of soil samples: Soil sample were dried at 60 ^{o}C for 72 hrs, powdered in pestle and mortar and filtered through 2 mm sieve and the sieved soil were dissolved in distilled water (2.5w/v) and vortexing for 5 minutes at 120 rpm

2.4 Isolation and characterization of fungi: Serial dilution agar plating (Apinis, 1963), Warcups soil plate and Waksman Direct inoculation methods were employed for the isolation of soil fungi. 1gm sample was suspended in 9 ml of sterilized distilled water and serial dilutions were made up to 10^{5}. One ml aliquot of each sample from appropriate dilution was poured in sterilized Petri-plates (in triplicate) and appropriate amount of Potato Dextrose Agar media was added in Petri-plates and mixed well. After solidification, plates were incubated at 25±2 ^{o}C and growth of fungal colonies was recorded at various time intervals. After five or seven days of incubation period (depending upon the types of fungal species appearing on plates), pure culture of each fungal isolate was prepared by using PDA media for identification purpose.

Colony forming units per gram (CFU/g) were calculated using following formula:

CFU = N×10-n where N= Total number of colonies, n= dilution

2.5 Fungal Identification

Isolated fungal species were identified on the basis of morphology (shape, size, growth rate and color of the colonies), presence of wrinkles and furrows, pigment production and microscopic characteristics (characteristics of mycelium, size, shape, color and arrangement of conidia, spore, conidiophores, sporangiophores, vesicle, sterigmata etc.) as described by Thom and Raper (1945) [18], Gilman (1971) [19], Barnett (1969) [20], Jamaluddin et al. (2004) [21], Samson et al. (2007a, b) [22, 23].

2.6 Calculations

Total incidence/abundance and frequency of occurrence of fungi isolated from the agricultural and barren lands was calculated by following formula:

$$\text{Frequency of occurrence} = \frac{\text{Number of samples containing a genera/fungal species}}{\text{Total number of sample evaluated}} \times 100$$

$$\text{Total incidence} = \frac{\text{Number of isolates of the species}}{\text{Total number of isolates}} \times 100$$

2.7 Inoculation of test fungi into the soil

On the basis of frequency of occurrence and total incidence, four most commonly occurring fungal species viz. *Aspergillus niger, A. flavus, A. terrius* and *Trichoderma* species were used as test organisms during the investigation.

6 mm disks from seven day old cultures were taken with the help of cork borer and shaken for 2 minutes in 10 ml of distilled water. After removal of disks, suspension was filtered through Whatman filter paper followed by drying. Spores were collected using brush and mixed with soil in the ratio of 10^5 spores /g. In one 6 mm disk, spores count was calculated by counting the approximate number of spores with the help of haemocytometer.

2.8 Pot preparation

For that experiment, soil containing seven pots each in triplicate was prepared. The first pot was inoculated with spores of *Aspergillus niger*; second with *A. flavus*, third with *A. terrius*, fourth with *Trichoderma* species, and fifth pot with mixed (all) fungus. The sixth pot was considered as control i.e. without any fungal infestation and seventh pot was considered as positive control (agricultural land soil served as without sterilization).

Sterilized soil were mixed with a dried spore mass of the each test fungi (1×10^5 spores/g) separately and mixed well for uniform distribution of spores. Two crop plant species; *Triticum aestivum* and *Vigna mungo* were grown in that soil and observed each day.

3. RESULTS AND DISCUSSION

In the present investigation, results reveal that total colony forming unit (CFU) of fungi was high (2.5×10^4) in agricultural land soil as compared to barren land soil where it was (2.1×10^4) (table 1). Total seven fungal species like *Aspergillus niger, A. flavus, A. terreus, A. nidulence, Curvularia* sp., *Alternaria* sp. and *Mucor* sp. were associated with agricultural land soil whereas five fungal species (*Aspergillus niger, A. terreus, A. nidulence, Curvularia sp.* and *Alternaria sp.*) were detected from barren land soil.

Total incidence of various isolated fungi from agricultural and barren land soil is presented in table 2. Total incidence of *A. flavus* was high in agricultural land soil where it was 32.72% whereas in barren land soil, very high total incidence (61.47%) of *A. terrius* was found.

In agricultural land soil, maximum total incidence was found in *A. flavus* (32.72%) followed by *A. niger* (17.33%), *Alternaria* sp. (9.26%), *Curvularia* sp. (7.78%), *Mucor* (7.43%), *A. terrius* (6.79%) and *A. nidulence* (6.06%). Whereas in barren land soil, highest total incidence was found in *A. terries* where it was 61.47% followed by 15.18% (*A. nidulence*), 6.02% (*A. niger*), and 2.5% (*Curvularia* sp. and *Mucor* sp.). *A. flavus* and *Mucor* sp. was not found in barren land soil (table 2).

The role of isolated fungal species (*A. niger, A. flavus, A. terreus*, *Trichoderma* sp. and mixture of all four fungus) in plant growth and development was tested on the germination and growth of two important crops viz; *Triticum aestivum* and *Vigna mungo*. In *Vigna mungo*, very fast germination was recorded in comparison to *Triticum aestivum*. Seeds of *Vigna mungo* germinated after two days whereas in *Triticum aestivum* germination was recorded after five days.

In *Triticum aestivum* maximum number of germinated seed (86.66%) was found in the treatment of *A. flavus* as compared to positive control (80%). In *Vigna mungo* hundred percent of germinated seed was found in the treatment of *A. niger*, *Trichoderma* sp. and mixed fungus (table 3).

Fresh and dried weight of shoot and root of *Triticum aestivum* and *Vigna mungo* was recorded after fifteen days. By the pot experiments, best result was recorded in the treatment of *A. flavus*, it was found very effective in enhancing the total fresh weight of shoot and root (0.847g) of *Triticum aestivum* in comparison to positive control (agricultural land soil without sterilization) where it was (0.821g). Maximum dry weight of shoot and root was recorded in *Triticum aestivum* by the treatment of *A. terreus* (0.610g) in comparison to positive control (0.577g).

Whereas in case of *Vigna mungo* best result was found in the treatment of *A. terreus*, it was found very effective in the growth and development of fresh weight of shoot and root of *Vigna mungo* (2.371g) in comparison to positive control (agricultural land soil without sterilization) where it was 2.316g. 0.310g dry weight of shoot and root of *Vigna mungo* was recorded by the treatment of *Trichoderma* sp. which was high from the positive control (0.265g).

Very few research papers are available in literature related to role of fungi in growth promotion. But many developing countries have been trying to increase the quality of the seed production by employing the culture of different fungal species. Dimic et al. (2008) isolated twenty three different fungi from the seeds of spices. This indicates the ability of fungi to develop association with broad spectrum of seed irrespective of their types.

Table 1: Total CFU of fungal species isolated from the agricultural and barren land soil.

S. No.	**Total CFU**	
	agricultural land soil	**barren land soil**
1	3.3×10^4	2.2×10^4
2	3.9×10^4	5.3×10^3
3	2.0×10^4	8.6×10^3
4	2.1×10^4	1.4×10^4
5	1.4×10^4	5.4×10^4
Total	$\mathbf{2.5\times 10^4}$	$\mathbf{2.1\times 10^4}$

Table 2: Total Incidence of various fungal species isolated from the agricultural and barren land soil.

S. No.	**Fungal species**	**Total Incidence (%)**	
		agricultural land soil	**barren land soil**
1.	*A. niger*	17.33	6.02
2.	*A. flavus*	**32.72**	-
3.	*A. terrius*	6.79	**61.47**
4.	*A. nidulence*	6.06	15.18
5.	*Curvularia* sp.	7.78	2.5
6.	*Alternaria* sp.	9.26	2.5
7.	*Mucor*	7.43	-

Table3: Role of various fungal species in seed germination of *Triticum aestivum* and *Vigna mungo* plant

S. No.	**Treatments**	**Germination period (in days)**		**Total no of germinated seed (%)**	
		In Triticum aestivum	**In *Vigna mungo***	***In Triticum aestivum***	**In *Vigna mungo***
1	*A. niger*	7	2	53.33	**100**
2	*A. flavus*	5	2	**86.66**	93.33
3	*A. terrius*	5	2	53.33	93.33
4	*Trichoderma* sp.	7	2	66.66	100
5	Mixed fungus	5	2	73.33	100
6	Positive control	7	2	**80**	**100**
7	control	3	2	100	100

Table 4: Fresh and dry weight of shoot and root of treated plant (*Triticum aestivum*) by various test fungi.

S. No.	Treatments	Fresh weight (g) of shoot and root of *Triticum aestivum*			Dry weight (g) of shoot and root of *Triticum aestivum*		
		Shoot	Root	Total weight	Shoot	Root	Total weight
1	*A. niger*	0.599	0.147	0.746	0.340	0.110	0.450
2	*A. flavus*	0.703	0.144	**0.847**	0.502	0.75	1.252
3	*A. terrius*	0.573	0.126	0.699	0.512	0.98	**1.492**
4	*Trichoderma* sp.	0.642	0.122	0.764	0.443	0.69	1.133
5	Mixed fungus	0.591	0.135	0.726	0.485	0.82	1.305
6	Positive control	0.674	0.147	**0.821**	0.498	0.079	**0.577**
7	control	0.942	0.369	1.311	0.706	0.295	1.001

Table 5: Fresh and dry weight of shoot and root of treated plant (*Vigna mungo*) by various test fungi.

S. No.	Treatments	Fresh weight (g) of shoot and root of *Vigna mungo*			Dry weight (g) of shoot and root of *Vigna mungo*		
		Shoot	Root	Total weight	Shoot	Root	Total weight
1	*A. niger*	1.926	0.066	1.992	0.250	0.035	0.285
2	*A. flavus*	1.808	0.060	1.986	0.195	0.045	0.240
3	*A. terrius*	2.259	0.112	**2.371**	0.162	0.069	0.231
4	*Trichoderma* sp.	2.166	0.080	2.246	0.265	0.045	**0.310**
5	Mixed fungus	2.132	0.135	2.267	0.182	0.047	0.229
6	Positive control	2.228	0.088	**2.316**	0.213	0.052	**0.265**
7	control	2.907	0.083	2.990	0.241	0.046	0.287

CONCLUSION

The results of the present investigation clearly established the concept of microbes as plant growth promoters. Some fungal species were even found more effective in growth promotion than positive control. The fungal species like *Aspergillus terreus* and *A. flavus* that have shown promising results can be utilized at large scale for initiation of seed germination and promotion of plant growth.

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