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# Antagonistic Behaviour of Soil Rhizosphere Fungus against Wheat Pathogens in Selected Fields of Bihar

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**ABSTRACT**

*The production of antifungal substances by rhizosphere fungi has long been recognized and this knowledge is entering practical life through the use of fungal antagonists to protect crops against their fungal pathogens. In this study rhizosphere fungi isolation in serial dilution plate technique, and their antimicrobial properties against plant pathogen tested by dual culture, disc diffusion method. Effect of these fungi in seed germination and growth promoting activity was measured in pot culture method.*

*Rhizosphere fungi it has become clear that in addition to diffusible substances evidence has accumulated that these bioactive compounds are not only able to promote seed germination and plant growth, but also to strongly inhibit pathogenic fungal growth. As the demand for organic products and the need to render agriculture more sustainable are rising, finding new environmentally friendly crop protection strategies is essential. In this perspective, the newly discovered capacity of fungal bioactive compounds to efficiently repel phytopathogenic fungi in laboratory experiments holds great promise.*

***Keywords: Rhizosphere, Biological control, Fusarium sp., Seed germination.***

## I. INTRODUCTION

Wheat (*Triticum vulgare*) is an important crop worldwide, with over half of the world population dependent on it for food. Wheat plants are attacked by many diseases caused by various phytopathogens, which result in low yield and quality of the crop. The common root rot caused by *Cochliobolus sativus (teleomorph), Bipolaris sorokiniana* (anamorph) and *Helminthosporium sativum*, while dryland root rot by *Fusarium spp* and pythium root rot by *Pythium spp* are common wheat disease in India.

Though the fungus is plant debris, and soil-borne, soil-borne inoculums are more important in causing infection and disease development. It was considered desirable to evaluate the efficacy of some chemical fungicides against the disease Application of chemical fertilizer to control the disease is not only very much effective, but also hazardous to environment and host plant resistance, which is often based on a single gene, may not be durable in the field, leading to frequent resistance breakdown. Fungicides can cause acute toxicity, and some cause chronic toxicity as well (Goldman, 2008). The use of chemical pesticides has been known to cause various environmental and health problems. Appropriate technological improvement, which results in the more effective use of natural resources, is required in agriculture. One of them is the use of microbial antagonists.

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Naturally, the majority of the microorganisms distributed around plant root surface have a role in the decomposition of organic matter, and some may suppress deleterious microorganisms, which could inhibit plant growth. A few of the root-associated microorganisms can promote plant growth, and they have been called "plant growth promoting fungi." Rhizosphere microorganism refers to a bacterial or a fungal microorganism that colonizes the region of the soil immediately adjacent (within 1 mm) to plant roots (Whipps, 2001). are different from those living in the non-rhizosphere surrounding soil, both in gross numbers of cells and the variety of strains. The rhizosphere microbial communities influence growth, resistance to disease or even death of the plant host depending on the degree of parasitism and pathogenecity.

The exploration of alternative methods has been a global effort to attain food security because of the public concern on pesticide use in crops. Many microbial antagonists have been reported to posses' antagonistic activities against plant fungal pathogens, such as *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Bacillus subtils*, *Bacillus cereus*, *Bacillus amyloliquefaciens*, *Trichoderma virens*, *Burkholderia cepacia*, *Sacharomyces* sp., *Gliocadium* sp. The successful control by these antagonists mainly against the diseases caused by following genera of pathogens: *Alternaria*, *Pythium*, *Aspergilus*, *Fusarium*, *Rhizoctonia*, *Phytophthora*, *Botrytis*, *Pyricularia* and *Gaeumanomyces* (Pal et al., 2006). Garrettson & Cornell (1975) made a survey of the mycroflora from the root zone of soil inhabited by *Phytophthora cinnamoni* they examined the antagonistic relationships between these fungi and *Phytophthora cinnamoni*. Eicker(1975) surveyed the antimycotic and antibacterial substance producing capabilities of *Fusarium*, *Cladosprium* and *Penicillium* on agar such ability was found associated with dominant fungi. Johri and Saksena (1975) studied the colonisation capacities and volatile fungiasis properties of Alternaria, Curvularia and Aspergillus on agar. The growth of inhibition varied among them which was suggested as equal chances of survival in soils. Kamyshko, Tupenvich, Chumakov and Shikunova (1976) reported the *Penicillium cyclopium*, the most frequent isolate from the soils investigated in active antagonist against *Fusarium avenacium*, *F. Culmovum*, *F. Oxysporium*, *Helminthosporium Sativum* and *Rhizoctinia solani*. *Trichoderma lignorum* is also highly antagonistic against the same pathogens. However, little information is available on the simultaneous effect of fungal antagonists on stem rot pathogens of rice.

These factors have led to the search for new and innovative approaches for plant disease management. Biological control has attained importance in modern agriculture to minimize the residual effects due to the continuous and indiscriminate use of toxic chemicals for the disease control. The addition of organic amendments to soil exerted a favourable effect on disease reduction due to its suppressive nature. The organic amendment not only increases the activity of bio-control agents but also acts as a source of nutrients to growth promote in crop plants. Present study isolation and identification of rhizosphere fungi and their antagonistic, seed germination activities were tested.

## II. METHODS AND MATERIALS

The pathogenic *Fusarium spp.* were isolated from root rot infected wheat plants collected from Gopalganj District using tissue segment method (Rangaswamy et al., 1999). Small pieces of tissues about 3 $mm^2$ from infected root region with some healthy tissue were cut with a sterile scalpel. Then the pieces were surface sterilized with one percent sodium hypochlorite solution for 30 seconds. The tissue pieces were subsequently

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washed in three changes of sterile distilled water to eliminate excess sodium hypochlorite and then pieces were transferred to potato dextrose agar (PDA) plated Petri dishes.

Plates were incubated at 28±2°C and were observed periodically for the growth of the fungus. The culture was purified by single hyphal tip method and maintained throughout the present investigation by periodical transfer onto PDA. The pathogen was identified as *Fusarium spp.* based on its mycelial and sclerotial characters (Barnett et al., 1972).

**2.1 Isolation of native antagonistic mycoflora from rhizosphere of wheat**

Serial dilution technique (Johnson et al., 1972) was used to isolate mycoflora from rhizosphere soil of wheat. Composite soil sample collected from the rhizosphere of healthy wheat plants was shade dried and then used for serial dilution. 10 g of this soil was dissolved in 100 ml of sterile distilled water to get $10^{-1}$ dilution. From this 1 ml of soil, suspension was taken and added to 9 ml of sterile distilled water to get $10^{-2}$ dilution. This is repeated until a final dilution of $10^{-4}$ was obtained.

Antagonistic mycoflora were isolated on Rose Bengal Agar medium by using a dilution of $10^{-4}$. 1 ml of soil suspension was taken in sterilized petriplates, melted and cooled medium was poured. Plates were rotated gently to get uniform distribution of soil suspension into the medium. Then the plates were incubated at 28±2°C and observed at frequent intervals for the development of colonies. 3-day-old colonies of mycoflora were picked up and purified by single hyphal tip method. Rhizosphere mycoflora were identified based on mycological keys described by Barnett and Hunter (1972). Mycoflora was maintained by periodical transfer on PDA.

**2.2 Dual culture technique**

This method (Baker and Cook, 1974) is used to study the efficacy of bio-control agent, against plant pathogens under laboratory conditions. PDA prepared and the medium sterilized in an autoclave at 121°C for 15 minutes and the medium (20 ml) poured into sterilized Petri-plate (90 mm diameter) when the medium is in lukewarm state than it should be allowed to solidify at room temperature. The culture discs (7-day-old) of the bio-agents and pathogen separated with the help of sterilized cork bores (5 mm). The culture discs of pathogen and bio-agent transferred aseptically and placed them at periphery of the Petri plate containing the medium Inoculate with culture disc of the pathogen alone in the Petri plates containing PDA, which serves as control. The inoculated Petri-plates transferred to an incubator and incubate at 27±2°C. The growth of the pathogen and antagonist in Petri plates observed periodically and the colony growth (diameter) in each Petri plate measured. The per cent inhibition of the pathogen by the bio-agent calculated when the growth of the pathogen is full in the control plates.

**2.3 Disc diffusion method**

*Preparation of crude fungal extracts*

Crude extracts of fungi were prepared as described by (Wang et al., 2011). All antagonistic culture were cultivated on potato dextrose broth by placing agar blocks of actively growing pure culture in 250 ml Erlenmeyer flaks containing 100 ml of the medium, the flasks were incubated at 26±2°C for 1-week with shaking 120 rpm incubator. Fungal cultures were filtered using filter paper to separate the culture broth and mycelia. All filtrates were transferred to separating fennel filled with equal amount of ethyl acetate stirred fully and left overnight, separating for the organic layer and then further concentrated in a vacuum rotary evaporator to dryness to remove organic solvents. EtOH extracts were dried by freeze drying, and then diluted with

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dimethyl sulfoxide to a concentration of 10 mg/ml and 5 mm disc were prepared for the antifungal activity assay.

A disc diffusion method pathogenic fungi was inoculated on the centre of the PDA plates. The crude extract containing discs were placed on PDA plate with the distance apart from the pathogenic fungi. The control disc was filled with DMSO. The plates incubate 26±2°C for 3 days. The fungal growth was determined by the inhibition distance between crude extract discs and the mycelium of the pathogen compared to the control disc inhibitory zone was measured.

**2.4 Fungal extract effect on seed germination**

Healthy wheat seeds were taken into sterile flasks treated with pathogenic fungal suspension, after test antagonistic fungal spore suspension 10, 20% were applied, and control seed were surface sterilized in 2% sodium hypo chloride. These seeds were dried in room temperature then after showing in sterile soil pored polythene bags. Ten days after planting the percentage of seed germination, rotting infected radicles were recorded there were three replicates of each treatment.

## III. RESULTS AND OBSERVATIONS

The pathogen was identified based on mycological characters as *Fusarium spp.* First the fungal mycelium was silky white in color and later turned to dull white with radial spreading giving fan-like appearance. Microscopic examination of the fungal culture revealed that the mycelium was hyaline, thin walled, septate, and profusely branched with clamp connections. When the fungus attained maturity, small mycelia knots were formed later turned to mustard seed like sclerotia. Initially, sclerotia were deep brown or brownish black shiny, hard, and spherical to irregular in shape. At maturity, the sclerotia showed honeydew like liquid material. The sclerotial bodies were concave on the side attached to the mycelium and were easily detachable from the mycelium. The sclerotia were bigger in size measuring about 1.0-1.3 mm in diameter.

Identification of native antagonistic rhizosphere mycoflora Rhizosphere mycoflora were identified based on colony and morphological characters. *Penicillium notatum, Rhizopus sp., Aspergillus flavus, Aspergillus niger, Penicillium sp., Trichoderma harizanum*. *Aspergillus sp.* cultures were isolated for observations regarding the colour and number of colonies per gram of rhizosphere soil (Table 1).

**Table 1. List of antagonistic mycoflora and percentage inhibition to pathogenic fungi.**

| S No | Rhizosphere fungi | Colonies/ Soil (gm) |
|---|---|---|
| 1. | *Penicillium spp* | 23 |
| 2. | *Penicillium notatum* | 10 |
| 3. | *Aspergillus flavus* | 24 |
| 4. | *T. harizanum* | 10 |
| 5. | *Aspergillus niger* | 07 |
| 6. | *Rhizopus spp* | 11 |
| 7. | *Alternaria spp* | 12 |

In vitro evaluation of antagonistic mycoflora against *Fusarium*, seven identified fungi viz., *Aspergillus flavus, A. niger, Penicillium sp., Penicillium notatum, Trichoderma harizanum, Alternaria sp.*, and *Rhizopus sp.* were

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isolated from rhizosphere samples of paddy. The antagonistic effect of these isolates was assessed based on their ability to inhibit the pathogen growth and sclerotial population by dual culture technique under in vitro. Moreover, the efficacy of antagonists against the test pathogen was assessed on the basis of the ability to form the inhibition zone. The reduction in the growth of the pathogen due to antagonistic mycoflora was calculated and expressed in percent inhibition.

The data pertaining to percent inhibition of mycelial of Fusarium due to antagonistic mycoflora are presented in Table 2.

**Table 2. Rhizosphere fungi inhibition of Fusarium spp in dual culture and diffusion methods.**

| Serial Number | Rhizosphere fungi | Inhibition% in Dualculture | Zone of Inhibition |
|---|---|---|---|
| 1. | *Penicillium spp* | 38.36±0.25 | 10.2±0.08 |
| 2. | *Penicillium notatum* | 48.50±0.15 | 10.5±0.26 |
| 3. | *Aspergillus flavus* | 70.86±0.22 | 12.6±0.28 |
| 4. | *T. harizanum* | 74.50±0.24 | 13.4±0.12 |
| 5. | *Aspergillus niger* | 35.12±0.15 | 10.5±0.14 |
| 6. | *Rhizopus spp* | 26.38±0.24 | 8.4±0.20 |
| 7. | *Alternaria spp* | 42.52±0.22 | 9.5±0.19 |

The data reveals that the *T. harizanum* was found to be superior followed by *Aspergillus flavus*, *Aspergillus niger* compared to others in reducing the mycelial growth and sclerotial population. The native *T.harizanum* isolate was superior with highest percent inhibition of mycelial growth by 74.50% and the microscopic examination revealed that the hyphae of antagonist coiled around the hyphae of the pathogen. *Aspergillus flavus* inhibited mycelial growth by 70.86%, *Pencillum notetum* inhibited mycelial growth by 48.50% The remaining fungi also inhibited mycelia growth indicate that overall percent inhibition of mycelial growth of *Fusarium* was maximum in case of *T. harizanum* and minimum in case of *Rhizopus* sp. Effect antagonists in dual culture were screened also inhibited to disc diffusion method. Maximum inhibition zone as observed *T. harizanum* extract followed by *A. flavus, Penicillium* sp. and *Fusarium* sp., was most effective inhibited to pathogenic fungi *Pythium.*

In pot culture experiments, the effect of seed germination, with fungal antagonists, on the stem rot fungus is shown in Table 3. In seeds naturally infected with *S. oryzae*, where seed infection was high, the biological treatment gave maximal germination and survival.

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**Table 3. Effect of seed treatment with fungal antagonists in Fusarium spp. As measured by seed germination % and infection.**

| S No | Treatments | % seed germination | Infection |
|---|---|---|---|
| 1. | *Fusarium sp.* | 40.50 | + |
| 2. | *Fusarium sp. +T.harizanum* | 85.06 | - |
| 3. | *Fusarium +A.flavus* | 76.42 | - |
| 4. | *Fusarium +Penicillium sp.* | 60.00 | - |
| 5. | *Fusarium +Rhizopus* | 46.00 | - |
| 6. | *Fusarium +A. niger* | 58.04 | - |
| 7. | *Fusarium + Alternaria sp.* | 55.56 | + |
| | **None** | 94.00 | - |

All treatments decreased significantly stem rot and radicle infection as compared with the control. However, seed germination was increased significantly only by *Trichoderma* sp. compared with control. In this test, *Fusarium* controlled most effective treatments for reducing seed infection were *T. harzianum* and *A. flavus* causing 85% and 76% seed rot reduction. However, *Penicillium* sp. Treatments was the least effective ones for reducing seed infection. The seed treated with antagonists ensure quicker and more effective utilization of the antagonists by the plants than the addition of antagonists to the soil. The action of biological agents at the seed surface seems to be more effective than soil application of fungal antagonists.

## IV. DISCUSSIONS

The colony characters and morphological characters of mycelium and sclerotia were in agreement with earlier reports (Chaudhary, 1997). Thus, the fungus under present investigation was identified as Fusarium.

Abdelmonem and Rasmy (2000) found that seed treated with *Trichoderma* spp. was the best biological treatment for reducing seed and seedling infections of mangrove caused by fungi and bacteria. The advantage of biological seed treatment is that protection can be prolonged, whereas chemical protects the seeds, the antagonists protect the seeds and roots. The rate of seed germination was increased only when seeds were either treated with culture filtrate or coated with a spore suspension of *T. harizanum* compared with controls. In this study, *Aspergillus flavus* appeared to be a more promising antagonist, as seed protecting bio-agent, than *Trichoderma* spp. Because it protected completely rice seeds and radicles against the infection of *Fusarium spp.*, the causal agent of dryland root rot of wheat.

*T. harzianum* capable of lysing mycelia of *Sclerotium rolfsi* and *Rhizoctonia solani* was isolated from a soil naturally infested with those pathogens (Elad et al., 1997). Under greenhouse conditions, incorporation of the wheat-bran inoculums preparation of *T. harzianum* in pathogen infested soil reduced significantly bean diseases caused by *S. rolfsi, R. solani,* or both, but its bio-control capacity was inversely correlated with temperature. The wheat-bran preparation of T. *harzianum* increased the growth of bean plants in a non-infested soil and its controlled *S. Rolfsi* more efficiently than a conidial suspension of the same antagonist. In naturally infested soils, wheat-bran preparation of *T. Harzianum* inoculums significantly decreased diseases caused by *S. rolfsi* or

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*R. solani* in three field experiments with bean, cotton, or tomato, and these increased significantly the yield of beans (Elad et al., 1997). Patale and Mukadam (2011) tested the antagonistic activities of three *Trichoderma* species, i.e. *T. viride*, *T. harzianum*, and *Trichoderma* sp. against seven pathogenic fungi, namely *Aspergillus niger*, *A. flavus*, *Phytophthora* sp., *Fusarium oxysporum*, *Rhizoctonia solani*, *Penicilium notatum*, and *Alternaria*

*solani*. They found that all three species of *Trichoderma* suppressed effectively the growth of seven pathogenic fungi.

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