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Biological Control of Common Root Rot of Wheat (*Bipolaris sorokiniana*) by *Trichoderma* Isolates

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Abstract: *Trichoderma viride* T112 and *T. viride* (MO), *T. harzianum* (M) and *T. harzianum* T194 were used as potential biological agent for control of common root rot caused by *Bipolaris sorokiniana.* Cell free and antifungal metabolites produced by all *Trichoderma* isolates inhibited growth of *Bipolaris sorokiniana*. The inhibition varied among isolates of *Trichoderma* and ranged from 58.20 to 93.93% using the cellophane overlay method and from 66.66 to 98.25% in volatile test. Mycelial growth of *B. sorokiniana* was numerically reduced more by *T. viride* T112 than the other isolate tested ($p<0.01$). The seed soaking treatment and also soil treatment with *T. vride* T112 and *T. viride* (MO) were the most effective in reducing infection by the pathogen compared with the corresponding control ($p<0.01$) All isolates of *Trichoderma* increased plant height, fresh and dry weight of roots and shoots of wheat seedling compared with the uninoculated control. Among *Trichoderma* isolates, *T. viride* T112 and *T. viride* (MO) proved to be the best in supporting the growth of wheat ($p<0.01$).

Key words: Biocontrol, wheat disease, pathogen, *Trichoderma harzianum*, *Trichoderma viride*

INTRODUCTION

Root and foot rots are major diseases of wheat when moisture is deficient. The causal fungi are widely distributed as unspecialized pathogen on most small-grain cereal and numerous grasses. One of the important soilborne disease of wheat is common root rot caused by *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. The most diagnostic symptoms caused by this pathogen is a dark brown or blackened subcrown internode. The infection and diagnostic discoloration may extend into the crown and a short distance up the culms. Diseased plants occur randomly or in irregular patches and appear stunted and chlorotic. Browning or blackening of the primary or secondary root system may be observed on close examination of washed roots. Seedling infection is reduced by shallow seedling and using clean or chemically disinfected seed. Also late autumn seedling of winter wheat is recommended to decrease seedling exposure to warm soil temperature. Reduced seedling infection, however is no guarantee against infection at the later growth stages Soil fertility must be adequate and balanced to support vigorous root and shoot growth. However, excessive fertilization, especially with nitrogen favors the disease by promoting vegetative growth, which in turn increases transportation and accelerates plant water stress. A few cultivars in Canada are resistant to *B. sorokiniana*[1], also *B. sorokiniana* on leaves can be controlled with conventional fungicides[2]. Control is difficult and expensive because the pathogen infects leaves, crown, rhizomes and roots of susceptible species and may be active on one or more plants of host plants throughout the growing season[3].

Biological agents could be an important components in control of *B. sorokiniana* if effective and reliable formulation were readily available and could be integrated with chemical fungicides. The antagonistic activities of *Trichoderma* and *Gliocladium* species against plant pathogens have been studied, extensively[4-10]. Some bacteria and fungi have been evaluated for antagonistic activity on *Bipolaris* and *Drechslera*, for example, effect of *Trichoderma harzianum* on sporulation of *Cochliobolus sativus* on excised wheat seedling leaves[11], potential biocontrol of *Bipolaris sorokiniana* on phyloplane of *Poa pratensis* with strains of *Pseudomonas* sp.[3], antagonistic effect of yeast against *Cochliobolus sativus* on wheat leaves[12]. There is no or little information on the efficacy of *Trichoderma* species against common root rot of wheat caused by *Bipolaris sorokiniana.*

The objective of this investigation was to evaluate the potential of *Trichoderma viride* (MO), *T. viride* T112, *T. harzianum* (M) and *T. harzianum* T191 for biological control of common root rot of wheat.

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MATERIALS AND METHODS

Pathogen and antagonist isolates: *Trichoderma viride* isolate T112 and *T. harzianum* T114 isolated from soil surrounding of wheat of Shahzand Arak in Markazi province of Iran and *T. viride* (MO) and *T. harzianum* (M) obtained from Dr. Rouhani Bo-Alisina University, Hamedan. Isolates SH and KH of *Bipolaris sorokiniana* obtained by Eng. Ghalandar, Agricultural Research Center of Markazi Province were used in this study. All isolates were maintained on potato dextrose agar at 5°C.

Effect of *Trichoderma* isolates on mycelial growth of *B. sorokiniana in vitro*: Dual culture[13] and cellophane overlays[14] were used to observe the effect of *Trichoderma* isolates on *B. sorokiniana*. All antagonist-pathogen combinations were examined on 10-15 mL of potato dextrose agar in 9 cm petri plates with 4 replicate plates per treatment. For dual culture mycelial plug (5 mm in diameter) taken from actively growing, 3 days old colonies of *B. sorokiniana* isolate or *Trichoderma* isolate were placed 5 cm apart on the agar, controls consisted of pure cultures.

For cellophane overlays, cellophane membranes (Australia Cellophanes, Victoria), 9 cm in diameter were boiled in distilled water, then interleaved with filter paper and autoclaved before being placed on the medium. One 5 mm diameter plug of *Trichoderma* species growing on PDA was placed on the center of each cellophane membrane. For controls, a plug of sterile PDA medium was used instead of the antagonist. The cellophane membrane and adhering fungus, or agar plug were removed after 2 days[9]. A plug of *B. sorokiniana* was placed on the agar in the center of plate and incubated for 6 days.

For antifungal activity of volatile, a petri plate containing PDA medium was inoculated with 5 mm diameter plug of *Trichoderma* isolates growing on PDA. A second petri plate containing PDA was inoculated with a 5 mm plug of the *B. sorokiniana* in the center of the plate and inverted over the *Trichoderma* culture. The two plates were sealed together with nescofilm and incubated at 25°C for 6 days. This ensured that both organisms were growing on the same atmosphere. For control instead of *Trichoderma* one plug of PDA was placed on agar surface[15].

The surface area of the colonies *B. sorokiniana* was recorded compared with controls and the percentage of growth inhibition was calculated. All experiments *in vitro* were arranged as Randomized Complete Design with 4 replications.

Biological control *of B. sorokiniana* on wheat in glasshouse condition

Seed treatment: The ability of *Trichoderma* isolates to reduce incidence of common root rot of wheat in glasshouse was investigated. Inoculum of the pathogen was prepared as follows. Wheat seeds were soaked for 16 h and then transferred to 125 erlenmyer flask and autoclaved for 1 h at 121°C on two successive days. *B. sorokiniana* isolates KH and SH were grown separately on Potato Dextrose Agar (PDA) and when they were grown 5 pieces of culture about 5x2 cm in size were added to each erlenmyer flask containing autoclaved wheat, mixed with wheat and incubated at 25°C for 30 days. The infested seeds were air-dried, powdered by grinding, then were added to autoclaved soil as a rate of 5%(w/w) and placed in 14 cm pots.

To obtain conidial suspension of *Trichoderma*, the fungus was grown on 9 cm petri plate for 7 days at 25°C. Conidia were harvested from surface of plates and washed several times in Sterile Distilled Water (SDW) and suspended in 0.01% tween 20. Seeds of cv. Alvand were surface disinfected by soaking in 0.5% sodium hypochlorite for 3 min then rinsed three times in SDW. The seed were soaked for 1 h in *Trichoderma* spore suspension and air dried in a laminar flow hood. For adhering of conidia to seed surface methyl cellulose was used[16]. The number of *Trichoderma* colonies was determined after 2 days of incubation at 25°C. Five T*richoderma* inoculated seeds were sown in each pot. Plants were maintained in the glasshouse at 22-25°C without supplementary lighting from April to May (spring). Pots were watered at 3 days intervals until emergence and daily thereafter. Forty five days after planting, plant height, fresh and dry weight of shoots and roots and percentage of infected roots were determined. Sample of tissue were taken from the root had discoloured. Segments of tissue 5 mm long, were surface-disinfected in 50% domestic bleach (0.5% available chorine) for 3 min, rinsed three times in SDW and plated on the PDA rose bengal peptone agar.

Soil treatments: The methods and materials for soil treatments were the same as described for seed treatment. In this experiment, density of conidia of *Trichoderma* was adjusted to 10^7 mL^{-1} with a hemacytometer. Seventy milliliter of spore suspension was added in each pot after planting of seeds. All experiments in glasshouse were arranged as randomized complete design with 4 replicate pots.

Statistical analysis: Data on percentage inhibition of growth and percentage of infected roots were subjected

to arcsin square root transformation before analysis. Data for plant height, fresh and dry weight of shoot and root were analysed directly. Analysis of variance was performed and means were separated using Duncan's Multiple Range Test at $p<0.05$[17].

RESULTS AND DISCUSSION

Effect of *Trichoderma* isolates on mycelial growth of *B. sorokiniana*: Dual culture, cellophane overly technique and volatile metabolite test showed that all isolates of *Trichoderma* tested inhibited the growth of 2 isolates of *Bipolaris sorokiniana* (Table 1). The inhibition varied from 29.56 to 69.82% in dual culture, from 66.66 to 98.25% in volatile metabolite test and from 58.20 to 93.93% using the cellophane overlay method. Results of dual culture, cellophane overlay indicated that mycelial growth of *B. sorokiniana* was numerically reduced more by *T. viride* T112 than the others isolate tested and also the percentage of growth inhibition of the pathogen by *T. viride* T112 was not less than 85% by means of volatile metabolite test.

Effect of *Trichoderma* isolates on control of common root rot of wheat in glasshouse: The results of seed-soaking with *Trichoderma* experiments indicated that common root rot was significantly reduced by *Trichoderma viride* T112 and *T. viride* (MO). For example, calculated mean in disease severity caused by isolate SH of the pathogen with these two antagonists were 31.50 and 27.75%, respectively compared to pathogen control (75.75%). All *Trichoderma* isolates used in this test reduced disease severity compared to control (Table 2). Plant height, dry weight and fresh weight of shoots and roots in treatment which the seed inoculated with *Trichoderma* was greater than pathogen control and also four *Trichoderma* isolates promoted plant growth and suppressed the *B. sorokiniana* (Table 2). *B. sorokiniana* was isolated from all inoculated plants showing symptoms.

All the *Trichoderma* isolates suppressed ($p<0.05$) common root rot severity when added to soil infested with *B. sorokiniana* compared to disease caused by *B. sorokiniana* alone. Isolate *T. viride* T112 and *T. viride* (MO) were more effective in reducing common root rot than the other isolates tested. Soil treatment with *Trichoderma* also promoted plant growth (Table 3).

The isolates of *T. harzianum* T114 and *T. viride* T112 which were isolated from soil surrounding of wheat in Arak of Iran and two isolates *T. harzianum* (M) and *T. viride* (MO) obtained from Dr. Rouhani, were tested for their antagonistic activity *in vitro* and *in vivo*. These isolate have not previously been evaluated for potential antagonists to *B. sorokiniana*. All isolates reduced

Table 1: Effect of *Trichoderma* isolates on mycelial growth of *B. sorokiniana* as % of colony area compared to control

Treatments	Dual culture	Volatile metabolites	Cellophane overlays
T112+KH	69.82a	85.71b	91.05a
T112+SH	62.53ab	98.25a	93.64a
T191+KH	60.69ab	76.52c	72.49b
T191+SH	55.49a-c	96.45a	58.20c
T114+KH	49.68bc	69.60d	93.93a
T194+KH	47.10bc	66.66d	77.70b
T114+SH	40.98cd	81.87bc	91.54a
T194+SH	29.56d	83.58bc	69.95b

Significant differences are denoted by different letters within each column according to Multiple Range Test at $p<0.01$. Data are expressed as % of control colonies without antagonist and values are average of 4 replicates. Data were subjected to arcsin square root transformation prior to analysis of variance, T112= *T. viride*, T194= *T. viride* (MO), T114=*T. harzianum*, T191= *T. harzianum* (M), KH= *Bipolaris sorokiniana* isolated from wheat field of Khomain, Iran, SH= *B. sorokiniana* isolated from wheat field of Shahzand, Iran

Table 2: Effect of *Trichoderma* isolates on control of *Bipolaris sorokiniana* at glasshouse condition (seed treatment test)

Seed treatments	Disease severity (%)	Plant height (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
T191 only	----	43.35cd	16.22bc	4.65b	8.63b	2.59bc
T114 only	----	44.41c	17.21b	4.39b-d	8.70bc	2.85cd
T194 only	----	51.65a	18.44a	4.95b	9.89a	3.20ab
T 112 only	----	46.90b	17.15 b	4.63b	9.38b	3.36a
Healthy control	----	41.77de	16.36bc	4.44bc	8.74bc	2.96bc
T191+KH	31.50de	36.12gh	13.68e	3.47g	7.02e	2.47fg
T114+KH	41.00cd	35.97gh	12.92e	3.82ef	7.25e	2.39fg
T194+KH	24.25e	40.95e	16.01c	4.25cd	8.29cd	2.55e-g
T112+KH	27.75 e	37.40fg	14.85d	4.09de	7.52 de	2.81c-e
KH only	59.25b	34.75h	10.66f	2.87h	5.104f	1.76h
T191+SH	35.25c-e	35.85gh	13.74f	3.49g	7.03e	2.46fg
T114+SH	43.25c	37.37fg	13.01e	3.68fg	7.27e	2.32g
T194+SH	27.75e	41.17e	15.67 cd	4.24cd	8.15cd	2.63d-f
T112+SH	31.50de	38.40f	15.27cd	4.17cd	7.76de	2.76c-e
SH only	75.75 a	34.30h	10.92 f	2.940h	4.89f	1.66h

Data represent the mean of 4 replicates, Significant differences are denoted by different letters within each column according to Multiple Range Test at $p<0.01$. T112= *Trichderma viride*, T194= *Trichoderma viride* (MO), T114=*T. harzianum*, T191= *T. harzianum* (M), KH= *Bipolaris sorokiniana* isolated from wheat field of Khomain, Iran, SH= *B. sorokiniana* isolated from wheat field of Shahzand, Iran

Table 3: Effect of *Trichoderma* isolates on control of *Bipolaris sorokiniana* at glasshouse condition(soil treatment test)

Soil treatments	Disease severity (%)	Plant height (cm)	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)
T114 only	----	43.42c	15.69bc	4.18bc	8.09bc	2.51bc
T191 only	----	42.40cd	16.10bc	4.29bc	7.91bc	2.46bc
T194 only	----	46.90b	17.12a	4.36b	8.66ab	2.69ab
T 112 only	----	51.15a	16.34bc	4.57a	9.07a	2.81a
Healthy control	----	41.25cd	15.48c	4.13c	7.89bc	2.45bc
T 191+KH	34.05e	35.15ef	11.78f	3.14f	6.30f	1.96e
T114+KH	39.8cd	37.15 e	13.36e	3.56e	6.6def	2.05de
T194+KH	31.64 e	36.47ef	14.38d	3.77d	6.80def	2.12de
T112KH	29.47e	40.27d	14.14d	3.84d	7.47cd	2.32cd
KH only	60.26b	34.72ef	10.00g	2.67g	4.44g	1.38f
T191SH	36.38cde	35.42ef	11.85f	3.16f	6.31f	1.96e
T114+SH	41.11c	35.80fe	12.95e	3.45e	6.78ed	2.11de
T194+SH	34.04de	37.12e	14.66d	3.84d	6.66ef	2.05de
T112+SH	31.77e	40.20d	14.38d	3.91d	7.32cd	2.28cd
SH only	76.25a	34.42f	9.66g	2.57g	4.28g	1.33f

Data represent the mean of 4 replicates, Significant differences are denoted by different letter(s) within each column according to Multiple Range Test at $p<0.01$. T112= *Trichoderma viride*, T194= *Trichoderma viride* (MO), T114=*T. harzianum*, T191= *T. harzianum* (M), KH= *Bipolaris sorokiniana* isolated from wheat field of Khomain, Iran, SH= *B. sorokiniana* isolated from wheat field of Shahzand, Iran

mycelial growth of Pathogen by means dual culture, cellophane overlay technique and volatile metabolite test. In most cases the effectiveness of *T. viride* T112 isolated from soil surrounding of wheat was superior to the other isolates and uniform among the above tests. Biocontrol activity of antagonist fungi and bacteria may partially be associated with production of antibiotic[9,18-20]. For example, the mode of action of *T. harzianum* appeared to be antagonism by the production Isonitrin, homothallin II, melanoxadin[18,21,22] and the other antibiotics Trichdermin[23], ergokonin[24], viridin[25,26] viridiofungin A, B and C[27] produced by different isolates of *Trichoderma viride* have been involved in biological control. However it has not been possible to extract these substances from *T. harzianum* and *T. viride* tested. The metabolites produced by *T. viride* T112 and *T. viride* T194 that provided biocontrol in these experiments is likely similar to metabolite produced by the other isolates tested by the other investigators mentioned above. Further research is needed to identify these metabolites and their properties.

T. viride and *T. harzianum* reduced disease severity of common root rot of wheat. Some investigator have tried to use *Tirichoderma* isolate and the other fungi for biocontrol of disease caused by *Cochliobolus sativus*[11] and *Drechslera sorokiniana* on wheat and rye leaves[28] and also successful antagonists against seed-borne *B. sorokinina* were *Chaetomium* sp., *Idriella bolleyi* and *Gliocladium roseum*[29]. Suppression of soil-borne fungi, including *B. sorokiniana* has been observed in the presence of Isothiocyanates released into soil by *Brassica* species[30]. Reduced symptoms could also be achieved, although not to be the full extent, after spraying with the bacterial biocontrol agent *Pseudomonas chloraphis* strain MA 342[31]. Hogdes *et al.*[3] indicated that *Pseudomonas* PSD-42 was antagonistic to *B. sorokiniana* on leaves of *Poa pratensis*. Plant height, fresh and dry weight of shoots and roots of wheat seedling from soil and seed treated with 2 isolates of *Trichoderma viride* plus *B. sorokiniana* were greater than those treated with pathogen alone. This study supports previous results showing that some *Trichoderma* isolates are capable of increasing plant growth and yield in green house[9,32-34].

Inoculation of soil and seed in the glasshouse with all four isolates of *Trichoderma* alone increased plant height, fresh and dry weight of shoots and roots compared with controls without *Trichoderma*. This is in accordance with results showing that some fungal isolates and bacterial strains are capable of promoting plant growth in greenhouse[4,35-37]. One of the mechanisms of disease suppression by *Trichoderma* isolates is competition with pathogen for infection site on the root surface. The degree of root colonization by the Plant Growth Promoting Fungal (PGPF) isolates depended upon the amount of pathogen inoculum present. The PGPF was found to colonize the epidermal and outer cortical cell layers of roots.

Another possible mechanism for suppression of common root rot could be induced resistance in wheat plants due to root colonization by PGPF isolates[4]. The production of pathogen inhibitory substance by *Trichoderma* during root colonization cannot be ruled out. Plant growth promotion due to PGPF has been attributed to the increase of mineral nutrient availability to roots during the growth activity of PGPF isolates in the rhizosphere. Induction of local or systemic resistance in wheat to pathogen following PGPF root colonization is yet another possible explanation for the suppression of common root rot. However the role(s) of other mechanisms in disease suppression cannot be rule out.

In conclusion *T. viride* T112 and *T. viride* (MO) tested here reduced severity in wheat in the glasshouse.

Future research will involve studies of the mechanisms involved. The isolates warrant further investigation for their ability to control common root rot of wheat, especially in commercial situations. An integrated approach using a combination of biocontrol agents and the fungicides which does not affect mycelial growth of these antagonists may allow reduction in the amount of fungicide needed to suppress common root rot.

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