

BIOLOGICAL CONTROL OF RICE BROWN SPOT WITH NATIVE ISOLATES OF THREE *TRICHODERMA* SPECIES

Elham Khalili^{1*}; Mehdi Sadravi²; Shahram Naeimi³; Vahid Khosravi⁴

¹Department of Plant Pathology, Gorgan University of Agriculture Science and Natural Resources, Gorgan, Golestan, Iran;

²Department of Plant Pathology, Yasouj University, Yasouj, Iran; ³Amol Biological Control Research Laboratory, Iranian Research Institute of Plant Protection, Tehran, Iran; ⁴Deputy of Rice Research Institute in Mazandaran, Amol, Iran.

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ABSTRACT

Brown spot caused by *Bipolaris oryzae* is an important rice disease in Southern coast of Caspian Sea, the major rice growing region in Iran. A total of 45 *Trichoderma* isolates were obtained from rice paddy fields in Golestan and Mazandaran provinces which belonged to *Trichoderma harzianum*, *T. virens* and *T. atroviride* species. Initially, they were screened against *B. oryzae* by antagonism tests including dual culture, volatile and nonvolatile metabolites and hyperparasitism. Results showed that *Trichoderma* isolates can significantly inhibit mycelium growth of pathogen *in vitro* by producing volatile and nonvolatile metabolites. Light microscopic observations showed no evidence of mycoparasitic behaviour of the tested isolates of *Trichoderma* spp. such as coiling around the *B. oryzae*. According to *in vitro* experiments, *Trichoderma* isolates were selected in order to evaluate their efficacy in controlling brown spot in glasshouse using seed treatment and foliar spray methods. Concerning the glasshouse tests, two strains of *T. harzianum* significantly controlled the disease and one strain of *T. atroviride* increased the seedling growth. It is the first time that the biological control of rice brown spot and increase of seedling growth with *Trichoderma* species have been studied in Iran.

Key words: Rice brown spot, Biocontrol, *Trichoderma harzianum*, *T. virens*, *T. atroviride*

INTRODUCTION

Rice (*Oryza sativa* L.) is among the most important cereals in Iran. On the basis of nutrition value, it is comparatively rated more than other cereals and plays a key role in nutrition (1). Rice brown spot caused by *Bipolaris oryzae* Breda de Hann (formerly, *Helminthosporium oryzae*) (Teleomorph: *Cochliobolus miyabeanus*) is occurred in all rice-growing areas of the world. The pathogen causes infection on all growth stages of rice plant from nursery to field and results in significant yield and grain quality losses. Rice brown spot was

a major factor for the "Great Bengal Famine" during 1942–1943 (27). In 1956, the disease was reported by Petrak at the first time from Iran and then it was reported by Sharif and Ershad from coastal area of Caspian sea in 1966 (16). The disease is prevalent in northern provinces of Iran and under environmental conditions conducive to disease; it can cause severe yield loss (30, 22).

Continuous, inappropriate and non-discriminative use of chemicals is known to cause undesirable effects such as residual toxicity, development of pathogen resistance to fungicides, environmental pollution, health hazards to humans

*Corresponding Author. Mailing address: MSc graduate student of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.; Tel: + 98 -171- 4426432 Fax: + 98 - 171- 4426432.; E-mail: elham.khalili_2007@yahoo.com

and animals and increased expenditure for plant protection. Instead, plant pathologists have focused their attention to develop environmentally safe, long-lasting and effective biocontrol methods for the management of plant diseases. Among various fungal and bacterial biocontrol agents, *Trichoderma* spp. was most frequently used against various plant diseases. Research during the previous two decades has led to the possibility of biological control as an increasingly realistic option for rice disease management (32). *Trichoderma* spp. has been shown to be effective for the control of brown spot disease and the increase of plant growth on rice (17). Rice plants sprayed with spore suspension of *T. harzianum* obtained a significant reduction in the severity of disease under greenhouse conditions (2). Also, *Trichoderma* species are able to colonize the root surface and rhizosphere from the treated seeds, protecting them from fungal diseases and stimulate plant growth and productivity (4). This study was accomplished to obtain indigenous *Trichoderma* isolates from paddy fields and to examine their biocontrol activities against *B. oryzae* *in vitro* as well as *in vivo* and also to evaluate their effects on rice growth parameters.

MATERIALS AND METHODS

Fungal isolates

In order to isolate *Trichoderma* spp., phyllosphere and soil samples were collected from paddy fields in Mazandaran and Golestan provinces from March to August 2007. Soil samples were taken at 0-30 cm depth. Isolation of *Trichoderma* from soil samples was performed by the soil dilution method (21). Ten grams of each soil sample was added to 90 mL distilled water on a shaker for one hour. Then, 10 mL aliquot from this suspension was transferred aseptically to 90 mL distilled water. This dilution process was repeated to get dilutions up to 10^{-5} . In the case of phyllosphere samples, rice stems and leaves were cut into 5 mm pieces and added to 150 mL distilled water on a shaker for 30 minutes. From each dilution suspension, one millilitre of aliquots (from soil and phyllosphere sample) was spread on RB-S-F selective medium

(9) in 9 cm Petri dishes. The dishes were incubated at $26 \pm 1^\circ\text{C}$ in the dark for 3-4 days. Putative *Trichoderma* isolates were purified using single spore technique.

For isolation of pathogen, naturally diseased leaves of different rice cultivars infected with varying degrees of brown spot were collected from nurseries and fields at different geographic locations in Mazandaran province, Iran. Eight *B. oryzae* isolates were obtained and purified using single spore technique (15, 30). For pathogenicity test 21-day-old rice seedlings of the most common cultivar in north of Iran *i. e.* Tarom, were grown under greenhouse conditions, and sprayed with spore suspension (4×10^5 spore mL^{-1}) of each *B. oryzae* isolate. Seven days after inoculation, the severity of brown spot disease was assessed following the standard evaluation system of International Rice Research Institute (20). The most aggressive *B. oryzae* isolate was selected for *in vitro* and *in vivo* tests of this study.

Identification of *Trichoderma* isolates

For morphological identification, *Trichoderma* isolates were grown on 2% malt extract agar under ambient laboratory conditions of light and temperature (about 21°C). Microscopic examination was carried out by mounting the culture in lactic acid 25%. Individual isolates were identified at the species level using morphological keys and fungal species descriptions (5, 6, 13, 35). For molecular identification, a nuclear rDNA region, containing the internal transcribed spacers 1 and 2 (ITS1 and 2) and the 5.8S rRNA gene was amplified using the primers ITS1 and ITS4 (34). PCR amplifications were performed as described previously (19). Amplicons were purified with the GenElute PCR Clean-up Kit (Sigma, USA) and sequenced at Macrogen Inc., South Korea. In order to identify the isolates at the species level, ITS sequence analysis was carried out with the aid of the program *TrichOkey 2.0* available online at <http://www.isth.info/>.

In vitro antagonism tests

Dual culture: Nine centimetres Petri plates containing potato dextrose agar (PDA) were inoculated with 5 mm

mycelial disks of either *B. oryzae* and *Trichoderma* isolates 10 mm away from the edge of the plate opposite to each other. Plates inoculated with *B. oryzae* alone served as control. Plates were incubated at $26 \pm 1^\circ\text{C}$ for seven days (12 h light/12 h darkness). The linear growth was measured. Three replicate plates were done for each treatment. The percentage of growth inhibition was calculated using the equation $\text{RI} = 100 \times (\text{R2} - \text{R1}) / \text{R2}$. Where RI was the percentage of reduction in mycelial growth, R1 was the averaged growth of pathogen in treated plates and R2 was the averaged growth of pathogen in control plates (12).

Production of volatile compounds: The bottoms of Petri dishes containing PDA were separately inoculated with mycelial plugs of pathogen and *Trichoderma* isolates. Then two bottoms held together with pathogen at top, sealed with adhesive tape and incubated at $26 \pm 1^\circ\text{C}$ in the dark for seven days. The radial growth of the pathogens was measured. Three replicates of each treatment were used (11).

Production of non-volatile compounds: A piece of autoclaved cellophane was placed on the surface of PDA medium in a 9 cm plate then a 7 mm disk of *Trichoderma* isolate was inoculated on the cellophane. The plates were incubated at $26 \pm 1^\circ\text{C}$ for 72 h. After the incubation period, the cellophane and the adhering *Trichoderma* mycelia were aseptically removed and the centre of each dish was inoculated with a 5 mm diameter disk of *B. oryzae* taken from an actively growing colony. The plates were incubated at $26 \pm 1^\circ\text{C}$ for a further five days. The control treatment was *B. oryzae* grown on PDA plate where previously there was a cellophane disc without antagonist. Three replicate plates were done for each treatment. The percentage of growth inhibition was calculated (12).

Mycoparasitism test: The slide culture method (28) was used to investigate the mycoparasitic nature of *Trichoderma* isolates against *B. oryzae*. A microscope glass slide covered with a thin layer of PDA in the agar plate was inoculated with

5 mm mycelial disks of *Trichoderma* spp. and pathogen, 1 cm apart from each other. All paired cultures incubated at 26°C and regions where the hyphae of *Trichoderma* isolates met the hyphae of the pathogen were periodically observed under a light microscope.

Evaluation of *Trichoderma* isolates on wet filter paper:

Based on *in vitro* antagonism test, 20 *Trichoderma* isolates were selected. Rice seeds were soaked in spore suspension of *B. oryzae* (10^5 spores mL^{-1}) containing 0.05% Tween 20 for 24 h. Then, seeds were air-dried and soaked in spore suspension of *Trichoderma* isolates (10^8 spores mL^{-1}) containing 0.05% Tween 20 for 2 h and then placed on the sterile wet filter paper in a seedling tray with 100 seeds per treatment (7). Plant height, root length and the percentage of disease control were determined. The number of infected seedlings was counted for each treatment and then compared to infected control with *B. oryzae*.

Glasshouse experiments

Seed treatment: According to wet filter paper test, 11 *Trichoderma* isolates were selected and used to inoculate infested rice seeds with pathogen and were sown in plastic pots containing autoclaved rice field soil. The pots were kept in glasshouse with the condition of 21 to 30°C temperature and 90% relative humidity. Three pots were used for each treatment. Rice seedlings growth parameters namely stem height, root length, stem and root wet weight, stem and root dry weight and percentage of disease control were determined 45 days after inoculation. Rice stem and root were dried in an oven at 80°C for 24 hours to a dry weight.

Foliar spray

Twenty one days old rice seedlings (cv. Tarom) were sprayed with spore suspension of 11 selected *Trichoderma* isolates (10^8 spore mL^{-1}) with 0.05% Tween 20 in greenhouse. After 24 h, the plants were sprayed with the spore suspension of *B. oryzae* (10^5 spore mL^{-1}). The seedlings which were sprayed with *B. oryzae* alone, served as control. Observations on the disease severity were recorded seven days after

inoculation of pathogen and the percentage of disease control was calculated for each individual treatment (12). The completely randomized design with three replications (pots) and five seedlings per pot was used.

Statistical analysis

The completely randomized design with three replications per treatment was used. Data were analyzed with statistical analysis software (6.0 SAS, 1999). All data were first subjected to analysis of variance (ANOVA). Means were compared using Duncan's multiple range test at $P = 0.01$.

RESULTS

A total of 45 *Trichoderma* isolates were obtained which

belonged to three species namely *Trichoderma harzianum*, *T. virens* and *T. atroviride*. The percentage of the total *Trichoderma* isolates obtained from the rice phyllosphere and soil samples were 63 and 38 percent, respectively. The most frequent species was *T. harzianum* with 31 isolates (69%). Eleven and three isolates belonged to *T. virens* and *T. atroviride*, respectively.

Based on the pathogenicity test, *B. oryzae* 3 (from a rice field in Amol, Mazandaran) showed highest disease severity, and this most aggressive isolate was used for all subsequent studies.

Data from dual culture tests showed that although all 45 *Trichoderma* isolates inhibited the mycelium growth of pathogen, there were significant differences among them at $P = 0.01$. *T. harzianum* isolates 38, 4, 27, 35, 20 and 7; *T. virens* isolates 8 and 13 and *T. atroviride* isolate 3 substantially reduced the growth of the pathogen (Table 1).

Table 1. *In vitro* effects of *Trichoderma* isolates on mycelial growth of *Bipolaris oryzae*.

The percentage of reduction in mycelial growth			
Non-volatile metabolite	Volatile metabolite	Dual culture	Isolates
84.5 b	70.2 abcd	69.1 cdef	<i>T. harzianum</i> 1
52.7 qr	64.8 cdefg	76.6 abcd	<i>T. harzianum</i> 4
56.0 pq	70.7 abc	70.0 cdef	<i>T. harzianum</i> 6
74.3 cde	72.4 ab	71.9 abcde	<i>T. harzianum</i> 7
70.8 efg	65.5 abcdefg	54.4 ijk	<i>T. harzianum</i> 9
100.0 a	61.0 bcdefghi	50.8 ijk	<i>T. harzianum</i> 10
61.8 lmn	67.7 abcde	53.2 ijk	<i>T. harzianum</i> 11
100.0 a	56.3 efghijk	64.4 efgh	<i>T. harzianum</i> 15
58.2 nop	61.0 bcdefghi	66.3 efgh	<i>T. harzianum</i> 19
70.2 efg	43.6 lm	71.9 abcde	<i>T. harzianum</i> 20
63.6 klm	47.1 jkl	65.4 efgh	<i>T. harzianum</i> 21
74.4 cde	65.2 abcdefg	68.2 def	<i>T. harzianum</i> 22
73.0 def	63.3 abcdefgh	71.0 bcdef	<i>T. harzianum</i> 23
60.7 mno	63.3 abcdefgh	69.1 cdef	<i>T. harzianum</i> 24
76.9 cd	69.0 abcd	57.9 hij	<i>T. harzianum</i> 26
65.4 hijkl	69.9 abcd	71.9 abcde	<i>T. harzianum</i> 27
76.1 cd	56.6 defghijk	67.2 efg	<i>T. harzianum</i> 28
63.0 klm	58.5 cdefghij	64.4 efgh	<i>T. harzianum</i> 29
74.0 cde	60.4 bcdefghi	66.4 efgh	<i>T. harzianum</i> 30
72.9 def	53.8 fghijkl	66.3 efgh	<i>T. harzianum</i> 31
48.8 r	64.6 abcdefgh	67.2 efg	<i>T. harzianum</i> 32
57.4 op	65.8 abcdef	65.4 efgh	<i>T. harzianum</i> 33
65.4 hijkl	67.1 abcde	71.9 abcde	<i>T. harzianum</i> 35
69.0 fghi	63.9 abcdefgh	68.2 def	<i>T. harzianum</i> 36
73.9 cde	63.9 abcdefgh	69.1 cdef	<i>T. harzianum</i> 37
69.3 fgh	52.5 hijkl	79.4 a*	<i>T. harzianum</i> 38
63.9 jklm	46.8 jkl	65.4 efgh	<i>T. harzianum</i> 40
67.9 ghij	60.1 cdefghi	64.4 efgh	<i>T. harzianum</i> 42
65.0 ijkl	30.9 on	68.2 def	<i>T. harzianum</i> 43
62.8 klm	64.5 abcdefgh	68.2 def	<i>T. harzianum</i> 44
51.4 r	58.4 defghij	65.4 efgh	<i>T. harzianum</i> 45
100.0 a	53.4 ghijkl	50.3 jk	<i>T. virens</i> 2
100.0 a	35.8 mn	58.9 ghi	<i>T. virens</i> 5
100.0 a	46.2 klm	77.5 abc	<i>T. virens</i> 8
100.0 a	54.4 fghijkl	52.2 ijk	<i>T. virens</i> 12
100.0 a	50.9 ijkl	71.9 abcde	<i>T. virens</i> 13

77.6 c	54.1 fghijkl	62.6 fgh	<i>T. virens</i> 14
76.9 cd	66.7 abcde	50.8 ijk	<i>T. virens</i> 16
69.7 fg	56.6 efg hijk	53.2 ijk	<i>T. virens</i> 18
76.6 cd	56.6 efg hijk	66.3 efgh	<i>T. virens</i> 25
66.6 ghijk	22.1 o	59.0 ghi	<i>T. virens</i> 39
100.0 a	62.6 bcdefghi	66.3 efgh	<i>T. virens</i> 41
42.6 s	21.8 o	78.4 ab	<i>T. atroviride</i> 3
64.9 ijklm	66.7 abcde	48.5 k	<i>T. atroviride</i> 17
100.0 a	75.0 a	69.1 cdef	<i>T. atroviride</i> 34

* Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.01)

* Each value represents the mean of three replicates.

Volatile metabolites of *Trichoderma* isolates also had inhibitory effects on the growth of *B. oryzae*. The most effective isolates were *T. harzianum* 4, 27, 35, 7, 23, 6, 24, 37, 1, 22, 44, 36, 32, 33, 26, 9 and 11; *T. virens* 16 and *T. atroviride* 34 and 17 (Table 1).

All tested *Trichoderma* isolates released non-volatile compounds that diffused through the cellophane membrane onto the agar medium, resulting inhibition growth of pathogen. The highest reduction in the mycelial growth of the pathogen (100%) was recorded for *T. harzianum* 15 and 10; *T. virens* 8, 13, 41, 5, 12, 2 and *T. atroviride* 34 (Table 1).

Light microscopic observations showed no evidence of mycoparasitic behaviour of the tested isolates of *Trichoderma* spp. such as coiling around the *B. oryzae*.

In wet filter paper test, significant differences were observed in plant height, root length and the percentages of disease control between treated and untreated rice seeds. The highest disease control was recorded for *T. harzianum* 7, 4, 1, 43, 38, 23, and 28; *T. atroviride* 17 and 3. Furthermore, *T. harzianum* isolates 7, 4, 43, 38, 23, 28, 27, 22, 20, 32, and *T. virens* isolate 8 showed increasing effects on the rice seedling growth (Table 2).

Table 2. Effect of *Trichoderma* isolates in controlling brown spot as well as on growth factors of rice seedlings in wet filter paper test.

Isolates	Disease control (%)	Stem height(cm)	root length (cm)
<i>T. harzianum</i> 1	59.0 ab	5.7 bcdefg	5.1 abcd
<i>T. harzianum</i> 4	60.3 a	6.6 abc	5.3 abc
<i>T. harzianum</i> 7	60.8* a	6.6 ab	5.5 ab
<i>T. harzianum</i> 20	40.2 efgh	6.7 ab	5.8 a
<i>T. harzianum</i> 22	41.2 defgh	6.4 abcd	5.7 a
<i>T. harzianum</i> 23	52.7 abcd	6.6 abc	5.1 abcd
<i>T. harzianum</i> 24	22.2 j	5.4 bcdefg	4.3 cde
<i>T. harzianum</i> 27	45.2 cdef	7.5 a	4.2 cde
<i>T. harzianum</i> 28	52.2 abcd	7.3 a	4.0 e
<i>T. harzianum</i> 32	35.7 fghi	6.4 abcd	4.5 bcde
<i>T. harzianum</i> 35	46.0 cdef	5.3 cdefg	4.8 abcde
<i>T. harzianum</i> 36	43.6 cdefg	5.9 bcdef	4.4 cde
<i>T. harzianum</i> 37	26.0 ij	5.2 defg	5.1 abcd
<i>T. harzianum</i> 38	54.8 abc	7.5 a	4.5 bcde
<i>T. harzianum</i> 43	58.2 ab	6.7 ab	5.5 ab
<i>T. harzianum</i> 44	31.5 hij	6.7 ab	4.3 cde
<i>T. virens</i> 2	33.5 ghi	5.4 bcdefg	4.4 cde
<i>T. virens</i> 8	35.9 fghi	6.2 abcde	5.1 abcd
<i>T. virens</i> 13	47.8 bcde	5.8 bcdef	5.0 abcde
<i>T. atroviride</i> 3	50.0 abcde	5.2 defg	5.1 abcd
<i>T. atroviride</i> 17	50.7 abcde	5.0 efg	4.8 abcde
<i>T. atroviride</i> 34	48.0 bcde	4.5 g	4.1 ed
Inoculated control(with <i>B. oryzae</i>)	—	4.8 fg	5.1 abcd
Non-inoculated control	—	5.9 bcdef	5.5 ab

* Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.01)

* Each value represents the mean of three replicates.

Seed treatment with *Trichoderma* isolates significantly decreased rice brown spot in glasshouse. *T. harzianum* 1 and 20 were the most effective isolates. In the case of growth factors, highest values were recorded for *T. atroviride* isolate 3 (Table 3).

Foliar spray with selected *Trichoderma* isolates in glasshouse showed that all treatments were significantly different in controlling the disease and *T. harzianum* 20 was the most effective isolate (Table 4).

Table 3. Effects of treatment of rice seeds by selected *Trichoderma* isolates on brown spot and growth characteristics of rice seedlings in glasshouse *.

Root dry weight (g)	Stem dry weight (g)	Root wet weight (g)	Stem wet weight (g)	Root length (cm)	Stem height (cm)	Disease control (%)	Isolate
0.2 e	0.2 f	0.8 ghi	1.1 fgh	16.7 a	48.4 de	61.2 a	<i>T. harzianum</i> 1
0.5 bc	0.4 bcd	2.0 ab	2.3 b	18.1 a	65.4 abcd	32.3 dce	<i>T. harzianum</i> 4
0.4 c	0.3 bcde	1.2 defg	1.4 defg	19.8 a	53.2 abcde	45.3 bc	<i>T. harzianum</i> 7
0.2 e	0.2 f	0.7 hi	0.9 gh	16.6 a	46.5 e	58.1 a	<i>T. harzianum</i> 20
0.4 cd	0.4 bc	1.3 def	1.9 bcd	20.6 a	67.1 abc	20.8 e	<i>T. harzianum</i> 27
0.3 e	0.3 bcde	0.5 i	0.8 h	17.8 a	54.7 abcde	50.3 ab	<i>T. harzianum</i> 28
0.4 cd	0.4 ab	1.6 bcd	2.1 bc	17.9 a	68.0 ab	34.7 cd	<i>T. atroviride</i> 34
0.3 de	0.3 def	1.2 defg	1.2 efgh	17.3 a	50.3 cde	44.7 bc	<i>T. harzianum</i> 38
0.6 a	0.3 bcde	1.8 bc	1.7 cde	17.5 a	60.5 abcde	34.6 cd	<i>T. harzianum</i> 43
0.3 de	0.3 bcde	1.1 efgh	1.4 defg	19.6 a	53.2 abcde	39.4 bc	<i>T. virens</i> 8
0.6 ab	0.5 a	2.3 a	3.0 a	20.4 a	69.6 a	24.0 de	<i>T. atroviride</i> 3
0.2 e	0.2 fe	1.0 fgh	1.2 efgh	15.8 a	50.3 cde	—	Infected check (with <i>B. oryzae</i>)
0.4 c	0.3 bcde	1.5 cde	1.6 cdef	17.0 a	59.2 abcde	—	Healthy check

* Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.01)

* Each value represents the mean of three replicates.

Table 4. Control of rice brown spot after foliar spray of *Trichoderma* isolates on Tarom cultivar *.

Treatment	Disease control (%)
<i>T. harzianum</i> 1	48.9 b
<i>T. harzianum</i> 4	35.0 ef
<i>T. harzianum</i> 7	44.7 bcd
<i>T. harzianum</i> 20	59.6 a
<i>T. harzianum</i> 27	32.2 f
<i>T. harzianum</i> 28	38.7 cdef
<i>T. atroviride</i> 34	43.6 bcde
<i>T. harzianum</i> 38	46.1 bcd
<i>T. harzianum</i> 43	37.4 def
<i>T. virens</i> 8	<i>T. virens</i> 8
<i>T. atroviride</i> 3	<i>T. atroviride</i> 3

* Values within a column followed by the same letter(s) are not significantly different according to Duncan's multiple range test (P = 0.01)

* Each value represents the mean of three replicates.

DISCUSSION

The majority of isolates (90%) recovered from the soil identified as *T. harzianum* and *T. virens* which is in agreement with the findings of Naeimi *et al.* (25). In addition, fungal species belonging to the genus *Trichoderma* are worldwide in

occurrence and they have been widely reported from rice fields in other countries such as Philippine, China and Bangladesh (23, 26, 36, 29). The results of the present study showed that the *Trichoderma* isolates could easily adopt and survive in irrigated rice ecosystems of northern Iran and this gives hope for future application of the promising native strains in paddy

fields in order to control brown spot and other rice diseases.

In vitro antagonism tests revealed that the native isolates of *Trichoderma* spp. significantly inhibited the mycelial growth of *B. oryzae* in several ways. The most effective isolates were belonged to *T. harzianum*. It is clear from the previous results that competition plays an important role in the *T. harzianum* and *B. oryzae* interaction (2, 17). *Trichoderma* isolates inhibited the growth of the target organisms through its ability to grow much faster than the pathogenic fungi thus competing efficiently for space and nutrients. Angelica *et al.* (3) reported that inhibition of pathogen growth is due to production of amylase by *Trichoderma* spp., which is partially responsible for the rapid growth of antagonists on potato dextrose agar medium. In addition to amylase, it has been reported that *Trichoderma* species also produce extracellular cellulose and pectinase enzymes that are capable of hydrolyzing the cell walls of other fungi (24). A comparison between the inhibition effects of volatile and nonvolatile metabolites of our *Trichoderma* isolates revealed that the nonvolatile metabolites seemed to be more effective. In a similar study, Abdel-Fattah *et al.* (2) also reported that the antifungal metabolites of *T. harzianum* strains completely inhibited the linear growth of *B. oryzae*. Gary *et al.* (14) identified five classes of volatile compounds, such as alcohols, esters, ketones, acids and lipids, produced by some fungi and bacteria. A large number of volatile and non-volatile antifungal substances such as diterpenes, peptaibols, butenolides, furanones, pyrones, and pyridones are produced by *T. harzianum* which have stimulus influence on plant growth and development. *Trichoderma* antifungal substances are also able to arrest the hyphal growth of different fungal pathogens. It is believed that these enzymes and antibiotics act synergistically on the host (8).

Light microscopic observations revealed that mycoparasitism did not appear to contribute to the aggressive nature of our *Trichoderma* isolates against *B. oryzae* which is in agreement with the findings of Abdel-Fattah *et al.* (2).

Results from seed treatments and foliar spray, demonstrated that significant reduction in disease severity is observed when *T. harzianum* 1 and 20 were applied in

glasshouse. In addition, *Trichoderma atroviride* isolate 3 had substantial influence on plant growth and development. Similarly, Harish *et al.* (17) stated that spraying of spore suspension of *Trichoderma* isolates on rice plant significantly inhibited the growth and spore germination of *B. oryzae* besides increasing seedling growth. There are high levels of nutrients in the rhizosphere and pathogen and the introduced biocontrol agent (antagonist) compete for the availability of space and nutrients. *Trichoderma* spp. has been reported to be more rhizosphere competent than most soil fungi (32). Abundant production and germination of conidia by *Trichoderma* spp. and their rapid mycelial growth may suppress the growth of the pathogen population in the rhizosphere and thus reduce disease development. Promotion of plant growth induced by *Trichoderma* spp. appeared to be due to both the control of minor pathogens and production of growth-regulating factors. A number of plant-associated microbes were found to produce poisonous compound cyanide which causes reduction of the plant growth. *Trichoderma* spp. could produce enzymes which detoxify waste cyanide and this process lead to increase in plant growth. Recent advances demonstrate that the effects of *Trichoderma* on plants, including induced systemic or localized resistance, are also very important. These fungi colonize the root epidermis and outer cortical layers and release bioactive molecules that cause to induction of pathways for resistance in plants, increased plant growth and nutrient uptake occurs (4, 188).

This study has demonstrated that a number of naturally occurring fungal isolates can inhibit growth of *B. oryzae*. Furthermore, this research raises some interesting possibilities for future research. These include testing whether the antagonist can show the same level of efficacy under natural field conditions; testing whether mixtures of biocontrol agents are more effective than a single strain on control of disease severity and testing for preparing the best formulation of promising *Trichoderma* isolates.

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