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Efficacy of *Trichoderma* spp as Biocontrol Agents against Rice Brown Spot Disease and Biochemical Approaches

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ABSTRACT

Fourteen isolates of *Trichoderma* spp were tested for their efficacy in controlling *Bipolaris oryzae* pathogen of rice brown spot disease in vitro and in vivo. Isolates of *T. hamatum* 4, *T. viride* 1, *T. harzianum* 1, 2 and 3 and *T. virens* were the best, recording 100% inhibition of the pathogen radial growth. Treatments of soil, and foliar spray with culture filterates of these antagonistic isolates were undertaken in the greenhouse and field during 2015 and 2016 seasons. A reduction in disease severity as well as an increment in chlorophyll content, POX, PPO and PAL enzymes activity and total phenol content were observed. *T. viride*1 and *T. koningii* were able to produce Indole Acetic Acid (IAA) with concentrations of 8.281, 1.359 ppm, gibberellic acid (GA_3) 70.176, 41.052 ppm and abscisic acid (ABA) 1.649, 0.45 ppm, respectively. Foliar application of the cultural filtrates of *T. harzianum* 1 and *T. viride* 1 gave the highest concentration of IAA (20.697 and 11.916 ppm, respectively) in rice seedlings. Under field conditions, soil treatments with *T. viride*1 were more effective in reducing both brown spot percentage and disease severity similar to the effect of Del cup (Copper Sulfate Pentahydrate) fungicide in the two seasons.

INTRODUCTION

Rice is one of the most important crops on which most of the world's population depend as a main source of energy. This need has more than doubled (e.g. 150 million tones in 1961 and 350 million tones in 2011) during the last five decades (IRRI, 2013). Rice brown spot disease is caused by the necrotrophic fungus, *Cochilobolus miyabeanus* (teleomorph) or *Bipolaris oryzae* (anamorph). It is one of the most divastating and prevalent diseases of rice (Ou 1985). Rice brown spot impairs grain quality and results in about 67% yield reduction (Jones *et al*., 1993). Treating rice plants with *Trichoderma* spp. has been shown to be effective for the control of brown spot disease and the increase of plant growth (Harish *et al*., 2007), enhance rice germination, vigor, seedling growth, vegetative growth, photosynthetic rate, stomatal conductance, internal CO_2 concentration and water use efficiency (Doni *et al*. 2014a; Doni *et al*., 2014b). Spraying rice plants with spore suspension of *T. harzianum* significantly reduces the severity of brown spot disease under greenhouse conditions (Abdel-Fataah *et al*., 2007). Also, *Trichoderma* spp are able to colonize the root surface and rhizosphere of treated seeds, protecting them from fungal diseases, stimulate plant growth and productivity (Baker, 1988). *Trichoderma* spp. have been exploited as plant growth enhancers and protectors against pathogen. *Trichoderma* spp. encourages rice plants to grow more with high productivity (Doni *et al*., 2013). *Trichoderma* spp. enhance some phytostimulation mechanisms such as improving root development and auxin production, increasing drought and salt tolerance (Shukla *et al*., 2012 and Contreras- Cornejo *et al*., 2014), expressions of defense protein within the plant (Thakur and Sohal, 2013). Chinmay, *et al*. (2010) reported that seed treatment with *T. viride* and *T. hazianum* reduces severity of brown spot disease. The treatments with bioagent as inducers also promoted the crop growth i.e. increasing in the lateral root development, the fresh root, biomass and chlorophyll concentration (Lee *et al*. , 2016). An increase in the content of endogenous defence and growth related plant hormones can be found when plants are treated with *Trichoderma* fungus due to the interaction between plant and the fungus. Treatment with T22 in cherry rootstocks 10 days after inoculation increases the content of IAA and GA_3 in leaves and in roots (Sofo *et al*. 2011). Several mechanisms may be interconnected in a complex network of cross-communicating plant hormone signaling pathways, as a result on plant growth and defense responses, (Mart´ınez-Medina *et al*. 2010, 2014)

The aim of this work was to evaluate and compare the efficacy of different isolates of *Trichoderma* spp in reducing brown spot disease incidence. Also, the physiological changes in rice plants were assessed.

MATERIALS AND METHODS

Laboratory, greenhouse and field studies:

Experiments were conducted at the Rice Pathology Department, laboratory and greenhouse at the Rice Research & Training Center (RRTC), while field experiment were performed at Sakha Agricultural Research Station, Egypt.

Pathogen inoculum preparation:

Isolate of rice brown spot fungus was obtained from Kalboush, 2007. The fungus was identified morphologically and microscopically as *Bipolaris oryzae* according to Barnett and Hunter (1972). The fungus was cultured in petri dishes containing PDA medium, and incubated at 26±2°C until full growth. Plates were exposed to continuous fluorescent light for two days to enhance sporulation. Inoculum of spore suspension was prepared by adding 10 ml sterilized water in each dish. Mycelia mats were gently scraped by spatula and filtered through cheese cloth. Spore suspension was adjusted to 10^5/ml.

Antagonistic isolates sources and preparation:

Fourteen antagonistic fungi i.e. *T. koningii, T. hamatum* (1,2,3 and 4), *T. verins, T. delyenses, T. viride* (1, 2 and 3) and *T. harzianum* (1, 2, 3 and 4) were kindly obtained from Kalboush, 2007. The isolates were identified at Laboratory of Mycology, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. Isolates of the antagonistic fungi and the pathogen were grown on PDA for 7 days at 26±2°C.

Antagonistic effect of *Trichoderma* isolates against *Bipolaris oryzae*:

The efficiency of the antagonistic fungal isolates in inhibiting the growth of *B.oryzae* was tested using the dual culture technique. PDA medium was poured into 9- cm Petri dishes, after solidification, plates were inoculated with 0.6 cm discs of *B.oryzae* at the periphery. Two days later, the same plates were inoculated with 0.6 cm discs of the antagonistic fungal isolates on the opposite sides. Four replicates were done for each treatment. Plates inoculated with the pathogen only were saved as a control treatment. Plates were incubated at 26±2^{o}C till full growth of the control treatment. The antagonistic activity was scored according to the scale developed by Alfredo and Aleli (2011) that involves four degrees: (+++), the antagonistic fungus was able to grow over the pathogen and pathogen growth was completely inhibited. (++), the pathogen growth was completely inhibited, but antagonist was not able to grow over the pathogen. (+), mutual inhibition initially, but antagonist was overgrown on pathogen. Finally, the pathogen growth was not inhibited, the antagonist was overgrown by pathogen

Effect of antagonistis on rice and brown spot disease incidence under greenhouse conditions:

A complete randomized design was performed in this experiment with eight antagonistic fungi. Methods of application and treatments were as follows:

1- Soil treatment:

The eight antagonistic fungal isolates were grown in 9 cm Petri dishes containig PDA medium and incubated for seven days at 26±2^{o}C. Thereafter, they were grown in a corn medium as a carrier for spores. Flasks of a sterilized corn medium (250g), were inoculated with the eight antagonistic isolates and then incubated at 26±2^{o}C for ten days. The dosage of antagonistic formulation was set at 5 g per 1 kg of autoclaved soil (Doni *et al*., 2014). The inoculated soil was placed in a 15 × 15 cm plastic trays. Seeds of Giza 177 rice cultivar were surface sterilized before planting by immersing in 0.5 % sodium hypochlorite solution for two minutes, then rinsed thoroughly with sterilized distilled water. Seeds were soaked in water for two days at 30^{0}C before sowing.

2- Foliar application:

PD broth medium was used for growing antagonistic fungi. The bioagent isolates were inoculated in 250 ml flasks containing 100 ml of PD broth medium and incubated at 26±2^{o}C for 7 days with continuous shaking. Colonized medium was filtered through a sterilized membrane (0.45µm mesh) (Lifshitz *et al*., 1986). Rice seedlings of 21- day old were sprayed with the filtrated cultural media of the eight trichoderma isolates, two days before inoculation with the pathogen. This study was performed to observe the effect of antagonistic fungi on the rice brown spot disease and changes of hormones. The recommended fungicide (Copper Sulfate Pentahydrate 5ml / L) was used as a control treatment to brown spot disease

Pathogensity test:

Giza 177 rice seedlings of 21- day old were sprayed with a pathogen spore suspension containing 10^5 spores/ml. Non inoculated plants were sprayed with water containing the same amount of Tween 20 (0.02%). Both inoculated and non-inoculated plants were kept in a moist chamber with 98−100% relative humidity for 24 hours and then transferred to the greenhouse. Sterilized seeds were planted in plastic boxes (15x15 cm diameter). Three replicates were used. Data was recorded 5 days after infection, when the rice plants reached the third leaf stage.

Disease assessment:

Brown spot infection was assessed as a percentage by counting the number of infected leaves of 100 randomly selected leaves per pot 5 days after infection. Disease severity was calculated as a total number of brown lesions/100 infected leaves, Kalboush (2007).

Biochemical studies:

The effect of using *Trichoderma* isolates as soil and foliar treatments on the biochemical changes (i.e. enzyme ctivities, total phenols, chlorophyll content and hormones) was determined in healthy and inoculated plants with *B. oryzae*. Determinations were carried out in both healthy and inoculated plants at 1 and 2 days at seedling stage. Seedlings of the Giza 177 rice cv. were artificially inoculated with spore suspension of *B. oryzae* (1 x 10^5 condia/ml).

Enzyme activities:

Rice leaves of Giza 177 rice cultivar were collected 24 and 48 hr after pathogen inoculation, quickly frozen in liquid nitrogen and stored at -20°C as powder samples.

POX activity:

Powder samples (1 g) were homogenized in 3 ml of 0.1 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 10,000 rpm at 5°C for 15 min and used within 2 to 4 h. Supernatant served as an enzyme source for POX and PPO. POX enzyme activity was determined according to the methods described by Allam and Hollis (1972) and Srivastava (1987). POX activity was expressed as changes in absorbance (optical density per 1 min/0.5g sample, OD/min/0.5g). The absorbance was measured at 425 nm, and recorded at 0, 1, 2, 3, 4 and 5 min. intervals using a spectrophotometer (Milton Roy, Spectronic, 1201 Digital). Three replicates were maintained for each treatment.

PPO activity:

The enzyme was determined according to the method adopted by Matta and dimond (1963). PPO activity was expressed as changes in absorbance (optical density/ min/0.5g), the absorbance was measured at 495 nm, and recorded at 0, 1, 2, 3, 4 and 5 min intervals using a spectrophotometer.

PAL activity:

PAL enzyme was assayed in powder samples prepared from leaves according to the method described by Zucker (1968). The enzyme activity was expressed as optical density at 290 nm (t – cinnamic acid) per 0.1 g sample powder.

Estimation of phenolic substances:

One gram of fresh sample was homogenized with 10 ml of 80% methanol and agitated for 15 ml at 70°C (Zieslin and Ben-Zeken., 1993), the absorbance of the blue was measured using a spectrophotometer (Milton Roy, Spectronic, 1201 Digital) at 725 nm and catachol was used as the standard (Kagale *et al*., 2004).

Chlorophyll content:

Total chlorophyll content of leaves was determined in mg using a chlorophyll meter (SPAD-502) (Kalboush, 2007).

Assay of hormones:

Gibberilic acid (GA3), indole acetic acid (IAA) and abscisic acid (ABA) hormones were estimated in both of filtered cultural media of two Trichoderma isolates (T.

viridi 1 & T. koningii) and samples of rice seedling treated with four Trichoderma isolates. The method of extraction was essentially similar to that adopted by Shindy and Smith (1975) and described by Hashem (2006). All the extraction and quantification of plant hormones was based on Food Technology Res. Institute, Agric. Res. Center (Hassanein *et al*, 2009).

Field experiment:

The effect of soil and foliar treatments of eight antagonistic agents on brown spot disease was studied. An experiment was performed during 2015& 2016 seasons under natural infection. Giza 177 rice cv. was used. Complete randomized block design with 9 m2 plot units (3x3 m) with four replicates was used. Corn media inoculated with each of the eight antagonistic trichoderma isolates was mixed in the soil. Rice seedlings, 30 days after transplanting, were sprayed by the cultural filterate media of the eight antagonistic fungi. Seeds of control (treated with tap water) were soaked in water for two days , then drained off and incubated for two days before sowing. Disease severity, % disease incidence and some agronomic characters (i.e. plant height, panicle length, number of discolored grain/panicle, number of healthy grain/ panicle and grain yield) were recorded.

Data Analysis: Data were statistically analyzed using standard statistical analysis with MSTATC. in the table of main treatments, Duncan's Multiple Range, T. (1955) was used to compare the significantly different averages.

RESULTS

Antagonistic effect of *Trichoderma* isolates against *B. oryzae*:

The fourteen isolates had variable efficiencies in inhibiting radial growth of Bipolaris oryzae (Table 1). The best antagonistic results were obtained by Trichoderma hamatum 4, T. viride 1, T. harzianum 1, T. harzianum 2, T. harzianum 3 and T. verins. These isolates scored high level (+++) in inhibiting the pathogen growth. These isolates prevented the growth of B. oryzae with 100% over growth as illusrtated in Fig. 1. Some of these isolates (T. hamatum 1, T. koningii, T. hamatum 3, T. viride and T. harzianum) caused moderate inhibition (++), while others (T. hamatum 2, T. delyenses and T. viride 2) resulted in low levels of inhibition (+).

Table 1. In vitro antagonistic activity of *Trichoderma* spp against *Bipolaris oryzae*.

No.	Treatments	Degree of antagonism
1	*T. koningii*	++
2	*T. hamatum 1*	++
3	*T. hamatum 2*	+
4	*T. hamatum 3*	++
5	*T. hamatum 4*	+++
6	*T. verins*	+++
7	*T. delyenses*	+
8	*T. viride*	++
9	*T. viride 1*	+++
10	*T. viride 2*	+
11	*T. harzianum*	++
12	*T. harzianum 1*	+++
13	*T.harzianum 2*	+++
14	*T. harzianum 3*	+++

***Mean of four plates (9- cm diameter) were used as replicates for each treatment.**

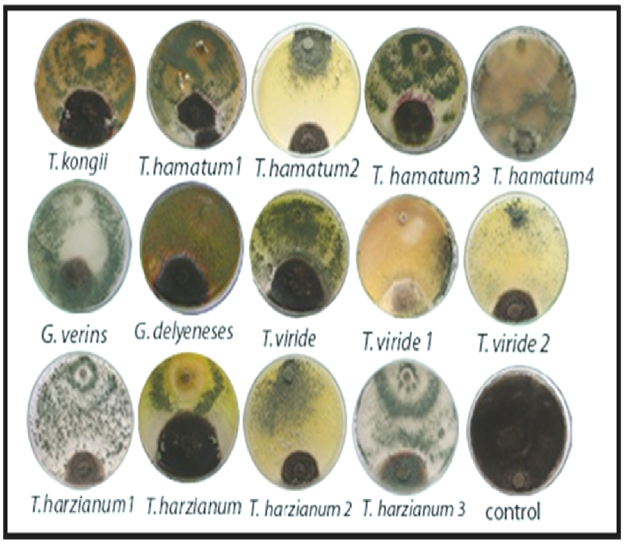

Fig. 1. Effect of different antagonistic fungi on growth of *B. oryzae*.

Effect of antagonistic fungi on rice brown spot disease incidence under greenhouse conditions:

1- Soil treatment:

Data in Table (2) show the effect of inoculated soil with the eight *Trichoderma* isolates on brown spot disease under greenhouse conditions. All the tested antagonistic fungi were effective against the pathogen, but following to the fungicide. *T. viride 1* and *T. hamatum* 4 were the best in this respect with efficiency of 83.84 and 83.14%, respectively followed by *T. koningii, T. hamatum 3* and *T. harzianum 3*, as they reduced the disease incidence & severity compared with the inoculated only. As regards to the soil treatment with *T. viride 1, T. hamatum 4, T. koningii, T. hamatum 3* and *T. harzianum 3*, data presented in Table (2) showed also the efficincy of these isolates in increasing the chlorophyll content and seedling length compared to the check treatment (inoculated only).

2- Foliar application:

The efficiency of cultural filterate media of the different antagonistic fungi obviously increased with spraying, two days before inoculation with spore suspension of the pathogen as showen in Table (3). *Trichoderma viride 1* and *T. harzianum 2* recorded the highest efficincy (92.76 & 91.34%, respectively). Chlorophyll content and seedling length increased with decreasing the disease severity.

Biochemical studies:

Effect of soil and foliar treatments with antagonistic fungi on enzyme activities and total phenols in rice plants infected with *B. oryzae*

All treatments with *Trichoderma* showed the highest values for all measured enzymes. Data presented in Figures (2 and 3) showed gradual increase in POX, PPO and PAL activities as well as total phenol contents in inoculated plants with the pathogen. However, the increment in the activity of each of POX, PPO, PAL as well as total phenol contents in inoculated plants treated by the tested antagonistic fungi were higher than in the untreated ones. The maximum increase in POX, PPO, PAL activities and total phenol content was recorded at one day after inoculation with the pathogen, and then significant decrease was observed. *T. viride 1* and *T. koningii* were the most effective antagonists, causing higher reductions in brown spot disease than the untreated inoculated control. Activity of all enzymes in healthy plants may be due to their presence in healthy plant tissues as constitutive enzymes.

Assay of hormones:

Trichoderma viride 1 and *T. koningii* were able to produce gibberellic acid (GA_3) with concentration of 70.176- 41.052 ppm, indole acetic acid (IAA) 8.281- 1.359 ppm, and abscisic acid (ABA) 1.649- 0.45 ppm, (Table 4) .

Table 2. Effect of soil treatment with antagonistic fungi on brown spot severity and chlorophyll content of Giza 177 rice cv. grown in greenhouse in 2015 season

Antagonistic fungi	Soil application				
	Disease incidence	Disease Severity	Efficiency	Chlorophyll content (SPAD) value)	Seedling length/cm
T. koningii	13.34 e	30.00 d	78.70	23.75 ab	41.33 def
T. hamatum 3	13.34 e	33.34 d	76.57	18.55 cd	44.00 cd
T. hamatum 4	10.00 e	24.00 e	83.14	23.90 ab	49.34 a
T. verins	54.00 b	70.00 b	50.82	20.50 bc	40.00 ef
T. viride 1	12.00 e	23.00 e	83.84	26.40 a	47.34 ab
T. harzianum 1	48.34 c	73.34 b	48.47	22.20b c	41.34 ef
T. harzianum 2	21.67 d	40.00 c	71.90	23.80 ab	44.67 bc
T. harzianum 3	15.00 e	33.34 d	76.57	23.40 ab	43.00 def
Del cup	5.000 f	10.00 f	92.76	18.90 cd	41.00 ef
Inoculated only	100.0 a	142.34 a	-	16.70 d	35.67 g
Tap water	0.000 f	0.000 g	-	18.70 cd	38.34 g

In a column, means followed by a common letter are not significantly different at the 5% level byDMRT

Table 3. Effect of foliar application with filtertes of certain antagonistic fungi on brown spot severity and chlorophyll content of Giza 177 rice cv. grown in greenhouse in 2015 season

Antagonistic fungi	Foliar application				
	Disease incidence	Disease Severity	Efficiency	Chlorophyll content (SPAD value)	Seedling length/cm
T. koningii	8.67 d	16.67 de	88.30	23.45 ab	54.34 a
T. hamatum 3	15.0 c	30.00 c	78.93	19.4 bcd	42.67 d
T. hamatum 4	23.0 b	45.00 b	68.40	20.50 bcd	46.67 bc
T. verins	23.4 b	45.00 b	68.40	23.15 abc	44.00 cd
T. viride 1	5.0 de	10.00 f	92.76	26.70 a	54.67 a
T. harzianum 1	11.0 d	21.67 d	84.78	20.85 bcd	45.00 cd
T. harzianum 2	6.34 d	12.34 ef	91.34	22.95 abc	53.34 a
T. harzianum 3	20.0 b	42.00 b	70.50	21.30 bc	48.34 b
Del cup	5.0 de	10.00 f	92.76	18.90 cd	41.00 e
Inoculated only	100.0 a	142.4 a	-	16.70 d	35.67 e
Tap water	0.00 e	0.00 g	-	18.70 cd	38.34 e

In a column, means followed by a common letter are not significantly different at the 5% level byDMRT

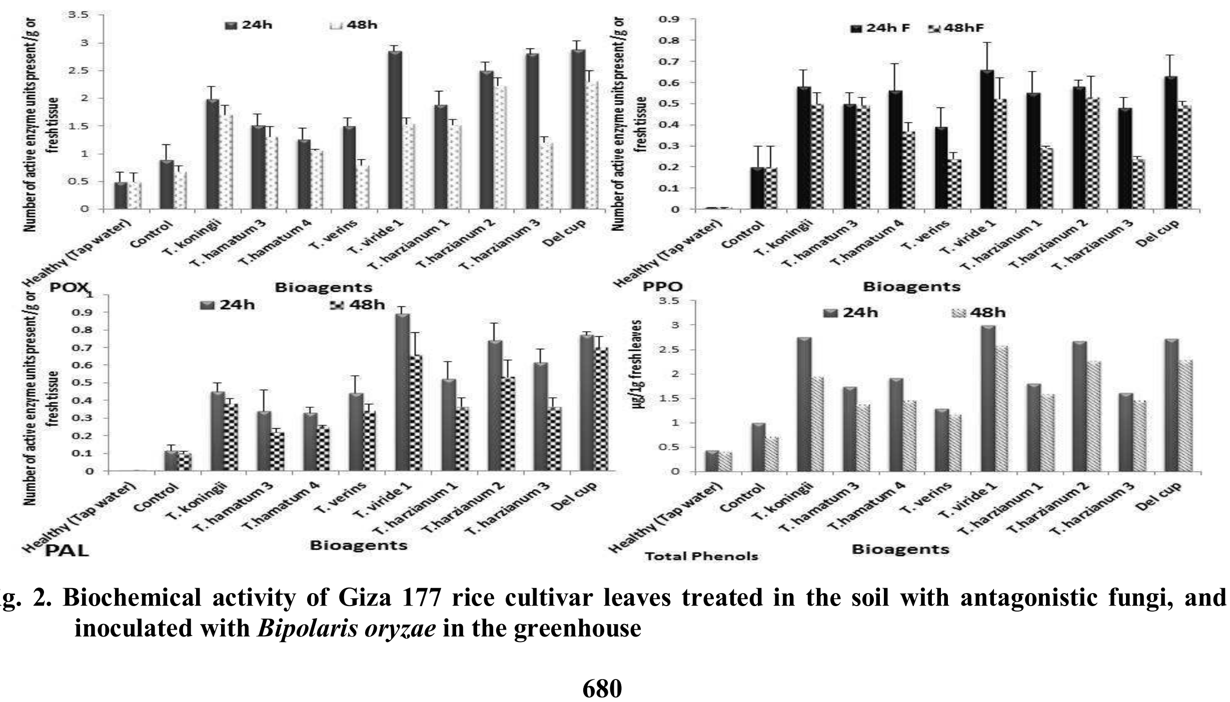

Fig. 2. Biochemical activity of Giza 177 rice cultivar leaves treated in the soil with antagonistic fungi, and inoculated with *Bipolaris oryzae* in the greenhouse

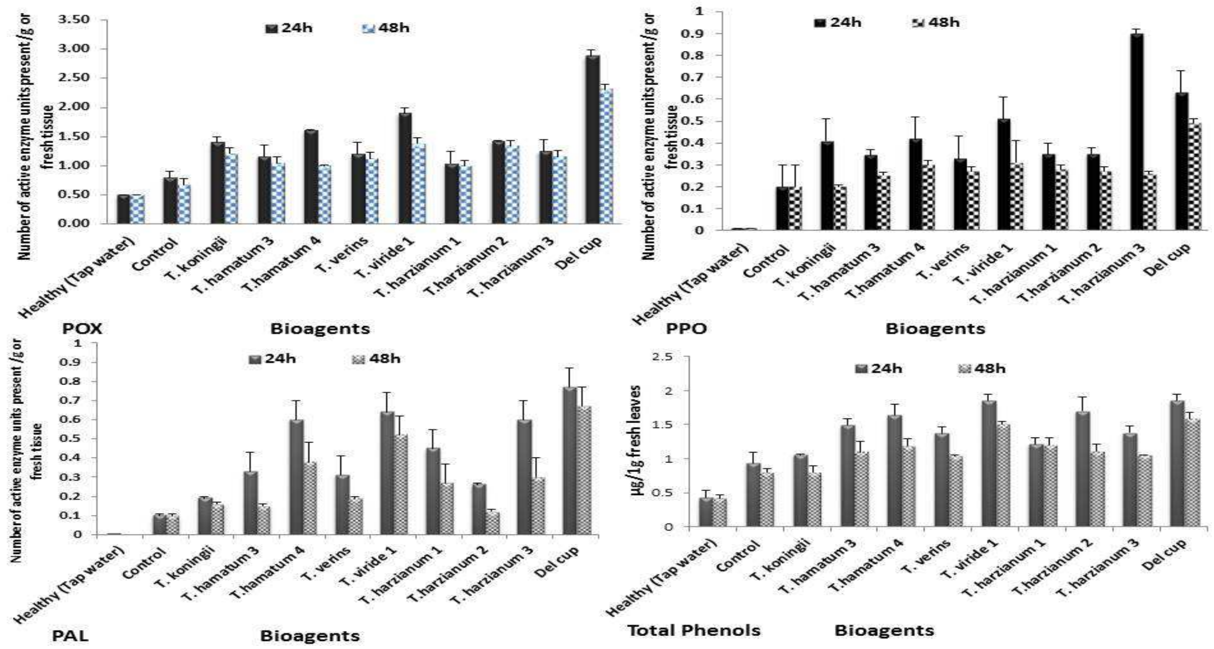

Fig. 3. Biochemical activity of Giza 177 rice cultivar leaves sprayed with cultural filtered antagonistic fungi, before inoculated with *Bipolaris oryzae* in the greenhouse

Table 4. Acidic hormones concentration on filtered cultural media for two *Trichoderma* isolates with HPLC chromatogram.

isolate	Hormone concentration (ppm)		
	Gibberelic acid	Indol acetic acid	Abscisic acid
T. viride1	70.176	8.281	1.649
T. koningii	41.052	1.359	0.450

Acidic hormones content of rice seedlings (Giza177 cv.) treated with filtered cultural media of antagonistic fungi by spraying and infected with *B. oryzae*:

The plant growth-promoting activities of *Trichoderma* isolates were assessed based on GA3, IAA and ABA production. The levels of GA3, IAA and ABA in rice leaves of treated plants increased, compared to control (inoculated only) or tap water. The highest concentration of GA3 was 217.717 and 215.105 ppm, respectively in plants sprayed with *T. viride 1* and *T. koningii*. IAA recorded 20.697 and 11.916 ppm, respectively on seedlings sprayed with cultural filtrates of *T. harzianum 1* and *T. viride 1*, (Table 5). ABA levels decreased in treated seedlings with *T. viride 1* (2.85 ppm) and then increased in control treatment (inoculated only with the pathogen 6.182 ppm).There were no differences between levels for ABA in other treatments, which ranged between 4.079 and 4.993 ppm.

Field experiment:

Effect of different biocontrol agents on rice brown spot incidence and severity:

The fungicide Del cup (Copper Sulfate Pentahydrate) with a dose of 1 kg/fedden as well as the untreated plants (tap water) were used for comparison. *T. viride* 1 was more effective than the other isolates and similar to the effect of the fungicide Del cup (Table 6) in reducing the percentage of brown spot disease as well as the disease severity in both growing seasons. Data presented in Tables (6 and 7) indicated that all antagonistic fungi were effective in controlling the disease. However, soil treatment with *T. hamatum 4* significantly reduced disease severity followed by *T. harzianum 2* (Table, 6). Foliar application with *T. koningii* and *T. harzianum 2* were the best amongst all tested antagonistic fungi compared with the untreated (Table 7). Of Giza 177 rice cultivar agronomic characters were significantly affected by soil antagonistic fungi treatments (Table 8). The treatments significantly affected on plant height, panicle length, number of discolored grain per panicle and grain yield. *T. viride 1* and *T. hamtum 4* produced the highest values of panicle length, number of healthy grain per panicle and grain yield. Tap water recorded the lowest values of panicle length, number of healthy grains per panicle and grain yield. Foliar applications with antagonistic fungi were the most effective to increase the agronomic characters. *T. viride 1, T. harzianum 2* and *T. koningii* increased the plant hight, panicle length, and grain yield and reduced the number of discolored grains per panicle (Table 9).

Table 5. Acidic hormones concentration on rice seedlings (Giza177 cv.) treated with antagonistic fungi before infection with *Bipolaris oryzae* in the greenhouse.

Isolate	hormone concentration (ppm)		
	Gibberelic acid	Indol acetic acid	Abscisic acid
T. viride1	217.7	11.92	2.85
T. koningii	215.1	6.74	4.84
T. harzianum 1	192.6	20.69	4.99
T. harzianum 2	179.5	6.80	4.079
Inoculated only	118.9	6.27	6.128
Tap water	68.60	1.50	0.59

Table 6. Effect of soil treatment with antagonistic fungi on brown spot percentage and severity in Giza 177 rice cultivar under field conditions.

Antagonistic fungi	Soil treatment							
	Second leaf %		Second leaf severity		Flag leaf %		Flag leaf severity	
	2015	2016	2015	2016	2015	2016	2015	2016
T. koningii	67.00b	63.4bcd	182.0b	184.0b	41.34b	71.00b	98.00b	104.0b
T. hamatum 3	55.00d	69.34b	169.6cd	172.0cd	27.00cd	36.00e	63.34d	64.00e
T.hamatum 4	41.34f	48.00f	76.67f	82.67f	26.0cde	24.67f	49.00e	56.00g
T. verins	53.67de	66.67bc	172.0c	177.3bc	42.67b	47.00c	50.00ef	87.00c
T. viride 1	40.34f	42.00g	55.34g	65.67g	19.34e	18.67g	23.34f	29.00h
T. harzianum 1	57.67c	61.34cd	132.0e	109.3e	30.00c	49.34c	70.00c	76.00d
T.harzianum 2	52.34e	56.67e	76.67f	84.0f	25.4cde	35.00e	51.34e	58.00f
T. harzianum 3	53.67de	59.34de	162.67d	165.0d	37.34b	38.67d	75.34c	58.67f
Del cup	35.34g	32.67h	54.34g	62.0g	20.34de	15.00h	23.34f	25.00i
Tap water	100.0a	82.67a	416.0a	421.0a	58.00a	100.0a	162.0a	170.0a

In a columns, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 7. Effect of foliar application with antagonistic fungi on brown spot percentage and severity in Giza 177 rice cultivar under field conditions

Antagonistic fungi	Foliar application							
	Second leaf %		Second leaf severity		Flag leaf %		Flag leaf severity	
	2015	2016	2015	2016	2015	2016	2015	2016
T. koningii	50.4e	26.7d	103.6f	98.6f	25.0cd	45.34d	45.0h	50.67g
T. hamatum 3	69.7c	29.4cd	143.0c	140.0c	27.34c	44.67d	51.34f	59.60f
T.hamatum 4	75.0b	30.7cd	119.0e	124.0e	32.34b	50.00c	100.0c	106.6c
T. verins	60.0d	40.0b	128.3d	133.3d	35.34b	65.00b	123.0b	130.6b
T. viride 1	33.0g	30.0cd	72.00g	79.0g	21.67de	23.34g	41.00g	46.67g
T. harzianum 1	68.4c	33.4c	127.0d	132.0d	30.34b	33.34f	62.00e	72.00e
T.harzianum 2	36.4f	30.7cd	75.00g	80.67g	27.67c	41.00e	55.00ef	60.00f
T. harzianum 3	68.4c	33.7c	190.0b	195.0b	27.00c	43.0de	73.00d	78.67d
Del cup	35.4f	32.7cd	54.34h	62.0h	20.34e	15.0h	23.34h	25.00h
Tap water	100a	82.67a	416.0a	421.0a	58.00a	100a	162.0a	170.0a

In a column, means followed by a common letter are not significantly different at the 5% level by DMRT

Table 8. Effect of soil treatment with different antagonistic fungi on agronomic characters of Giza 177 rice cultivar under field conditions

Antagonistic fungi	Soil treatment									
	Plant height (cm)		Panicle length (cm)		No. of discolored grain $panicle^{-1}$		No. of healthy grain $panicle^{-1}$		Grain yield t ha^{-1}	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
T. koningii	91.0c	92.0abc	23.2ab	22.6b	26.3g	23.6e	73.7b	76.4ab	8.80c	8.0b
T. hamatum 3	87.0d	82.0bc	21.0b	20.5c	37.3b	39.0b	62.6d	61.0e	8.43d	7.96bc
T.hamatum 4	97.0ab	98.0a	23.4a	23.8a	29.3f	26.3e	70.6bc	73.7bc	8.90c	8.0b
T. verins	85.0d	85.6cd	21.5b	19.0e	33.0d	33.3c	65.6cd	66.7d	7.70f	7.8bc
T. viride 1	99.0a	98.0a	23.5a	23.4ab	22.6h	24.3e	76.0ab	75.7abc	9.03b	9.06a
T. harzianum 1	95.0abc	94.6ab	22.8ab	20.3cd	35.0c	31.0de	65.0cd	69.0d	8.46d	7.56bc
T.harzianum 2	91.6bc	90.6bc	22.3ab	22.8b	29.6e	22.3e	70.3bc	77.7d	8.43d	7.73bc
T. harzianum 3	91.0c	92.6ab	19.2c	19.6de	19.3i	27.0de	80.7a	73.0c	7.83e	7.30bc
Del cup Pentahydrate)	80.0d	80.0d	19.0c	19.0e	20.0i	23.0e	80.0a	77.0a	9.20a	9.03a
Tap water	80.0d	80.0d	19.0c	19.00e	48.0a	66.0a	52.0e	44.0f	7.13g	6.85d

In a column means followed by a common letter are not significantly different at 5% level by DMRT

Table 9. Effect of foliar application with different antagonistic fungi on agronomic characters of Giza 177 rice cultivar under field conditions

Antagonistic fungi	Foliar application									
	Plant height (cm)		Panicle length (cm)		No. of discolored grain $panicle^{-1}$		No. of healthy grain $panicle^{-1}$		Grain yield t ha^{-1}	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
T. koningii	95.0ab	96.0ab	22.5ab	22.0ab	21.0d	17.0e	79.0a	83.0a	9.30b	8.86b
T. hamatum 3	85.0cd	88cde	21.5ab	19.5ab	28.6b	21.0cd	71.4e	79.0d	7.66h	8.13c
T.hamatum 4	93.0ab	94.0bc	20.5b	19.8ab	29.0b	25.6bc	71.0e	74.4f	8.70e	8.16c
T. verins	90.3abc	85.0e	20.0b	20.0ab	24.0bc	28.0b	76.0bc	72.0g	8.50f	7.86d
T. viride 1	97.0a	97.0a	23.5a	23.0a	20.0de	16.0e	80.0a	84.0a	9.40a	9.10a
T. harzianum 1	89.0bc	87.0de	21.5ab	20.0ab	25.0bc	23.0cd	75.0cd	77.0e	8.26g	8.83b
T.harzianum 2	92.0abc	93abc	20.5b	21.8ab	21.0d	19.0e	79.0a	81.0b	8.90d	8.93b
T. harzianum 3	90.3abc	91bcd	23.0a	21.0ab	22.0d	18.4e	78.0ab	81.6a	8.43f	8.80b
Del cup	80.0d	80.0f	19.0bc	19.0ab	20.0de	23.0cd	80.0a	77.0e	9.20c	9.03a
Tap water	80.0d	80.0f	19.0bc	19.0ab	48.0a	66.0a	52.0f	44.0h	7.13i	6.85e

In a column means followed by a common letter are not significantly different at 5% level by DMRT

DISCUSSION

As fungicide application has many harmful side effects on human and animals, thus biological control method is a promising strategy for controlling foliar and soil diseases in various crops. Biological application is safe, ecofriendly, long-lasting and promotes plant growth. *Trichoderma* genera have been considered biocontrol agents against phytopathogens since 1920s (Harman, 2006). Several decades ago, the mechanisms of *Trichoderma* against diseases were interpreted as antibiosis, mycoparasitism and competition for nutrient and space.

Recently, attention has been focused on the ability of *Trichoderma* to induce plant defence reactions including localized or systemic resistance. In this work, we studied the effect of some *Trichoderma* isolates in vitro and in vivo to control rice brown spot disease. Data revealed the efficacy of all tested bioagent isolates in reducing *Bipolaris oryzae* mycelial growth and growing over it competing for space and nutrients inside PDA medium.

Results were consistent with Angilica *et al*., (2001) and Marco, *et al*., (2003) who reported that Trichoderma spp could produce enzymes like extracellular cellulose or pectin that are capable of hydrolyzing the cell walls of other fungi or amylase enzyme which is partially responsible for the faster growth of antagonistic fungi on PDA medium, inhibiting the pathogen. Many workers reported releasing of hydrolytic enzymes i.e., chitinase and β-1,3- glucanase which degrade the pathogen cell wall, resulting in pathogen control (Parmer *et al*., 2015; Dildey *et al*., 2016). Also, Gomothinayagam *et al*., (2012) indicated that *T.harzianum* could override *B.oryzae* at the fifth day of antagonism with secretion of lytic enzymes, while *T. viride* secreted gliotoxin on the ninth day, so the pathogen is ceased. On the other hand, Abdelfatah *et al*., (2007) and Khalili *et al*., (2012) showed no evidence of *Trichoderma* spp mycoparasitic behavior against *B.oryzae* under light microscope where the hypha intermingled.

In vivo experiments showed the efficiency of *Trichoderma* isolates in reducing both disease incidence and severity through the two tested treatments (soil and foliar spray). Significant increases in agronomic characters (i.e., leaf chlorophyll content, seedling length, plant height, panicle length, number of discolored or healthy grains and grain yield) were recorded under greenhouse or field conditions during 2015 and 2016 seasons. Lo and Yin lin (2002) suggested several possible mechanisms to interprete the phenomenon of plant growth increase. These factors are: 1- control of harmful root microorganisms, 2- encourage production of growth stimulating factors (i.e., plant hormones or growth factors), 3- increase in uptake of nutrients via root growth enhancement and 4- reduce concentrations of plant growth inhibitory substances in soil. Nawrocka *et al*., (2011) indicated that *Trichoderma* can capture the useful molecules to be less available to pathogens and it has the ability to convert those poorly soluble in water, making them more available to plants.

Our data are in accordance with those of Abdelfatah *et al*., (2007) who showed significant increases in rice seedling length and chlorophyll content (a & b and carotenoids) as a result of chloroplast enzyme activities (Gautam *et al*., 2015). Also, Khalili *et al*., (2012) found significant differences in brown spot control between *Trichoderma* treated and untreated treatments besides increase in plant height, panicle length and grain yield when rice plants were sprayed with *Trichoderma* spp spore suspension. They suggested that plant growth enhancement induced by *Trichoderma* spp might be due to both producing growth regulating factors and management of minor pathogens. In addition, *Trichoderma* may detoxify cyanide particles produced by some microbes associated with plants, encouraging plant growth.

Recent reports suggested that the genus *Trichoderma* might stimulate the production of biochemical compounds of phenoilc nature associated with the host defense which are responsible for induced plant resistance pathways when it colonizes the epidermis and outer cortical layers (Khaledi and Parissa, 2016). The current study revealed that the bioagent *Trichoderma* spp isolates were able to induce defense responses in rice plants against *B. oryzae.*

Increments in POX, PPO, PAL enzyme activity and total phenol contents were more observed in inoculated plants treated with the tested antagonistic fungi than in the untreated ones. Yedidia *et al*. 2000 observed higher activities of chitinase, beta -1,3-glucanase, cellulase and peroxidase, for up to 72 h post-inoculation compared to untreated control in treated *T. harzianum* T-203cucumber roots. Similar findings were observed by Solanki *et al*., 2011 who found greater accumulations of total phenols, peroxidase, polyphenoloxidase and phenylalanine ammonia lyase in tomato chitin-supplemented Trichoderma/Hypocrea formulation-treated plants challenged with *Rhizoctonia solani.*

Peroxidase catalyses the condensation of phenolic compounds into lignin which has role in plant disease resistance and increase in host plants following pathogen infection (Scott-Craig *et al*., 1995). Induction of phenylalanine ammonia lyase might lead to increased levels of signaling molecule salicylic acid in the leaves thereby contributing to disease resistance (Christopher *et al*., 2010 and Ojha and Chatterjee 2012) and it also increases lignification (Navaveetha *et al*., 2015).

Nawrocka *et al*., 2011 reported that treating plants with various *Trichoderma* strains increases reac-tive oxygen species (ROS) generation and activity of antioxidant enzymes involved in response to oxidative stress as well as of peroxidases, chitinases and phenylalanine ammonia lyase. The antimicrobial compounds accumula-tion e.g. phenolic compounds, phytoalexins and different PR pro-teins expression have been detected (Ahmad *et al*., 2008 and Sreelakshmi & Sharma 2008).

Our data referred to the ability of two different *Trichoderma* isolates to produce indole acetic acid (IAA), gibberilic acid (GA) and absicic acid (ABA) in their filterates. Also, the hormones in *Trichoderma* treated plants increased.

The observed increase in IAA could interprets the increase in seedling length or plant height growth observed in treated plants. Seelding length changes were induced in treated seedlings by spraying with cultural filtered media of *Trichoderma* isolates.

GA hormone is involved in the promotion of elongation in axial organs in combination with auxins, and induces mitotic division in leaf buds and leaves (Blake *et al*. 2000; Srivastava 2002). Sofo *et al*., (2010) found that increases in the number of leaves and in stem diameter of T22-treated GiSeLa6 rootstocks. abscisic acid (ABA) as a general inhibitor of growth and metabolism, and negatively affects the synthesis of proteins and nucleic acids, even though these effects vary with tissue and developmental stage (Kobashi *et al*., 2001 and Srivastava 2002). ABA levels between leaves and roots in plant treated with T22 did not induce a higher ABA accumulation in both the tissues and thus did not determine growth inhibition (Sofo, *et al*., 2011). Indole-3-acetic acid is the most widely naturally-occurring auxin in vascular plants, and it is involved in lateral and adventitious roots initiation and emergence, as well as in shoot development by changes in cell division, expansion and differentiation (Hedden and Thomas, 2006). Many *Trichoderma* spp are now commercially available with strain mixes becoming increasingly common due to their greater consistency of performance. Various mechanisms have been proposed to explain growth promotion including control of minor pathogens, enhanced nutrient uptake, increased carbohydrate metabolism and photosynthesis, and phytohormone synthesis. There is a strong evidence for a role for microbe-produced indole acetic acid (IAA), although it is most likely that *Trichoderma* stimulates growth by influencing the balance of hormones such as IAA, gibberellic acid and ethylene. (Alison S. and R. Hill, 2014).

Outcomes of the current results can motivate further studies to test the usefulness of *Trichoderma* isolates, and to prepare applicable formulations for rice disease control.

REFERENCES

Abdel-Fataah, G.M.; Y.M. Shabana; A.E.Ismail and Y.M. Rashad (2007). *Trichoderma harzianum*: a biocontrol agent against *Bipolaris oryzae*. Mycopathol., 164: 81-99.

Ahmad, P.; M. Sarwat,. and S. Dharma(2008). Reactive oxygen species, antioxidants and signaling in plants. J. Plant Biol., 51:167-173.

Alfredo M.S. and C. R.P. Aleli (2011). Biological control of sheath blight of upland rice with *Trichoderma species*. J Trop. Plant Pathol., 69: 1-9.

Alison, S. and H. Robert (2014). Applications of *Trichoderma* in plant growth promotion. Biotechnology and Biology of Trichoderma 415–428. https://doi.org/10.1016/B978-0-444-59576-8.00031-X.

Allam, A. I. and J. P. Hollis (1972). Sulphide inhibition of oxidase in rice roots. Phytopathology, 62(3): 634-639.

Angelica, M.; G. Barbosa; K.G. Rehn; M. Menzes and R.R.Mariano (2001). Antagonism of *Trichoderma* spp. on *Cladosporium herbarum* and their enzymatic characterization. Braz. J. Microbiol., 32: 98–104.

Baker, R. (1988). Trichoderma spp. as plant-growth stimulants. Biotechnol., 7(2): 97–106.

Barnet, H.L. and B.B. Hunter (1972). Illustrated genera of imperfect fungi. 3rd End. Burgess, Minneapolia, Minnesota, 241p.

Blake, P.S.; G. Browning; L.J. Benjamin and L.N. Mander (2000). Gibberellins in seedlings and flowering trees of Prunus avium L. Phytochemistry, 53:519–528.

Chinmay, B.; S.S.L. Srivastava and S.K. Biswas (2010). Effect of biotic, abiotic and botanical inducers on crop growth and severity of brown spot in rice. Indian Phytopath., 63 (2) : 187-191.

Christopher, D. J.; T. S.Raj; S. U.Rani and R.Udhayakumar (2010). Role of defense enzymes activity in tomato as induced by *Trichoderma virens* against *Fusarium* wilt caused by *Fusarium oxysporum f sp. lycopersici*. Journal of Biopesticides, 3(1): 158–162.

Contreras-Cornejo, H.A.; L. Macías-Rodríguez; R. Alfaro-Cuevas and Lopez-Bucio (2014) .*Trichoderma* spp. improve growth of Arabidopsis seedlings under salt stress through enhanced root development, osmolite production, and Na+ elimination through root exudates. Mol Plant Microbe Interact, 27(6):503–514, doi:10.1094/MPMI-09-13-0265-R.

Dildey, O.D.F.; Broetto, L.; Rissato; B.B.; Gonçalves-Trevisoli; E.D.V.; Coltro-Roncato, S.; Dal'Maso, E.G; Meinerz, C.C.; Henkemeier, N.P.; Stangarlin, J.R.; Kuhn, O.J. and Webler, T.F.B.(2016). *Trichoderma*-bean interaction: Defense enzymes activity and endophytism. African Journal of Agricultural Research, 11(43): 4286-4292.

Doni, F.; A. Isahak; C. R. Che Mohd Zain; S. M. Ariffin; W. N.W.Mohamad and W. M. W.Yusoff (2014). Formulation of *Trichoderma* spp. SL2 inoculants using different carriers for soil treatment in rice seedling growth. SpringerPlus, 3:532 http://www.springerplus.com/content/3/1/532.

Doni, F.; I. Anizan; C.M.Z. Che Radziah; A.H. Salman; M.H. Rodzihan and W.Y. Wan Mohtar (2014a). Enhancement of rice seed germination and vigour by *Trichoderm*a spp. Res J App Sci Eng Technol, 7(21):4547–4552.

Doni, F.; I. Anizan; C.M.Z. Che Radziah and W.Y. Wan Mohtar (2014b) Physiological and growth response of rice (Oryza sativa L.) plants to *Trichoderma* spp. inoculants. AMB Express 4:45, doi:10.1186/s13568-014-0045-8.

Doni, F.; N.K.N. Al-Shorgani; E.M.M.Tibin; N.N. Abuelhassan; I. Anizan; C.M.Z. Che Radziah and W.Y. Wan Mohtar (2013). Microbial involvement in growth of paddy. Curr Res J Biol Sci, 5(6):285–290

Dubey, S.C.; Tripathi, A.; Dureja, P. and Grover, A. (2011). Characterization of secondary metabolities and enzymes produced by *Trichoderma* species and their efficacy against plant pathogenic fungi. Ind.J.Agric.Sci., 81(5): 455-61.

Duncan, M. R.T. (1955). Multiple rang and multiple F. test. Biometrics. 11: 1-42.

Gautam, S.S.; Kanchan, K. and Satsangi, G.P.(2015). Effect of *Trichoderma* species on germination and growth of mungbean (*Vigna radiate* L.) and its antagonistic effect against fungal pathogens. Int. J. Advanced Res., 3(2): 153-158.

Gomathinayagam, S.; Persaud, S.A. and Rekha, M.(2012). Comparative study of biological agents, *Trichoderma harzianum* and *Trichoderma viride* for controlling brown spot disease in rice. J. Biopest, 5:28-32.

Harish, S.; D.Saravavakumar; R. Radjacommar; E.G. Ebenezar and K. Seetharaman (2007). Use of plant extracts and biocontrol agents for the management of brown spot disease in rice. Biocontrol, 53(3): 555-567.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol., 96: 190–194.

Hashem, H.A., (2006). Physiological and molecular actions of jasmonic acid on soybean plant. Ph.D Thesis, Fac. of Sci., Ain Shams Univ., Cairo, Egypt.

Hassanein, R. A.; Amira A. Hassanein; A. B. El-din; M. Salama and Hanan A. Hashem.(2009). Role of Jasmonic Acid and Abscisic Acid Treatments in Alleviating the Adverse Effects of Drought Stress and Regulating Trypsin Inhibitor Production in Soybean Plant. Australian Journal of Basic and Applied Sciences, 3(2): 904-919, ISSN 1991-8178.

Hedden, P and S.G. Thomas (2006). Plant hormone signaling. Blackwell, Oxford, pp 37–66.

IRRI International Rice Research Institute (2013). Rice today. International Rice Research Institute. Metro Manila, Philippines., 12(1): 8- 44.

Jones. M.P.; F. Jeutong and J. Tchatchoua (1993). A survey of rice diseases in Cameroon. Plant Dis., 77:133-136.

Kagale, S.; T. Marimuthu; B. Thayumanavan; R. Nandakumar and R. Samiyappan (2004). Antimicrobial activity and induction of systemic resistance in rice by leaf extract of Datura metel against *Rhizoctonia solani* and *Xathomona oryzae pv. oryzae.* Phys. Mol. Plant Pathol., 65: 91-100.

Kalboush, Zeinab,A.A. (2007. Integrated management of brown spot disease of rice in Egypt. Ms. Thesis, Faculty of Agric., Kafr El-Sheikh Univ. Egypt.pp98.

Khaledi, N. and Parissa Taheri. (2016). Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. Journal of plant protection research, 56(1):21-31.

Khalili,E.; M. Sadravi; S. Naeimi and V. Khosravi (2012). Biological control of rice brown spot with native isolates of three *Trichoderma* species. Brazilian J. Microbiology 297-305. ISSN 1517-8382.

Kobashi, K.: S. Sugaya; H. Gemma and S. Iwahori (2001). Effect of abscisic acid (ABA) on sugar accumulation in the flesh tissue of peach fruit at the start of the maturation stage. Plant Growth Regul 35:215–223.

Lee, S. , M. Yap, G. Behringer, R. Hung and J. W. Bennett1(2016). Volatile organic compounds emitted by *Trichoderma* species mediate plant growth. Fungal Biol Biotechnol 3:7. DOI 10.1186/s40694-016-0025-7.

Lifshitz, R.; M.T.Windham, and R.Baker (1986). Mechanism of biological control of pre-emergence damping-off of pea by seed treatment with *Trichoderma* spp.Phytopathology 76:720-725.

Lo, C.T. and Lin, C.Y.(2002). Screening strains of *Trichoderma* spp for plant growth enhancement in Taiwan. Plant Pathology Bulletin, 11: 215-220

Marco, J.L.; M.C.Inglis and C.R. Felix (2003). Production of hydrolytic enzymes by *Trichoderma* isolates with antagonistic activity against *Crinipellis perniciosa*, the causal agent of witches, broom of cocoa. Braz. J. Microbiol., 34(1): 148–54.

Mart ́ ınez-Medina A, J.A. Pascual, F. P ́ erez-Alfocea *et al.*(2010). *Trichoderma harzianum* and *Glomus intraradices* modify the hormone disruption induced by *Fusarium oxysporum* infection in melon plants. Phytopathology, 100:682–8.

Mart ́ ınez-Medina A, M. Del Mar Alguacil, J.A.Pascual *et al.*(2014). Phytohormone profiles induced by *Trichoderma* isolates correspond with their biocontrol and plant growth-promoting activity on melon plants. J Chem Ecol, 40:804–15.

Matta, A. and A. E. Dimond (1963). Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology, 53: 574-575.

Navaneetha, T.; Prasad, R.D. and Rao, V.(2015). Liquid Formulation of *Trichoderma* Species for Management of Gray mold in Castor (Ricinus communis L.) and Alternaria ster Leaf Blight in Sunflower (Helianthus annuus L.). J Biofertil Biopestici, 6:1.

Nawrocka, J.; Snochowska, M., Gajewska, E.; Pietrowska, E. Szczech, M.and Malolepsza, U.(2011). Activation of defense responses in cucumber and tomato plants by selected polish *Trichoderma* strains.vegetable crops research bulletin, 75:105-116.

Ojha, S., and N.Chatterjee (2012). Induction of resistance in tomato plants against *Fusarium oxysporum f. sp. lycopersici* mediated through salicylic acid and *Trichoderma harzianum*. J. plant protection research, 52(2): 220–225.

Ou, S.H. (1985). "Rice Diseases". 2nd Ed. Commonwealth Mycological Institute, Kew, England, 380 pp.

Parmar, H.J.; Bodar, N.P.; Lakhani, H.N.; Patel, S.V.; Umrania, V.V. and Hassan, M.M. (2015). Production of lytic enzymes by *Trichoderma* strains during in vitro antagonism with *Sclerotium rolfsii*, the causal agent of stem rot of groundnut. Afric.J.Microbiol. Res., 9(6):365-372.

Scott-Craig, J.S.; Kerby, K.B.; Stein, B.D. and Sommerville, C.S. (1995). Expression of an extracellular peroxidase that is induced in (*Erysiphe graminis f. sp. hordei*). Physiological and Molecular Pl. Pathol., 47 (6): 407–418.

Shindy, W.W. and O. Smith, (1975). Identification of plant hormones from cotton ovules. Plant Physiol., 55: 550-554.

Shukla, N.; R.P. Awasthi; L. Rawat and J. Kumar (2012). Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. Plant Physiol Biochem, 54:78–88.

Sofo, A .; A. Scopa; M. Manfra; M.; D. Nisco; G. Tenore; J. Troisi; R. Di Fiori and E. Novellino (2011). *Trichoderma harzianum* strain T-22 induces changes in phytohormone levels in cherry rootstocks (Prunus cerasus 3 P. canescens). Plant Growth Regul, 65:421–425 . DOI 10.1007/s10725-011-9610-1

Sofo, A.; L. Milella and G. Tataranni (2010). Effects of *Trichoderma harzianum* strain T-22 on the growth of two Prunus rootstocks during the rooting phase. J Hortic Sci Biotechnol, 85:497–502.

Solanki, M.K.; Singh, N.; Singh, R.K.; Singh, P.; Srivastava, A.K.; Kumar, S.; Kashyap, P.L. and Arora, D.K. (2011). Plant defense activation and management of tomato root rot by a chitin-fortified Trichoderma/ Hypocrea formulation. Phytoparasitica, 39(5): 471-481.

Sreelakshmi Y., Sharma R. 2008. Differ-ential regulation of phenylalanine ammonia lyase activity and protein level by light in tomato seedlings. Plant Physiol. Bioch., 46: 444-451.

Srivastava, L.M. (2002). Plant, growth and development-hormones and environment. Elsevier, San Diego, pp 307–314.

Srivastava, S.K. (1987). Peroxidase and polyphenol-oxidase in Brassica juncea plants infected with *Macrophomina phaseolina* (Tassi: Goid of and their implication in disease resistance. Phytopathology, 120: 249-254.

Thakur, M.; B.S. Sohal (2013). Role of elicitors in inducing resistance in plants against pathogen infection:areview. ISRN Biochem 1–10. doi:10.1155/2013/762412.

Yedidia, I., Benhamou, N., Kapulnik, Y. and Chet, I. (2000). Induction and accumulation of PR proteins activity during early stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. Plant Physiology and Biochemistry, 38: 863–873.

Zieslin, N. and R. Ben-Zeken (1993). Peroxidase activity and presence of pehnolic substances in peduncles of rose flowers. Plant Phys. Biochem., 31: 333-339.

Zucker, M. (1968). Sequential induction of phenylalanine ammonia layse and lyase in inactivating system in potato tuber disks. Plant physiol., 43: 365-374.

كفاءة عزلات الترايكوديرما كعوامل حيوية ضد مرض التبقع البنى فى الأرز والتغيرات البيوكيميائية

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أجريت تجارب حقلية ومعملية لأختبار كفاءة اربع عشرة عزلة من فطر *Trichoderma* spp ضد فطر *Bipolaris oryzae* المسبب لمرض التبقع البنى فى الأرز. وكانت عزلات *T. hamatum* رقم 4 ، *T. viride* رقم 1 و*T. harzianum* رقم 1 ، 2 و 3 وعزلة *T. virens* هى الأفضل فى تثبيط نمو الفطر الميسليومى بنسبة 100%. تم اختبار معاملات التربة والرش الورقى برواشح المزرعة لهذه الفطريات المضادة تحت ظروف الصوبة والحقل خلال موسمى 2015 , 2016. لوحظ انخفاض فى الشدة المرضية مع زيادة فى محتوى الكلوروفيل ونشاط أنزيمات البيروكسيديز، البولى فينول اوكسيديز ، الفينايل الانين امونيا لاييز ومحتوى الفينولات الكلية. كانت عزلات *T. viride* رقم 1 ، *T. koningii* *لها* القدرة على انتاج أندول حامض الخليك بتركيزات 8,281- 1,359 وحامض الجبريلك 70.176- 41,052 و حامض الأبسيسك 1,649- 0,45 جزء فى المليون على التوالى.اعطت معاملة رش الاوراق براشح مزرعة عزلات *T. harzianum* رقم 1 و *T. viride* رقم 1 أعلى تركيزات لاندول حامض الخليك 20,697- 11,916 جزء فى المليون على الترتيب فى بادرات الأرز. تحت ظروف الحقل،كانت معاملة التربة بعزلة *T. viride* رقم 1 هى الأكثر فعالية فى خفض كل من النسبة المئوية للتبقع البنى والشدة المرضية بما يشابه اثر مبيد الدل كب فى كلا الموسمين.