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ISOLATION AND EVALUATION OF *Trichoderma* SPP. AS A BIOCONTROL AGENT AGAINST LEGUMES SEED BORNE FUNGI FROM EAST DELTA REGION

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ABSTRACT: Twenty-three isolates of the genus *Trichoderma* were recovered from 63 soil samples collected from East Delta Region. The isolates percentage to number of soil samples/ *Trichoderma* isolates in respect to location were 37%, 45%, 30%, 10%, 46%, and 17% in Sharkia, Ismailia, Port Said, Suez, North Sinai, and South Sinai Districts, respectively. Six different morphologically *Trichoderma* isolates were selected. The average linear growth rate of *Trichoderma* isolates ranged between 41.78 mm/day for *Trichoderma* isolate G5 PP1 and 53.41mm/day for isolate G4 IF2. Antagonism activity *in vitro* between legumes seed borne fungi, *i.e. Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani*, (isolated from faba bean, beans, and peas) and selected *Trichoderma* isolates were done. *Trichoderma* isolates G4 IF2, G2 NA and G4 IF2 caused a remarkable reduction in linear growth of *F. solani*, *M. phaseolina* and *R. solani*, respectively. *Trichoderma* isolates G5 PP1, G3 IQ2 and G5 PP1 recorded highly survival of faba bean seedlings were sown in infested soil with *F. solani*, *M. phaseolina* and *R. solani*, respectively. *Trichoderma* isolates G4 IF2 and G1 SA1 recorded highly survival beans seedlings in infested soil with *F. solani* and *M. phaseolina*, respectively. While, *Trichoderma* isolates G2 NA, G4 IF2 and G6 SoR1 were similar of highly survival seedling in infested soil with *R. solani*. *Trichoderma* isolates G1 SA1, G2 NA, and G3 IQ2 were identical of highly survival of peas seedlings in infested soil with *F. solani*. Whereas treatment with *Trichoderma* isolates G2 NA, G4 IF2, G5 PP1, and G6 SoR1 showed, highly survival seedling. *Trichoderma* isolates G2 NA showed highly survival peas seedlings in infested soil with *R. solani*. In general, *Trichoderma* isolates G2 NA and G5 PP1were the most effective, which had the highest survival plants. This study suggested that, all selected isolates of *Trichoderma* needed a molecular identification and genetically improvement to obtain one isolate used as highly effective biocontrol agent against most legumes seed born fungi.

Key words: *Trichoderma*, biocontrol agents, seed borne fungi, growth rate.

INTRODUCTION

Trichoderma species are saprophytic, filamentous fungi belonging to the Ascomycota division (Chaverri and Samuels, 2003). Genus *Trichoderma* is nearly ubiquitous in the environment, found in most ecosystems (farm lands, forests, grasslands, deserts, salt marshes, water lakes and airborne particles) and have been isolated from a wide range of organic materials, including soils, dead plant matters, live roots (of most plant species) and seeds (Monte, 2001). Genus of *Trichoderma* has been identified as opportunistic, a virulent plant symbionts, many are parasites of and compete against phytopathogenic fungi, and therefore can play an important role in suppressing plant diseases (Vinale *et al.*, 2008). *Trichoderma* spp. has been investigated as biological control agent for more than 70 years (Samuels, 1996). Jin *et al.* (1992) emphasized on three critical components of the biocontrol system as applicable to the development of seed treatments. Seed is the most important input for

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crop production. Pathogen-free seed is urgently needed for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses; reduction in plant growth and productivity of crops (Kubiak and Korbas, 1999; Dawson and Bateman, 2001 ; Islam *et al.*, 2009). Legume seeds have comparatively higher protein content than non-legume plants. The high protein content gives them especial importance in agriculture. All varieties of pulses are excellent sources of easily digestible protein, but there are several factors responsible for their low production. Plant diseases play an important role (Nine, 1986). Seed-borne diseases have been found to affect the growth and productivity of crop plants (Kubiak and Korbas, 1999; Dawson and Bateman, 2001; Weber *et al.*, 2001). Load of seed borne fungi is one of the important aspects that determine the quality of seed. Harris (1999) evaluated two isolates of *Trichoderma koningii* for controlling damping-off diseases in seedlings of *Capsicum annuum.* Both isolates of *T. koningii* reduced seedling death caused by *R. solani* and *P. ultimum.* Rajappan and Ramaraj (1999) evaluated the efficacy of four fungal biocontrol agents *(Trichoderma viride, Trichoderma harzianum, T. hamatum* and *Gliocladium virens)* against the cauliflower wilt pathogen, *F. moniliforme in vitro. T. harzianum* produced the maximum inhibition zone of 15 mm compared with the minimum of 7 mm produced by *T. hamatum.* Biswas *et al.* (2000) tested eleven isolates of *T. harzianum* in dual culture, three isolates, T8, TID and T2, were effective against *Sclerotium rolfsii*, the causal agent of stem rot of groundnut, and they overgrew the pathogen up to 92, 85 and 79%, respectively, *in vitro.* Isolates T8 and Tl0 reduced stem rot incidence significantly when delivered as seed dressing or soil application in pot trials. Burns and Benson (2000) evaluated four isolates of *T. virens* for biocontrol of pre-emergence damping-off of *Catharanthus roseus* caused by *P. ultimum.* Biocontrol of pre-emergence damping-off with the four isolates of *T. virens* was variable. The isolates of *T. virens* were as effective as metalaxyl at 0.01 ml for control of *P. ultimum* in lots of mix amended one to sex days before seeding. Harman (2000) stated that some strains of *Trichoderma* become commercially available. This is largely a result of the change in public attitude towards the use of chemical pesticides, together with eased knowledge of their harmful side effects. Biological control is widely considered by the public as "natural" and therefore non-threatening products. *Trichoderma* species act against target organism in several ways. *Trichoderma* is one the most studied genus of fungi. Species belonging to this genus are used worldwide as bio-pesticides, biofertilizers, growth enhancers and stimulants of resistance. Species such as *T. asperellum, T. atroviride, T. gamsii, T. hamatum, T. harzianum, T. polysporum, T. virens*, and *T.viride* are used as main ingredients, alone or in combination with other biological control agents, for commercial products across the world (Woo *et al.*, 2014). Ahmad *et al.* (2000) studied *in vitro* biological against effects on mycelial growth of *R. solani, M. phaseolina* and *F. oxysporum,* the causal pathogens of root-rots and wilt diseases. *Trichoderma harzianum, T viride* and *Bacillus subtilis* inhibited the mycelial of all fungi tested *in vitro.* Soil infestation with each of the tested control agent reduced the percentage of infected plants and disease severity. *Trichoderma harzianum* was the most effective one followed by *T. viride* and *B. subtilis.* Naseby *et al.*, (2000) tested five strains of *Trichoderma (T. harzianum* and *T. pseudokoningii)* known with biological control activities during pea growth with antagonistic effect against large *P. ultimum* inocula. All *Trichoderma* strains decreased the number of lesions caused by *P. ultimum* and increased number of lateral roots. The effect of *P. ultimum* on emergence and growth was significantly reduced by all *Trichoderma* strains. The first real generic description of *Trichoderma* was proposed based on colony growth rate and microscopic characters by Rifai (1969). *Trichoderma asperellum* Tri-5 strain was remarkable for the greater inhibition of mycelial and radial growth. The average PRGI of the action of all *Trichoderma* strains over Rsol-1 was lower than the general average; however, due to its antagonistic capacity, *T. asperellum* was able to invade 90% of the root phitopathogenic fungus of the common bean. The formation of *T. asperellum* haustoria facilitated the entry and mycoparasitism of the pathogen hyphae (Sánchez-García *et al.*, 2017). Good results *in vitro* assay are not always good indicators of positive antagonistic effects *in vivo* (Campanile *et al.*, 2007). Some species of *Trichoderma* (*T.*

harzianum, *T. viride*, *T. virens* and *T. koningii* are well known antagonists and are being utilized to control plant pathogens under field conditions (Galarza *et al*., 2015). Kumari *et al*. (2016) found that, the antagonistic potential of 26 isolates of *Trichoderma* spp. against *R. solani* were varied in their inhibitory effect on *R. solani*.

Pastrana *et al*. (2016) demonstrated the antagonistic effects of *T. asperellum* and *Bacillus* spp. by inhibiting radial growth of *M. phaseolina* and *F. solani* by more than 36%. Preventive application of *T. asperellum* by root-dipping reduced the incidence of charcoal rot and also reduced disease progression and the percentage of crown necrosis

The aim of the present work was to obtain different *Trichoderma* isolates from East Delta Region and evaluate their effect as biocontrol agent against some important legume seed-borne pathogenic fungi.

MATERIALS AND METHODS

Isolation, Purification and Identification of *Trichoderma* Isolates

Collection of soil samples

Sixty three soil samples were collected from twenty one locations at six governorates in East Delta Region (Sharkia, Ismailia, Port Said, Suez, North Sinai and South Sinai). One kilogram soil samples were collected from each of irrigated legumes fields at a depth of 5-10 cm of soil surface, open fields and plastic tunnels. Each soil sample was placed in a plastic bag and mixed thoroughly. Seventeen samples were collected from Sharkia Governorate, 16 from Ismailia Governorate, 10 from Port Said Governorate, 7 from Suez Governorate, 8 from North Sinai Governorate, 5 from South Sinai Governorate. Soil samples were collected randomly from the above mentioned areas.

Isolation of *Trichoderma* species from soil

Trichoderma had been isolated from soil samples as follows: 25g of each soil sample was suspended in 250 ml of water. Samples were shacked for 20-30 minutes on a rotary shaker at 250 rpm. Serial dilutions (10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}) were prepared from each soil sample then 0.1 ml of 10^{-4} soil and suspension was transferred *Trichoderma* selective media (TSM) and spread with a glass rod. ($MgSO_4$97 H_2O, 0.2g; K_2HPO_4,0.9g KC1, 0.15g; NH_4NO_3, 1.0g; glucose, 3.0g; chloramphenicol, 0.25g; p-dimethylaminobenzenediazo sodium sulfonate 0.3g; pentachloronitrobenzene, 0.2g; rose-bengal 0.15g; agar, 15g and 1000 ml desalt water) according to Elad *et al*. (1981). Then plates were incubated at 25ºC for 5-7 days. After incubation, *Trichoderma* spp. isolated and purified on potato dextrose agar using hyohal tip and/or single spore techniques (Dhingra and Sinclair, 1973) and then transferred on PDA slants at 5°C for further studies. For each soil suspension, five plates were considered as replicates.

Identification of *Trichoderma* isolates

Trichoderma spp. isolates were identified according to Barnett and Hunter (1987). Identification of *Trichoderma* spp. was kindly done by Plant Pathology Institute, Agriculture Research Center, Giza, Egypt.

Isolation, Purification and Identification of the Legumes Seed-Borne Fungi

Samples of one hundred seeds of different legumes cultivars *i.e.* beans *cv.* pulsta, faba bean cv. local and peas cv. local were collected from East Delta Region. Seeds were surface sterilized for 5 minutes in a 2% sodium hypochlorite solution followed and rinsed three times with sterile distilled water. The sterilized seeds were directly placed in sterilized petri-dishes containing PDA complemented with 0.06 mg/ml chloramphenicol (PDAC), five seeds/plate. Plates were incubated at 28 ± 2^{o}C and observed daily. Then fungi that appeared have been isolated and purified using hyphal tip and/ or single-spore technique (Dhingra and Sinclair, 1973). The purified cultures were incubated on PDA slant medium for 7days at 28^{o}C then stored in a refrigerator at 5^{o}C. Identification was carried out using appropriate methods for each genus (Raper and Fennel, 1973; von Arx, 1981 and Barnett and Hunter 1998). Identification was kindly done by Plant Pathology Institute, Agriculture Research Center, Giza, Egypt. The identified fungi were *Fusarium solani* (Mart.), *Macrophomina phaseolina* (Tassi) and *Rhizoctonia solani* (Kühn).

Evaluation of *Trichoderma* Isolates as Biocontrol Agent Against Legumes Seed Borne Fungi

Growth rate

Mycelial growth rate of *Trichoderma* isolates was done on plates containing PDA as four petri dishes (90mm diameter) containing PDA was centrally inoculated with a 5-mm *Trichoderma* disc of 7-day *Trichoderma* isolates. Plates were incubated at 25 ±2°C for three consecutive days. Radial mycelial growth was recorded every 24 hours. The colony diameter was measured as the mean of two perpendiculars. Average linear growth rates (ALG) were calculated using the following formula (Elad *et al.*, 1981): ALG (mm /day) = [C3–C1]/T Where C3: colony diameter in mm after three days, C1: colony diameter in mm after one day of incubation and T: the difference in time (day). Four replicates (plates) for each treatment were used.

Antagonism *in vitro*

The antagonistic effect of optioned *Trichoderma* isolates against *F. solani*, *M. phaseolina* and *R. solani* was carried out under laboratory conditions. Discs (5 mm in diameter) were taken from the marginal growth of *Trichoderma* isolates. Discs were placed on the opposite side to the tested pathogenic fungi were grown on PDA media in petri-dishes for 5 days at 25°C. Three replicates were used for each particular treatment. Percentage of growth reduction of tested pathogenic fungi was determined using the following formula (Ferreira *et. al.*, 1991).

$$GR + (\%) = \frac{A - B}{A} \times 100$$

Where:

G.R (%): percentage of growth reduction

A: The distance of mycelial growth of pathogenic away from the antagonistic one.

B: The distance of mycelial growth of the pathogenic fungi towards the antagonistic one.

Antagonism *in vivo*

A greenhouse experiment was carried out in complete randomized block design with three replicates. Pots (25 cm in diameter) filled with 4 kg sandy-clay soil (1:1) infested with 3% inoculum per pot of three selected seed borne fungi *F. solani*, *M. phaseolina* and *R. solani* each fungi alone. Surface sterilized seeds of bean cv. pulsta, faba bean cv. local and peas cv. Local were immersed for one hour in spore suspension of *Trichoderma* isolates at concentration of 6×10^8 CFU /ml prepared from 7-10 days old culture grown on PDA medium according to (Elad *et al.*, 1982 ; Abd El-Kader, 1997). Then treated seeds were left 1-2 hours to dry. Inoculated seed were sown in infested pots at the rate of 5 seeds/pot. Percentage of the pre, post-emergence damping-off and the survival plants were recorded 15, 30 and 60 days after sowing, respectively.

Statistical Analysis

All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significance difference (LSD) at P = 0.05 (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Isolation, Purification and Identification of the Biological Control Agents

Twenty-three isolates of *Trichoderma* species were isolated from 63 soil samples collected from different locations of the East Delta Region in Egypt. Number of *Trichoderma* isolates from the rhizosphere of different soil types (Tables 1 and 2) were 7, 7, 3, 3, 2 and 1 from Sharkia, Ismailia, Port Said, Suez, North Sinai and South Sinai, respectively. Trichoderma isolates were isolated from different habits (Elad *et al.*, 1981; Shalaby and Atia 1996; Atia *et al.*, 2005; Kovács *et al.*, 2014). The percentage of *Trichoderma* isolates to soil samples in respect to location were 37%, 45%, 30%, 10%, 46%, and 17% in Sharkia, Ismailia, Port Said, Suez, North Sinai, and South Sinai, respectively.

Evaluation of *Trichoderma* Isolates

Growth rate mm/day

Mean average of linear growth rate for isolates ranged from 41.78 and 53.41 mm/day for isolate G5 PP1 and G4 IF2, respectively (Fig. 1). Results showed that significant differences between isolates G1 SA1, G2 NA, G3 IQ2, G5 PP1, and

Table 1. Number of *Trichoderma* isolated from soil samples collected from different locations in the East Delta Region

Area	Site	No. of soil samples collected	No. of isolates collected	(%) of isolates collected/ soil sample	Codes *Trichoderma* isolates
Sharkia	**Abu Hammad**	5	2	40	SA1, SA2
	Al-Qarin	2	1	50	SQ1
	Zagazig	3	1	33	SZ1
	Bilbes	5	3	60	SB1, SB2, SB3
	Abu Kabir	2	0	0	-
Average				37	
Ismailia	**Al-Tall Al-Kabir**	3	1	33	IT1
	Al-Qasasin	5	2	40	IQ1, IQ2
	Abu Sawyer	2	1	50	IS1
	Fayed	3	2	67	IF1,IF2
	Mahsmh	3	1	33	IM1
Average				45	
Port Said	**Sahl Alttayna**	2	1	50	PS1
	Sahl Alhasaynia	3	0	0	-
	South Port Said	5	2	40	PP2,PP1
Average				30	
Suez	**Shandoura**	2	0	0	-
	Al-Jinayin	5	1	20	SzA1
Average				10	
North Sinai	**Nakhl**	2	1	50	NH1
	Bir al-Abed	3	1	33	NB1
	Al-Hasana	1	0	0	-
	Al-Aarish	2	2	100	NA1,NA2
Average				46	
South Sinai	**Ras Sidr**	3	1	33	SoR1
	Al-Tur	2	0	0	-
Average				17	

Table 2. Classification *Trichoderma* isolates into six groups according to their morphological characters and isolates selected of groups

Group	Isolate	Selected isolate	Isolate code
Group1	SA1, , PS1, NH1	SA1	G1 SA1
Group 2	SZ1, SB3, SzA1, NA, NB11	NA	G2 NA
Group 3	SA2, IQ2, IF1	IQ2	G3 IQ2
Group 4	SB2, IF2, PP2	IF2	G4 IF2
Group 5	SQ1, IS1, PP2	PP1	G5 PP1
Group 6	SB1, IT1, IM1, SoR1, NA2	SoR1	G6 SoR1

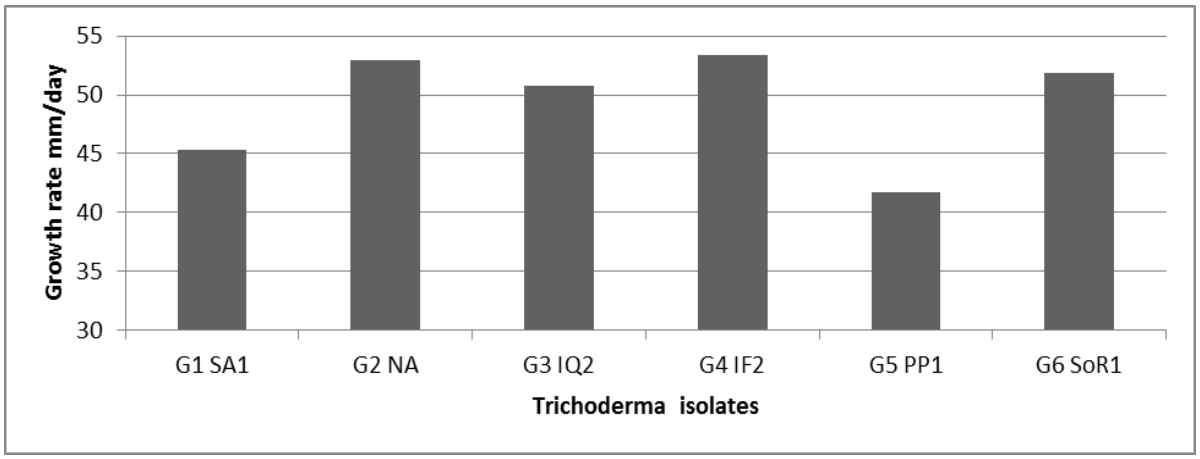

Fig. 1. Rate of liner growth of the six selected *Trichoderma* isolates measured in mm/day for 3 days on PDA at 25 ±2 **°C**

G1 SA1and G6 SoR1; as well as between G5 PP1 and G6 SoR1. There were no significant differences between isolates G2 NA, G4 IF2, and G6 SoR1, isolates G3 IQ2 and G6 SoR1. Similar results were obtained by Kovács *et al*. (2014). They found that, *Trichoderma* isolates TR05 and TR06 are capable for active growth under broad temperature.

Antagonism *in vitro*

Isolate G4 IF2 significantly reduced reduction (%) of *F. solani* linear growth followed by G6 SoR1 and G2 NA, which recorded 57.11, 54.02 and 51.07%, respectively. While, G2 NA caused significantly reduced linear growth of *M. phaseolina* followed by G4 IF2 and G6 SoR1which, recorded 71.11%, 68.15 and 66.67%, respectively. On the other hand, G4 IF2 significantly reduced linear growth of *R. solani* followed by G6 SoR1 and G2 NA, Table, 4 and Fig. 2 (49.56, 47.01 and 45.22% of growth reduction, respectively).

Significant differences were detected in linear growth reduction of *F. solani* between isolates G1 SA1 and G2 NA, G4 IF2, and G6 SoR1. Also, Significant differences were detected in linear growth reduction of *M. phaseolina* between isolates G1 SA1 and G5 PP1, isolates G2 NA, G3 IQ2, G4 IF2 and G6 SoR1. As well as, significant differences were detected in linear growth reduction of *R. solani* between isolates G1 SA1 and isolates G2 NA, G3 IQ2, G4 IF2, G5 PP1and G6 SoR1. G4 IF2 significantly reduced linear growth of *F. solani*, *M. phaseolina* and *R. solani*, followed by G6 SoR1 and G2 NA (58.27, 55.90 and 55.8%, respectively) (Table 3).

Effect of *Trichoderma* isolates on growth inhibition of the pathogens may be due to mycoparasitism, competition for space and

nutrients and production of volatile compounds (Reddy *et al.*, 2014). Mycoparasitism includes hyphal interaction and parasitism, is the most

Table 3. Percentage of reduction in the mycelial growth of some pathogenic seed-borne fungi by *Trichoderma* isolates

Growth reduction (%)	Seed borne fungi			
***Trichoderma* isolates**	***Fusarium solani***	***Macrophomina phaseolina***	***Rhizoctonia solani***	**Mean**
G1 SA1	33.67	25.19	22.43	27.10
G2 NA	51.07	71.11	45.22	55.80
G3 IQ2	45.69	62.22	39.06	48.99
G4 IF2	57.11	68.15	49.56	58.27
G5 PP1	46.67	42.96	35.68	41.77
G6 SoR1	54.02	66.67	47.01	55.90
LSD at 5%	15.66	17.82	4.60	12.69

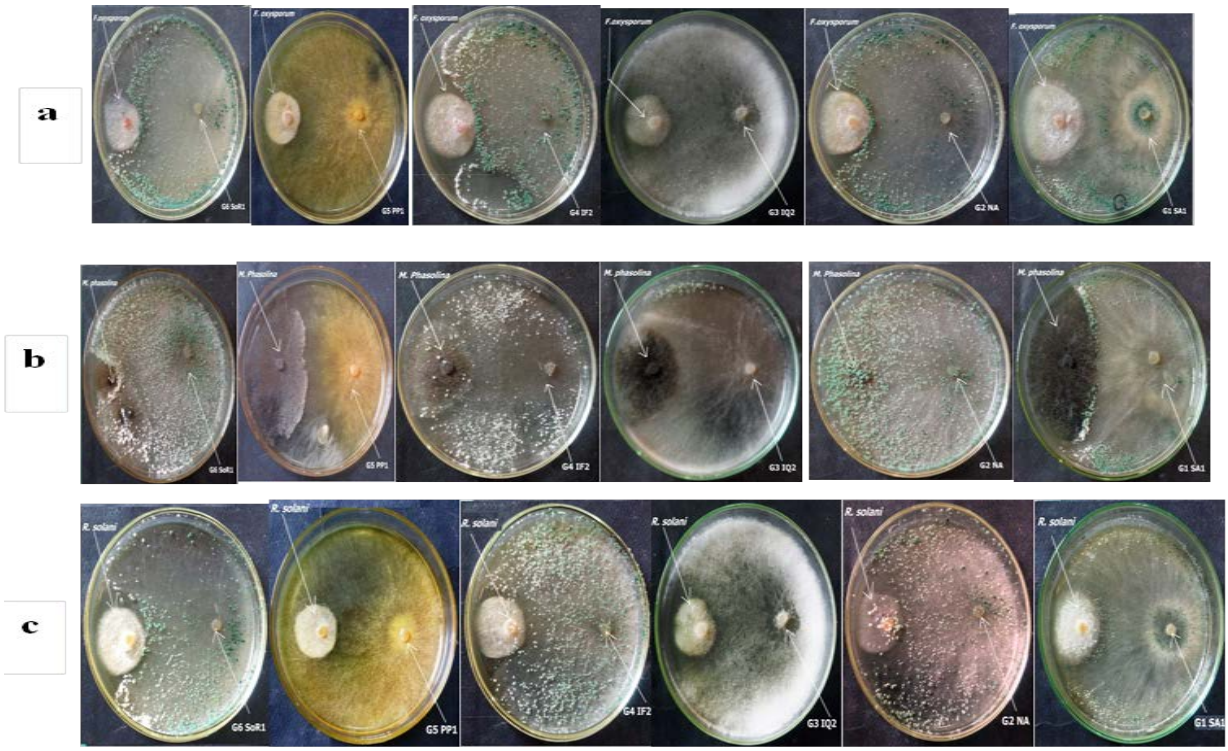

Fig. 2. Antagonistic activity of *Trichoderma* isolates on mycelium growth of: a) *Fusarium solani*, b) *Macrophomina phaseolina* c) *Rhizoctonia solani* isolated from some legumes seeds using potato dextrose ager

vital mechanism of the fungal antagonist (Elad *et al.*, 1982; Pan and Bhagat, 2007; Reddy *et al*., 2014). Different species of *Trichoderma* were effectively against pathogenic fungi (Elad *et al*., 1982; Reddy *et al.*, 2014). Harman *et al.* (2004a) suggested that, mycoparasitism was the principle mechanism involved in controlling *Pythium* damping-off of pea seed. This phenomenon may probably be correlated with the differences in levels of hydrolytic enzymes produced by each isolates when they attach the mycelium of the pathogens. *Trichoderma* spp. was capable of producing extra cellular lytic enzymes that are responsible for their antagonistic activity (Elad *et al.*, 1982). Antagonism by *Trichoderma* spp. against a range of seed borne plant pathogens have been reported earlier by Papavizas (1985) and Elad *et al. (*1982). Observations on the growth and colonization of the test pathogens in dual culture screening by the antagonistic isolates proved that different isolates of *Trichoderma* has variation in their ability to inhibit the growth of the pathogen (Cherkupally *et al*., 2017).

Antagonism activity *in vivo*

Results presented in Table 4 show that, the treatment with G5 PP1 isolate recorded highly survival seedling of faba bean sowing in infested soil with *F. solani* followed by G1 SA1, G2 NA and G6 SoR1 were recorded 93.33, 86.67.0 and 80.0%, respectively. Significant differences were detected in faba bean survived seedlings between G5 PP1 and G4 IF2 treatments.

Isolate G3 IQ2 recorded highly survival seedling of faba bean sowing in infested soil with *M. phaseolina* (86.67) followed by G2 NA and G6 SoR1 which recorded 80.0 and 80.0%, respectively.

Isolate G5 PP1 recorded highly survival seedling of faba bean sown in soil infested with *R. solani* followed by G3 IQ2 and G6 SoR1 which recorded 86.67, 73.33 and 73.33%, respectively.

Results in Table 4 also show that, the treatment with G4 IF2 recorded highly survival seedling in bean sowing in infested soil with *F. solani* (93.33%) followed by G2 NA, G5 PP1 and G6 SoR1 (recorded 86.67%). Also, isolates G2 NA, G4 IF2 and G6 SoR1 were equal of highly survival seedling in bean sowing in infested soil with *R. solani* were recorded 86.67% followed by G1 SA1 and G3 IQ2 which were equal and recorded, 80.0%. Treatment with G1 SA1, G2 NA, and G3 IQ2 were identical of highly survival seedling of peas sowing in infested soil with *F. solani* (93.33%) and followed by G4 IF2, G5 PP1 and G6 SoR1 were equal (86.67.0%). Treatment with G2 NA, G4 IF2 G5 PP1 and G6 SoR1were matching with highly survival seedling of peas sowing in infested soil with *M. phaseolina* (86.67%) followed by G1 SA1 and G3 IQ2 which were equal (86.67.0%). Isolates G2 NA showed highly survival seedling of peas sowing in infested soil with *R. solani* (86.67%) followed by G4 IF2 (80.0%) and G3 IQ and G5 PP1, were equal (73.33%). Significant differences were detected survival seedling of bean sowing in infested soil with *R. solani* between isolates G2 NA, G1 SA1 and G6 SoR1. *Trichoderma* isolates G2 NA and G5 PP1 were the most effective ones showed highly survival plants in general in infested soil with *F. solani, M. phaseolina* and *R. solani*, followed by G6 SoR1 recorded 82.22, 82.22 and 80.79%, respectively.

Trichoderma isolates were found to be an effective biological control agent for protecting number of crops from infection with *F. solani, M. phaseolina,R. solani* and other soil borne fungi under *in vivo* (Marzano *et al*., 2013; Pastrana *et al*., 2016; Kumari *et al*., 2016). Several mechanisms may explain the biocontrol activity of these isolates (Elad, 1996). Hyperparasitism and volatile metabolites may be involved in the inhibition of *R. solani* (Naeimi *et al*., 2010). Cell wall degrading enzymes such as chitinase, glucanase and proteases are thought to be closely related to the mycoparasitism of *Trichoderma* strains (Harman, 2006;

Figure2. Antagonistic activity of *Trichoderma* isolates on mycelium growth of: a) *Fusarium* *Macrophomina phaseolina* c) *Rhizoctonia solani* isolated from some legumes seeds using potato dex

Saravanakumar *et al.*, 2016). Inhibitory volatile substances such as alkylpyrons may also contribute to the biocontrol activity of some *Trichoderma* strains (Devaki *et al.*, 1992; Papavizas and Lumsden, 1982; Claydon *et al.*, 1987). Harman *et al.* (2004b) reported that, *Trichoderma* have ability to antagonize seed-borne phytopathogens as well as it also induced plant growth promotion and protect plants form biotic and abiotic stresses

Table 4. Effect of *Trichoderma* isolates collected on the survival plants of faba bean (cv. local), bean (cv. Pulsta) and peas (cv. Local) sowing in soil infested with three pathogenic fungi under greenhouse conditions

Seed borne fungi / *Trichoderma* isolates	Survived faba bean plants (%) in soil infested with			Survived bean plants (%) in soil infested with			Survived peas plants (%) in soil infested with			Mean
	Fusarium solani	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Fusarium solani*	*Macrophomina phaseolina*	*Rhizoctonia solani*	*Fusarium solani*	*Macrophomina phaseolina*	*Rhizoctonia solani*	
G1 SA1	86.67	66.67	73.33	80	93.33	80	93.33	80	66.67	80.00
G2 NA	80	80	73.33	86.67	66.67	86.67	93.33	86.67	86.67	82.22
G3 IQ2	73.33	86.67	80	80	73.33	80	93.33	80	73.33	80.00
G4 IF2	66.67	66.67	60	93.33	66.67	86.67	86.67	86.67	80	77.04
G5 PP1	93.33	73.33	86.67	86.67	86.67	66.67	86.67	86.67	73.33	82.22
G6 SoR1	80	80	73.33	86.67	80	86.67	86.67	86.67	66.67	80.74
LSD At 5%		21.67			19.92			18.57		20.05

(Maurya *et al.*, 2008). Isolate G4 IF2 caused a remarkable mean reduction linear growth *in vitro* between *F. solani, M. phaseolina* but *in vivo* it was the lowest isolates in mean percentage of survival plants. Similar results were found by Companile *et al*. (2007) who found that, means good results *in vitro* assay are not always good indicators of positive antagonistic effects *in vivo*.

Thus, it can be concluded that, *Trichoderma* isolates obtained proves to be an effective biocontrol agent and native isolates of it may be further explored as biocontrol agent against *F. solani, M. phaseolina* and *R. solani*. *Trichoderma* isolates collected needed molecular identification and genetically improvement to obtained one isolate used as highly effective biocontrol agent against legumes seed born fungi.

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عزل وتقييم فطر التريكوديرما كعامل المقاومة الحيوية ضد الفطريات المنقولة عن طريق بذور البقوليات من منطقة إقليم شرق الدلتا

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تم الحصول على ٢٣ عزلة من فطر التريكوديرما من ٦٣ عينة تربة والتي تم الحصول عليها من منطقة شرق الدلتا، وكانت نسبة العزلات المتحصل عليها بالنسبة لعدد عينات التربة هو ٣٧%، ٤٥%، ٣٠%، ١٠%، ٤٦% و١٧% في محافظات الشرقية والإسماعيلية وبورسعيد والسويس وشمال سيناء وجنوب سيناء على التوالي، تم اختيار عدد ستة عزلات مختلفة في الشكل المظهري لإجراء التقييم عليها، وقد تراوح متوسط معدل النمو الخطي لعزلات التريكوديرما من ٤١.٧٨ مم/يوم لعزلة G5 PP1 و ٥٣.٤١ مم/يوم لعزل G4 IF2، تم قياس التضاد الحيوي معمليا بين عزلات التريكوديرما المختارة وضد الثلاثة فطريات الممرضة والمعزولة من بذور بعض البقوليات وهي فيوزاريم سولاني، ماكروفومينا فاسولينا وريزوكتونيا سولاني، وقد سببت العزلات G4 IF2 وG2 NA وG4 IF2 انخفاض كبير في النمو الخطي لفطر فيوزاريم سولاني، ماكروفومينا فاسولينا، ريزوكتونيا سولاني على التوالي، تم استخدام ثلاثة انواع من البقوليات هي الفول البلدي، الفاصوليا، البسلة لدراسة تأثير التضاد الحيوي لعزلات التريكوديرما ضد مرض موت البادرات في الصوبة، وقد سجلت العزلاتG5 PP1 و G3 IQ2 و G5 PP1 أعلى نسبة من البادرات السليمة للفول البلدي في التربة المعدية بالفطريات الثلاثة المختبرة، وقد سجلت العزلات G4 IF2 و G1 SA1 أعلي نسبة من بادرات الفاصوليا السليمة في التربة المصابة بالفطريات، في حين أن العزلات G2 NA و G4 IF2 و G6 SoR1 كانت متساوية في نسبة البادرات السليمة في التربة المصابة بفطر ريزوكتونيا سولاني علي محصول الفاصوليا، وقد كانت المعاملة بالعزلاتG1 SA1 وG2 NA و G3 IQ2 متطابقة في نسبة البادرات السليمة عند زراعة بذور البسلة في التربة المصابة بالفيوزاريوم سولاني، في حين كانت المعاملة بالعزلات G2 NA و G4 IF2 و G5 PP1 و G6 SOR1 كانت متساوية في نسبة البادرات السليمة للبسلة، وقد أظهرت المعاملة بالعزلة G2 NA أعلى نسبة من البادرات السليمة عند زراعة بذور البسلة في التربة المصابة بالريزوكتونيا سولاني وعموما كانت العزلة G2 NA والعزلة G5 PP1 أعلى العزلات تأثيراً مع الفطريات الثلاثة المختبرة مع محاصيل البقول تحت الدراسة، وتقترح هذه الدراسة إحتياج العزلات الست المختبرة إلى التعريف علي المستوي الجزئي للحامض النووي والتطوير الوراثي للوصول إلى عزلة واحدة قادرة علي مقاومة معظم الفطريات المنقولة عن طريق البذور في البقوليات.