In vitro Antagonistic Activity of *Trichoderma harzianum* against *Rhizoctonia solani* The Causative Agent of Potato Black Scurf and Stem Canker

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IN EGYPT Potato (*Solanum tuberosum* L.) is considered one of the most important vegetable crops as well as many others countries in the world. It plays an important role in the Egyptian agricultural economy, not for local consumption but also for exportation especially to Europe, Russia and Arabic countries. However, potato crop suffer from more than 40 pests and diseases caused by insects, nematodes, viruses, bacteria and fungi, of which black scurf and stem canker induced by *Rhizoctonia solani* is probably the most serious disease. The world yield losses caused by *R. solani* were estimated to 5-15%. *R. solani* is an unspecialized parasite, survive in soil in the absence of host plant and make itself a very difficult pathogen to manage. Reduction or elimination of soil borne inoculum is the only effective solution to overcome the problem and this may be achieved through the application of various control measures of which fungal antagonists consider among the most important tactic. Although *R. solaniis* usually controlled through the application of chemicals, the serious ecological and financial toll of this fungus has prompted for research on biopesticides as a viable alternative. *Trichoderma* spp. were well-known fungi often used for the biological control of crop pests, whose anti-fungal mechanisms include competition for the substrate, antibiosis and/or mycoparasitism. Five isolates of *Trichoderma harzianum* were tested *in vitro* for their antagonistic potential against *Rhizoctonia solani*. Both *Trichoderma harzianum* and *Rhizoctonia solani* were identified by molecular and morphological methods. In dual culture of all isolates were found antagonistic to the growth of *R. solani*. The hyphal interaction studied using light microscopy revealed destructive mycoparasitism of *R. solani* by *T. harzianum.* The method of mycoparasitism was sparse to intense coiling of *R. solani* followed by disintegration, disorganization and death of *R. solani* mycelium.

Keywords: Potato, Black scurf, *Rhizoctonia solani,* Biocontrol, *Trichoderma* spp.

Introduction

The Food and Agriculture Organization (FAO) proposed highlighting the potato (*Solanum tuberosum* L.) for (1) Its key role in the world global food system as it is the world's fourth most produced food commodity, (2) Its ability to grow worldwide, (3) Its convenience for farming systems in developing countries – potato crops harbour a high ratio of yield productivity to soil occupation (85% of the plant is comestible compared with only 50% in cereals), and (4) Its nutritive qualities, with a higher amount of vitamins than grass plants (FAO, 2008).

Potato however, suffer from many pets of which stem canker and black scurf diseases caused by *Rhizoctonia solani* Kuhn (teleomorph, *Thanatephorus cucumeris* (A. B. Frank, Donk), consider one of the most important cosmopolitan necrotrophic soil-borne fungus. These diseases have worldwide distribution wherever potato is grown but their etiology varies depending on the predominance of distinct *R. solani* anastomosis groups (AGs) in a particular area. Although the disease is superficial on tuber, negative economic impact of sclerotia on potato, both as a tuber blemish and a source of inoculum on seed and in soil is very important and considered.

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On worldwide basis different anastomosis groups (AGs) of *R. solani* that have different virulence level associated with potato stem canker and black scurf diseases, has been identified (Jehtonen, 2009). AG3 group of *R. solani* is responsible disease in potato however the other anastomosis groups such as AG-2 type 1, AG-2 type 2, AG-4 and AG-5 are also included in infection both in tuber and stems (Demirci & Doken, 1993). Several researchers now recognize *R. solani* as a complex of species which can be best delimitedon the basis of their hyphal fusion *i.e.* anastomosis (Anderson, 1982; Ogoshi, 1987 and Balali et al., 1995). In general, these groups of species share the following characteristics: (i) Multinucleate cells, pale to dark brown pigment, rapidly growing mycelium of relatively large diameter with branching near the distal septum of hyphae, (ii) Formation of monilioidcells which are barrel-shaped cells, chain of these cells aggregate to produce vegetative resting structure, the sclerotia, (iii) Sclerotia are usually of uniform texture and of varying size and shape, and (iv) Lack of conidiaand sexual spores.

Planning to control *Rhizoctonia* diseases are restricted because of its ecological behavior. It is extremely wide host range, having high survival rate of sclerotia under various environmental condition (Grosch et al., 2006).Moreover, cultivars with complete resistance are not available at now (Li et al., 1995). Thence, effective strategies to control the pathogen are demanding. In the other hand, increasing use of chemical application causes several negative effects, such as the development of pathogen resistance to the applied agents and their non-target environmental impacts (Gerhardson, 2002). A growing awareness of agricultural practices in using chemicals have a great impact on human health and on the environment has spawned research into the development of effective biocontrol agents to protect crop against diseases.

Application of antagonistic fungi as biological control agents is considered a non-polluting approach for alternative plant protection. This biological control relies on a reduction of the pathogen population size by the control organism, thus keeping pathogen density below the threshold necessary for disease formation. There is now unequivocal evidence that antibiotics and/or direct parasitism play a key role in the suppression of various soil-borne plant pathogens by antagonistic microorganisms (Braun et al., 2010 and Wensing et al., 2010).

Diverse species belonging to the genus *Trichoderma* are capable of parasitizing fungal plant pathogens such as *R. solani*, producing antibiotics effective against soil-borne pathogens and competing for infection sites against pathogens (Bénítez et al., 2004 and Vinale et al., 2008). *Trichoderma virens* and *T. harzianum* have been shown to be effective at controlling stem canker and black scurf, as well as increasing tuber yield (Tsror et al., 2001; Brewer & Larkin, 2005; Wilson et al., 2008a and 2008b).). Kurzawińska (2006) also demonstrated an antagonistic effect of several *Trichoderma* species on *Helminthosporium solani,* providing evidence for the potential suppression of silver scurf by *Trichoderma*. Wang et al. (2009) reported that the use of an antagonistic microorganism of a *Bacillus* sp. strain CHM1 against *R. solani* on horse bean (*Vicia faba*), and Kumar et al. (2013) who also reported a *Bacillus* sp. strain N antagonized *R. solani, Fusarium oxysporum*, and *Penicillium expansum. R.solani* causes various kinds of diseases on such as bottom rot on lettuce, black scurf on potato, damping-off of cucumber, pine, and tomato (Tunlid et al., 1989; Huang et al., 2012 and Golinska & Dahm, 2013).

Comprehension the mechanisms of biological control of plant diseases through the interactions between antagonists and pathogens may allow us to select and construct the more effective biocontrol agents and to manipulate the soil environment to create a conducive condition for successful biocontrol. The mechanisms of biocontrol may involve and be divided into (i) Antibiosis, (ii) Competition, (iii) Mycoparasitism, (iv) Cell wall degrading enzymes, and (v) Induced resistance. However, these mechanisms of biological control are probably never mutually exclusive. They may include one and more processes (Adams, 1990).

The aim of the present study was to isolate and identify fungal biota as well as testing strains of *Trichoderma harzianum* isolated from healthy and infected potato tubers to determine the efficacy of their potentiality as biocontrol agents against *R. solani* causative agent of potato stem canker and black scurf under laboratory conditions (*in vitro*).

Materials and Methods

Source of potato tubers

Samples of potato (cultivar spounta) showing typical symptoms were taken from different parts

of plant (stem, tuber, root, stolon and sprout) were collected from Ismailia Governorate (El-Shabab Region). Some fields were examined randomly through potato plantation (summer crop). The samples were transferred to laboratory in tight polyethylene bags and kept at low temperature until plating out; for isolation of the black scurf causal agent and other tuber associate fungal biota.

Isolation of Rhizoctonia solani

Pieces of potato plant (stem, tuber, root, stolon) that showed symptoms of *R. solani* disease were submerged in 2 % sodium hypochlorite for 3 min then soaked in 70 % ethanol for 1 min (Melgarejo et al., 1985). After rinsing with sterile water several times, infected potato tuber peel were divided into small pieces, transferred to plates of potato-dextrose-agar (PDA) supplemented by Rose bengal and chloramphenicol and incubated at 28ºC for four days. Plates were daily observed for mycelial growth (Fig. 1). Hyphal tips emerging from the peel pieces, were transferred to fresh plates of PDA and CYA (Czapek's yeast extract agar). The isolated *R. solani* strains were stored at 5ºC in slant containing PDA.

Fig. 1. Plate containing isolated *Rhizoctonia* showing hyphal growth.

Disease Incidence

Fifty potato tubers were randomly selected from each surveyed location to determine disease incidence using formula proposed by Ahmed et al. (1995):

$$\text{Disease Incidence (black scurf)} = \frac{\text{No.of tubers infected}}{\text{Total Tubers Observed}} \times 100$$

Disease severity

Black scurf severity disease was determined by using 0-5 disease severity grades based on percent tuber surface showing disease symptoms (Ahmed et al., 1995).

Isolation of fungal biota

The dilution plate technique as described by Waksman (1927) was adopted throughout this investigation for isolation and counting of fungal biota associated with healthy and infested potato tubers.Czapek's Yeast Extract Agar (CYA) supplemented with a combination of rose bengal (1/15.000) and Chloramphenicol (50 ppm) to suppress bacterial growth was elected during this study where it showed the best diversity as well as the most reasonable colony count. For each tubers sample six plates has been prepared thereafter incubated at 29 ºC, after 7 days, growing colonies were identified and counted.

Morphological identification of R. solani & T. harzianum

Macro-morphology (colony diameter, colony color, reverse and exudates) and micro-morphology (conidia & phialides if present, mycelium) of both *R. solani* & *T. harzianum* were used as criterions for Morphological identification of the fungal biota.

Antagonistic efficiency of Trichoderma isolates

Fifth *Trichoderma harzianum* strains were selected from the isolated fungi based on prior *in vitro* and *in vivo R. solani* suppression, or on their previous success in controlling plant diseases, including soil-borne plant pathogens in other biocontrol programs.

Trichoderma harzianum isolates from healthy and infested potato tubers were tested in a dual culture assay against *R. solani* representing AG-3 (isolate RS -1), which was recovered from potato tubers infected by black scurf. The isolates were cultivated in Petri dishes with PDA media for seven days. Disks of 5mm of diameter were cut and removed from the growing borders of the colonies and transferred to another Petri dish with PDA. Each plate received two disks, one with *Trichoderma* mycelium and another with *Rhizoctonia* mycelium, placed at a distance of 7 cm from each other, in time intervals according the growth speed test organisms. The plates were incubated at 29 °C for five days in according to the growth rate of

the two competitors. The experiment was entirely at random with three replicates. The percentage inhibition of radial growth I = [(C – T) / C] × 100 of *R. solani* and the width of the zone of inhibition between both colonies were recorded as described by Royse & Ries (1978). Mode of inhibition was assessed on a scale from 1 to 4, in which 1 = Mycelial growth of *R. solani* ceased due to overgrowth of *T. harzianum*, 2 = Partial inhibition of *R. solani* and interacting fungus but both grow slowly over each other, 3 = Mutual inhibition at a distance <2 mm, 4 = Growth of *R. solani* inhibited at a distance > 2 mm (Chand & Logan, 1984).

$$I = [(C – T) / C] \times 100$$

where, I = Inhibition of radial mycelial growth; C = Pathogen Radial growth measurement in control; T = Pathogen radial growth in the presence of *Trichoderma* spp.

Molecular identification of R. solani and T. harzianum

Anastomosis group identification of R. solani

Isolates were assigned to AGs by conventional PCR assays using specific primers for AG-3, sequencing of the ITS-rDNA and hyphal interactions.

Mycelium for DNA extraction from *R. solani* isolates was obtained from six days old cultures on PDA. After incubation at room temperature, mycelium was harvested by scraping the mycelia from the surface of PDA plates, followed by freezing and lyophilization. DNA was extracted with a QIAGEN® DNeasy Plant Mini-Kit. The anastomosis group was determined by selective amplification of the ribosomal DNA (rDNA) region using specific primers for *R. solani* AG-3 (Lees et al., 2002). Polymerase chain reactions (PCR) were carried out in 10 µl volumes containing 10-50 ng of genomic DNA, 10 mM of KCl, 10 mM of $(NH_4)_2SO_4$, 20 mM of Tris-HCl, 2 mM of $MgSO_4$, 0.01% of Triton X-100, pH 8.8 (NEB), 0.2 µM of each primer (Microsynth), 0.1 mM of each dNTP and 0.06 U of Taq polymerase (NEB). PCR conditions comprised initial denaturation of 2 min at 96°C, followed by 35 cycles of denaturation for 30 s at 96°C, annealing for 45 s at 57°C and elongation for 45 s at 72°C, with a final extension step of 5 min at 72°C. In both cases, PCR products were visualized with UV on agarose gels.

Genomic DNA extraction from Trichoderma strains

Genomic DNA was extracted from the mycelium of *Trichoderma* isolates using the method described by Wijesinghe et al. (2010).

PCR amplification of ITS region of Trichoderma isolates

To confirm the species of strain *Trichoderma* at the molecular level, ITS region was amplified using universal primers ITS 1(5'-TCTGTAGGTGAACCTGCGG3') and ITS 4(5'-TCCTCCGCTTATTGATATGC-3') according to White et al. (1990) and Gardes & Bruns (1993). Genomic DNA was amplified using a DNA thermal cycler of Applied BioSystems (USA).The reaction mixture contains 38.5 µl deionized water, 5µl 10 X Taq polymerase buffer, 0.5 µl of 1 U Taq polymerase enzyme, 3 µl 2 mM dNTPs, 1 µl of 100 mM reverse and forward primers and 1 µl of 50 ng template DNA. PCR conditions were as follows; an initial denaturation of 3 min at 94°C followed by 35 cycles of 1 min denaturation at 94 °C, 1 min primer annealing at 50 °C, 1 min extension at 72 °C and a final extension of 10 min at 72 °C. PCR products were checked by electrophoresis using 2% agarose gel in 1X TAE buffer.

Results

Results of surveyed fields showed that the prevalence of disease at all visited areas. The above-ground parts of potato plants showed aerial tubers, rolling of leaves and chlorosis before harvesting while the most obvious symptoms of the black scurf were observed as sclerotial masses on tubers after harvesting of crop (Fig. 2).

Data of Table 1 clearly showed that maximum disease incidence for black scurf and stem canker was (23 % and 26 %) in field no. 4, minimum was (8 % and 9 %) in field no. 1.Data represent the average of two consecutive years 2013/2014 in surveyed fields.

Black scurf severity disease was determined by using 0-5 disease severity grades based on percent tuber surface showing disease symptoms (Ahmed et al., 1995), where: 0 = no symptoms on the potato tubers, 1 = less than 1% of the tuber area affected, 2 = 1-10% of the tuber area affected, 3 =11- 20 % of the tuber area affected, 4 =21- 51 % of the tuber area affected and 5 = 51 % or more of the tuber area affected (Fig. 3).

TABLE. 1. Percentage of potato stem canker and black scurf in investigated fields.

Governorate	Season	Field No.	Potato cultivar	% of disease incidence 2013 / 2014	
				Black scurf	Stem canker
Ismailia	Summer	1	Spounta	8	9
		2		17.6	16.3
		3		18.6	17.6
		4		23	26
		5		12.3	13
		6		17	15.3
		7		14.3	16.3
LSD (0.05%)				5.96	4.5

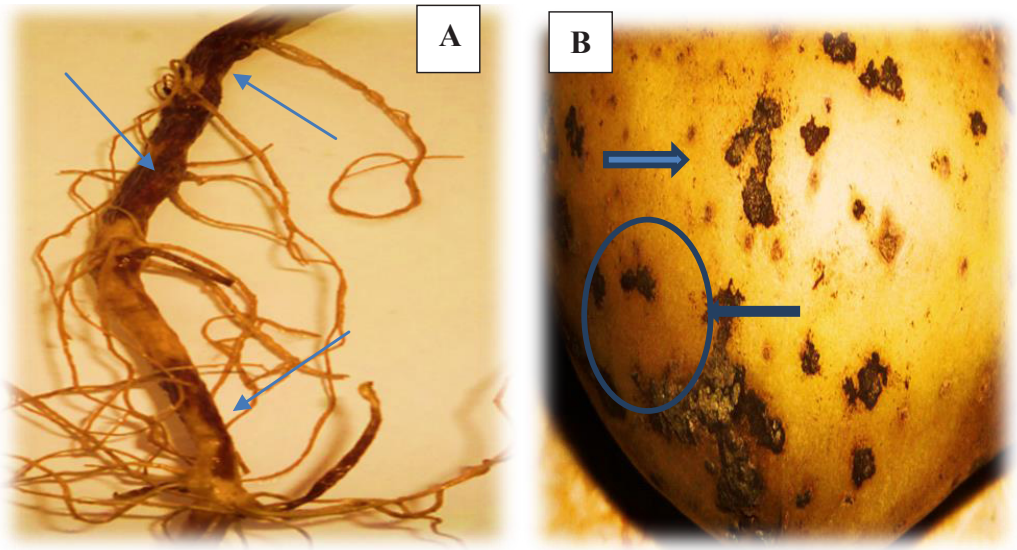

Fig. 2. Symptoms of R. solani infected potato plant and tuber.
A- Lesions of stem canker (arrows), X= 25 B- Sclerotia on tuber (arrows), X= 25.

Fig. 3: Black scurf index in potato according to Ahmad et al. (1995), X= 25.

The *Rhizoctonia solani* was initially identified by the presence of thread-like whitish mycelial growth, which gradually turned to dark brown followed by production of sclerotia on the culture that seemed to be submerged in the agar plates; large aggregates of sclerotia were spherical and irregularly shaped (Fig. 4).

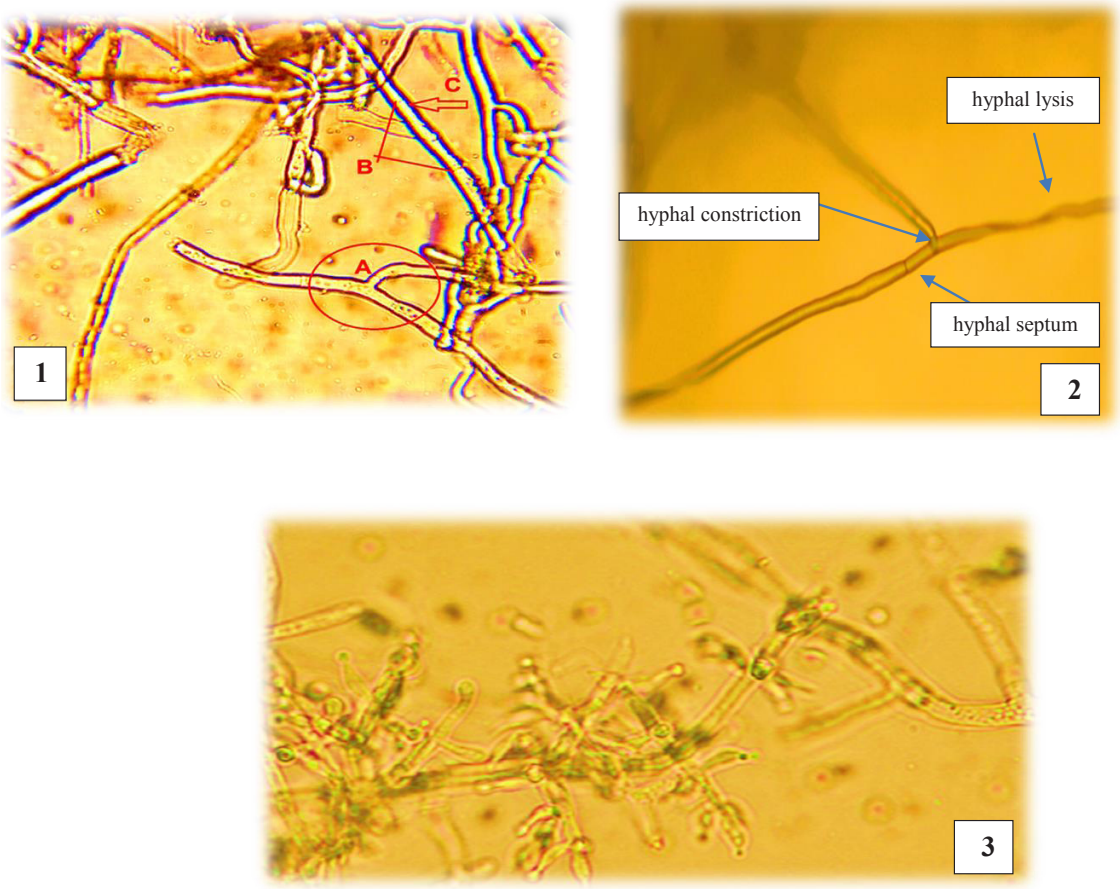

Fig. 4. Morphological characteristics to *R. solani* and *T. harzianum* under light microscope (1): A branching hypha B- septum C- main hyphae (X= 25 %) (2): hyphal branching and mycelium septum (arrow; X= 25 %). (3): T. harzianum phialides & conidia (X= 50 %).

Fungal biota of healthy and infected tuber

A spectrum of 14 genera represented by 23 species have been reported from the peel tubers of healthy and infected potato tubers (Table 2). In view of total count the most abundant species comprise the following taxa (in decreasing order of dominance): *Aspergillus niger*, *A. terreus*. *A. flavus*, *A. versicolor*, *Fusarium solani*, *Penicillium chrysogenum*, *Cladosporium cladosporioides*, *Scopulariopsis brevicaulis* and *Botryotrichum piluliferum*. These species are common to both healthy and infected tubers but with some difference in the order of dominance.

Antagonistic efficiency of Trichoderma isolates

Data of *in vitro* dual culture clearly demonstrated that all five tested *T. harzianum* isolates were potentially active against *R. solani* with a ratio ranged from 67 % to 73 %. Some strains overgrowth upon *R. solani* with producing clear zone (Fig. 5 a), other strains overgrowth on target pathogen with formation of coiling around *R. solani* mycelium as well as lysis of hyphae (Fig. 5 b, c, d).

Molecular identification of R. solani and T. harzianum

Data of PCR and DNA sequencing proved that most *R. solani* isolates evaluated were assigned to AG-3PT (99 %), while almost *Trichoderma* isolates were allocated to *T. harzianum* (98 % to 99 %).

TABLE 2. Mean count (cfu/g)* and frequency of fungal biota isolated from healthy and infected potato tubers.

No.	Fungal species / Potato tuber	Healthy tuber		Infected tuber	
		Count**	Frequency %	Count**	Frequency %
1	*Aspergillus niger*	87	85	83	71
2	*A. terreus*	65	71	80	57
3	*A. flavus*	43	57	94	85
4	*A. versicolor*	46	43	65	57
5	*Fusarium solani*	68	43	25	29
6	*Penicillium chrysogenum*	52	43	7	29
7	*Cladosporium cladosporioides*	18	43	21	43
8	*Scopulariopsis brevicaulis*	16	29	23	14
9	*Botryotrichum piluliferum*	15	29	21	29
10	*Penicillium citrinum*	33	29	-	-
11	*Chaetomium globosum*	19	29	6	14
12	*Penicillium* sp.	17	14	5	14
13	*Trichoderma harzianum*	11	43	6	57
14	*A. ochraceus*	13	14	-	-
15	*Fusarium oxysporum*	-	-	13	43
16	*Gliocladium roseum*	9	29	-	-
17	*Emericella nidulans*	8	14	-	-
18	*Absidia corymbifera*	-	-	7	43
19	*Chaetomium* sp.	6	14	-	-
20	*Acremonium terricola*	3	29	2	14
21	*Paecilomyces variotii*	-	-	5	29
22	*Trichoderma koningii*	-	-	5	29
23	*Myrothecium* sp.	-	-	3	14

*(cfu/g) = Colony forming unit
**Mean of 7 samples.

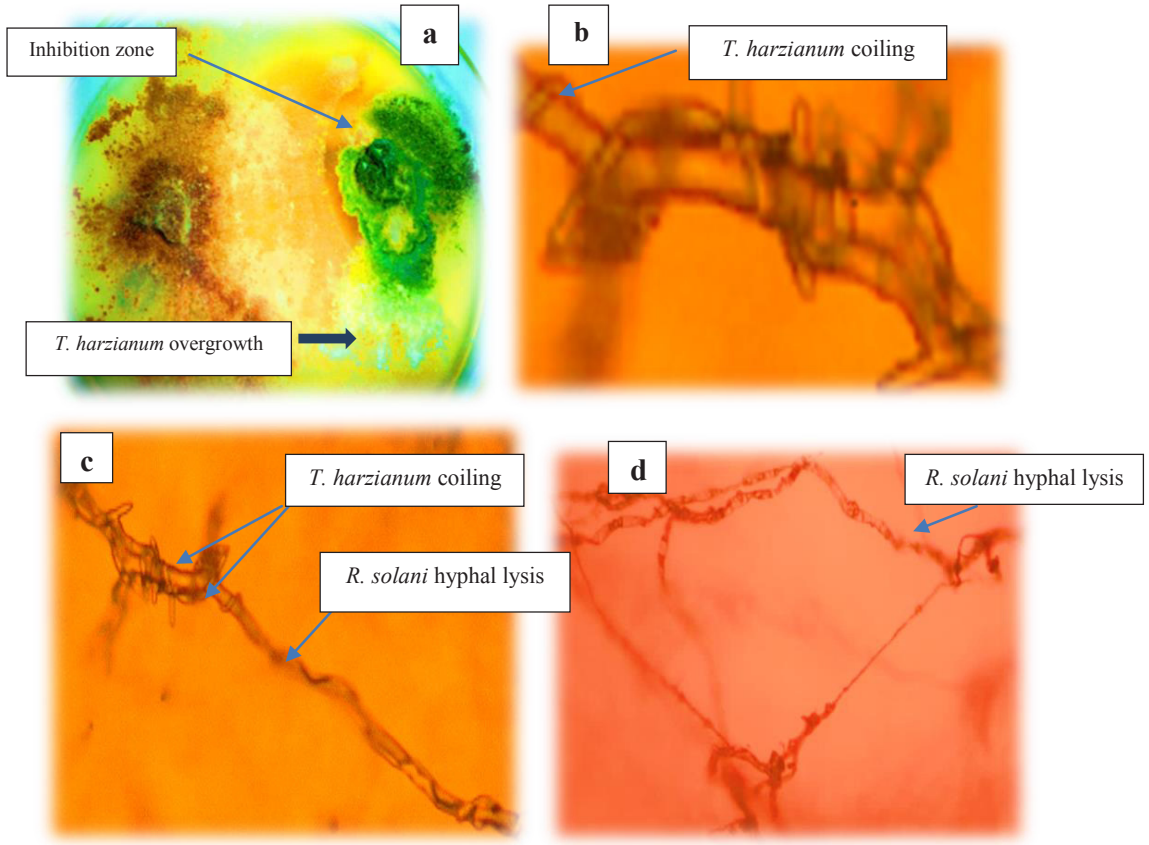

Fig. 5. Antagonistic efficiency of *T. harzianum* isolates against *R. solani*
i: (a) plate show inhibition zone & overgrowth (X= 12.5 %). ii: (b, c, d) interaction between the two opponent coiling of *T. harzianum* hyphae around *R. solani* hyphae (mycoparasitism X= 200 %, 100|%) & hyphal lysis of *R. solani* (X= 25%)

Discussion

R. solani is widespread and responsible for severe damage to many worldwide economically important agricultural and horticultural crops (Grosch et al., 2006). As for potato high yield losses were reported reaching up to 20 % (Grosch et al., 2005). Strategies to control *Rhizoctonia* diseases are limited because of its ecological behavior. Control of the pathogen is difficult because of its ecological behavior, extremely broad host range and the high survival rate of sclerotia under various environmental conditions (Groth & Bond, 2006). In agriculture, crop protection depend heavily on chemical pesticides. However, there is a growing concern for negative health and environmental effects of such pesticides. Biological control is an alternative strategy which may provide effective and sustainable control of *R. solani* on potato or other host crops (Larkin & Brewer, 2005).

Indeed, a lot of fungal biota have been reported to be effective biocontrol agents of *R. solani* on potato. Among these are species of *Gliocladium* (Papavizas, 1985 and Van den Boogert, 1996), *Trichoderma* (Brewer & Larkin, 2005; Grosch et al., 2006 and Wilson et al., 2008a), and *Verticillium* (Jager & Velvis 1985 and Turhan, 1990). *Chaetomium olivaceum, Cylindrocarpon destructans, Epicoccum nigrum, Gliocladium viride, Gliocladium roseum, Penicillium cyclopium, Penicillium nigricans, Trichoderma harzianum,* and *Trichothecium roseum* were frequently isolated from sclerotia of *R. solani* (Chand & Logan, 1984).

In Egypt diverse *T. harzianum* isolates have been shown to be effective antagonists of *R. solani*. These isolates contain many mycoparasitic strains that are considered good biocontrol agents against soil-borne pathogens. However, many investigators reported that within the genus *Trichoderma*, species such as *T. hamatum*, *T.*

harzianum, *T. reesei*, *T. virens*, and *T. viride* have demonstrated efficient antagonistic activity against *R. solani* under laboratory conditions as well as under pots and field conditions (Chand & Logan, 1984; Beagle-Ristaino & Papavizas, 1985; Brewer & Larkin, 2005; Grosch et al., 2006 and Wilson et al., 2008a). *Trichoderma* isolates are well adapted to survival in crop soils, compatible with the indigenous soil microflora, and capable of colonizing the zone immediately adjacent to plants roots. *Trichoderma* spp. are mycoparasitic on *R. solani* (Elad et al., 1983). In addition, *T. harzianum* isolates are known to secrete several extracellular enzymes which are potentially antagonistic to *R. solani* (Bertagnolli et al., 1996). The first event in decay of fungal pathogens, however, may be due to the secretion of small molecular weight antifungal compounds by the *Trichoderma* spp. (Benhamou & Chet, 1996).

During this investigation twenty three fungal taxa, representing different frequency classes, have been isolated. Among these taxa there are many species having antagonistic potentiality against plant pathogens and *R. solani* viz: *Penicillium* spp., *Chaetomium* spp., *Trichoderma* spp., *Acremonium* sp. And *Gliocladium* sp. All isolated *T. harzianum* (5 isolates) have been tested on agar plates, for their ability to antagonize the target organism (*R. solani* AG-3PT). Results of screening tests revealed that the antagonistic potentiality of *T. harzianum* isolates differ one strain to another by showing maximum inhibition ranged between 67 % and 73 %.

In pair culture technique, some *T. harzianum* succeeded to surround the target organism colony completely and prevented it from any radial spread *i.e.* the tested candidates strains were unable to produce antifungal antibiotics but were able to compete with the pathogen for nutrients (especially carbon) and for space. The hyphal interaction studied using light microscopy revealed destructive mycoparasitism of *R. solani* by *T. harzianum*. The method of mycoparasitism was sparse to intense coiling of *R. solani* followed by disintegration, disorganization and death of *R. solani* mycelium.

Another third finding an inhibition zone was observed, which indicates the presence of fungistatic metabolites secreted by these fungi. Ahmad & Baker (1987) and Ghisalberti & Sivasithamparam (1991) have been demonstrated that the capacity of some *Trichoderma* isolates to produce antifungal compounds which inhibit the growth of the soil-borne fungal pathogen *R. solani* is a useful property to exploit in the use of *Trichoderma* isolates as biocontrol agents. A biocontrol agent may act against pathogens by using one or more of the following mechanisms: competition, antibiosis, and parasitism as well as activating host defense mechanisms (Papavizas & Lumsden, 1980).

Results of the present study agree with the finding of many researchers all over the world as well as in Egypt: several species belonging to the genus *Trichoderma* are capable of parasitizing fungal plant pathogens such as *R. solani*, producing antibiotics effective against soil-borne pathogens and competing for infection sites against pathogens (Bénítez et al., 2004 and Vinale et al., 2008). *T. virens* and *T. harzianum* have been shown to be effective at controlling stem canker and black scurf, as well as increasing tuber yield (Tsror et al., 2001; Brewer & Larkin, 2005 and Wilson et al., 2008a, 2008b). Van den Boogert & Luttikholt (2004) by using *Verticillium biguttatum*, Wilson et al. (2008a) by applying *T. harzianum* and Lahlali & Hijri (2010) by testing *T. artroviride* have been reported the efficiency of these antagonistic fungal biota agents against AG3-PT in potato. Bertagnolli et al. (1998) show that *T. harzianum* secretes the antimycotic compound trichodermin and a short peptide which inhibit mycelial growth of *Rhizoctonia solani.* Antagonistic activity by *Penicillium* species against *R. solani* has been observed, and it has been reported in relation to the production of toxic metabolites (Nicoletti et al., 2004). Madbouly et al. (2014) able to biocontrol of *R. solani* causing stem canker disease of potato by using rhizosphere fungal biota.

Non-pathogenic isolates of of *R. solani* have demonstrated potential as biological control agents (Tsror et al., 2001), including a naturally occurring hypovirulent isolate known as Rhs1A1. Rhs1A1 reduced black scurf and stem canker of potato when applied in field experiments (Bandy & Tavantzis 1990 and Larkin & Tavantzis (2013).

This *in vitro* test seems attractive as it shows clear and visible results (inhibition or lysis of the pathogen). It has also another advantage, *i.e.* relatively easy and quick to perform with large number of isolates. It is very suitable for selecting organisms with a particular mode of action, however it is considered a very poor predictor of the activity of any organism in the field because

what happens *in vitro* cannot signify what exist *in vivo* (Andrews, 1985).

Conclusion

Since certain isolates of *T. harzianum* proved antagonistic to *R. solani* AG-3, these isolates will be further tested for their pathogenicity to *R. solani* on tuber seedling, under field conditions, in order to screen for the best biocontrol strains.

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النشاط التضادى للتريكوديرما هارزيانوم ضد ريزوكتونيا سولانى العامل المسبب لمرض تقرح الساق و القشرة السوداء فى البطاطس وذالك تحت ظروف المختبر

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تعتبر البطاطس (سولانوم توبيروسوم) في مصر واحدة من أهم المحاصيل النباتية وكذلك فى العديد من البلدان الأخرى في العالم. وهو يلعب دورا هاما في الاقتصاد الزراعي المصري، وليس للاستهلاك المحلي فقط، بل أيضا للتصدير خاصة إلى أوروبا وروسيا والدول العربية. ومع ذلك، يعاني محصول البطاطس من أكثر من ٤٠ آفة وأمراض ناجمة عن الحشرات والديدان الخيطية والفيروسات والبكتيريا والفطريات، التي من المحتمل أن تكون فيها مرض القشرة السوداء وتقرح الساق التي يسببها ريزوكتونيا سولاني هي أخطر الأمراض. وتقدر الخسائر في العالم الناتجة عن ريزوكتونيا سولاني بنسبة 5,15٪. و يعد ريزوكتونيا سولاني طفيلي غير متخصص، يقضي الفطر فترة بقائه من موسم إلى آخر في التربة على الدرنات المصابة في صورة أسكلروشيات (أجسام حجرية)، ويوجد أيضاً في بقايا النباتات المصابة على صورة ميسيليوم وهو فطر صعب المقاومة. ويعد تقليل أو القضاء على بقايا الفطر فى التربة هو الحل الفعال للتغلب علىهذ الفطر ويمكن تحقيق ذلك من خلال تطبيق طرق مقاومة متعددة والتي يعتبرمن أهمها هو إستخدام الفطريات التضادية. والجدير بالذكر أن ريزوكتونيا سولاني يتم التحكم فيه عادة من خلال إسستخدام المبيدات الكيميائية والتى تسبب أضرار و خسائر بيئية و مالية كبيرة لذلك تم إجراء بحوث عن المبيدات الحيوية كبديل قابل للتطبيق للقضاء على هذا الفطر. ويعتبر استخدام أنواع من فطر التريكوديرما وهو من فطريات التربة المعروفة للسيطرة البيولوجية على آفات المحاصيل. و قد تم اختبار خمس عزلات من التريكوديرما هارزيانوم في المختبر لإمكاناتها التضادية ضد ريزوكتونيا سولاني. تم تعريف كل من التريكوديرما هارزيانوم و ريزوكتونيا سولاني بواسطة الطرق الجزيئية والمورفولوجية. وفي تجربة أطباق الآجار المزدوجة وجدت جميع عزلات التريكوديرما هارزيانوم مقاومة لنمو ريزوكتونيا سولاني. و باستخدام المجهر الضوئي تم توضيح أن عزالات فطر التريكوديرما هارزيانوم المختلفة تضاد فطر ريزوكتونيا سولاني عن طريق ١- التطفل المباشر، ٢- وكذلك عن طريق إفراز مضادات حيوية وسموم وإنزيمات فطرية.