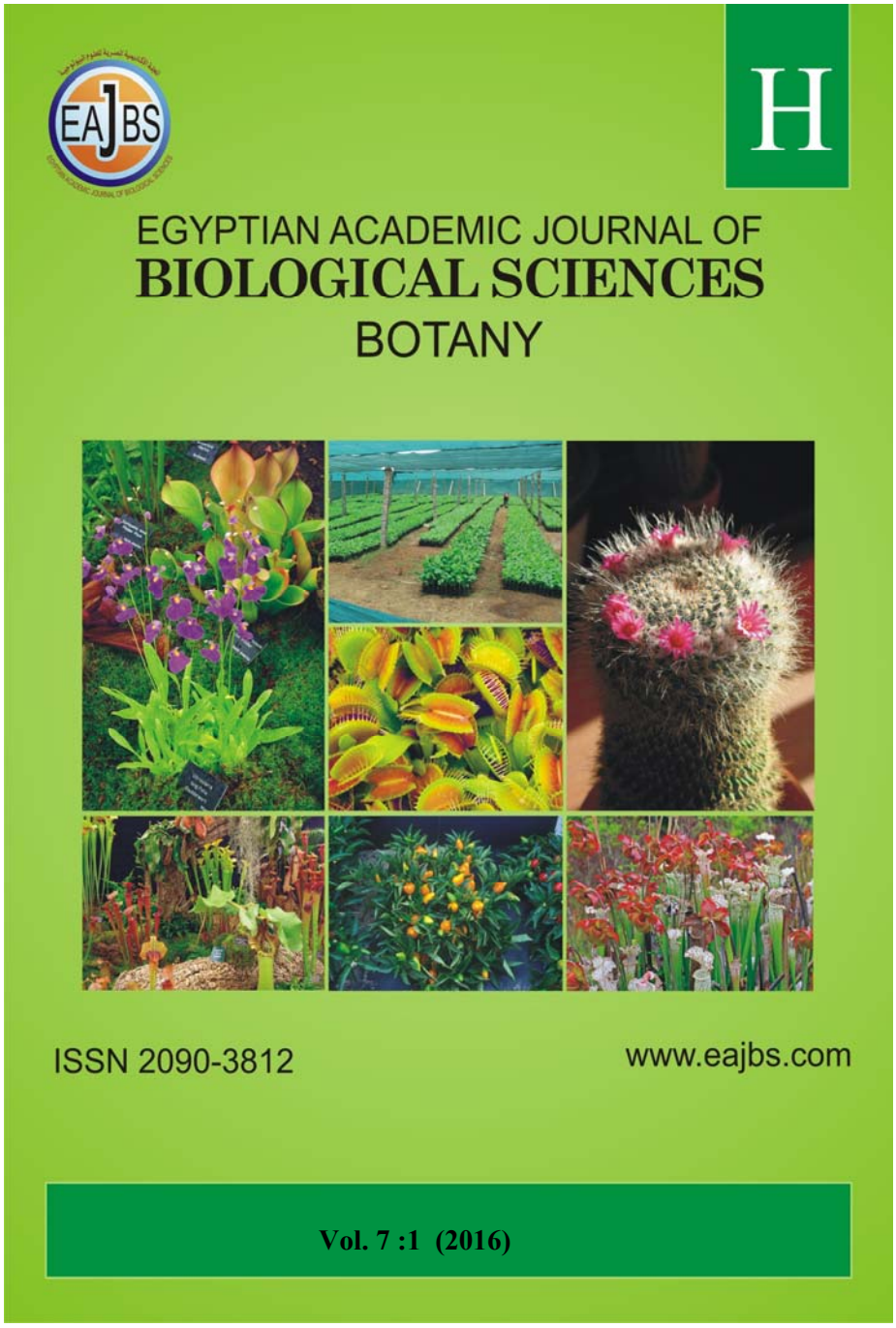

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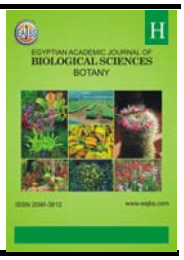

The Role of Antagonism in the Rhizospheric Region for *Chaetomium globosum* and *Trichoderma harzianum* against *Fusarium* spp. Attacking Tomato Plant.

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ABSTRACT

Chaetomium globosum and *Trichoderma harzianum* were isolated from healthy tomato plants at the rhizospheric area. These isolates were evaluated as potential bio-agents in control of the soil-borne fungi, *Fusarium solani* the causal agent of root rot, *Fusarium oxysporum f. sp. lycopersici*, *Fusarium oxysporum f. sp. radicis-lycopersici* and *Fusarium semitectum* were isolated from diseased tomato plants in Alexandria and Giza Governorates. The Pathogenicity test on tomato plant was conducted under greenhouse conditions. It indicated that, all tested fungi have a pathogenic effect on tomato plant. After one week the death percentages caused by *F. solani*, *F. oxysporum f. sp. lycopersici*, *F. oxysporum f. sp. radicis-lycopersici* and *F. semitectum* were 43.8, 31.3, 68.8 and 56.3%, respectively. However, after 45 days, the obtained data percentages for the same fungi were 62.5, 73, 87.5 and 83.8, respectively. The tested bio-agents affected all tested pathogens ,however, *C. globosum* at zero time of the pathogen inoculation reduced the radial growth of *F. oxysporum f. sp. lycopersici*, *F. oxysporum f. sp. radicis-lycopersici, F. semitectum* and *F. solani* by 53, 24.4, 48.9 and 22.2%, respectively, while *C. globosum (*3 days before the pathogen inoculation) reduced the radial growth of *F. oxysporum f. sp. lycopersici*, *F. oxysporum f. sp. radicis-lycopersici, F. semitectum* and *F. solani* by 68.9, 48.9, 72.2 and 38.8%, respectively, on the other hand, *T. harzianum* reduced the radial growth of the same tested pathogens (*F. oxysporum f. sp. lycopersici*, *F. oxysporum f. sp. radicis-lycopersici*, *F. semitectum* and *F. solani*) by 66.7, 67.8, 100 and 61.1, respectively.

T. harzianum was the most effective bio-agent against *Fusarium* spp. Comparing with *C. globosum*. It had recorded the complete growth reduction When it had been tested on *Fusarium semitectum* with 100% percentage.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is arguably the world's most important vegetable crop. In the vegetable world market, it is ranked not only in the second place, next to potato, in world total production but also in the first position in international commodity prices (FAO, 2012).

Egypt is among the top five tomato producing countries worldwide with about 8.6 millions ton (FAO, 2012), the cultivated area in Egypt is over a (216,400,000) hectare (Vijendra, 1987).

Tomato, aside from being tasty, is very healthy because of its high nutritive value as it provides important nutrients such as vitamin C, beta-carotene, lycopene, and flavonoids. Furthermore, antioxidant substances available in tomato may actually represent a modern-day "fountain of youth." Recent evidences suggested that lycopene has a strong anti-oxidative activities and anti-cancer functions (Wu *et al*.

2011; Raiola *et al*. 2014).It is noteworthy that tomato is not only sold fresh, but also processed and canned as soups, sauces, juices, ketchup and other products. Therefore, the production and consumption of this nutritious, health-promoting vegetable is constantly increasing. However, this crop is attacked by many pathogens causing a considerable reduction in yield quantity and quality (De Curtis *et al*., 2010).

Among these diseases was two symptomologically distinct forms of the pathogen can cause either a vascular wilt *(Fusarium oxysporum f. sp. lycopersici* W. C. Snyder & H. N. Hans.) or a crown and root rot (*F. oxysporum f. sp. radicis-lycopersici* W. R. Jarvis & Shoemaker). Both of these pathogens occur throughout most tomato growing areas and either can devastate crop. The Crown root rot disease is caused by *F. oxysporum* which reduces yield to 50%; this fungus causes severe root rot and finally plant death (De Araujo *et al.,* 2009; Nihorimbere *et al*., 2010). The Interaction between *F. oxysporum* and *F. solani* causes a root-rot disease complex that severely damages this important crop (Klotz, 1973).

Diseases are known to limit worldwide production of tomato. Controlling such diseases mainly depend on fungicides treatments (Rauf, 2000). However, fungicidal applications cause hazards to human health and increase environmental pollution. Biological control had attracted the interest because of increasing regulation and restriction of chemical pesticides or unsuccessful control attempts by other means. Biological control for pathogens by antagonistic microorganisms is potential especially for soil-borne diseases because these pathogens are difficult to be controlled with specific fungicides (Moussa *et al*., 2007). Potential agents for bio-control activity are rhizosphere competent fungi and bacteria, which in addition to their antagonistic activity are capable of inducing growth responses by either controlling minor pathogens or by producing growth stimulating factors (Akrami *et al.,* 2011). It has been suggested that microorganisms isolated from the root or rhizosphere of a specific crop may be better adapted to that crop and may provide better control of diseases than organisms originally isolated from other plant species (Cook, 1993). Breeding of *Trichoderma* is directed to achieve effective mycoparasitic strains for bio-control against plant fungal pathogens under a wide range of adverse environmental conditions (Manczinger *et al*., 2002), *Chaetomium globosum* has been reported effective in reducing damage caused by seed rot and damping off of several seed- and soil-borne plant pathogens (Aggarwal *et al*., 2004).

Our study aimed at isolation and identification of soil-borne pathogenic fungi in tomato Plants in Egypt as well as examination of a bio-control method to control these pathogens for the reduction of diseases.

MATERIALS AND METHODS

Tomato samples

Ten intact plants (either healthy or infected) representing two cultivars of tomato (*S. lycopersicum* L.) plants i.e. (448) and (765) that were collected from Bain El-Bahrain Island in Giza Governorate and a farm in Alexandria Governorate during the growing season 2010. The healthy tomato plants were used for isolation of non-pathogenic fungi for the antagonistic study, while the pathogenic fungi were isolated from the infected plants showing typical symptoms of crown rot, root rot, vascular wilt diseases.

Isolation and identification of pathogenic fungi

The method described by Burr *et al.* (1978) was used for isolation of pathogenic fungi. Each plant was washed carefully with tap water to remove the adhering soil particles. The stems and roots were cut into small pieces (1*1 cm) and surface sterilized by immersing in 5% sodium hypochlorite solution for 5 min. The segments were rinsed three times in sterilized distilled water, dried between two folds of sterilized filter papers and transferred under aseptic conditions to sterilized Petri dishes containing potato dextrose agar medium (PDA) **(**Difco**,** 1984**)**. Plates were incubated at 27^{o}C and the developed colonies examined after 5 days. The developed fungal colonies were picked up and transferred to PDA slants then purified using the single spore method and / or hyphal tip technique (Dhingra and Sinclair, 1984). The isolated fungi were microscopically identified to the species level according to Nelson *et al.* (1983) and Barnett and Hunter (1987). Stock cultures were maintained on PDA slants and kept at 4^{o}C in refrigerator for further experiments. Isolates were verified either in Plant Pathology Department., Fac. Agri., Cairo Univ. or in Mycology Research and Plant Diseases Survey Department, Plant Pathology Research Institute, Agricultural Research Center.

Isolation and identification of the bio-control agents

The same procedure described above was used for the isolation of the bio-control agent fungi from the roots of the healthy plants.

Pathogenicity of soil borne fungi

Pathogenicity test was carried out using four isolates of suspected fungal isolates, e.g. F1, F2, F3, and F4 to evaluate their infection efficiency under greenhouse conditions. The test was carried out at the Central Agriculture Pesticide Laboratory of A.R.C., Giza, Egypt during the growing season 2014 according to Whitehead (1957).

Pots of 20 cm diameter were sterilized by immersion in formalin solution (0.05%) followed by aeration for 2 weeks. The pots were filled with a mixture of clay and sand (3:1). The soil mixture was sterilized by mixing with formalin solution (0.5%) and covered with polyethylene sheets before3 days, the cover was removed and aeration was continued for 14 days. The isolated fungi were grown on autoclaved corn sand meal medium **(**Abd El-Ghany, 2001). Flasks containing corn sand meal were inoculated with discs (5 mm diameter) taken from 7 day old cultures of each tested fungus. The inoculated flasks were incubated at 27 ^{o} C for 14 days. The sterilized soil was individually infested with the tested fungi at the rate of 5% of soil weight. The added inoculum was thoroughly mixed with the soil and regularly watered for ten days before planting to insure the distribution of inoculum growth. One tomato cultivar e.g. Castle rock was used. Four tomato seedlings were cultivated in each pot and four pots were used for each treatment. Other group of pots, contained un-inoculated medium was kept as control. The death percentage of seedlings was determined by the equation:

$D = (C-L/C) \times 100.$

Where, D is the Death percentage; C is the total count of seedlings in control and L is the count of live seedlings in treatments.

Antagonistic effect of isolated bio-control agents against pathogenic fungi:

Two plugs of mycelium (6 mm diameter) were cut from the margins of actively growing PDA cultures, one carries the stock of bio-agent fungal isolates, while the other carries either of the pathogenic fungal isolates. The plugs were then placed at the periphery of Petri plate (9 cm in diameter) at the same distance on PDA medium. One plug of the pathogenic fungi was maintained as control (culture without bio-

agent). Each replicate has three plates. Both the dual and control cultures were incubated at 25 ° C ± 2 for 7 days (Mokhtar and Aid, 2013). The percentage of inhibition growth (I) was calculated by using the following formula: [I (%) = (I–T /C) x 100]; where: I= inhibition Percentage of pathogen growth by antagonists, C= Radial growth in control and T=Radial growth in the treatment.

Statistical Analysis

All experiments were replicated three times. The obtained Data in the present study were subjected to Statistical Analysis according to Gomez and Gomez (1984), and L.S.D. values of 0.05 level of significance were used for comparison between means.

RESULTS

Identification of the fungal isolates

Table (1) summarizes the results of identification of the fungal isolates. Four isolates representing different species could be obtained from the infected plants, while only two genera were obtained from the healthy plants.

Table 1: Occurrence of different fungi isolated from infected and healthy plants in two locations in Egypt

Isolates No.	Source of isolation		Identification	Code
	Plants	Location		
F1	Infected	Alex. Governorate, Egypt	*F. oxysporum f. sp. radicis*	FORL
F2	Infected	Alex. Governorate, Egypt	*F. oxysporum f. sp. lycopersici*	FOL
F3	Infected	Giza Governorate, Egypt	*F. solani*	F.s
F4	Infected	Giza Governorate, Egypt	*F. semitectum*	F. semi
F5	Healthy	Alex. Governorate, Egypt	*T. harzianum*	T. h
F6	Healthy	Alex. Governorate, Egypt	*C. globosum*	C. g

Pathogenicity of soil-borne fungi

Greenhouse studies were carried out for the pathogenicity test of some fungi isolated from tomato rhizosphere, which were evaluated by the percentage of plant death by each fungus (Table 2).

Table 2: Effect of isolated pathogens on tomato plant death

	% Plant death after treatment				
Tested fungi / Time (d)	***F. oxysporum f. sp. radicis***	***F. oxysporum f. sp. lycopersici***	***F. semitectum***	***F. solani***	**control**
After one week of transplantation	43.8	56.3	31.3	68.8	6.3
After 45 days of transplantation	62.5	93.8	75.0	87.5	6.3

The obtained data indicated that, all tested fungi have a pathogenic effect on tomato plant. The highest infection (68.8% after 7 days) was observed when *F. solani* was applied. However, *F. oxysporumf. sp. lycopersici* was responsible for higher death percentage (93.8%) after 45 days of transplantation.

In spite of, the most pathogenic effect was recorded by *F. solan*i, the fungus *F. semitectum* recorded the lowest pathogenic one after one week. On the other hand, the obtained data after 45 days indicated that, FOL fungus had the most pathogenic effect and the FORL had the lowest one.

The obtained data in Table (3) proved that, the efficiency of *C. globosum* at 0 time of the pathogen inoculation and *C. globosum* before 3 days of the pathogen inoculation and *T. harzianum* as bio-agents in controlling FOL, FORL, *F. semitectum* and *F. solani* under laboratory conditions.

The efficacy of *C. globosum* at zero time was determined against the four tested pathogens. The means of radial growth in FOL, FORL, *F. semitectum* and *F. solani* were 4.8, 2.2, 4.4 and 2 mm in diameter, respectively, compared to 9 mm in diameter in the control treatment as shown in Table (3) and illustrated in Figure 1 (a, b, c and d). The corrected reduction in the growth of the tested pathogens was calculated according to Abbott's formula as 53, 24.4, 48.9 and 22.2, respectively, compared to the control. It is obvious from Table (3) and Figure 2 (a, b, c and d) that, *C. globosum* before 3days the pathogen inoculation affected FOL, FORl, *F. semitectum* and *F. solani* as 6.2, 4.4 ,6.5 and 3.5 mm, respectively compared to 9 mm in the control treatment. However, the calculated reduction percentages were 68.9, 48.9, 72.2 and 38.9, respectively.

Table 3: Antagonistic effect of *C. globosum* (at zero time and before 3 days of the pathogenic inoculation) and *T. harzianum*.

Pathogens / **Bio-agents**		***F. Oxysporum f. sp. Lycopersici***	***F. oxysporum f. sp. radicis***	***F. semitectum***	***F. solani***	**Mean of reduction (%)**	**F value**	**L.S.D.**
***C. globosum* (0 time pathogen inoculation)**	M	4.8	2.2	4.4	2	37.12 c	70.75	0.627
	R%	53b	24.4c	48.9c	22.2c			
***C. globosum* (3days before pathogen inoculation)**	M	6.2	4.4	6.5	3.5	57.2b		
	R%	68.9a	48.9b	72.2b	38.9b			
T.harzianum	M	6	6.1	9	5.5	73.9a		
	R%	66.7ab	67.8a	100a	61.1a			
Control		0	0	0	0			
F value		3.95	33.44	337.7	131.42			
L.S.D.		1.35	1.13	0.44	0.52			

M: Mean of radial growth (mm) for pathogenic fungi.
R %: Reduction percentage in growth of the tested pathogens.
-Means of reduction percentage within a column followed by the same letter are not significantly different (Duncan's Multiple Range Test at $P< 0.05$).

It is shown from Table (3) that *T. harzianum* reduced the radial growth of the tested pathogens (FOL, FORl, *F. semitectum* and *F. solani*) as 6, 6.1, 9 and 5.5 mm respectively, compared to 9 mm for the control treatment as seen in Figures 3 (a, b, c and d).

Accordingly, the calculated growth reduction percentages in FOL, FORL and *F. solani* by *T. harzianum* were 66.7, 67.8 and 61.1, respectively. The promising result was obtained by *T. harzianum* when tested against *F. semitectum*. The recorded growth reduction percentage reached 100%. It can be concluded that, *T. harzianum* was the most effective bio-agent against *Fusarium* spp.

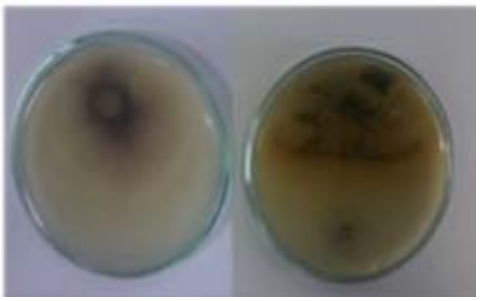

Fig. 1(a): *C. globosum* against *F. solani*

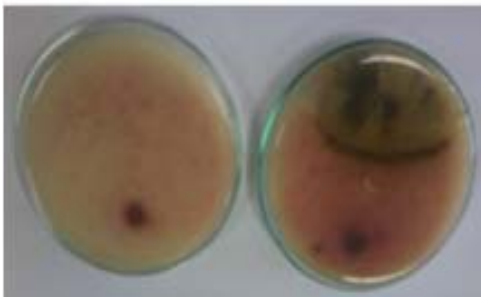

Fig. 1(b): *C. globosum* against FOL

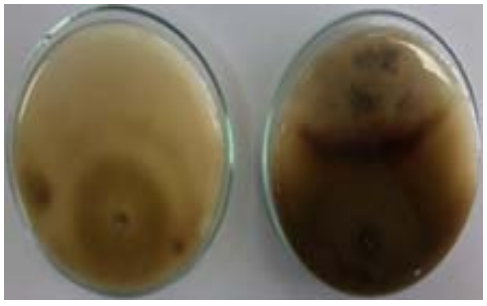

Fig. 1(c): *C. globosum* against *F. semitectum*

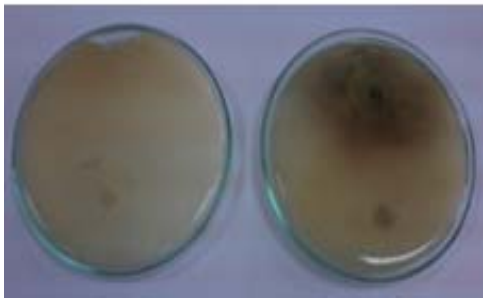

Fig. 1(d): *C. globosum* against FORL

Fig. 1: Antagonistic effect of *C. globosum* on *Fusarium* spp. at zero time of the pathogen inoculation.

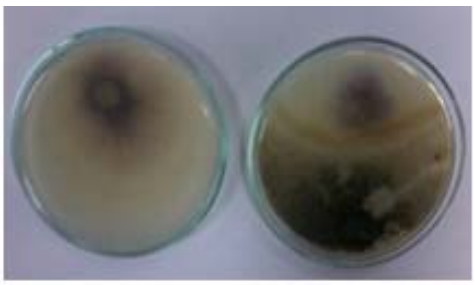

Fig. 2(a): *C. globosum* against *F. solani*

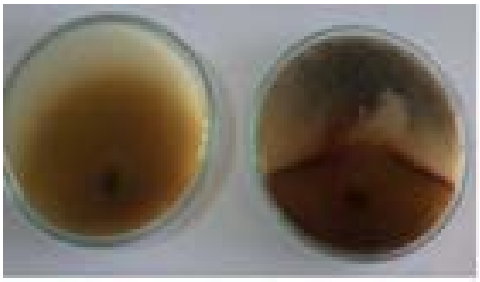

Fig. 2(b): *C. globosum* against *F. semitectum*

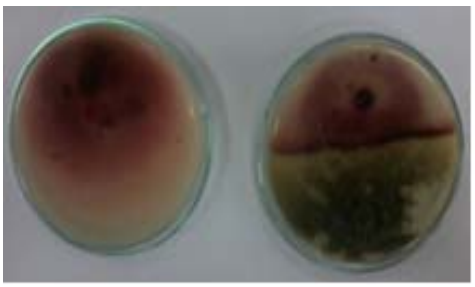

Fig. 2(c): *C. globosum* against FOL

Fig. 2(d): *C. globosum* against FORL

Fig. 2*:* Antagonistic effect of *C. globosum* on *Fusarium* spp. (3 days before the pathogen inoculation

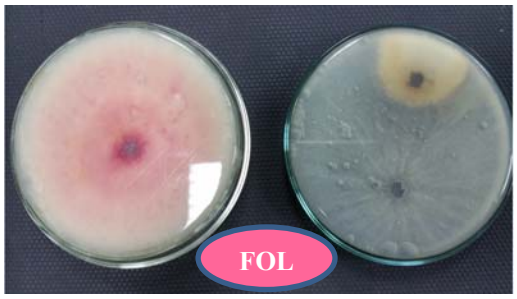

Fig. 3(a): *T. harzianum* against FOL

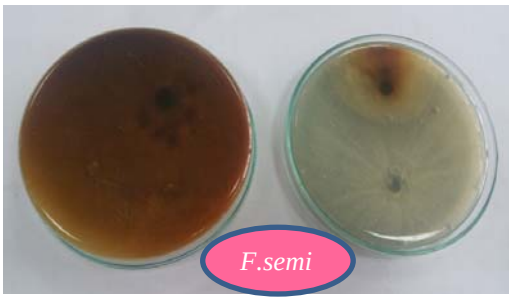

Fig. 3(b): *T. harzianum* against F. *semitectum*

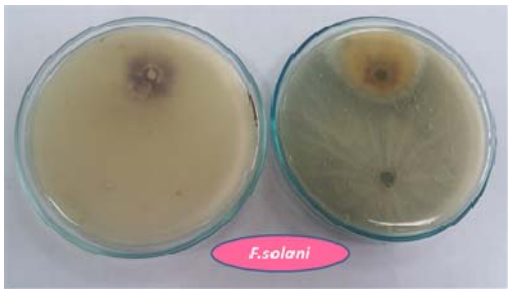

Fig. 3(c): *T. harzianum* against *F. solani*

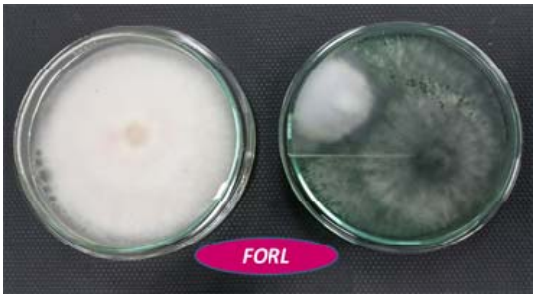

Fig. 3(d): *T. harzianum* against FORL

Figure 3. Antagonistic effect of *T.harzianum* on *Fusarium* spp.

DISCUSSION

The aim of the present study was to investigate the ability of two bio-agents in order to control the four soil borne fungi e.g. FOL, FORL, *F. semitectum* and *F. solani* under laboratory conditions.

It is quite evident from the recorded data in Table (3), the first tested bio-agent C. *globosum* at 0 time was effective against the above mentioned fungi.

However, the lowest reduction percentage was observed when it was tested on *F. solani.* The recoded reduction was 22.2%, on the other hand, the highest percentage was recorded on FOL (53%).

C. globosum 3 days before the pathogen inoculation controlled all fungal diseases, but the highest reduction was recorded on *F. semitectum* which was 72.2%. The lowest reduction was on *F. solani* (38.9 %). These results agreed with Alabouvette *et al*. (2006) who studied the antagonistic activity of bio-control microorganisms that is often demonstrated by the inhibition of growth, infection or reproduction of pathogen. In addition, these results are similar to those obtained by Sun *et al.* (2006) and Longoni *et al*. (2012) who reported that, the degradation of mycelia of tested pathogen probably resulted from the lytic enzymes which are commonly secreted by *Chaetomium* species.

The highest reduction percentage at all was recorded when *T. harzianum* was applied against *F. semitectum* (100%).The lowest percentage was recorded on *F. solani* (61.1%).

The growth of *T. harzianum* was faster than *C. globosum* and this may explain the highest recorded percentage in the pathogen growth reduction (100 %),

compared to the lowest one (22.2 %) recorded by *C. globosum* which had lower growth. The obtained results are in harmony with Harman *et al*., (2004)., He used *Trichoderma* fungi in agriculture and found that, it can provide numerous advantages; 1) colonization of the root and rhizosphere of plant, 2) control of plant pathogens by different mechanisms such as parasitism, antibiosis production and inducing systemic resistance, 3) improvement of the plant health by promoting plant growth , and 4) stimulation of root growth.

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ARABIC SUMMERY

دور التضاد لكل من فطرىّ *Chaetomium globosum* و *Trichoderma harzianum* فى منطقة الريزوسفير المحيطه بنبات الطماطم أمام جنس *Fusarium* الممرض

زينب حسن وهبه [١] و ألفت سيد بركات [٢] - رفاعى إبراهيم رفاعى [٢] و سامى شفيق رمسيس [١]
١- مركز البحوث الزراعية – المعمل المركزى للمبيدات – الدقى – الجيزة.
٢- جامعة القاهرة – كلية الزراعة – قسم الميكروبيولوجيا الزراعية.

تم عزل *Chaetomium globosum* و*Trichoderma harzianum* من منطقة الريزوسفير المحيطه بنبات الطماطم السليم وتعتبر تلك العزلات مواد حيوية فعاله ضد فطريات التربة الممرضه. وقد تم عزل تلك الفطريات من محافظتى الجيزه والإسكندرية.

وعند دراسة التأثير الممرض للفطريات تحت ظروف الصوبه الزجاجية أوضحت التأثير الممرض للفطريات على نبات الطماطم وكانت نسب الموت المتحصل عليها بعد أسبوع من المعامله بالعدوى الصناعية لتلك الفطريات *Fusarium solani* , *Fusarium. oxysporum f. sp. lycopersici* , *Fusarium. oxysporum f. sp. radicis-lycopersici* و*Fusarium. semitectum* هى ٤٣.٨ و٣١.٣ و٦٨.٨ و ٥٦.٣ % على الترتيب وبعد ٤٥ يوم من الزراعه كانت النتيجه لنفس الفطريات ٦٢.٥ و ٧٣ ٨٧.٥ و ٨٣.٨ % على التوالى.

جميع المواد المضادة أثرت على الفطريات المختبرة وكان *C. globosum* الموضوع فى نفس وقت وضع الكائن الممرض على طبق بترى (عند وقت الصفر) قد أحدث نسبة خفض فى النمو الميسيليومى لكلا من فطر *F. oxysporum f. sp. lycopersici* و *F. oxysporum f. sp. radicis-* و *F. semitectum* و *Fusarium solani* هى ٥٣ و ٢٤,٤ و ٤٨,٩ و ٢٢,٢ % على الترتيب فى حين ان *C. globosum* (الموضوع على سطح طبق بترى قبل ثلاثة أيام من وضع الكائن الممرض) قد تسبب فى إحداث نسبة خفض لكلا من فطر *F. oxysporum f. sp. lycopersici* و *F.oxysporum f. sp. radicis- lycopersici* و *F. semitectum* و *F. solani* تصل إلى ٦٨,٩ و ٤٨,٩ و٧٢,٢ و ٣٨,٨ % على الترتيب . بينما تسبب فطر *T. harzianum* فى نسب خفض تصل إلى ٦٦,٧ و ٦٧,٨ و ١٠٠ و ٦١,٦ % فى النمو الميسيليومى لكلا من فطر *F. oxysporum f. sp. lycopersici* و*F.oxysporum f. sp. radicis- lycopersic* و *F. semitectum* و *F. solani* على الترتيب.

ويستخلص من الدراسة أن *T. harzianum* كان الأكثر فاعلية ضد أمراض الفيوزاريوم محل الدراسة مقارنة ب *C. globosum* حيث سجل خفض كامل للنمو الميسيليومى لفطر *F. semitectum* بنسبة ١٠٠%.