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Use of *Trichoderma* spp. For Biological Control of Sugar Beet Damping-Off Caused by *Fusarium* spp.

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ABSTRACT

Antagonism between biocontrol agents *Trichoderma viride*, *T. harzianum* and *T. hamatum* against three phytopathogenic fungi: *Fusarium solani*, *F. oxysporum* and *F. dimerum* which cause damping-off on sugar beet cultivars in Egypt. *In vitro*, percentage reduction of *T. viride* was more than the other fungi for the untreated with *Fusarium solani* and *F. oxysporum*, while *Trichoderma hamatum* was effective antagonist for the control of *F. dimerum*. *In vivo*, *T. viride* was the most antagonist effective for untreated with *F. solani* (85%) survival plants, while *T. hamatum* was the most antagonist effective for the control of *F. oxysporum* and *F. dimerum* (87.5, 82.5) % survival plants. This result shows that *Trichoderma* spp. are very effective biocontrol agents against *Fusarium* spp. involved in sugar beet damping-off.

Keywords: Antagonism, Biological control, *Trichoderma viride*, *T. harzianum*, *T. hamatum*

INTRODUCTION

Sugar beet (*Beta vulgaris* L.) is one of the most important sugar crops in many countries of the world. In Egypt, due to the great consumption of sugar, the production of sugar beet must be increased to cover the requirements of sugar, which depends on sugar cane production.

Under Egyptian conditions, sugar beet plant is attacked by numerous foliar and root diseases, which have been recorded in several reports (El-Kholi 1979, El-Kholi 1984, Mosa and El-Kholi 1996).

The most important of soil diseases are damping-off and root-rot caused by different pathogens such as *Rhizoctonia solani* Kuhn, *Sclerotium rolfsii* Sacc., *Phoma (Pelospora) beta* Berl. Several species of *Fusarium* and *Pythium* were also recorded, i.e. *Fusarium solani* (Mart) Sacc, *F. oxysporum* f. sp. *conglutinans* Wollenw, *F. oxysporum* Snyder & Hans, *F. moniliformum* Sacc. and *F. meresmoides* Corda, *Pythium aphanidermatum*, *P. mamillatum* Meurfd, *P. ultimum* and *P. debaryanum* Hesse (El-Kholi 2000).

Damping-off disease is one of the economically most important diseases causing heavy losses in different parts of the world. The disease complex is caused by species of *Fusarium*, *Pythium* and *Rhizoctonia* (Cook and Baker 1983).

Many rhizospheric microorganisms such as *Trichoderma* spp. are known to be equipped with antagonistic potential against soil borne pathogens (Chet and Baker 1981).

The aim of the present study was to evaluate potential antagonists (*Trichoderma* spp.) against three species of *Fusarium* involved in sugar beet damping-off.

MATERIALS AND METHODS

Isolates and morphology of biocontrol agents

A total of three fungal isolates divided into : two of isolates were obtained from Microbiol. Dept. Fac. of Agric. Mansoura Univ., Mansoura , Egypt. These two isolates are namely *Trichoderma harzianum* and *T. hamatum* as stander fungal isolates . While , third isolate (coded No. 1) was isolated from rhizosphere of sugar beet cultivar and screening with *T. harzianum* and *T. hamatum* to controlling *Fusarium* wilt fungi by *in vitro* antagonism.

Fungal pathogens

Fungi as the pathogens were *Fusarium* spp.(*Fusarium solani*, *F. oxysporum* and *F. dimarium*). The cultures of these fungi were obtained from Plant Pathol. Res. Dept., Agriculture Research Center (ARC) , Giza , Egypt .

Antagonism between biocontrol agents and the cusals pathogens fungi

This work was carried out *in vitro* to manage the causes damping-off diseases of sugar beet *Fusarium* spp. by applyiang bioagents. PDA plates (9 cm diam.) were inoculated peripherally with each of the pathogens and the bioagents under study at the opposite side to each other and incubated at 28 OC for 7 days. After the elapse of the incubation period, the colony diameter of pathogens were measured and recorded as the percentage of reduction over the control. Three replicates plates were used. A complete randomized design was used in this experiment. The percentage of reduction (R %) in the mycelial growth was calculated according to the following formula adopted by Ferreira *et al.* 1991 as follows:

$$R \% = \frac{A - B}{A} \times 100$$

where

R%=Percentage of growth reduction.

A=The distance of mycelial growth of the pathogenic fungi in the control.

B=The distance of mycelial growth of the pathogenic fungus towards the tested bioagents

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Identification of fungal isolate

The fungal isolate No.1 was identified by microscopic observation according to (Domsch *et al.* , 1993 and Samson *et al.*, 2000) .

In vivo experiments

The pot experiment was carried out in greenhouse of Mycology Research & Plant Disease Survey Department , Plant Pathology Research Institute , Agriculture Research Center (ARC) , Giza , Egypt.

This experiment aimed to investigate the role of biocontrol agents were the most effective *in vitro* with pathogen fungi (*Fusarium* spp.) to control damping-off of sugar beet.

Greenhouse experiment

Inocula of the *Fusarium* spp. 5mm mycelial disk from a 5 days old culture of *Fusarium* spp. on PDA were prepared by growing in sterilized glass bottles (500ml) containing barley medium (150g seeds, 50g clean sand, 4g glucose and 200 ml water) and autoclaved in two consecutive days and incubated at 27±10C for 15 days. Pots (35 cm. in diameter) were sterilized by immersing in 5% formalin solution for 10 min. and left to dry in open air. Treated pots were left for one week.

At the same day of sowing, surface sterilized seeds were soaked for one hour before sowing in a 5-day-old culture of *Trichoderma* spp. (10⁸-10⁹cfu/ml) grown in liquid potato dextrose broth medium. Ten seeds were sown in each pot and four replicates were used for each treatment. Surface sterilized un-soaked seeds were served as a control check.

Assessment of disease incidence was calculated as

a percentage of pre- and post-emergence damping-off after 15 and 45 days of sowing. These experiments were carried out under greenhouse conditions for growing season 2014/2015.

Percentages of pre and post-emergence damping off as well as survival plants were calculated up to 45 days from planting as follows:

1. % of pre-emergence damping-off = (No. of non-emerged seeds/No. of sown seeds) x 100
2. % of post-emergence damping-off = (No. of killed seedlings/ total No. of emerged seedlings) x 100
3. % of survival plants = (No. of un-infected plants/total No. of plants) x 100

Statistical analysis

Analysis of variance (ANOVA) of the data was performed with (Steel and Terrie 1960). Duncan's multiple range test (DART) were applied for comparing means under study (Duncan 1955).

RESULTS AND DISCUSSION

Antagonistic effect of fungal isolate (No.1) and *Trichoderma* spp. against *Fusarium* spp. *in vitro* .

The results are recorded in Table (1) indicate that the biocontrol agents actively affected the growth of all fungal pathogens under study . Isolate No.1 was the most potent inhibitors to the growth of *F. solani*. Data shown in Table (1) recorded that, isolate No.1 reduced growth of *Fusarium oxysporum* (78.11%) more than and *Trichoderma harzianum* (73.33%), *Trichoderma hamatum* (72.56%).

Table 1. Effect of fungal isolate No.1 and *Trichoderma* spp. on the reduction on (R %) of linear growth fungal pathogens.

Bioagents	<i>F. solani</i>		<i>F. oxysporum</i>		<i>F. dimerum</i>	
	L.G "cm"	Reduction%	L.G "cm"	Reduction%	L.G "cm"	Reduction%
Isolate No.1	1.83 d	79.51	1.97 c	78.11	2.70 b	69.76
<i>T.harzianum</i>	2.87 b	67.86	2.40 b	73.33	2.93 b	67.19
<i>T. hamatum</i>	2.20 c	75.36	2.47 b	72.56	1.73 c	80.63
Control	8.93 a	0.00	9.00 a	0.00	8.93 a	0.00

L.G: Linear growth of the fungal pathogen (cm)

Values with different letters are significantly different

Identification of fungal isolate No.1

Fungal isolate No.1 ,was obtained for identification because it showed very high antagonism against *Fusarium* spp. The fungal isolate No.1 was identified as *Trichoderma viride* according to culture examination that shows colonies reaching 6 – 7 cm diameter in ten days at 28 °C , on Malt , green color .



Photo. Microscopic observation of *Trichoderma viride* using an image analysis system.

At the same time microscopic observation in photo shows that conidia are : sub – spherical , green in mass 3.8 x 2.0 µm.

Percent of damping-off and survival plants between *Trichoderma* spp. and *Fusarium* spp.

T. harzianum is bio-controlling agents as combination in integrated control system of damping-off disease (Afify & Ashour 1995). Also, (Tondje et al. 2007) recorded that genus *Trichoderma* has been applied as biocontrol agents against many commercial fungal pathogens. Recently, (Afify et al. 2017) found that *T. viride* are quite important and effective as biocontrol agents.

a) Percent of damping-off and survival plants between *Trichoderma* spp. and *F.solani*

Results are recorded in Table (2) that all treatments reduced plant disease and increased healthy plants compared with the untreated treatment. *Trichoderma viride* was the most effective (85%) survival plants. Ushamalani *et al.* (1997) reported the great effects of antagonists *Trichoderma* spp. against *M. phaseolina* and *F. oxysporum* *in vitro*.

Table 2. Effect of the *Trichoderma* spp. on growth of *F. solani* caused damping-off of sugar beet seedling disease.

Treatments	Damping – off (%)		Survival plants (%)
	Pre emergence	Post emergence	
	15 days	45 days	
<i>T. viride</i>	12.5 b	15 b	85 a
<i>T. harazianum</i>	15 b	25 b	75 a
<i>T. hamatum</i>	10 b	25 b	75 a
control	35 a	55 a	45 b

Values with different letters are significantly different

b) Percent of damping-off and survival plants between *Trichoderma* spp. and *Fusarium oxysporum*

While *F. oxysporum* the results are recorded in Table (3) that *Trichoderma hamatum* and *Trichoderma harazianum* were the high effective (87.5% and 77.5%) survival plants, respectively. Gouda (2001) showed the possibility of controlling these sugar beet diseases by several bioagents under field conditions.

Table 3. Effect of the *Trichoderma* spp. on growth of *F. oxysporum* caused damping-off of sugar beet seedling disease.

Treatments	Damping – off (%)		Survival plants (%)
	Pre emergence	Post emergence	
	15 days	45 days	
<i>T. viride</i>	15 b	30 b	70 b
<i>T. harazianum</i>	17.5 b	22.5 c	77.5 ab
<i>T. hamatum</i>	7.5 b	12.5 c	87.5 a
control	35 a	60 a	40 c

Values with different letters are significantly different

c) Percent of damping-off and survival plants between *Trichoderma* spp. and *Fusarium dimerum*

In the case of *F. dimerum* the results are recorded in Table (4) that *T. hamatum* was the high effective (82.5%) survival plants. Esh (2000) showed that *Trichoderma hamatum* a most activity in reducing the tested pathogenic fungal growth in field experiments.

Table 4. Effect of *Trichoderma* spp. on growth of *Fusarium dimerum* caused damping-off of sugar beet seedling disease.

Treatments	Damping – off (%)		Survival plants (%)
	Pre emergence	Post emergence	
	15 days	45 days	
<i>T. viride</i>	5 c	20 b	80 a
<i>T. harazianum</i>	10 bc	25 b	75 a
<i>T. hamatum</i>	15 b	17.5 b	82.5 a
control	35 a	52.5 a	47.5 b

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إستعمال فطريات التريكوودرما لمقاومة مرض موت بادرات بنجر السكر الناجم عن الإصابة بفطريات الفيوزاريوم
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تم اختبار مقدرة ثلاث أنواع من فطريات التريكوودرما معمليا ضد ثلاثة فطريات مسببة للذبول الفيوزاريومي لنبات بنجر السكر في مصر وتشمل فيوزاريوم سولاني فيوزاريوم أوكسيبوريم، و فيوزاريوم ديمريم، قد سجلت هذه الاختبارات قدرة فائقة للتريكوودرما على احداث ظاهرة التضاد في المعمل حيث كانت التريكوودرما فردى (المعزولة والمعرفة) اقوى العزلات في مقاومة الفيوزاريوم سولاني والفيوزاريوم اوكسيبوريم بينما التريكوودرما هامتيم اكثر السلالات كفاءه في مقاومة الفيوزاريوم دايمريم وقد اختبرت النتائج ايضا في الصوبة فكانت كما يلي: نسبة النباتات الحية بعد المعاملة بالتريكوودرما فردى في حالة المقاومة لفطر الفيوزاريوم سولاني بنسبة (85%) بينما اظهرت التريكوودرما هامتيم كفاءتها في مقاومة الفيوزاريوم اوكسيبوريم والفيوزاريوم دايمريم بنسبة (5, 87) % على التوالي وبالتالي استخدام التريكوودرما لمعاملة بنجر السكر أدى الى تخفيض النسبة المئوية لموت بادرات بنجر السكر تحت ظروف الصوبة الزراعية. وبهذا تعتبر أنواع فطر التريكوودرما من العوامل الفعالة في المقاومة الحيوية لفطريات الفيوزاريوم.