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Photosynthetic Efficiency Promotion of Sugar Beet by Formulation of Trichoderma and Control of Some Sugar Beet Disease Seedling

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Abstract

Four isolates of *Fusarium sambucinum*(Fuckel) (isolates 1,2,3,4)and four isolates of *Fusarium solani* (Mart.) Sacc .(isolates5, 6, 7, 8)were isolated from different localities of sugar beet in *Assiut Government* .The tested isolated were pathogenic to sugar beet oskarpoly variety causing damping off and root rot. Isolates 2 and5 had the highest pathogen city to sugar beet isolates 3 and 8 had the lowest pathogen city pathogen city. *Trichoderma viride* have been used for their potential antagonism for controlling *Fusarium* sp damping off and root rot disease of sugar beet. *In vitro* studies showed that the culture filtrate of *Trichoderma viride* significantly decreased the growth of the tested isolates of both *Fusarium sambucinum* and *Fusarium solani* Treating the soil with formulation of *Trichoderma viride* before planting decreased damping off and root rot of sugar beet compared with untreated and untreated soil with formulation of *Trichoderma viride* under greenhouse conditions during growing seasons 2010and 2011.Chlorophyll a chlorophyll b and total chlorophyll carotenoids decreased when treatment the infested soil with either *Fusarium sambucinum* or *Fusarium solani* compared with untreated and untreated soil with formulation of *Trichoderma viride*.

Keywords: Fusarium sp; Trichoderma virid; Photosynthetic; Sugar beet

Introduction

Sugar beet (*Beta vulgaris L.*) is one of the important sugar crops in Egypt as well as all over the world. Sugar beet is considered the second most important source of sugar. Sugar beet is attacked by some *Fusarium* species causing damping – off and root rot disease which is considered to be one of most destructive and serious disease in many parts of the world as well as in Egypt [1-3] Biocontrol technologies have gained momentum in disease control of crop plants in recent times as these technologies not only minimize of replace the usages of harmful chemical pesticides but also found to be cheaper and efficient in certain disease control programmers. Successful use of fungal biocontrol agents like *Trichoderma spp* agents that commercially produced to prevent development of several soil pathogenic fungi [4-6]. Different mechanisms control soil born disease caused by pathogens. *Rhizoctonia ,Sclerotium ,Fusarium ,Pythium and Phophthora* in several crops have been reported [7]therefore using biological control such as *T.viride* which is one the efficient biocontrol have been suggested as being responsible for their bio–control activity which includes mycoparasitism, antibiosis, competition for nutrients and space .Secretion of chitinolytic enzymes[8].The major aspects of successful biological control technologies include the establishment of product, formulation and delivery system for microorganism that enable them for efficient disease control .The mass production systems should be complete with industrial and commercial development methods and field application [9] 2010. *Trichoderma spp* can be formulated as pellets [9-10] dusts and powders [11] Striking changes in the amount and distribution of photosynthetic pigment result from the infection by obligated parasite [12] This work aimed to study the effect of *Trichoderma viride* on growth activates of *Fusarium sambucinum* or *Fusarium solani* in vitro .The effect of formulation Trichoderma viride in reducing caused by *Fusarium sambucinum* and *Fusarium solani* disease incidence Assess Photosynthetic efficiency changes associated with disease development after treatment with *Trichoderma viride.*

Material and methods

Isolation:

Natural diseased sugar beet plants showing damping off symptoms were collected from different locations of Assiut Governorate for pathogens isolations. Isolation technique was carried out according to [1] isolated fungi were purified using single spore and hyphen tip technique and identified according to descriptions in the manual of[13-14] Then confirmed by Assiut University Mycological center (AUMC).

Soil infestation

Inoculums of the isolated Fungi *Fusarium sambucinum* (isolate1 Assiut isolate 2 Assiut isolate 3 Manfulate isolate and 4 Assiut) and *Fusarium solani*, (isolate 5Assiut isolate 6 Manfulate isolate 7 Assiut isolate and 8 Assiu isolate T, were prepared individually on barley grain medium [15] for artificial infestation. Fourteen days old culture of each pathogen was used for the infestation of sterilized soil 7 days before sowing. Inoculums of each pathogen were applied to the autoclaved soil at the rate 5% by weight. Ten sugar beet seeds of variety oscarpoly were sown in each pot (25 cm in diameter) and watered. Four replicates represented each treatment and four pots filled with uninfected soil were used as control. The percentage of pre and post emergence damping off, survival seedlings and disease index for

seedlings were recorded after 21 and 45 days, respectively from planting.

Trichoderma isolation

Evaluation of antagonistic activity of Trichoderma viride, In dual culture technique (in virto)

The microorganism was isolated from *rhizosphere* of sugar beet according to [16] *T. viride* was carried out according to [17] by using dual culture technique. *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani*, (isolates 5, 6, 7,8) separately, on PDA medium for 7 days at 25°C. Disc (5mm – diameter) from each bio-control fungus was inoculated on surface of PDA medium in side of Petri dish. A disc (5 mm – diameter) of *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani*, (isolates 5,6,7,8) separately was inoculated at equal distance of the opposite side of Petri dish. Petri dishes were inoculated with each pathogenic fungus only as control. Three Petri dishes for each bio-control-pathogenic fungus treatment as well as the control were used as replicates. The inoculated Petri dishes were incubated at 25°C at 7 days when the pathogen fungi covered the plate surface of the control treatment, and then *T. viride* and pathogens were evaluated based on radial growth of colony of pathogen, over growth of *Trichodermia*.

Antagonistic effect of *T. viride* as decrease of the mycelia growth of pathogenic fungi was determined using the following formula:

Where,

A: The diameter of mycelia growth of pathogenic fungus in control

B: The diameter of mycelia growth of pathogenic fungus with Trichoderma fungus.

Culture filtrate (Nonvolatile metabolites) and early volatile metabolites tests

Mycelia disks of each *Trichoderma* isolate grew on 1/4-strengh PDA was separately inoculated into 100ml flasks containing potato dextrose liquid and incubated at 20 to 29°C and 120 RPM in rotary shaker incubator for 10 days. The cultures were then filtered through 0.22mm Millipore filters and 15ml of these filtrates were added into sterile Erlenmeyer flasks containing 50ml 1/4-strength PDA with 25% further agar at 45°C. After medium solidifying, mycelia disks of *Fusarium sambucinum* (isolates 1,2,3,4). and *Fusarium solani, (*isolates 5,6,7,8) separately individually agent derived from actively growing colonies were placed on one edge of medium plates and were incubated at 25 ± 3°C;[18-19]. For early volatile metabolites test, pathogen and *Trichoderma* actively growing colonies were subculture on PDA and incubated in dark condition at 25°C. Then, opened Petri dishes containing 48h old colony agent *Fusarium sambucinum*(isolates 1,2,3,4) and *Fusarium solani,(*isolates 5, 6, 7,8) separately placed on 24h old colony of Trichoderma and were airtight using parapylm. Control was Petri dishes containing PDA medium. The Petri dishes were incubated in the same temperature and dark conditions .Radial growth on pathogen was measured daily in both tests. Inhibitory percentages were calculated by Above Formula.

Inoculums preparation of antagonistic *Trichoderma*

For mass production they were grown in 1000 ml conical flasks each containing 250 ml vermiculate 250 wheat bran and 250 ml Medium(C ZDIFCO) and autoclaved for 20 min at 121 on two consecutive days .The flasks were inoculated with the antagonist fungus and incubated at 20C°.After incubation period, contents of flask were transferred to plastic plate under sterile conditions left to dry and then mixed in a blander to dry then mixed in a blender to become powder *T.viride* mixture contains 10x108 and was kept in poly ethylene bags at room temperature until used

Antagonistic effect of formulation of *T. Viride* against The causative pathogen of sugar-beet damping off and root rot disease under greenhouse conditions

Applied formulation of *T. viride* at the rate 5% weight of the soil one week before planting the seed .The same method preparation the inoculum pathogens and infestation the soil as is described in pathogenicity test

Chemical Assessment

Photosynthetic pigment (chlorophyll a, chlorophyll b and carotenoids) were determined in treated plants with pathogens only or with formulation of T.viride and untreated control according to [20]

Statistical analysis

Data were subjected to statistical analysis and means were compared using L.S.D. test [21]

Results

Pathogen city test

Identification of isolated fungi shown in Table (1) indicated that isolated fungi were identified as *Fusarium sambaucinum* and *Fusarium solani* isolates were varied in their virulence on sugar beet

Fungi	Isolates	Damping off%			
		Pre	Post	Survival%	Disease index %
Fusarium sambucinum	I1	50	5	45	55
	I2	72.5	5	22.5	67.5
	I3	42.5	5	52.5	47.5
	I4	67.5	0	32.5	64.5
Fusarium solani	I5	65.5	5	30	65
	I6	57.5	2.5	40	47.5
	I7	57.5	5	37.5	52.5
	I8	45	2.5	52.5	40
Control		0	0	100	0
L.S. D 5%		17.056	7.195	16.616	5.135

Table 1: Pathogen city tests of Fusarium sambucinum (isolates1,2,3,4) and *Fusarium solani* (isolates5,6,7,8) the causal pathogens damping off and root rot oskarpoly sugar beet variety under greenhouse conditions during growing season2009

oskarpolay variety the data revealed that in general there were significant difference between the isolates in both fungal species either pre–post emergence and survival compared with control .data also indicate that *Fusarium sambucinum*. isolate No 2 was the highest virulent one in case of pre emergence damping off and less survival followed by isolate No4 and isolate No.1while isolate No 3 was the lowest pathogenic one and the highest survival *F. solani* also varied in their pathogenic isolate No 5 and 6, 7 were highly pathogenic when compared with isolate 8.

In vitro antagonistic effect of T. *Viride* against the cause of sugar beet damping off and root rot disease

Dual culture assays *T. viride* substantially reduced the growth of *Fusarium sambucinum*(isolates 1,2,3,4)and *Fusarium solani*, (isolate 5,6,7,8) . The causal pathogens of damping off and root rot sugar beet compared with the control. The *Trichoderma viride* grow over and sporulated of the different *Fusarium spp* isolates. Resulting of complete degradation (Tables 2, 3).

Isolates	Inhibition Zone
I1	-
I2	-
I3	
I4	-

Table 2: Reaction and antifungal of *Trichoderma viride* on *Fusarium sambucinum*(isolates1,2,3,4)causing damping off and root rot sugar beet *in vitro*

Isolates	Inhibition Zone
I5	-
I6	-
I7	
I8	-

Table 3: Reaction and antifungal of *Trichoderma viride* on *Fusarium solani* (isolates, 5, 6, 7,8) causing damping off and root rot sugar beet *in vitro*

Isolates	% growth reduction
I1	50
I2	27
I3	55
I4	37

Table 4: Effect of culture filtrates of *T. viride* on *Fusarium sambucinum* (isolates 1,2,3,4) causing damping off and root rot sugar beet *in vitro;* - L.S.D. 5%1.2

Data also indicate that the *T. viride* and its filtrate inhibited the growth of the pathogens *Fusarium sambucinum*(isolates 1,2,3,4) and *Fusarium solani,* (isolates, 5, 6, 7,8) Tables (4) and (5). Isolate 1 and isolate 8. Showed the highest percentage of growth reduction while the Isolate 2 and Isolate 5 showed the lowest percentage of growth reduction.

Isolates	% growth reduction
I5	31.3
I6	35
I7	32.6
I8	39.8

Table 5: Effect of culture filtrates of *T. viride* on *Fusarium solani* (isolates, 5, 6, 7,8) causing damping off and root rot sugar beet *in vitro;* - L.S.D 5% 0.1.29

In general data also indicate that filtrate of *T. viride* showed the highest percentage of growth *Fusarium sambucinum* reduction compared with *Fusarium solani*

Data also indicate that filtrate of *T. viride* significant difference inhibited between isolates1, 2, 3, 4 of *Fusarium sambucinum*. Data also indicate that filtrate of *T. viride* significant difference inhibited between (*Fusarium solani* isolates 5, 6, 7, 8) the causal pathogen of damping off and root rot sugar beet

Antagonistic effect of T. *Viride* against the causative pathogen of sugar-beet damping off and root rot disease under greenhouse conditions

Data in Table (6,7) indicated that soil treatment with formulation T. viride resulted in protection against the causal pathogen *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani*, (isolates, 5, 6, 7,8) at the seedling stage. Minimal amount of disease were observed on plants inoculated with pathogen and bio agent compared with untreated control. *T. viride* reduced the percentages of disease incidence and disease severity of *Fusarium spp*. the causal pathogen of sugar beet compared with control.

The highest percentage of seedling survival and the least disease severity were associated with Isolates 3 and 8 but the lowest percentage of seedling survival and highest disease severity were associated with Isolates 2 and 5. Data also indicate that T. viride reduce significantly damping off and disease severity compared with control caused by the pathogens *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani,* (isolate, 5, 6, 7,8)during two growing seasons.

Treatment	2010	2011

	Pre%	Post%	Survival%	Disease index %	Pre%	Post%	Survival%	Disease index %
I1	72.5	12.5	15	77.5	62.5	7.5	30	80
I2	87.5	10	2.5	76.2	82.5	10	7.5	70
I3	65	10	25	78.7	55	7.5	37.5	75
I4	92.5	0	7.5	63.5	77.5	0	22.5	63.5
I1+Trichoderma viride	45	5	50	63.7	35	2.5	62.5	62
I2+Trichoderma viride	70	5	25	61.5	60	2.5	37.5	65.2
I3+Trichoderma viride	40	0	60	62.2	32	50	67.5	61
I4+Trichoderma viride	60	7.5	32.5	67	52	55	42.5	54
Control	0	0	100	0	0	0	100	0
L.S. D 5%	28.183	8.965	26.469	3.145	25.942	6.06	24.616	1.406

Table-6: Effect of formulation of *T. viride* on damping off and root rot sugar beet caused by, *Fusarium sambucinum* (isolates 1,2,3,4) under greenhouse conditions during growing seasons 2010

Effect of treatment with formulation of *T. viride* on photosynthetic pigments ,chlorophyll a, chlorophyll b, carotenoid of sugar beet infected with, *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani*, (isolates, 5, 6, 7,8) under greenhouse conditions during growing seasons 2010.

Treatment	2010				2011			
	Pre	Post%	Survival%	Disease index %	Pre%	Post%	Survival%	Disease index%
I5	77.5	7.5	15	80.62	75	5	20	75
I6	70	5	25	73.75	67.5	5	27.5	72
I7	72.5	5	22.5	75	70	5	25	73.5
I8	70	30	27.5	66.25	65	0	35	63.7
I5+Trichoderma viride	65	2.5	32.6	62.5	62.5	2.5	35	65
I6+Trichoderma viride	60	2.5	37.5	61.8	55	2.5	42.5	45
I7+Trichoderma viride	62.5	0	37.5	61	60	2.5	37.5	61
I8+Trichoderma viride	55	2.5	42.5	58.75	52.5	2.5	45	58.75
Control	0	0	100	0	0	0	100	0
L.S. D 5%	17.943	4.865	15.67	5.072	12.502	4.865	13.155	5.043

Table 7: Effect of formulation of *T. viride* on damping off and root rot sugar beet caused by *Fusarium solani* (isolates, 5, 6, 7,8) under greenhouse conditions during growing seasons 2010 and 2011

Treatment	2010				2011			
	Chl.a	Chl.b	Total chloraphall	cardonides	Chl.a	Chl.b	Total chloraphall	cardonides
I1								
I2								

I3								
I4								
I1+Trichoderma viride								
I2+Trichoderma viride								
I3+Trichoderma viride								
I4+Trichoderma viride								
Control								

Table 8: Effect of treatment with formulation of *T. viride* on photosynthetic pigments , chlorophyll a, chlorophyll b, carotenoid of sugar beet infected with *Fusarium sambucinum* (isolates 1,2,3,4) under greenhouse conditions during growing seasons 2010 and 2011

Treatment	2010				2011			
	Chl.a	Chl.b	Total chloraphall	Cardonides	Chl.a	Chl.b	Total chloraphall	cardonides
I5	0.019	0.105	0.124	0.046	0.018	0.01	0,032	0.043
I6	0.250	0.001	0.251	0.001	0.251	0	0.252	0.001
I7	0.129	0.004	0.133	0.056	0.128	0	0.132	0.053
I8	0.279	0.002	0.271	0.105	0.278	0	0.28	1.3731
I5+Trichoderma viride	0.337	0.189	0.526	0.165	0.336	0.19	0.524	0.164
I6+Trichoderma viride	0.319	0.235	0.554	0.159	0.138	0.23	0.372	0.158
I7+Trichoderma viride	0.299	0.243	0.545	0.128	0.298	0.24	0.541	0.127
I8+Trichoderma viride	0.643	0.003	0.643	0.004	0.643	0	0.0643	0.004
Control	0.743	0.222	0.963	0.169	0.743	0.22	0.967	0.169

Table 9: Effect of treatment with formulation of *T. viride* on photosynthetic pigments, chlorophyll a, chlorophyll b, carotenoid of sugar beet infected with, *Fusarium sambucinum* (isolates 1,2,3,4) under greenhouse conditions during growing seasons 2010 Discussion

Fusarium sambucinum (isolates 1,2,3,4) and *Fusarium solani*, (isolates 5,6,7,8) cause damping of f and root rot of sugar beet this results agree with [1-2].

The biological control of plant pathologic fungi has received considerable attention as an alternative strategy. The use of the antagonistic properties of *Trichoderma spp* in the biological control of many plant diseases has been a subject of many studies [22-3]in vitro culture filtrate of *T .viride* varied decrease % growth reduction of *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani*, (isolates, 5,6,7,8) .The suppressive effect varied according to the antagonistic filtrate. These results agree with Chet and Baker1981 [23].

Trichoderma spp is known to have the ability to produce some extra cellular, lactyic enzymes that are involved in the process of antagonism against a variety of pathogenic organisms [24]. Biocontrol technologies have gained momentum in disease control of crop plants in recent times as these technologies not only minimize of replace the usages of harmful chemical pesticides but also found to be cheaper and efficient in certain disease control programmers. Successful use of fungal bio control agents like *Trichoderma spp*. for the agents that commercially produced to prevent development of several soil pathogenic fungi [4,5,6]. Different mechanisms control soil born disease caused by pathogens like Rhizoctonia, Sclerotium, Fusarium, Pythium and Phophthora in several crops have been reported [7] therefore using biological control such as *T.viride* which is one the efficient bio control have been suggested as being responsible for their bio–control activity which includes mycoparasitism, antibiosis, competition for nutrients and space and secretion of chitinolytic enzymes [8]. The mass production systems should be complete with industrial and commercial development methods and field application [9]. *Trichoderma spp*. can be formulated as pellets [10] dusts an powders [11] Formulation *T.viridi* have a positive effect against the tested pathogenic sugarbeet could be explained by hyper parasitism. Treatment the soil with formulation of *T.viridi* reduce damping off and root rot caused by *Fusarium sambucinum* (isolates 1,2,3,4) and *Fusarium solani*, (isolates 5,6,7,8) compared with control and untreated and untreated with *T.viridi* these results agreement with those recorded by [25,26,3]. They reported that the bio control agent characterized by faster metabolic rates anti-microbial metabolites and physiological conformation are key factors which contribute to

antagonism of these fungi. The antagonistic effect of bio-control agent also may be due to mycoparasitism, spatial and nutrient competition antibiosis by enzymes and secondary metabolites and induction of plant defense system are typical bio-control actions of these fungi. The obtained results revealed that frequency of some saprophytic fungi such as *A.niger, Aspergillus spp., Penicillium spp.* and *Trichoderma spp*. as well as pathogenic. Fungi of *Fusarium spp*., *F. solani* and *R. solani* in treated rhizosphere of sugar beet were affected by *T.viride*. The saprophytic mycoflora could be playing an important role in increasing the antagonistic effect of the bio control agent [8]. Photosynthetic pigments chlorophyll a and chlorophyll b and carotenoids were lower in the infected leaves than healthy one and in infected leaves than healthy one and in infected leave and treated the soil with formulation of *T.viride*. The occurrence of changes in leaf color as a result of infection by most disease was proved by many investigation .The chlorophyll pigment plays an important role in metabolic activity in the plant extremely affected by disease incidence [12] stated that striking changes in the amount and distribution of photosynthetic pigment resulted from the infection by obligated parasite.

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