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EVALUATION OF GRAFTING AND BIOLOGICAL CONTROL AGAINST SQUASH DAMPING OFF

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ABSTRACT

Fusarium solani Mart. and Rhizoctonia solani Kuhn. are among the most common pathogens causing damping off disease of cucurbits in Egypt. Grafting is a method for controlling number of soil borne pathogens. Tabark variety (Cucurbita pepo L.) on Pumpkin (Cucurbita maxima Duch.) as rootstock is strong and resistance to F. solani and R. solani while, Alexandria variety (Cucurbita pepo L.) on Gourmet (Citrullus colocynthis L.) as rootstock is susceptible. On the other hand, grafting Alexandria on Pumpkin and Tabark on Gourmet rootstocks are moderate susceptible. The antagonistic effect of two Trichoderma species, i.e. T. harzianum Rifai., T. viride Harz. or mixture of them and Bacillus subtilis were tested aganist F. solani and R. solani under artificial infestation in greenhouse and natural infection in field conditions for two successive seasons. In greenhouse experiment, mixture of T. harizianum and T. viride, as soil treatments significantly reduced the pre- and post-emergence damping off compared with control treatment. All of treatments used as biocontrol agents significantly reduced the incidence of damping off compared with control treatments under field conditions. Data obtained in 2011/2012 growing seasons also indicated that the mixture of T. harizianum and T. viride showed the highest effect on reduction disease incidence and also increased the vigority of treated rootstock plants compared with control.

Key words: Squash, damping off, grafting, Trichoderma harzianum, T. viride, and Bacillus subtilis.

INTRODUCTION

Squash (Cucurbita pepo L.) is considered as one of the most popular vegetable crop in Egypt while attacked by numerous pathogenic fungi, wherever the crop is grown (Wien, 1997 and Maynard and Hochmuth, 2007). Damping off and root rot are considered as destructive diseases, and cause great losses in many parts of the world (Madkour et al., 1983; Abd-El-Rehim et al., 1987; Fantino et al., 1989 and Celar, 2000). Many pathogenic fungi were isolated from diseased plants belong to cucurb4itace i.e. squash, pumpkin and gourmet. These pathogens were considered as seed borne or soil borne pathogens attacked young and old seedlings (5-15days old). They were also found that severities of these fungi on plants suffering

from unfavorable conditions were similar as when compared with the effect of the same pathogens on plant growing under favorable conditions.

Fusarium solani usually survives as thickwalled chlamydospores in soil (Nagy, 2007). *Rhizoctonia solani* is one of the most economically important damping off pathogens in squash. Rhizoctonia survives at absence of hosts as sclerotia or as fungal mycelia in the soil (Summer, 1976). Young plants are more susceptible to infect than older plants. Application of the fungicides is not economic because they pollute the environment, leave harmful residues and can lead to the development of resistant strains of the pathogen in the long time (Summer, 1976; Hall *et al.*, 1981; Rasmy, 1986 and Vinale *et al.*, 2008).

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Grafting is a method of asexual plant propagation that joins plant parts to live together. Normally the method has applied for trees propagation. Stocks are selected due to the good characteristics include size of root system or resistance to some diseases or nematodes whereas cuts are selected for their good characteristics of foliage and fruits. Intensive agriculture system in cucurbits led to spread out of different soil borne and root rot pathogens. Grafting of vegetables is common in several Mediterranean and Southeast Asian countries mainly to control Fusarium diseases and number of other soil borne pathogens (Koren, 2002 and Lee, 2003). Vegetable grafting was also being utilized to develop plants resistant to drought stress or salty soil (Wien, 1997).

Biological control of plant diseases especially soil borne plant pathogens has been handled in many scientific papers .In the last two decades, Trichoderma spp., are well documented as effective biological control agents of plant diseases caused by soil borne fungi (Sivan and Chet, 1994, Basim et al., 1999 and Whipps and Lumsden, 2001) .They are believed that many of these species originated from tropical regions in Africa, the Americans, and Asia (Wien, 1997 and Maynard and Hochmuth, 2007). One of the most important bio-control agents is Trichoderma spp., that the most frequently isolated as soil fungi and present in plant root ecosystems (Harman et al., 2004). Trichoderma spp., also are commercially marketed as biocides, bio-fertilizers and soil amendments. The use of Trichoderma fungi in agriculture can provide numerous advantages; colonization of root and rhizosphere of plant, control plant pathogens by different mechanisms such as parasitism, antibiosis production and induced systemic resistance, improved plant health by promoting plant growth hormone and stimulation of root growth. The antagonistic activity of the genus Trichoderma spp., is well documented as effective biological control agents of plant diseases caused by soil borne fungi (Sivan and Chet, 1994; Basim et al., 1999; Whipps and Lumsden, 2001 and Mclean et al., 2004). Although, biological control of damping off disease fungi are studied by many researchers, few studies are done on the control of squash damping off and root-rot disease using Trichoderma spp. Hadar et al. (1979) and Elad et al. (1980) observed that the application of wheat bran colonized by T. harzianum to soil infested with R. solani reduce the incidence of disease caused by this pathogen in squash. Considerable researchers have been done to investigate antagonistic microbes to be used as seed treatments as reported by Callan et al. (1990); Baird et al. (1994) and Howell and Stepanovic (1995). Mixing antagonists with each others may be lead to antagonistic effect consequently decrease efficacy of treatment (Robinson et al., 2009) or lead to synergistic effect and increase the efficacy (Latha et al., 2009 and Yobo et al., 2011). This increase or decrease is due to harmony and compatibility factors between bioagents.

Several *Bacillus* spp., including *B. subtilis* are antagonistic to plant pathogenic fungi and bacteria. *Bacillus* spp.. produce at least 66 different antibiotic compounds (Ferreira *et al.*, 1991). The antagonistic effect of *B. subtilis* against many fungi *in vitro* and *in vivo* was reported by (Asaka and Shoda, 1996; Farahat, 1998; Abd-El-Moity *et al.*, 2003; Abd El Moneim *et al.*, 2005 and Hussein *et al.*, 2007).

The present study is conducted to identify the pathogens causing damping off of squash seedlings in Egypt. Grafting is used to come over this problem either alone or in combination of using some biocontrol agents, stocks figure out the most suitable stock in production and protection of cucurbits. Four different stocks are used to show their effects on squash cuts protection (Tabark and Alexandria) against damping off disease. Different biocontrol agents (Trichoderma harzianum, T. viride, mixture of them and Bacillus subtilis) are also in combination with grafting on different stock to improve and increase protection effect in these stocks and also increase yield. To determine effect of these biological treatments compare with control or blank treatment.

MATERIALS AND METHODS

Samples Collection

Two squash varieties, Pumpkin and Gourmet exhibited damping off and root-rot diseases were collected from growing areas, in Tag Elezz station at El-Dakhliya Governorate.

Isolation, Purification and Identification of Pathogenic Fungi

Squash, Pumpkin and Gourmet diseased seedlings and plants were thoroughly washed using running tap water to remove soil particles, cut into small pieces and surface sterilized in 1% sodium hypochlorite solution for 2 minutes. The sterilized parts washed twice in sterilized distilled water and dried between two sterilized filter papers. The sterilized parts placed onto plain agar medium and incubated at 27°C for 7 days. All the developed fungi were purified using hyphal tip and \ or single spore techniques (Dhingra and Sinclair, 1995). The purified fungi were transferred to slant medium and kept at 5°C for further studies. The isolated fungi were microscopically identified according to their morphological characteristics using the description by Burnett and Hunter (2003).

Pathogenicity Tests under Greenhouse Conditions

Seeds of Pumpkin (*Cucurbits maxima* Duch.), Gourmet (*Citrullus colocynthis* L.) in addition to two local squash varieties (*Cucurbita pepo* L.) (Tabark and Alexandria) were obtained from Vegetable Crops Research Dept., Agricultural Research Centre, Giza, Egypt.

Pathogenicity tests for the isolated fungi were carried out under greenhouse conditions in 2011 growing season to select the most pathogenic fungus. Inocula were prepared by growing each of the isolated fungi F. solani and R. solani in 500 ml conical flask containing 200 ml of autoclaved corn meal broth media and incubated at 25°C for 10-15 days (Abd-El-Moity, 1985). The fungal growth was blended in the blender for two minutes using sterilized water to homogenize the inocula. Plastic pots 30 cm in diameter were sterilized by immersing them in 5% formalin solution for 15 minutes, then left to dry for 7 days to ensure getting rid off and evaporation the excess poisonous of formalin. The sterilized plastic pots were filled with sterilized sand soil. Infestation was carried out by adding the blended homogenized fungal inocula to sterilized soil at the rate of 3-5% of soil weight (v\w). The infested soil then watered and left for 15 days to ensure its distribution in the soil. Control pots were watered with the used

medium free from the fungus at the same rate. Four pots were used for each particular treatment. All plants were observed daily and watered as needed. Pre and post emergence as well as root rot were recorded after 5-10 days from planting (Tousson and Snyder 1961 and Paternote 1987). Healthy survival plants were recorded after 15 days from inoculation.

Biocontrol Agents

Three different biocontrol agents were kindly obtained from the Organic Agricultural Central Laboratory. These biocontrol agents are namely Trichoderma harzianum Rifai. (T1), Trichoderma viride Harz.(T2), mixture of them and Bacillus subtilis. These bioagents were used as granules using method developed by Abd-El-Moity (1981). The concentrations of different biocontrol agents were adjusted to be 30×10^6 c f u /ml. This preparation is mixed with soil at concentration of 5g/kg of soil, while the untreated seedlings grown in soil free from the pathogen were also used as a negative control. The experiment contains four treatments design with three replicates. Two pathogens Fusarium solani and Rhizoctonia solani were grown on corn meal agar media for 9 days (Abd-El-Moity, 1985). The pathogens were added to the clay soil at the rate of 5g/kg.Grafted squash (rootstocks) were set up in plastic pots each containing 2.5 kg of soil sown with 5 rootstocks squash plants.

Grafting was carried out by placing cuts obtained from 7 old days (Tabark and Alexandria varieties) in 10 old days stock Pumpkin or Gourmet. Grafted plants are planted under greenhouse and field conditions. To improve surviving grafted plants methods were undertaken developed by Edelstein and Ben-Hur (2006).

During the course of the biocontrol evaluation study, seedlings were planted at depth of 2 cm in clay pot (25 cm). The untreated seedlings were grown in the soil free of the pathogen and used as a negative control. The percentage of diseased plants and survivals due to the different seedling treatments calculated based on the percentage of infection relative to the positive control (untreated seedling sown in infested soil) were determined.

Field Experiment

The efficacy of soil treated with T. harzianum and T. viride and mixture of them as well as Bacillus subtilis used in separated blocks, to evaluate their effect on the percentage of survival and diseased plants, on grafted squash varieties. This experiment was carried out under field conditions in Tag Elezz station at El-Dakhliya Governorate in two tested growing seasons 2011 / 2012 were examined. The field trial (20 plots) designed in complete randomized block with three replicates. Each plot was 3x3 m and had four rows of 3m in length and 75 cm in width. Inocula of each tested bio-control agent applied at the rate of 50g/m² from soil incorporated at 20 cm depth from the soil surface. The infested soil irrigated 7 days before sowing date. Grafted squash were planted at the rate of 2 plants / hill at 20 cm space.

Plant Growth and Yield Parameters

Random samples of ten grafted squash plants were collected after 60 days of sowing for each bio-control agent treatment as well as the control plants. The plant growth parameters measured as number of fruits per plant, average weight of fruits per plant and yield were determined and calculated.

Statistical Analysis

Obtained data were subjected to Computer Statistically analyzed according to the procedures reported by Snedecor and Cochran (1980). The means of all treatments were compared by the least significant difference value "L.S.D." at 5% level of probability).

RESULTS AND DISCUSSION

Fusarium solani Mart. and Rhizoctonia solani Kuhn. was the most frequently isolated fungi from root rot and damping off pumpkin (Cucurbita maxima Duch.), gourmet (Citrullus colocynthis L.) and Tabark and Alexandria squash varieties (Cucurbita pepo L.) These fungi were previously reported to be associated with squash damping off and root-rot disease in other countries (Martyn and Mclaughin, 1983 and Pushpa et al., 1999). The obtained data from pathogenicity test showed that squash plants were highly attacked by F. solani and R. solani, in descending order. Data obtained in Table 1 show that all the tested fungi caused dampingoff disease incidence to their respective hosts. where it significantly decreased the survival seedlings. Fusarium solani isolate (3) was highly pathogenic while, Rhizoctonia solani isolate (1) was the most aggressive to all varieties. Alexandria variety was the most susceptible one while, Tabark was resistance. It is also clear that. Fusarium solani was more pathogenic than Rhizoctonia solani. These results agreed with those obtained by Rasmy (1986) who found that, F. oxysporum Rhizoctonia solani and F. solani were isolated from seeds of squash and caused damping-off disease of certain cucurbits.

Evaluation of used antagonistic microorganisms (T. harzianum and T. viride and mixture of them as well as*Bacillus subtilis*) in controlling damping off diseases in squash plants under greenhouse conditions revealed that all bioagents significantly reduced the percentage of disease incidence compared with control treatment in rootstocks squash plants.

Data obtained in Table 2 indicate that the most widely used rootstock is good when grafting between Tabark (Cucurbita pepo L.) with Pumpkin (Cucurbita maxima). This stock was exerting strong resistance to the Fusarium solani and Rhizoctonia solani (40% and 46.7%). Grafting success rate can also be affected by morphology, *i.e.* differences in hypocotyle diameters of rootstocks and scions (Lee, 2003). He reported that minimizing the difference between the hypocotyle diameters of the rootstock and scion might enhance survival of squash grafted on Pumpkin. Differences in stem diameter between rootstocks and scions reduced the survival ratio of grafts and existence of a large central space on the stem of cucurbitaceae negatively affects grafting success. Good graft compatibility with Alexandria (Cucurbita pepo L.) Gourmet (Citrullus colocynthis L.) and rootstocks were susceptible to Rhizoctonia solani and Fusarium solani (80% and 73.3%) while, grafting Tabark with Gourmet (43.33% and 50%) and Alexandria with Pumpkin (50% and 70%) were moderate susceptible. Pumpkin was the most resistance variety against two pathogens (50% and 60%). Fusarium root rot of squash, Gourmet and Pumpkin is caused by

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Taalada	% D	isease	incide	nce	of squ	ash vai	rietie	es as da	mping	off an	d surv	vival
Isolate		Pre Post Surviva								v ival		
						Vari	etie	•				
Varieties	Tabark	Alexandria	Gourmet	Pumpkin	Tabark	Alexandria	Gourmet	Pumpkin	Tabark	Alexandria	Gourmet	Pumpkin
					Rĥ	izocto	nia s	olani				
Isolate 1	10	40	20	10	20	20	30	30	70	40	50	60
Isolate 2	10	40	20	30	20	30	20	20	70	30	60	50
Isolate 3	10	30	20	20	10	30	10	20	80	40	70	60
Mean	10	36.67	20	20	16.67	26.67	20	23.33	73.33	36.67	60	56.67
					F	usariu	m so	lani				
Isolate 1	10	40	10	10	20	20	10	20	70	40	80	70
Isolate 2	20	30	20	20	10	30	20	30	70	40	60	50
Isolate 3	20	60	20	30	20	20	30	30	60	20	50	40
Mean	16.67	43.33	16.67	20	16.67	23.33	20	26.67	66.67	33.33	63.33	53.33
L S D at 0.05 Variety (V) Isolate (I)		9.8 8.1				10.(5.9				2.7 5.5		<u></u>
(I x V)		16.2				16.2				11.		

Table 1. Pathogenicity test of three different isolates of *Rhizoctonia solani* and *Fusarium solani* on squash varieties

Table 2. Evaluation of squash rootstocks and varieties against damping off under greenhouse conditions

Rootstocks and varieties	%Damp	ing off	%Survival			
ROUGIUCKS and varieties	F. solani	ni R. solani F. solani 50.00 56.67 46.67 60.00 80.00 26.67 70.00 50.00 76.67 33.33 80.00 23.33 73.33 50.00 60.00 50.00 60.00 50.00 18.72 18	R. solani			
Tabark + Gourmet rootstocks	43.33	50.00	56.67	50.00		
Tabark + Pumpkin rootstocks	40.00	46.67	60.00	53.33		
Alexandria + Gourmet rootstocks	73.33	33 80.00 26.67		20.00		
Alexandria+ Pumpkin rootstocks	50.00	70.00	50.00	30.00		
Tabark variety	66.67	76.67	33.33	23.33		
Alexandria variety	76.67	80.00	23.33	20.00		
Gourmet variety	50.00	73.33	50.00	26.67		
Pumpkin variety	50.00	60.00	50.00	40.00		
Mean	56.25	67.08	43.75	32.92		
L S D at 0.05 Variety (V)	9.3	7	9.37			
Isolate (I)	18.3	72	18.72			
(I x V)	13.2	25	13.	25		

Fusarium solani (Mart.) Sacc. Fusarium root rot and damping off squash described first in detail in South Africa in 1932 (Rasmy, 1986). It also reported in Australia, Canada, and the United States; however, its known distribution in the United States is limited. Fusarium solani can cause seedling diseases of cucurbits. Numerous strains of F. solani have been found and there is a high degree of variability in the fungus (Nagy, 2007). Rhizoctonia solani is a soil borne fungus with a very broad host range. This fungus can survive by infecting and thriving on a great number of plant hosts, besides cucurbits, and persist in the soil as a saprophyte. The disease is generally problem only in squash and certain Pumpkin varieties; however, Pumpkin variety was less susceptible than other cucurbits, but all are equally susceptible in the seedling stage under field conditions (Summer, 1976). Grafting is not used alone but as a component of an IPM program which includes other control methods such as fumigation, sanitation, pathogens free seeds and seedlings weed control, to increase improvement of plant growing conditions etc... (Oda, 2002).

Data obtained in Table 3 indicated that mixture of T. harzianum and T. viride were the most effective treatment against damping off pathogen F. solani in rootstock Tabark with Pumpkin and Alexandria with Gourmet (31.33% and 34.67%). T. viride was the most effect on Alexandria with Pumpkin rootstock (33.67%). These effects might be due to direct mycoparasitism as in T. viride or effect through enzymes and / or antifungal substance (Turner, 1971 and Paderes et al., 1992) or also stimulate resistant in the host (Elad et al., 1998; Bolar et al., 2000 and Howell et al., 2000). On the other hand, Mixture of Trichoderma spp., gave the was grafting with best result when Tabark Pumpkin and Alexandria with Pumpkin rootstocks (33% and 51%) to control R. solani while, Alexandria with Gourmet and Tabark with Gourmet rootstocks recorded (31.33% and 57.67%). This antagonistic effect might be due to the number of species within the genus Trichoderma are well known for their biological control capabilities against a wide range of pathogens important plant commercially (Whipps and Lumsden, 2001 and Mclean et al., 2004). They are known to produce a number of antibiotics, such as trichodermin, trichodermol and harzianolide (Elad et al., 1980).

Data obtained in Table 4 indicate that grafting Tabark with Gourmet and Pumpkin variety were the most resistance to damping off diseases in the first season (31.67% and 40%). While, grafting Tabark with Gourmet, Tabark with Pumpkin and Tabark variety recorded the same reduction percentage (23.33%) in the second season under field conditions. One of the major advantages of using grafted plants is the control of many pathogens like Fusarium diseases. Grafting is common in several Mediterranean and South East Asian countries mainly for the control Fusarium spp., and R. solani (Lee, 2003). He added that there are many combinations of pumpkin and squash, or their rootstocks that still need to be evaluated for quality yield and might need less fertilizer, which should help control production costs. There were different degree of susceptibility to all tested varieties and rootstocks against damping off pathogens. The differences between varieties in their resistance and susceptibility might be due to the differences in their genetic make up which affected on some morphological factors affected host-pathogen relationship which played a role in varieties (Edelstein and Ben-Hur, 2006). Also, the variability of varietals reaction among squash varieties might be attributed to the plant growth stage, root oxidates and locality (Lee, 1994).

Data presented in Table 5 show that, all tested bioagents significantly reduced percentage of disease incidence compared with control. In addition grafting Tabark with Gourmet and Tabark with Pumpkin were the most resistance rootstocks against damping off diseases in two tested seasons. Data also indicated that mixture of T. harzianum and T. viride in the first season gave the best result to control damping off diseases on Tabark with Gourmet rootstock, (20.00%). It is also clear that, grafting Tabark with Pumpkin decreased the percentage of to 22.67% while, grafting damping off Alexandria with Ggourmet recorded 33.33% might be due to spp. (Trichoderma mycoparasitism, lytic enzymes and competition.

Abd-El-Moity and Shatla (1981) stated that the effect of different isolates of *T. harzianum*

Pathogenic fungi		Fusariu	m solan	Rhizoctonia solani						
	Varieties + Stocks									
Bioagents	Tabark + Gourmet	Tabark + Pumpkin	Alexandria + Gourmet	Alexandria + Pumpkin	Tabark + Gourmet	Tabark + Pumpkin	Alexandria + Gourmet	Alexandria + Pumpkin		
Bacillus subtiles	31.67	34.33	40.33	38.67	35.67	35.33	59.00	53.00		
Trichoderma harazianum (T1)	32.67	35.33	39.67	39.00	35.00	36.33	59.67	52.00		
Trichoderma viride(T2)	34.33	32.00	35.00	33.67	34.00	36.00	64.67	56.67		
Mixture of (T1+T2)	30.00	31.33	34.67	35.67	31.33	33.00	57.67	51.00		
Control	43.33	40.00	73.33	50.00	50.00	46.67	80.00	70.00		
Mean	34.40	34.59	44.6	39.40	37.20	37.47	64.20	56.53		
L S D at 0.05 Variety (V) Isolate (I) (I x V)	3.07 3.05 6.10					5	.83 .57 .14			

Table 3. Effect of different bioagents on rootstocks squash plants against damping off disease under greenhouse conditions

Table 4. Evaluation of squash rootstocks and varieties on damping off in 2011/ 2012 growing seasons under field conditions

Rootstock and varieties	First seaso	n 2011	Second season 2012			
Rootstock and varieties	%Damping off	%Survival	%Damping off	%Survival		
Tabark +Gourmet rootstocks	31.67	8.33	23.33	76.67		
Tabark +Pumpkin rootstocks	33.33	66.67	23.33	75.00		
Alexandria +Gourmet rootstocks	43.33	56.67	25.00	61.67		
Alexandria +Pumpkin rootstocks	46.67	53.33	38.33	46.67		
Tabark variety	43.33	56.67	23.33	76.76		
Alexandria variety	56.67	43.33	43.33	56.67		
Gourmet variety	46.67	53.33	33.33	66.67		
Pumpkin variety	40.00	60.00	26.67	73.33		
Mean	42.27	49.79	29.58	66.68		
LS D at 0.05	9.95	9.95	10.62	10.62		

Bioagents]	First sea	uson 201	1	Second season 2012				
	Tabark + Gourmet rootstocks	Tabark + Pumpkin rootstocks	Alexandria+ Gourmet rootstocks	Alexandria+ Pumpkin rootstocks	Tabark + Gourmet rootstocks	Tabark + Pumpkin rootstocks	Alexandria+ Gourmet rootstocks	Alexandria+ Pumpkin rootstocks	
Bacillus subtilus	24.33	26.33	35.33	45.00	23.33	10.00	30.00	41.67	
Trichoderma harazianum (T1)	31.67	24.33	35.00	33.33	26.67	13.33	26.67	31.6 7	
Trichoderma viride (T2)	21.00	25.00	38.00	35.33	23.33	10.00	30.00	40.00	
Mixture of (T1+T2)	20.00	22.67	33.33	35.00	35.00	11.67	23.33	33.33	
Control	31.67	33.33	43.33	46.67	30.00	23.33	38.33	53.33	
Mean	25.73	26.33	36.99	39.07	27.67	13.67	29.67	40	
L S D at 0.05 Variety (V) Isolate(I) (I x V)		4.	.04 .05 .10		6.06 3.80 7.61				

Table 5. Effect of different bioagents on rootstock squash plants against damping off in 2011 and 2012 growing seasons under field conditions

affect the growth of the pathogenic fungi through different mechanisms, i.e. production of gliotoxin, mycoparasitism and grow very fast and act as barrier between susceptible plant tissue and virulent pathogen. T. harzianum recorded the best percentage on Alexandria with Pumpkin rootstock (33.33%). Tronsmo et al. (1993) mentioned that, T. harzianum isolates produces chitinase and 1.3B.gluconase enzymes which are responsible for dissolving cell wall of the pathogenic fungi, than it can grown and consume the inner content causing destruction of the pathogen. Data also indicated that, in the second season treatment, B. subtilis led to the best reduction of damping off disease as well as T. viride when grafting Tabark with Gourmet and also Tabark with Pumpkin rootstocks (33.3% and 10% respectively). Effect of B. subtilis might be due to the production of antibiotics iturin and increase surfactin (Asaka and Shoda 1996; Hwang et al., 1996 and Ryder et al., 1999). These effects might be due to direct mycoparasitism as in T. viride or effect through enzyme and/or antifungal substance

(Turner, 1971 and Paderes *et al.*, 1992). or also stimulate resistant in the host plants (Elad *et al.*, 1998; Howell *et al.*, 2000 and Bolar *et al.*, 2000) as well as through production of antifungal substances (Hayes, 1992). The Trichoderma resistance induced is systemic (Harman *et al.*, 2004). The systems for induced resistance appear to be in at least some ways similar to those induced by rhizobacteria.

Data in Table 6 indicate that all the tested bioagents significantly increased the number of fruits /plant when compared with control while, grafting Tabark with Gourmet and Tabark with Pumpkin were the most rootstocks increased average number of fruits in two tested seasons. Data in first season indicated that, T .viride increased the number of fruits/plant when grafting Tabark with Gourmet (8%). While, B. subtilis recorded the best effect when Tabark grafting with Pumpkin as well as mixture of T. harzianum and T. viride recorded (6.67%) when grafting Alexandria with Pumpkin. On the other hand, mixture of T. harzianum and T. viride

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		Average number of fruits/plant								
]	First sea	son 2011	Second season 2012						
Bioagents	Tabark +Gourmet rootstocks	Tabark +Pumpkin rootstocks	Alexandria +Gourmet rootstocks	Alexandria+ Pumpkin rootstocks	Tabark +Gourmet rootstocks	Tabark +Pumpkin rootstocks	Alexandria+ Gourmet rootstocks	Alexandria +Pumpkin rootstocks		
Bacillus subtilus	6.67	6.67	5.67	5.00	8.00	7.67	6.33	6.33		
Trichoderma harazianum (T1)	7.67	6.00	6.00	5.67	7.67	8.00	6.67	6.00		
Trichoderma viride (T2)	8.00	6.00	6.00	5.67	8.33	7.67	6.67	5.67		
Mixture of (T1+T2)	7.67	6.33	6.67	7.00	8.00	8.33	6.67	6.67		
Control	6.00	6.00	5.33	5.33	7.00	6.67	5.67	5.00		
Mean	7.20	6.20	5.93	5.73	7.80	7.67	6.40	5.93		
L S D at 0.05 Variety (V)		0.3	33		0.44					
Bioagents (Bio) (Bio x V)		0.62 1.24				0.62 1.24				

Table 6. Effect of different bioagents on average number of fruits/plant of squash rootstock plants against damping off in 2011 and 2012 growing seasons under field conditions

recorded (7%) when grafting Alexandria with Gourmet. The great reduction of the pathogen population densities in the rhizosphere soil could be a result of lower proliferation rate of the pathogen in the rhizosphere already colonized by the antagonist (Muhammad and Amusa, 2003). B. subtilis also grow very fast and occupies the court of infection and consume all available nutrients. These actions prevent pathogen spores to reach susceptible tissues. Also its effect might be due to competition for spaces or nutrients (Wolk and Sorkar, 1994). Data in the second season indicated that, mixture of T. harzianum and T. viride led to increase in average number of fruits when grafting Alexandria with Gourmet and Alexandria with Pumpkin (6.67% and 7%, respectively). Trichoderma spp., might also induce resistance of plants against the tested diseases. In several cases, biological agents stimulate plant growth and development which increase crop yields (Hussein et al., 2007). T. *viride* led to the good result when grafting Tabark with Gourmet while, mixture of T. harzianum and T. viride recorded the same percentage when grafting Tabark with Pumpkin (8.33%). On the other hand, mixture of T. harzianum and T.

viride, recorded the same percentage when grafting Alexandria with Gourmet (6.67%). Generally, application of *T. harzianumm* and *T. viride* as soil treatments infested with *F. solani* and *R. solani* increased the total microbial count during the first 30 days, while after that opposite trend data was observed. Increase in fungal population is attributed to root exudates and sloughs which supplied the fungi with nutrients to grow and proliferate. Similar results were also obtained on onion in Egypt by Abd El-Moneim *et al.* (2005).

Data obtained in Table 7 show that in the first season mixture of *T. harzianum* and *T. viride* gave the highest effect on weight of fruits when grafting Tabark with Gourmet, Tabark with Pumpkin and Alexandria with Gourmet (1429.7, 1576.47 and 761.93 g, respectively) while, *T. viride* gave the best result when grafting Alexandria with Pumpkin (930.53 g). This effect could be attributed to some growth regulators produced by these antagonistic microorganisms as mentioned by Abd-El-Moity (1981); Tronsmo *et al.* (1993); Hwang *et al.* (1996) and Sankar and Jeyarajan (1996).

	Average weight of fruits/plant (g)									
		First sea	son 2011	Second season 2012						
Bioagents	Tabark+ Gourmet rootstocks	Tabark +Pumpkin rootstocks	Alexandria +Gourmet rootstocks	Alexandria+ Pumpkin rootstocks	Tabark +Gourmet rootstocks	Tabark + Pumpkin rootstocks	Alexandria+ Gourmet rootstocks	Alexandria+ Pumpkin rootstocks		
Bacillus subtilus	1166.83	1506.37	706.07	778.03	1141.47	1387.47	954.73	824.33		
Trichoderma harazianum (T1)	1127.27	1507.23	731.10	792.33	1140.93	1509.60	910.87	842.33		
Trichoderma viride(T2)	1270.83	1468.00	697.50	930.53	1106.90	1589.87	917.63	790.17		
Mixture of (T1+T2)	1429.70	1 576.4 7	761.93	892.73	1156.03	1498.57	898.57	889.83		
Control	943.07	957.67	671.67	712.60	1076.87	1211.80	860.70	749 .17		
Mean	1187.54	1403.15	713.65	821.24	1124.44	1439.46	908.5	819.17		
L S D at 0.05 Variety (V)	60.01				72.76					
Bioagents (Bio)			.80			75.3	-			
(Bio x V)		111	60			150.	61			

Table 7. Effect of different bioagents on average weight of fruits/plant (g) of squash rootstocks
plants against damping off compared with control in 2011 and 2012 growing seasons
under field conditions

On the other hand, in the second season mixture of T. harzianum and T. viride were the most effective treatment increasing the average weight fruits when grafting Tabark with Gourmet and Alexandria with Pumpkin (1156.03 and 889.83 g) while, T. viride caused the most effect when grafting Tabark with Pumpkin (1589.87 g). This might be due to that all used antagonists have different effect against pathogenic fungi. These effects might be due to these actions prevent pathogen spores to reach to the susceptible tissues. Also its effect might be due to competition for spaces or nutrients (Wolk and Sorkar, 1994 and Hussein et al., 2007). While, Bacillus subtilis gave more of average weight when grafting Alexandria with Gourmet. Bacillus subtilis also grow very fast and occupies the court of infection and consume all available nutrients. These actions prevent pathogen spores to reach susceptible tissues. Also its effect might be due to competition for spaces or nutrients Bacillus subtilis produces more antibiotics (Bacteriocin and subtilisin) which act as inhibitors to pathogenic fungi (Ferreira et al., 1991; Asaka and Shoda, 1996 and Farahat, 1998).

Data obtained in Table 8 show that in the first season mixture of *T. harzianum* and *T. viride* gave

the best effect to increase yield on grafted plants (Tabark with Gourmet, Alexandria with Gourmet and Alexandria with Pumpkin) (10.93, 4.90 and 6.27 kg/plant, respectively). Bacillus subtilus also increasing the yield when grafting Tabark with Pumpkin (10.07 kg/plant) .On the other hand, in the second season data indicated that, mixture of T_{i} harzianum and T, viride led to the best treatment to increase yield when grafting plants (Tabark with Gourmet, Tabark with Pumpkin and Alexandria with Pumpkin) (9.23, 12.47, 5.93 kg/plant, respectively). T. viride recorded the most effective treatment when grafting Alexandria with Gourmet (6.14 kg/plant). This effect could be attributed to some growth regulators produced by these antagonistic microorganisms as mentioned by Sankar and Jeyarajan (1996). When grafted plants were used, a higher yield is obtained with this half density population. In 2011 and 2012 growing seasons, the total production of grafted plants was significantly higher than non-grafted plants. The quality, expressed as percentage of exported production was also higher. In Morocco; experiments have been conducted in the main cucurbits producing areas to compare yields of fruits/plant, average weight of fruits /plant and yield of grafted plants were much higher than the yield of the non-grafted plants (Cohen et al., 2005).

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		Average yield (Kg/Plant)									
]	Second season 2012									
Bioagents	Tabark + Gourmet rootstocks	Tabark + Pumpkin rootstocks	Alexandria + Gourmet rootstocks	Alexandria + Pumpkin rootstocks	Tabark + Gourmet rootstocks	Tabark + Pumpkin rootstocks	Alexandria + Gourmet rootstocks	Alexandria + Pumpkin rootstocks			
Bacillus subtilus	7.80	10.07	4.00	3.90	9,13	10.38	6.07	5.17			
Trichoderma harazianum (T1)	8.43	9.07	4.40	4.53	9.14	12.10	6.07	5.07			
Trichoderma viride(T2)	10.27	8.80	4.17	5.30	9.20	10.90	6.14	4.10			
Mixture of (T1+T2)	10.93	9.87	4.90	6.27	9.23	12.47	5.94	5.93			
Control	5.70	5.83	3.53	3.87	7.50	8.10	4.90	3.71			
Mean	8.63	8.73	4.2	4.77	8.84	10.74	5.82	4.79			
L S D at 0.05 Variety (V) Bioagents (Bio) (Bio x V)	0.61 0.94 1.87				0.60 0.92 1.84						

Table 8. Effect of different bioagents on average yield (Kg/Plant) of squash rootstock plants against damping off in 2011 and 2012 growing seasons under field conditions

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تقييم التطعيم والمقاومة الحيوية لمكافحة موت البادرات على نباتات الكوسة تحت ظروف الصوبة والحقل

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يعتبر فطري الريزوكتونيا سولاني والفيوزاريم سولاني من أكثر المسببات الممرضة شيوعا والتي تسبب الموت الفجائي في العائلة القرعية في مصر. وجد أن أكثر الطعوم المقاومة لفطرى الريزوكتونيا والفيوزاريم سولاني هو طعم تبارك مع اليقطين بينما أكثر هم قابلية للإصابة هو طعم اسكندرية مع الجورمة بينما الطعمين الآخرين تبارك مع الجورمة واسكندرية مع اليقطين كانا متوسطين القابلية للإصابة.وجد أن باسيلس ستلس وترايكودرما هاريزيانم وترايكودرما فيردي وخليط منهما من الكائنات ذات التأثير التصادي في الصوبة والحقل على الفيوزاريم ولاني عندما يتم مقارنتهما بالكنترول أما في تجارب الصوبة وجد أن خليط من الترايكودرما هاريزيانم وترايكودرما فيردي معنوية في خفض شدة مرض موت قبل الظهور وبعد الفلهور فوق سطح التربة تحت ظروف العدوى الصناعية بالفيوزاريم والريزوكتونيا سولاني أيضا. وجد أن المعاملة بالكائنات الحيوية للتربة تحت بالفيوزاريم والريزوكتونيا معنولي ألماني أطعور وبعد الظهور فوق سطح التربة تحت فروضابة المناعية في الحقل في كلا الموسمين عند المقارنة بالكنترول. وأعطى خليط من الترايكودرما هاريزيانم وفيردي معاملة للتربة أحدث في الفيوزاريم وليزي ألماني ألفين موت قبل الظهور وبعد الظهور فوق سطح التربة تحت فروف العدوى الصناعية في الحقل في كلا الموسمين عند المقارنة بالكنترول. وأعطى خليط من الترايكودرما هاريزيانم وفيردي حماية من مرض الموت الفجائي وتحسن في نمو المحصول والقياسات الحقاية في كل الطعوم المستخدمة.

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