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Biocontrol of cantaloupe damping-off disease caused by *Fusarium semitectum* by using formulations of antagonistic fungi

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Abstract

Antagonistic capability of 19 isolates of fungi isolated from rizosphere of cantaloupe plants was tested in *vitro* against growth of *Fusarium semitectum* isolate the causal pathogen of damping- off of cantaloupe. *Trichoderma viride* (isolate no. 17), *T. harzianum* (isolate no. 19) and *Fusarium concolar* (isolate no.4) showed significant percentage of inhibition against *F. semitectum*. The effect of carrier formulations of antagonistic fungi (talc based powder and rice bran) on damping-off of cantaloupe were tested under greenhouse and field conditions. In greenhouse experiments, application of antagonistic fungi with rice bran formulation two weeks before planting caused the highest percentage of survival plants in pre and post damping-off (83.33% and 75%, respectively), whereas application of talc based powder formulation significantly increased percentage of plant survival at the time of planting in pre and post damping-off (91.67 and 75%, respectively). In field experiments, application of tested formulations of antagonistic fungi to infested soil with *F. semitectum* two weeks before planting resulted in higher percentage of plant survival in pre and post damping-off in both tested seasons (2009 and 2010) than application at time of planting. The numbers of antagonistic fungal propagules decreased gradually by prolonging storage. After five months of storage period, population of fungal propagules showed high reduction in approximately 50%.

Key words: Damping-off, cantaloupe, *Trichoderma* spp, *Fusarium semitectum*, *F. concolar* formulation

Introduction

Cantaloupe (*Cucmis melo* var. *cantaloupensis*) is one of the most important vegetable crops in Egypt. The fruits are rich in vitamins such as vitamin C and A. In Egypt, the cultivated cantaloupe area is about 75408.8 feddan production is about 7677541 tons (Riad Shaimaa et al., 2011). Damping-off disease caused by *Fusarium semitectum* causes severe economic losses in cantaloupe plants (Shekari et al., 2006). *Fusarium semitectum* attacks the entire plant and affects vascular system causing pre and post emergence mortality (Gupta et al., 2011). Various methods for controlling this disease have been investigated, including resistant varieties (Brisa et al., 2007), chemical control and cultural practices (Punja et al., 1986).The use of fungicides is the most common mean to control fungal disease in field and greenhouse (Washington & McGee, 2000; Fravel et al., 2005). Although this method has been very effective in controlling plant diseases; nevertheless some major problems arose from the extensive use of fungicides, such as

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some fungi have developed resistance to chemicals; fungicides are not readily biodegradable and tend to persist for years in the environment and affect human health (Brady, 1984). Therefore, many trials on using biocontrol compromise this problem were carried out (Sallam Nashwa et al., 2009). Many researchers have demonstrated the potential of *Trichoderma* spp. in controlling damping-off disease caused by *Fusarium* spp. (Dubey et al., 2007). Saprophytic species of *Fusarium* have been found to be effective in suppression of *F. oxysporum*, the causal agent of wilt disease of cucumber (He et al., 2002; Reid et al., 2002). The development of formulation and delivery systems for biocontrol by using antagonistic microorganisms to suppress soil borne pathogens is of great importance (Çiğdem & Merih 2005). Several approaches optimized the formulation of biocontrol agents, such as the application of appropriate carrier materials (Vidhyasekaran et al., 1997; Krishramuthy and Grarananickam, 1998; Ali et al., 2001; Sallam Nashwa et al., 2013) and formulation additives (Schmidt et al., 2001).
The objectives of the present study were 1) to demonstrate the capability of certain antagonistic fungi against *F. semetictum in vitro*, 2) to investigate the effect of different carrier formulations on damping-off of cantaloupe in greenhouse and filed conditions, and 3) to correlate the effects of storage period on biological activity of formulated antagonistic fungi.

Materials and methods

Preliminary test for antagonistic capability of certain isolated microorganisms against *Fusarium semetictum in vitro*: Nineteen fungal isolates were obtained from rhizosphere of cantaloupe plants according to the method described by Dhingra and Sinclair (1995). They were tested against *F. semitectum*, this pathogen was isolated by the authors from diseased cantaloupe plants. The pathogenicity of this isolate was previously tested and it showed high virulence as recorded by authors (Riad Shaimaa et al., 2011). Sterilized Petri dishes (9 cm. in diameter), each containing 10 mL of PDA (200 g potato, 20 g dextrose, 20 g agar and one-liter water) were used in this study. Disks (5 mm) from each isolate of antagonistic fungi and *F.* semitectum were taken from 5 days old culture and transferred onto opposite sides of PDA plates (9 cm distance) and incubated at 25°C. Each treatment contained three replicates. Inoculated plates with the pathogen only were used as control. Linear growth of the tested pathogen was recorded when the control treatments covered the plate surface. The percentage of inhibition of mycelial growth was calculated according to following formula (Sallam Nashwa et al., 2013).

Percentage of mycelial growth inhibition = [A-B/A] x 100. Where, A= length of the mycelial growth in control. B = length of mycelial growth in the treatment.

The antagonistic fungi which caused high percentage of mycelia growth inhibition were purified by single-spore isolation and identified according to their morphological characteristics of mycelia and conidiophores as descried by Domsch et al. (1980).

Preparation of formulated antagonistic fungi

Talc-based formulation: The highly antagonistic isolates of *Trichoderma*

harzianum Rifai no.19, *T. viride* Rifai no.17 and *F. concolar* no.4 were selected for this study. For mass production, *Trichoderma harzianum* and *Trichoderma viride* were cultured on Oat Meal Medium (30 g oat in one liter water to boiling and simmer gently for 2 h., filtered through cloth and fill up to one liter and sterilized by autoclaving at 121°C for 15 min.). Flasks (1000 ml) containing 500 ml medium were used. *Fusarium concolar* was cultured in Potato Dextrose Broth medium (PDB) (200 g potato; 20 g dextrose and one-liter water) in flask (1000 ml) containing 500 ml medium. The culture of antagonistic fungi was shaken at 150 rpm at 28°C for seven days. The suspension was blended in a coffee blender at a low speed for 20 seconds. The population of propagules was adjusted to be $2x10^8$ cfu/mL, and the suspension was used for preparing formulation. Five milliliters of fungal spore suspension was mixed with100 g sterilized fine grade-Talc (magnesium trisi licate) and sterilized 1g carboxy methyl cellulose (CMC).The talc–based powder formulation was dried at 28°C for 24 h in sterile plastic trays. The dried formulation was homogenized in a coffee blender for 30 sec at low speed and packed in polypropylene bags (50 g/bag) as described by Jayaraj et al. (2006).

Rice bran formulation: For mass production, 1000-mL conical flasks each containing 250 ml vermiculite, 250 ml rice bran and 250 ml Cz medium, were autoclaved for 20 min at 121°C, in two consecutive days. The flasks were inoculated with antagonistic fungi and incubated at 25°C. After 15 days of incubation, contents of flasks were transferred to sterilized plates (60x40 cm) under sterile conditions, left to dry at 28°C for 24 hour then mixed in a blender to become powder and kept in polyethylene bags.

Effect of application of formulated antagonistic fungi on incidence of cantaloupe damping-off under greenhouse and field conditions

Greenhouse experiments: This experiment was carried out in 2009 in the greenhouse of Plant Pathology Dept., Faculty of Agriculture, Assiut University. Inocula of *F. semetictum* was grown on barley medium (150g barley + 50 g clean sand + 4 g glucose + 0.2 g yeast extract + 200 mL water) in 500-mL flasks and incubated at 25± 2°C for 15 days. Sterilized pots (25 cm in diameter) were filled with sterilized soil and infested by isolates of *F. semitectum* at the rate of 3% (w/w) two weeks before planting. The formulated antagonistic fungi were added to infested soil at the rate of 1.5 % (w/w) in each pot two weeks before or at the time of the planting. Each pot was sown with 4 sterilized seeds of cantaloupe cultivar "Paquito". Three pots were used for each treatment as replicates and untreated pots with antagonists were used as control. Percentages of survived plants were recorded after 3 and 6 weeks of sowing as pre- and post-emergance dampping-off, respectively.

Field experiments: These experiments were carried out in 2009 and 2010 growing seasons. Cantaloupe cv "Paquito seeds were sown in plots (3 x 3.5 m) contained 2 rows (1.5 m). Each row contained 2 hills 50-cm spaced. Each hill was sown with 4 sterilized seeds and three replicates were used for each treatment. Thirty-five grams of *F. semitectum* inoculum was placed in each hill two weeks before planting and each formulation of antagonistic fungi was added to infested soil (approximately 17g / hill) 15

days before planting or at the time of planting. Plots containing inoculum of *F. semitectum* without antagonistic formula were used as control. Percentages of survival plants were recorded after 3 and 6 weeks from sowing (as pre- and post-emergence damping off, respectively).

Effect of storage period on biological activity of formulated antagonistic fungi: The effect of storage period on the biological activity of formulated antagonistic fungi at 4°C was tested in the laboratory from 1 to 9 months. One gram of each formulation was suspended in 200 ml of sterile distilled water every month. One hundred micro liters of the respective dilution of formulations was plated onto PDA medium for *Trichoderma* spp. and *Fusarium concolar* with 3 replicates per-treatment. Fungal propagules were counted after 3 days incubation period at 25°C.

Statistical analysis: All experiments were performed twice. Analyses of variance were carried out using the MSTAT-C, 1991 program version 2.10. Duncan's multiple range test was employed to test for significant differences between treatments at p = 0.05 (Gomez & Gomez 1984).

Results

Preliminary test for antagonistic capability of certain d fungal isolates against growth of *F. semitectum in vitro*: Antagonistic capability of 19 isolates of fungi isolated from healthy cantaloupe plants were tested *in vitro* against *F. semitectum* on PDA medium. The percentage of linear growth of the pathogen was recorded when its growth covered the plate surface in control treatment.

Data (Table 1) indicated that, the tested antagonistic fungi showed different percentage of inhibition against growth of the tested pathogenic isolate. Isolates no. 19 and 17 showed the highest percentage of growth inhibition against *F. semitectum* followed by isolates nos. 18 and 6. Isolates nos. 17, 19 and 4 were selected for further studies. They were identified according to their morphological characteristics of mycelia and conidiophores as reported by Domsch et al. (1980) as *Trichoderma viride*, *T. harzianum* and *F. concolar*, respectively.

Table 1: Preliminary test for antagonistic fungi against *F. semitectum* in *vitro*

Fungal isolates	% mycelia growth inhibition *F. semitectum*
1	39.33 ^{d}
2	47.0 ^{c}
3	50.0 ^{c}
4	49.33 ^{c}
5	48.33 ^{c}
6	59.66 ^{b}
7	46.33 ^{c}
8	39.33 ^{d}
9	54.33 ^{b}
10	48.33 ^{c}
11	46.33 ^{c}
12	34.66 ^{d}
13	31.0 d ^{e}
14	31.0 d ^{e}
15	26.0 ^{e}
16	29.66 ^{e}
17	72.0 ^{a}
18	58.0 ^{b}
19	74.6 ^{a}

Number in column followed by the same letter do not significantly differ according to Duncan's multiple range test ($P< 0.05$)

Application of formulated antagonistic fungi to infested soil with *Fusarium semitectum*

Greenhouse experiments: Results in Table 2 indicated that, application of formulated antagonistic fungi *Trichoderma viride* (no.17), *T. harzianum* (no.19) and *F. concolar* (no.4) into infested soil with the pathogen resulted in significant increase in

survival plants compared with the control in pre- and post-emergence damping off. Application of rice bran formulation 15 days before planting showed greater effect on percentage of survival plants than application at the time of planting in both pre significantly superior in percentage of survival plants than addition 15 days before planting. Data also showed that talc formulation was superior in increasing percentage of survival plants than rice bran formulation in case of pre-emergence damping-off (83.33 and 75.00%), respectively; while, the reverse was true in case of post-emergence damping-off (69.44 and 62.50%, respectively). In pre-emergence damping-off, formulated isolate of *T. harizanum* caused the highest increase in survival plant percentage (91.66%) followed by *T. viride* (85.42%) then *F. concolar* (66.66%). While, in post-emergence damping-off, formulated isolate of *T. viride* (79.17%) showed significantly higher number of survival plants than *T. harizanum* (66.66%) followed by *F. concolar* (52.08%).

Field experiments: The efficiency of formulated antagonistic fungi (*Trichoderma viride*, *T. harzianum* and *F. concolar*) with different carrier formulations (rice bran and talc based powder) on incidence of pre and post damping-off caused by *F. semitectum* was tested under field conditions in 2009 and 2010 growing seasons. Results presented in Tables 3 & 4 showed that, application of formulations of antagonistic fungi into infested soil with *F. semitectum* caused significant increase survival number of plants percentage in pre- and post-damping off compared to the control. In general, application of rice bran formulation at the time of planting showed higher percentage of survival plants in pre and post emergence damping-off (65.2 and 54.79% respectively in 2009 season and 61.33 and 56.63%, respectively in 2010 season) than its application two weeks before planting (50.69 and 40.28%, respectively in 2009 and 50.00 and 48.30% respectively in 2010). Also, application of talc powder formulation at the time of planting showed greater effect of survival of plant percentage than its applied two weeks before planting in pre- and post- damping off. The two carrier formulations exhibited the same effect in survival of plant percentage in pre emergence damping off. But, rice bran formulation showed greater survival of plant percentage than talc powder formulation in post emergence damping off. In pre emergence damping-off, formulation isolate of *T. harzianum* showed the best effect of survival plant percentage followed by *F. concolar* and *T. viride*. Each formulated of antagonistic fungi was the same effect of number of survival plants percentage in post emergence damping off in 2009 season.

Effect of storage period on biological activity of formulated antagonistic fungi: Results in Table 5 showed the effect of storage period on the biological activity of the antagonistic fungi. The numbers of antagonistic fungal propagules were decreased gradually by prolonging storage period. After five months of storage, population of fungal propagules showed high reduction in approximately 50%. In the case of *formulated* isolate of *T. viride* with talc formulation showed high reduction of propagules after five months compared with other formulated isolates.

Table 2: Effect of application of formulated antagonistic fungi on incidence of cantaloupe damping off caused by *F. semitectum* under greenhouse conditions.

Antagonistic fungi	Survival plants %									
	Pre emergence damping off					Post emergence damping off				
	Rice		Talc		Mean	Rice		Talc		mean
	Before*	With**	Before	With		Before	With	Before	With	
T.viride	91.67a[y]	75b	75b	100a	85.42b	91.67a	75b	75b	75b	79.166a
T.harzianum	91.67a	75b	100a	100a	91.66a	66.67b	75b	25d	100a	66.66b
F. concolar	66.67d	50c	75b	75b	66.66c	66.67b	41.67b	50c	50c	52.083c
Control	0 f	0 f	0 f	0 f	0 f	0f	0 f	0 f	0 f	
Mean	83.33b	66.67c	83.33b	91.67a		75a	63.89b	50c	75a	
Mean	75b		83.33a			69.44a		62.50 b		

*Formulated antagonistic fungi added to soil two weeks before planting.

** Formulated antagonistic fungi added to soil at the same time of planting.

Number in each column followed by the same letter did not significantly differ according to Duncan's multiple range test ($P< 0.05$). Each mean in a column followed by the different letter was significantly differ according to Duncan's multiple range test ($P< 0.05$).

Table 3: Effect of application of formulated antagonistic fungi on damping off of cantaloupe caused by *F. semitectum* under field conditions in season 2009

Antagonistic fungi	Survival plants %									
	Pre emergence damping off					Post emergence damping off				
	Rice		Talc		mean	Rice		Talc		mean
	Before*	With**	Before	With		Before	With	Before	With	
T.viride	52.08b	50b	50b	52.08b	51.04c	43.75b	43.75b	45.83b	41.67b	43.75a
T.harzianum	54.17b	75a	47.92c	72a	63.02a	45.83b	58.33a	25d	58.33a	46.875a
F.concolar	45.83c	70.83a	45.83c	64.58a	56.77b	31.25c	62.3a	37.5c	37.5c	42.137a
Control	0 f	0 f	0 f	0 f		0 f	0 f	0 f	0 f	
Mean	50.69b	65.28a	47.92b	63.89		40.28b	54.79a	36.11c	45.83b	
Mean	57.99a		55.902a			47.536a		40.972b		

*Formulated antagonistic fungi added to soil two weeks before planting.

** Formulated antagonistic fungi added to soil at the same time of planting

Number in each column followed by the same letter did not significantly differ according to Duncan's multiple range test ($P< 0.05$). Each mean in a column followed by the different letter was significantly differ according to Duncan's multiple range test ($P< 0.05$).

Table 4: Effect of application of formulated antagonistic fungi on damping off of cantaloupe caused by *Fusarium semitectum* under field conditions in season 2010

Antagonistic fungi	Survival plants %									
	Pre emergence damping off					Post emergence damping off				
	Rice		Talc		mean	Rice		Talc		Mean
	Before*	With**	Before	With		Before	With	Before	With	
T.virde	53.00	49.00	47.00	53.00	50.50a	51.00b	48.7cb	45.93b	51.00c	49.55a
T.harizanum	56.00c	75.00a	45.00	75.00	62.75a	51.70b	63.2a	35.00d	60.43a	52.50a
F.concolar	47.00d	60.00	53.00	62.00	55.50a	42.00d	58.00a	44.00d	48.60b	48.40a
Control	0 f	0 f	0 f	0 f	0 f	0 f	0 f	0 f	0 f	
Mean	50.00b	61.33a	48,3b	63.30a		48.30b	56.63a	41.63b	53..34 a	
Mean	63.20a		59.74a			52,47a		47.49b		

*Formulated antagonistic fungi added to soil two weeks before planting.

** Formulated antagonistic fungi added to soil at the same time of planting.

Number in each column followed by the same letter did not significantly differ according to Duncan's multiple range test ($P< 0.05$). Each mean in a column followed by the different letter was significantly differ according to Duncan's multiple range test ($P< 0.05$).

Table 5: Effect of storage period on viability of fungal formulation

	CFU /ML after storage period (months)						
Formulation	Zero	One	Two	Three	Four	Fife	Si x
F. concolar +rice	2 x 108	1.9 x 107	1.8 x 107	1.5 x 107	1 x 107	3.3 x 106	1.9 x 106
F. concolar +talc	2 x 108	4.8 x 106	4 x 106	3.5 x 106	1.8 x 106	1.4 x 106	1 x 106
T. harzianum+ rice	2 x 108	1 x 107	7.5 x 106	5.65 x 106	4.7 x 106	4.5 x 106	2.5 x 106
T .harzianum +talc	2 x 108	9.3 x 106	8.5 x 106	5.8 x 106	5.4 x 106	4 x 106	3.5 x 106
T. viride +rice	2 x 108	2. x 106	2 x 106	1.7 x 106	1.5 x 106	1 x 106	6.5 x 105
T. viride+ talc	2 x 108	5 x 106	4 x 106	3.5 x 106	2.5 x 106	7 x 105	2 x 105

Discussion

A capability of 19 isolates of fungi isolated from soil rhizosphere of cantaloupe plants were tested against growth of *F. semitectum*. Results of this study revealed that all tested fungal isolates inhibited growth of *F. semitectum*. Isolates no. 17 of *T. viride*, no. 19, *T. harzianum* and no. 4 of *Fusarium concolar* gave the greatest percentage of growth inhibition. Such results are in agreement with those reported by Rose et al., (2003) and Nahed, (2007**)**. Antagonistic effect may be due to direct influence of antagonistic fungi against the pathogens through cloning their hyphae around the hyphae of the pathogens to prevent their continued growth **(**Chu & Wn, 1981; Adekunle et al., 2006) and/or produce antagonistic substance which can play an important role in lyses of cell wall components of the pathogenic fungi to help the antagonists to penetrate the host hyphae and grown on it as hyper parasite (Papavizas et al., 1984). To improve biological control of the disease, antagonistic fungal isolates of *T. viride*, *T. harzianum* and *F. concolar* with different carriers (talc based powder and rice bran) were tested on incidence of cantaloupe damping-off caused by *F. semitectum* in greenhouse and field on conditions. Under greenhouse conditions, application of antagonistic fungi two weeks before planting with rice bran formulation into infested soil with *F. semitectum* showed higher percentage of survival plant of pre and post emergence damping-off than applied at the time of planting. However, talc based powder formulation increased percentage of survival plants when applied at the time of planting compared with two weeks before. In pre emergence damping-off formulation of isolate *T. harzianum* gave the highest number of survival plants precentage followed by *T. viride* then *F. concolar*. Several researchers have reported that *T. viride* and *T. harzianum* were superior as antagonistic fungi against several soil and seed borne plant pathogens (Poddar et al., 2004; Lee et al., 2008; 2011). The potentiality of *Trichoderma* spp. as biocontrol agents of phytopathogenic fungi in several crops is well known especially to *Fusarium* spp. The two primary mechanism of action associated with nonpathogenic *Fusarium* spp. are induced systemic resistance and competition for nutrients in the soil and parasitic competition for infection sites on the roots (Kaur et al., 2010).

Under field conditions in the two growing seasons, applied formulations of antagonistic fungi into infested soil with *F. semitectum* at the time of planting showed the higher percentage of survival plants in

the case of pre and post emergence damping-off than applied two weeks before planting. Such results agree with those reported by Lewis and Lumsden (2001). This may be due to that application of biocontrol formulations at the time of planting has avoided the spread of the pathogen in soil. Coley-Smith et al. (1991) reported that formulations of biocontrol against soil-borne fungi were more effective when added at the time of planting compared to those applied two weeks before planting. Lewis et al., (1998) reported that, the ability of a biocontrol agent in formulation to inhibit the spread of the pathogen is perhaps more important than the effectiveness of the formulation to eliminate pathogen propagules evenly distributed in the soil. Also, application of *Trichoderma* spp. as powder formulation into soil provides nutrient sources for other soil microorganisms such as growth promoting rhizobacteria (Sallam Nashwa et al., 2008).

The effect of storage time at 4°C on the viability of fungi with different carrier formulations showed gradual decrease in its viability along with storage period. After five months of storage period at 4°C, antagonistic formulated fungi showed high approximately 50% reduction in propagules population, in case of *T. viride* with talc formulation which showed high reduction of propagules population compared to other tested isolates. Only propaguls of *F. concolar* appeared on rice and talc formulations up to eight months. Similar results were obtained by Jayaraj et al., (2006), who declared that, after six months of storage period of carrier formulations of *Trichoderma* spp., the population of propagules reduced to approximately 50% at 24°C. The number of propagules population of formulated *Trichoderma* spp. was decreased gradually by prolonging storage time up to four months (Sallam Nashwa et al., 2008).

References

Adekunle AT, Ikotun T, Fiorini DA, Cardwell KF, 2006. Field evaluation of selected formulations of Trichoderma species as seed treatment to control damping- off of cowpea caused by Macrophomina phaseolina. African Journal of Biotechnology **5**: 419-424.

Ali NI, Siddiqui LA, Shaukat SS, Zaki MJ, 2001. Survival of *Pseudomonas aerugiginosa* in various carious for the inhibition of root rot knot disease complex of mung bean. Phytopathologia Mediterranea **40**: 108-112.

Brady NC, 1984. *The Nature and Properties of Soils,* P, 528. MacMillan Publishing Company, New York.

Brisa R, Fernando MA, Asunción GS, Noemí MR, Arturo PE, José MD, 2007. The gene coding for a new transcription factor *ftf1* of *Fusarium oxysporum* is only expressed during infection of common bean. Fungal Genetics and Biology **44**: 864-876.

Chu FF, Wn WS, 1981. Antagonistic action of *Trichoderma* spp. and *Penicillium* spp. On *Rhizoctonia solani*. *Memoirs of the College of Agriculture, National Taiwan University,* **212**: 4- 18.

Çiğdem K, Merih K, 2005. Effect of formulation on the viabılıty of biocontrol agent, *Trıchoderma harzıanum* conidia. African Journal of Biotechnology **4**: 483-486.

Coley-Smith JR, Ridout CJ, Mitchell CM, Lynch JM, 1991.Control of bottom rot disease of lettuce *Rhizoctonia solani* using preparations of *Trichoderma viride, T. harzianum* or tolclofos-methyl. Plant Pathology **40**: 359-366.

Dhingra OD, Sinclair JB, 1995. Basic plant pathology method second edition, Lewis Publishers, CRC Press, USA, 400-450.

Domsch KH, Gams W, Anderson TH, 1980. Compondiuum of soil fungi. Academic press. A Subbsidiary of Harcout Barce Jovanovich, Bublishers , London, **1**: 859 pp.

Dubey SC, Suresh M, Birendra S, 2007. Singh Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management ofchickpea wilts. Biological Control **40***:* 118–127.

Fravel DR, Deahl KL, Stommel JR, 2005. Compatibility of the biocontrol fungus *Fusarium oxysporum* strain CS-20 with selected fungicides. Biological Control **34**: 165–169.

Gomez KA, Gomez, AA, 1984. Statistical procedures for Agricultural Research. A. Lviley. Interscience Publication. New York, Pp. 678.

Gupta S, Dubey A, Singh T, 2011. *Fusarium semitectum* as a seed-borne pathogen in Dalbergia sissoo Roxb., its location in seed and its phyttopathological effects. Indian Journal of Fundamental and Applied Life Sciences, 2231-6345 online.

He CY, Hsing T, Wolin DJ, 2002. Induction of systemic disease resistence and pathogen defense response in *Asparagus officinalis*inoculated with nonpathogenic strains of *Fusarium oxysporum*. Plant Pathology **51**: 225-230

Jayaraj J, Radhakrishnan NV, Velazhahan R, 2006. Development of formulations of *Trichoderma harzianum* strain M1 for control of damping- off of tomato caused by *Pythium aphanidermatum*. Phytopathology and Plant Protection **39**: 1-8.

Kaur J, Rama S, Singh T, 2010. Nonpathogenic Fusarium as a biological control agent. Plant Pathology Journal **9**: 79- 91.

Krishramuthy K, Grarananickam SS, 1998. Biological control by *Pseudomonas fluorescens* strain pf7-14, evaluation of a marker gene and formulations. Biological Control **13**: 158-165.

Lee TO, Khan Z, Kim SG, Kim YH, 2008. Amendment with peony (*Paeonia suffructicosa*) root bark powder improves the biocontrol efficacy of *Trichoderma harzianum* against *Rhizoctonia solani*. Journal of Microbiology and Biotechnology **18**: 1537-1543.

Lee HM, Khan Z, Kim SG, Baek NI, Kim YH, 2011. Evaluation of biocontrol potential of some medicinal plant materials alone and in combination with *Trichoderma harzianum* against *Rhizoctonia solani* AG 2-1. Plant Pathology Journal **27**: 68-77.

Lewis JA, Lumsden RD, 2001. Biocontrol of damping-o! of greenhouse grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. Crop Protection **20**: 49-56.

Lewis JA, Larkin RP, Rogers DL, 1998. A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in

soil less mix. Plant Disease **82**: 501-506.

Nahed ZH, 2007. Improving biological control of Fusarium root–rot in cucumber *Cucumis sativus* L. by allopathic plant extracts. International Journal of Agriculture and Biology **9**: 459-461.

Papavizas GC, Dunn MT, Lewis JA, Beagle- Ristaino J, 1984. Liquid fermentation technology for experimental production of biocontrol fungi. Phytopathology **74**: 1171-1175.

Poddar RK, Singh DV, Dubey SC, 2004. Integrated application of *Trichoderma harzianum* mutants and carbendazim to Manage chichpea wilt *Fusarium oxysporum f.sp. ciceri*. Indian Journal of Agricultural Sciences **74**: 346-348.

Punja ZK, Carter JD, Campbell GM, Rossell EL, 1986. Effects of calciumand nitrogen fertilizers, fungicides, and tillage practices on incidence of *Sclerotium rolfsii* on processing carrots *Daucus carota*. Plant Disease **70**: 819–824

Reid TC, Hausbeck MK, Kizilkaya K, 2002. Use of fungicides and biological controls in suppression of Fusarium crown and root rot of asparagus under greenhouse and growth chamber conditions Plant Disease **86**: 493-498.

Riad Shaimaa N, Sallam Nashwa M, Mohamed MS, Seef Eleslam A, 2011. Fungi Associated With Cantaloupe Root Rot Disease and Reaction of Some Cultivars to the Disease. Assiut J. of Agric. Sci., **42**:453-460.

Rose S, Parker M, Punja ZK, 2003. Efficacy of biological and chemical treatments for control of Fusarium root rot and stem rot on greenhouse cucumber. Plant Disease **87**: 1462-1470.

Sallam NMA, Abo-Elyousr KAM, Hassan MAE, 2008. Evaluation of *Trichoderma* species as biocontrol agent for damping- off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. Egyptian Journal Phytopathology **36**: 81-93.

Sallam Nashwa MA, Abd Elrazik AA, Hassan M, Koch E, 2009. Powder formulations of *Bacillus subtilis, Trichoderma* spp and *Coniothyrium minitans* for biocontrol of white rot of onion. Archives of phytopathology and plant protection **42**:142-174

Sallam Nashwa M, Riad Shaimaa N, Mohamed MS, Seef Eleslam A, 2013. Formulations of *Bacillus* spp and *Pseudomonas fluorescens* for biocontrol of cantaloupe root rot caused by *Fusarium solani* Journal of Plant Protection Research **53**: 275-300.

Sallam Nashwa MA, Abd-El-Razik AA, Hassan MHA, Koch E, 2009. Powder formulations of *Bacillus subtilis*, *Trichoderma* spp and *Coniothyriu m minitans* for biocontrol of OnionWhite Rot. Archive of Phytopathology and Plant Protection **42**: 142-147.

Schmidt CS, Lorenz D, Wolf GA, Jager J, 2001. Biological control of grapevine dieback fungus. Eutypa latall, influence of formulation additives and transpajon mutagenesis on the antagonistic activity of *Bacillus subtilis* and *Erwinia herbicola*. Journal of Phytopathology **149**: 437-445.

Shekari A, Mirabolfathi M, Mohammadi-Pour M, Zad J, Okhovvat M, 2006. Phytophthora rot and crown rot of several field and vegetable crops in east Azarbaijan province. Iranian Journal of Plant Pathology **42**: 293-308.

Vidhyasekaran P, Sethuraman K, Rajajappan K, Vasumathi K, 1997. Powder formulations of *Pseudomonas fluorescens* to control Pigeopea wilt. Biological Control **8**: 166-171

Washington WS, McGee P, 2000. Dimethomorph soil and seed treatment of potted tomatoes for control of damping-off and root rot caused by *Phytophthora nicotianae* var *micotianae*. *Australian* Plant Pathology **29**: 46–51.

Wiyono S, Schulz DF, Wolf GA, 2008. Improvement of the formulation and antagonistic activity of *Pseudomonas fluorescens* B5 through selective additives in the pelleting process. Biological Control **46**: 348-357.