Egypt. J. Phytopathol., Vol. **44**, No. 2, pp. 17-34 (2016)

Use of Beneficial Microorganisms to Minimize the Recommended Rates of Macronutrients to Control Cucumber Damping off

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Rhizoctonia solani is a major problem causing damping-off of seedlings and root rot in mature plants and thus reducing nutrient uptake and plant stand. The aim of this study is to evaluate the effect of some bioagents in combination with the lowest doses of macronutrients on management of *R. solani* in cucumber plants. Four cucumber hybrids, *i.e.* hybrid 6, hybrid 9, hybrid 14 and Beit-Alpha F1 were tested for their susceptibility to the pathogenic fungi. Data indicated that Beit-Alpha, was highly susceptible to infection by all the pathogenic fungi and *R. solani* isolate No.1 was highly aggressive. The double recommended dose of each of potassium and phosphorous was the most effective treatment. Half dose of phosphorous gave the lowest reduction of damping-off. *T. harzianum, T. viride*, a mixture of them, *Bacillus subtilis* and *Pseudomonas fluorescens*, as well as arbuscular mycorrhizal fungi (AM), were used. The maximum reduction in disease incidence was recorded due to using *P. fluorescens* followed by AM fungi. They significantly reduced damping-off and enhanced the vegetative growth of cucumber plants and the levels of oxidative enzymes compared to control.

Keywords: AM fungi, biological control, cucumber cultivars, damping-off, macronutrients and oxidative enzymes.

Many species belonging to genera *Trichoderma*, *Pseudomonas*, *Bacillus*, as well as the arbuscular mycorrhizal fungi are responsible for sustaining plant health and the fertility of the soil. They affect plant growth either by reducing the deleterious effects of phytopathogenic organisms, increasing nutrient uptake, nutrient cycling, the synthesis and release of growth promoting hormones (increasing plant vigour) or by preventing pathogen attack and thus acting as biocontrol agents (Jeffries *et al.*, 2003). Cucumber is one of the most economic vegetable crops grown for both local consumption and exportation. The crop is susceptible to serious losses from soil borne and foliar diseases. The production volumes are 40-50% lower when diseases are more prevalent (Abd-El-Kareem, 2009). Damping-off is a frequent disease that kills seedlings. *Rhizoctonia solani* Kuhn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk is a widespread and ecologically diverse soil-borne fungus, causing different types of diseases in many plant species. It causes root rot, stem rot, fruit and seed decay, damping-off, foliar blight, stem canker and crown rot in various crops (Guleria *et al.*, 2007). Control of this sclerotial pathogen is difficult

because of its ecology; it has an extremely broad host range and a high survival rate of sclerotia under diverse environmental conditions. Efficient strategies for control are therefore needed. The requirement to reduce pesticide usage in food crops and the concern for a healthy environment mean that alternative methods of disease control are needed. One such method is the use of fertilization which provides almost an infinite number of ways to modify the soil and plant environment or the plant itself and plays a critical role in plant disease control (Kataria and Grover, 1987).

Balanced nutrient keeps plant healthy and some nutrients that act as fertilizers may influence the rhizosphere mycoflora of plants which may have a positive role in disease suppression (Duffy and Defago, 1999). Some bacteria show benefice effects on plants such as some of *P. fluorescens* and *B. subtilis* (Plant Growth Promoting Rhizobacteria PGPR). The positive effects of PGPR are normally divided into two categories: growth promotion and biological control (Kloepper, 1997). The arbuscular mycorrhizal (AM) symbiosis is association formed between plants and fungi. As all beneficial co-operation, both partners (fungi and plant) have advantages of the symbiosis. (Hafez *et al*., 2013) found that pre-colonization of bean plants by AM fungi significantly reduced disease severity and disease incidence caused by *R. solani*. *Trichoderma* spp. also solubilize various plant nutrients such as Fe, Cu, Mn and Zn in the soil and even some natural fertilizers such as rock phosphate and pyrite (Altomare *et al.*, 1999); this ability enhances plant growth and productivity. For improving crop yield and growth and other beneficial effects of rhizosphere activity, the use of *Trichoderma* appears to be a promising alternative to pesticides and chemical fertilizers.

The present work was aimed to investigate the positive effect of AM fungi and bioagents on minimizing the recommended rates of macronutrients and reducing the damping off of disease. Moreover, changes in the oxidative enzymes in the treated plants were also determined.

Materials and Methods

Isolation of the causal pathogens:

Isolation from soil:

Soil samples from the rhizosphere of infected cucumber roots were collected and the fungal isolation method of (Dhingra and Sinclair, 1985), was followed. Dilution plate's technique of the soil samples 1:10, 1:100, 1:1000 and 1:10000 were applied. Ten grams of the collected rhizospheric soil were transferred to a flask containing 100 ml sterilized water and was shaken for 30 minutes. One ml of the suspension was added to 99 ml sterilized water in a plugged flask. One ml of this suspension was added to the surface of Petri dish containing Potato Dextrose Agar (PDA) amended with Rose Bengal (0.003%) and Streptomycin Sulphate (0.01%). Five replicates were used for each dilution. The plates were incubated in the dark at 25^{o}C

with daily observation until developing single colonies. The resulted colonies were counted and recorded as colony forming units (cfu) and imputed to each sample. The isolated fungi were individually transferred to Potato Dextrose Agar (PDA) plates. Purification of fungi was carried out using the single spore or the hyphal tip technique.

Isolation from diseased roots of cucumber plants:

Diseased cucumber plants at various growth stages showing severe damping-off and seedling rot were collected from different locations of Dakahliya governorate. The diseased roots were carefully washed in tap water and cut into small pieces. Infected samples were surface sterilized in 2% sodium hypochlorite solution for three minutes, then thoroughly rinsed in sterilized water and dried between two sterilized filter papers. The treated plant pieces were transferred into a Petri dishes containing PDA amended with (0.01%) Streptomycin sulfate in order to minimize bacterial contamination. Plates were incubated at 25^{o}C for 48-72 hours. Pure cultures were obtained using the single spore or the hyphal tip technique. The isolated fungi were identified, following the descriptions of Booth (1971), Singh (1982), Sneh *et al.* (1992) and Burnett and Hunter (2003), and confirmed in the Department of Mycological Research and Plant Disease Survey, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Cultivars susceptibility:

Seeds of cucumber cultivars were obtained from the Horticultural Research Institute, A.R.C. Four cucumber cultivars namely hybrid 6, hybrid 9, hybrid 14 and Beit-Alpha were screened for their reactions against the dominant fungi isolated from the diseased roots of the different cultivars with high frequency. These fungi, *i.e.*, two isolates of *F. oxysporum*, two isolates of *F. solani*, five isolates of *R. solani*, two isolates of *Verticillium* spp., *Cephalosporium* spp. and *Myrothecium* spp. were selected according to their pathogenic capabilities.

Inoculum preparation and soil infestation:

Inocula of the tested fungi were prepared by growing each fungus for 2 weeks at 25˚C in bottles containing sorghum grains sand medium (25 g clean sand and 75g sorghum grains and enough water). The cultures were thoroughly mixed with clean soil (clay- sand soil 2:1 w/w) in pots, 25 cm in diameter at the rate of 2%. The infested soils were moistened for one week. Soil mixed with uninoculated sterilized sand sorghum medium, at the same rate, was used as control. Seeds of each cultivar were sown in the prepared pots till the plants reached 28 days-old, (five seeds per pot) and covered with clay-sand mix slightly. Three replicates were employed for each treatment. All the pots were kept in the greenhouse. The plants received the normal agricultural practices of irrigation and fertilization.

Pathogenicity test:

Two sets of pots filled with disinfested soil were prepared. Pots of the first set (25 cm diameter) were divided into sub-groups, each consisted of 3 pots. Potted soil of each 3 pots was infested with one of the isolated fungi (Table 4) at the rate of 2% of soil weight. Pots of the second set were left uninfested to serve as control. Seeds of Beit Alpha were sown in the infested pots (five seeds per pot). Three pots were employed for each fungus. In each treatment, the numbers of pre- and post-

emergence and survived plants were recorded after 10, 20 and 30 days from planting (Paternote, 1987). According to the obtained results, the most virulent isolate of any tested pathogen was used in the further studies.

Antagonistic microorganisms:

Bio-control agents used in this study (*B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride*) were obtained kindly from the Central Lab of Organic Agriculture, ARC, Giza, Egypt. *T. harzianum* and *T. viride* were grown in liquid gliotoxin fermented medium (GFM) under complete darkness to stimulate toxin production (Abd El-Moity and Shatla, 1981) for 14 days. The suspensions of *T. harzianum* and *T. viride* were prepared by adjusting number of *Trichoderma* propagules in the suspension to be $30x10^6$/ml. *B. subtilis* and *P. fluorescens* were grown on nutrient glucose broth (NGB) prepared as described by (Dowson, 1957) for 48 h. The bacterial suspensions were also adjusted to contain $30x10^6$ cfu/ml.

In vitro studies:

Different bioagents were evaluated under laboratory conditions for their antagonistic effect against *R. solani*. Petri dishes 9.0 cm in diameter each contained 15 ml of GM were used to determine the antagonistic effect. On the other hand, plates containing NGB medium were used to determine the effect of bacteria against the pathogenic fungus. *R. solani* (6 mm disk) was inoculated at one side and bioagent, obtained from 3 days old culture, at the other side. A loop full of antagonistic bacteria grown on liquid NGB medium for 48 hours was streaked. Five plates were used for each treatment. Plates inoculated only with the pathogenic fungus served as a control treatment. Inoculated plates were incubated at 25^{o}C. When mycelia growth covers the entire medium surface in any treatment, all plates were examined and percentages of reduction in mycelia growth of pathogenic fungus were calculated using the formula: Percent inhibition = C2−C1/C2 × 100 (Fokkema, 1973).

Greenhouse experiments:

The role of macronutrients on controlling damping off disease:

The plastic pots 30 cm in diameter were filled with sand and clay soil. Infestation was carried out by homogenized fungal inocula to sterilized soil at the rate of 2% (v\w). Control pots were watered with the used medium free from the fungus at the same rate. Pre and post-emergence and survived plants were recorded after 10, 20 and 30 days from planting. Nitrogen, and potassium were used in the form of ammonium sulfate (21.0% N) and potassium sulfate (52% K_2O), respectively. Phosphorus was applied in the form of calcium phosphate (15.5% P_2O_5). The amounts of each fertilizer required were calculated in correspondence with the field rate. Table (1) represents the rates of NPK amended to each pot containing 8 Kg infested or un-infested soil (which served as control) with the pathogenic fungus. The control treatment was left without fertilization. All the pots were maintained under the greenhouse conditions.

Table 1. Levels and amounts of NPK (gm) amended to the pots

Rates of NPK	Forms of used nutrition and their amounts (g)*		
	Nitrogen (N)	Phosphorus (P)	Potassium (K)
	Ammonium sulfate	Calcium phosphate	Potassium sulfate
1N:1P: ½ K	2.8	2.4	1.0
1N:1P:2K	2.8	2.4	4.0
1N: ½ P:1K	2.8	1.2	2.0
1N: 2P:1K	1.2	4.8	2.0
½ N: 1P:1K	1.4	2.4	2.0
2N:1P:1K	5.6	2.4	2.0
1N:1P:1K*control (1)	2.8	2.4	2.0
0N:0P:0K control (2)	0.0	0.0	0.0

The recommended dose/feddan for: N=350 kg, P=300 kg and K=260 kg.

Five seeds were sown in each pot and three pots were used for each treatment. Pots were carefully watered with water and NPK fertilizers were applied at the recommended dose. All the pots were kept under the greenhouse conditions for 60 days. The numbers of pre- and post-emergence damping off and plant survival were recorded after 10, 20 and 30 days from planting.

The effect of different bioagents on controlling damping off disease:

The mixtures of AM fungi were obtained kindly from (Mycol. Res. and Dis. Survey Dept., Plant Pathol. Inst., A. R. C., Giza, Egypt). This mixture consisted of spores of *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. intraradices* Schenck & Smith, *G. clarum* Nicol. & Schenck, *Gigaspora gigantea* (Nicol. & Gerd.) Gerd. & Trappe, and *Gigaspora margarita* (Becker & Hall). AM fungi inocula were used as 50 g/pot according to El-Sharkawy (2010). Selected biocontrol agents (*T. harzianum*, *T. viride*, mixture of them (at the rate of 1:1), *B. subtilis*, and *P. fluorescens* were used as seed soaking and macronutrients with level of (½N: ½ P: ½K), were applied compared with the recommended dose of NPK without addition of any biocontrol agents. The pots were sown with cucumber seeds (10 seeds per pot) and three pots were used for each treatment. Experiments were designed in complete randomized plots.

Physiological analysis:

Determination of oxidative enzymes activity:

Activities of oxidative enzymes, *i.e.* peroxidase (POX) and polyphenoloxidase (PPO) were determined. The extraction procedure was essentially based on the methods described by (Biles and Martyn, 1993) as follows; one gram of leaf tissues was ground in 2 ml of sodium phosphate buffer (pH 6.5) using a mortar and a pestle. Samples were transferred to eppendorf tubes and then centrifuged for 20 min at 12000 rpm at 4°C. Supernatant was stored at -8°C. Three replicates were prepared for each treatment.

POX activity:

The POX activity was determined according to the method described by (Hammerschmidt *et al*., 1982). The reaction mixture consisted of 2.9 mL of a 100 mM sodium phosphate buffer (pH 6.5) containing 0.25% (v/v) catechol and 100 mM H_2O_2. The reaction started by adding 100 µL of the crude enzyme extract. Changes in the absorbance at 495 nm were recorded every 30 sec intervals for 3 min. Enzyme activity was expressed as increase in absorbance $min^{-1}g^{-1}$ fresh weight.

PPO activity:

The PPO oxidase activity was determined according to the method described by (Maliak and Singh, 1980). The reaction mixture contained 3.0 ml buffered catechol solution (0.01 m), freshly prepared in 0.1 ml phosphate buffer (pH 6.5). The reaction started by adding 100 µl of the crude enzyme extract. Changes in the absorbance at 495 nm were recorded every 30 sec intervals for 3 min.

Plant growth and yield parameters:

Random samples of cucumber plants were collected after 30 days from sowing. Plant height (cm) and plant fresh weight (gm) were determined. Cucumber fruits at premature stage were picked at the mid harvesting season (60 days after sowing) and then weighted and the values were recorded in grams.

Statistical analysis:

Data were analyzed the statistical analysis system CoStat (CoHort Software, U.S.A.) version 6.4 (CoStat, 2005). The means comparison was done using Duncan's multiple range test (Duncan, 1995) at $P \leq 0.05$.

Results and Discussion

Isolation from soil:

Isolation trials from the rhizosphere of cucumber plants recovered *Cephalosporium* sp., *Cladosporium* sp., *Fusarium solani* (Mart.) Sacc., *F. oxysporum* Schl., *Myrothecium* sp., *Rhizoctonia solani* Khun and *Verticillium* spp. The occurrence and frequency of the isolated fungi from the rhizosphere were differed from one location to another within the Nile Delta. Data in Table (2) show that *R. solani* was the prevalent in the soil. The highest frequency *R. solani* was $8.5x10^4$ followed by *F. oxysporum*, being $6.6x10^4$ colony forming units per gram soil (cfu/g), and *F. solani* recorded $6.5x10^4$ (cfu/g soil). *Cladosporium* sp. showed the lowest rank among the isolated fungi ($1.0x10^4$). However, *R. solani* was the most dominant fungus with high frequency. Generally, this may be due to that *R. solani* survives between crops as sclerotia or as mycelia in the soil. Similar findings of the destructive action of such fungus on cucumber were reported by Jarwis (1992).

The variation in fungal counts determined from different localities may be due to the susceptibility of the cultivated cultivars and/or the differences of meteorological conditions prevailing in these localities. The type of soil, the crop rotation in the location or the intensive growth of cucumber in the field for a number of successive seasons may be considered.

Table 2. The fungal density in the rhizosphere of infected cucumber plants (cfu/g)

Fungi	% frequency (cfu/g soil)
Fusarium solani (Mart.)Sacc.	6.5×10^4
Fusarium oxysporum Schl.	6.6×10^4
Cephalosporium sp.	1.8×10^4
Myrothecium sp.	2.5×10^4
Verticillium sp.	1.0×10^4
Rhizoctonia solani Khun	8.5×10^4
Cladosporium sp.	1.0×10^4

Isolation from diseased cucumber plants:

Isolation trials were carried out from diseased cucumber plants collected from different localities of Nile Delta. The isolated fungi were identified according to their morphological features as *Alternaria tenuis* Fr., *Fusarium oxysporum* Schl., *Fusarium solani* (Mart.) Sacci, *Rhizoctonia solani* Khun, *Cephalosporium* sp., *Cladosporium* sp, *Verticillium* sp, and *Pythium* sp. The occurrence and frequency of fungi associated with diseased plants differed from one locality to another. Among the isolated fungi *R. solani* was the most prevalent in the inspected locations. The percentage of its frequency was 16.3% followed by *F. solani* (15.1%). The interaction between the environmental conditions of localities and the genetic makeup of tested varieties led to high percentage of diseases (Eastburn *et al.*, 2011).

Table 3. Occurrence and frequency (%) of fungi isolated from diseased cucumber roots collected from different locations of Egypt

Fungi	Frequency (%)
Alternaria tenuis Fr.	7.00
F. oxysporum Schl.	12.8
F. solani (Mart.) Sacc	15.1
Cladosporium sp.	3.50
Cephalosporium sp.	10.5
Rhizoctonia solani Khun	16.3
Verticillium sp.	8.13
Pythium sp.	7.00

Pathogenicity tests:

Four cucumber hybrids, *i.e.* hybrid 6, hybrid 9, hybrid 14 and Beit-Alpha (F1) were tested for their susceptibility to the pathogenic fungi. Table (4a,b) illustrates a wide range of variations among the cultivars in their reactions. It was found that all isolates caused pre-emergence damping-off and post-emergence seedling mortality in infested pots with root rot pathogens 10 and 30 days after sowing, respectively. Hybrid 14 was the least susceptible one against all pathogens except isolates 3 and 4 of *R. solani*. Data also indicated that the Beit-Alpha was highly susceptible to infection and gave the least percentage of seedling stands against all pathogenic fungi. It was clear also that *R. solani,* isolate No.1 was highly aggressive and caused damping off. These results are in accordance with previous studies carried out on the pathogenic and genetic characterization of fungi (Lakhdar *et al*., 2004). The differences between cultivars in their resistance might be due to the differences in genetic makeup which effect on some morphological factors that affected host-

pathogen relationship which played a role in cultivars. Variation in susceptibility or resistant was reported by many workers. Source of variation might be due to morphological differences and/or physiological differences. Similar results on cucumber varieties and genotypes were reported by many investigators (Buriev *et al*., 2004).

Table 4a. Percentages of damping-off and seedling survival of cucumber cultivars as affected by different isolates, under greenhouse conditions

Tested Fungi	Damping-off and seedling survival %					
	Hybrid 6			Hybrid 9		
	pre	post	survival	pre	post	survival
F. oxysporum (Isolate 1)	52.0d	2.4de	54.4e	52.0c	1.7b	46.3j
F. oxysporum (Isolate 2)	29.7h	0.0f	70.3b	31.7h	0.3c	68.0b
F. solani (Isolate 1)	44.0e	10.4a	44.6g	52.0c	1.0bc	47.0i
F. solani (Isolate 2)	30.4h	1.0ef	68.6c	45.0e	1.4b	53.6f
Cephalosporium sp.	56.0c	0.0f	44.0g	32.0h	0.0c	68.0b
Myrothecium sp.	60.0b	0.0f	40.0h	36.0g	0.0c	64.0d
R. solani (Isolate 1)	63.0a	6.7b	30.3i	60.0a	13.0a	27.0i
R. solani (Isolate 2)	59.0b	1.0ef	40.0h	55.0b	2.0b	43.0k
R. solani (Isolate 3)	53.0d	3.0d	44.0g	42.0f	1.3bc	56.7e
R. solani (Isolate 4)	34.3f	2.0d	63.7d	30.0h	2.0b	68.0b
R. solani (Isolate 5)	32.4g	4.6c	63.0d	31.0h	2.0b	67.0c
Verticillium sp. (Isolate 1)	52.0d	0.7ef	47.3f	52.0c	0.0c	48.0h
Verticillium sp. (Isolate 2)	51.7d	0.7ef	47.6f	47.0d	0.0c	53.0g
Control without fungus	28.0i	0.0f	72.0a	15.0i	0.0c	85.0a

Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

Table 4b. Percentages of damping-off and seedling survival of cucumber cultivars as affected by different isolates, under greenhouse conditions

Tested Fungi	Damping-off and seedling survival %					
	Hybrid 14			Beit-Alpha		
	pre	post	survival	pre	post	survival
F. oxysporum (Isolate 1)	32.0f	2.7b	65.3h	35.0d	2.0bc	63.0i
F. oxysporum (Isolate 2)	22.0i	0.7de	77.3d	22.0j	0.0d	78.0c
F. solani (Isolate 1)	32.0f	2.0bc	66.0g	35.0d	2.0bc	63.0i
F. solani (Isolate 2)	30.0h	1.0cde	69.0e	31.0j	0.4d	68.6f
Cephalosporium sp.	20.0j	0.0e	80.0b	21.0k	1.0cd	78.0c
Myrothecium sp.	22.0i	0.0e	78.0c	21.0k	0.0d	79.0b
R. solani (Isolate 1)	55.0a	5.0a	40.0l	68.0a	9.0a	23.0l
R. solani (Isolate 2)	52.7c	1.0cde	46.3j	51.0b	2.4b	46.6k
R. solani (Isolate 3)	54.7b	1.7cd	43.6k	50.0c	1.0cd	49.0j
R. solani (Isolate 4)	32.4e	0.6de	67.0f	31.7f	0.7d	67.6g
R. solani (Isolate 5)	31.7g	1.0cde	67.3f	33.0e	0.0d	67.0h
Verticillium sp. (Isolate 1)	32.0f	0.7de	67.3f	25.0i	1.0cd	74.0d
Verticillium sp. (Isolate 2)	35.0d	1.0cde	64.0i	27.0h	0.7d	72.3e
Control without fungus	7.0k	0.0e	93.0a	8.0l	0.0d	92.0a

Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

Effect of the antagonistic microorganisms on the linear growth of R. solani:

Data presented in Table (5) show that all tested bioagents reduced significantly the percentage of linear growth of *R. Solani* except *P. fluorescens*, compared to the control treatment. Effect of *P. fluorescens* might be due to production of some antifungal substances, *i.e.,* pyrrolintrin, pyoluteorin and 2, 4 diacetylploroglucinol

(Sharifi-Tehrani *et al*., 1998). Data in Table (5) indicate that *T. harzianum* (T1) was the most effective antagonist against *R. solani* (81.5%) without significant differences between *T. harzianum* (T1) and *T. viride* (T2). This high potentiality in suppression might be due to that *Trichoderma* spp., act through different actions as production of antifungal substances (Hayes, 1992). *Trichoderma* spp. also acts through production of destructive enzymes, *i.e.* chitinase (Harman, 2006) which attacks the fungus structures. *Trichoderma* spp. also attacks through mycoparasitism (Hafez *et al.*, 2012 and El-Sharkawy *et al*., 2015).

Table 5. Effect of different bioagents on the mycelial growth of *R. solani,* No.1 under laboratory conditions

Tested bioagents	Reduction in the linear growth
Bacillus subtilis	54.82 b
Pseudomonas fluorescens	0.0 c
T. harzianum (T1)	81.85 a
T. viride(T2)	80.16 a
Control	0.0 c

Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

The role of macronutrients NPK on the incidence of damping off under greenhouse conditions:

Fertilizer salts were chosen because of their ready availability and use in commercial greenhouse and their relatively nontoxic effects on people and plants (Ehret*et al*., 2002). Results in Table (6) indicate that all NPK levels applied to the infested soil with *R. solani* significantly decreased the occurrence of damping-off disease in comparison with the non-fertilized soil (check treatment) on cucumber plants. Data showed that potassium at the double recommended dose (4g/pot) was the most effective treatment, where survived plants recorded 81.3%. However, half dose of phosphorous (1.2 g/pot) gave the lowest reduction of damping-off. Phosphorus is part of the "energy currency" of the cells of all living things (ATP). It encourages root development, encourages rapid strong growth, hastens the maturity of plants and stimulates the blooming by promoting early cell development. It helps the plant to build resistance to disease. The role of potassium in decreasing the susceptibility of host plants to parasites can be explained by its ability to play a central role in the development of thick cuticles, a physical barrier to infection or penetration. It is well known that potassium acts as a catalyst or activator of certain enzymes. It helps encourage healthy root development and has a lot to do with the vigor and health of the overall plant. Enhanced synthesis of organic compounds could act as phytoalexins that can inhibit growth and spread of pathogens, in addition to accumulation of polyphenols (Ruan *et al*., 1999).

The nutrients have a significant impact on all aspects of the disease cycle through four possible mechanisms. Nutrients play a major role in the plant ability to develop strong cell walls and other tissues, as shown by resistance. The germination of spores is stimulated by compounds exuded by the plants. The amount and composition of these exudates are affected by the nutrition of the plant. When plants

have low levels of certain nutrients, these exudates will contain higher amounts of compounds such as sugars and amino acids that promote the establishment of the fungus. As a plant becomes infected by a fungus, its natural defences are triggered. The infection causes increased production of fungus inhibiting phenolic compounds and flavonoids, both at the site of infection and in other parts of the plant. The production and transport of these compounds are controlled in large part by the nutrition of the plant. Another plant response to infection is the formation of oxygen radicals (O= and OH-) and hydrogen peroxide. These products and compounds can be destructive to the plants cells as well as the pathogen. Some nutrients act to detoxify oxygen radicals and hydrogen peroxide, thus limiting damage to plant cells (Engelhard, 1993). It has been found that fertilizer may influence the rhizosphere mycoflora of plants which may have a positive role on inhibited many pathogens and influence on the production of other antibiotics in biocontrol strains (Duffy and Defago, 1999). The resistance can be also increased by some changes in plant anatomy (thicker epidermal cells and a higher degree of lignification or the silification), and in physiological and the biochemical properties (higher production of inhibitory or repelling substances) through enhanced formation of mechanical barriers (lignification) and the synthesis of toxins (phytoalexins). The minerals work by directly reducing the inoculum potential, or by improving host tolerance, or by both (Von-Broembsen and Deacon, 1997).

Table 6. Percentage of seedling stands of Beit-Alpha cucumber amended with different levels of (NPK) under greenhouse conditions after 30 days from sowing

Treatments	Damping-off%		Plant Survival%
	Pre-	Post-	
Control, un-infested	3.7h	0.0h	96.3a
Control, infested	48.4a	18.3a	33.3g
½NPK	19e	17d	64.0d
2NPK	22d	17.4c	60.6e
N½ PK	28.6b	17.8b	53.6f
N2PK	7.0g	16e	77.0 c
NP ½ K	26.7c	12.7f	60.6 e
NP2K	13.7f	5.0g	81.3b

[a] Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).
1N= recommended dose. ½ P= half recommended dose. 2K= double dose.

Evaluation the effect of bioagents on the incidence of damping-off on Beit-Alpha cucumber amended with 1/2 level of (NPK) under greenhouse conditions:

Data presented in Table (7) show that all tested bioagents decreased significantly the percentage of damping off with half dose NPK compared to the control treatment with full recommended dose from NPK. Data indicated that *P. fluorescens* showed the highest effect in controlling cucumber damping- off disease. This effect might be due to the fact that *P. fluorescens* can inhibit disease by many of actions, *i.e.* chelated iron and prevent other microorganisms to utilize this element, as a result to iron starvation the pathogen cannot grow or penetrate and cause disease (Becker and Cook,

1998). AM Fungi occupied the second rank after *P. fluorescens*, this agrees with (Abdel-Fattah *et al.*, 2011) who found that the application of AM fungi as a biocontrol agent played an important role in enhancing plant resistance against *R. solani.* Mechanisms that can account for the disease control ability of AM fungi may include competition for infection sites and host photosynthesis, root damage compensation. Enhancement of plant resistance through various physical and physiological mechanisms, such as increasing the cell-wall thickness of the host or the accumulation of some antimicrobial substances. Increased the activity of enzymes responsible of induced resistance, *i.e*, polyphenoloxidase and peroxidase enzymes and production of phenolic compounds (El-Sharkawy, 2010) or changes in lignin formation (Saldajeno *et al*., 2008) and induction of new isoforms of the hydrolytic enzymes, *i.e.*, chitinase and β-1, 3-glucanase (El-Khallal, 2007). Harman (2006) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones. Mixing more than one species has led to combination of different mode actions and improving the efficacy of the mixture.

Table 7. Evaluation of some bioagents on the incidence of damping-off on Beit-Alpha cucumber amended with the level (½NPK) under greenhouse conditions

Treatments	Damping-off%		Plant Survival%
	Pre	Post	
Bacillus subtilis	30c	16a	54d
Pseudomonas fluorescens	16e	10b	74a
T. harzianum (T1)	40b	3c	57c
T. viridie (T2)	40b	10b	50e
T1+T2	40b	10b	50e
AM Fungi	26d	3c	71b
Control	43a	16a	41f

[a] Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

Effect of using different bioagents on the activity of oxidative enzymes of Beit-Alpha cucumber amended with (½NPK) under greenhouse conditions:

Data in Table (8) indicate that, all tested bioagents increased the enzyme activities in cucumber Beit-Alpha cultivar plants compared to control. Data also showed that, *P. fluorescens* was the most effective bioagents in increasing peroxidase and polyphenoloxidase (1.7 and 1.8) followed by AM fungi (1.7 and1.7). Bacillus subtilis was the least (1.0 and 0.97) in this regard. Many plant phenolic compounds are known to be antimicrobial, functioning asprecursors to structural polymers such as lignin which inhibits disease development through different mechanisms involving the inhibition of extracellular fungal enzymes (cellulose, pectinase, ..), inhibition of fungal oxidative phosphorylation, nutrient deprivation (metal complications, protein in solubilisation (Hammerscmidt, 2005).

Polyphenoloxidase enzyme is involved in the oxidation of polyphenols into quinones and lignification of plant cells during microbial invasion. Also, peroxidase enzyme is oxido-reductive enzymes that participate in the cell wall polysaccharides processes such as oxidation of phenols, suberization and lignification of host plant cells during the defense reaction against pathogenic agents. Induction of resistance using bioagents triggers defense mechanisms nevertheless the pathogen itself. Also, (Nandkumar*et al*., 2001) reported that peroxidase might stimulate the interaction Ca^{2+} signals required for induction of defense responses. The increase in the activities of these enzymes plays an important role to help the plants to defense themselves from the pathogens.

Bioagents increased phytoalexin accumulation after the initial suppression points towards an extra stimulation of host cell metabolism. In addition to phytoalexin accumulation leads to gum formation and compositional changes in the cell wall. It appears that changes in host cell wall composition apparently are more prominent or start earlier than an increase in phytoalexin accumulation. As such, these cell wall alterations and/or related phenomena such as increased phenolic turnover could be responsible for slowing down fungal development. However, it still remains to be proven. Polyphenol oxidase is a widespread enzyme found in plant cells located in the chloroplast, this enzyme is capable of dehydrogenating of O-diphenols to produce O-quinones. However, it indicates the highest activity toward hydroxylation of monophenols to diphenols. Moreover, polyphenol oxidase induces metabolization of these phenolic compounds into toxic forms (Chranowski *et al*., 2003 and Fayzalla *et al*., 2009).

Table 8. Effect of different bioagents on the activity of oxidative enzymes of Beit-Alpha cucumber cultivar an amended with level of (½NPK) for controlling damping-off disease

Bioagents with amended level of (½NPK)	Oxidative enzyme in Beit-Alpha cultivar	
	Peroxidase	Polyphenoloxidase
B.subtilis	1.0c	0.97d
P. fluorescens	1.7a	1.8a
T. harzianum(T1)	1.3b	1.3c
T. viride(T2)	1.2b	1.2c
(T1+ T2)	1.4b	1.5b
AM Fungi	1.7a	1.7a
Control infested	1.4b	1.5b
Control noninfested	1.1c	1.3c

[a] Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

Effect of bioagents on some agronomic characters of Beit-Alpha cucumber amended with (½ NPK) under greenhouse conditions:

Data in Table (9) indicate that all growth parameters were increased due to using all bioagents compared with control treatment. Treated plants gave more fruits weight/plant than the control. Direct effects of bioagents on plant growth have been demonstrated and were considered to be due to increased availability of minerals or other ions or to microbial production of plant growth regulators which improve plant

growth and production (Kashif *et al.*, 2008). AM fungi were the most effective bioagents and root colonization by AM-fungi frequently enhanced root growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients (Arora *et al.*, 1992). These results are similar to these obtained by (El-Sharkawy, 2010) who found that AM fungi led to the improvement of growth parameters and reduced the effect of disease in the case of addition of half recommended dose and without adding amount of mineral fertilization and increased the total weight of fresh and dry weight of shoot and root, plant length, number of leaves and leaf area of tomato plants infected with *F. oxysporum* f.sp. *lycopersici.* He found that AM fungi affected on enhancing uptake of some inorganic nutrients (N, P, K) which play a role in a decrease in the incidence of wilt Fusarium of tomato and increased alkaline and acid phosphatase. Thus, improvement in plant nutrition can enhance plant development and also might make the plant more resistant. These results are similar to those obtained by (Yedidia *et al.*, 2001) who showed that treatment of cucumber plants in soil with *T. harzianum* (T-203) resulted in large increase in root area and cumulative root lengths, and a significant increase in dry weight, shoot length the leaf area over that of the untreated control.

Increased growth response has been demonstrated by several other investigators. (Altomare *et al.*, 1999) demonstrated the ability of *T. viride* and *T. harzianum* to solubilize insoluble tri calcium phosphate *in vitro.* They showed clearly the positive effect of potassium fertilization on cucumber yields. Another study has shown a clear positive response of cucumber productivity to potassium nutrition of the plants. An over optimal level of either potassium or nitrogen may induce or accentuate a phosphorous deficiency.

Table 9. Effect of bioagents on agronomic characteristics of the Beit-Alpha cucumber amended with level of (½NPK)

Bioagents with amended level of (½NPK)	Vegetative growth		Yield (g/plant)
	Fresh weight (g)	Plant height (cm)	
B. subtilis	13.3e	28e	378.0e
P. fluorescens	20a	38a	513.0a
T. harzianum (T1)	12.5f	26f	327.0f
T. viride (T2)	14d	32d	410.0d
(T1+ T2)	16c	35c	456.9c
AM Fungi	19b	37b	480.4b
Control	10.5g	24g	310.1g

[a] Values in each column followed by the same letter(s) are not significantly different according to Duncan multiple range test ($P \leq 0.05$).

The current study indicates that the application of AM fungi and bioagents led to the uptake of the mineral nutrients and reduced the quantity of the chemical fertilizers used. In addition to reductions in the incidence of the disease, the production of the vigorous seedlings with more resistance to soil-borne plant pathogenic fungi and reduced the burden of chemical pesticides and increased the productivity of the cucumber.

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(Received 03/01/2016;
in revised form 07/03/2016)

استخدام الكائنات الدقيقة النافعة لتقليل المعدلات الموصى بها للمغذيات الكبرى لمقاومة مرض سقوط البادرات في الخيار

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**المعمل المركزي للزراعة العضوية- مركز البحوث الزراعية الجيزة – مصر.

يعتبر فطر الريزوكتونيا سولاني من أكثر المسببات الممرضة شيوعا والتي تسبب موت البادرات في نباتات الفصيلة القرعية في مصر. وجد أن أكثر الأصناف القابلة للإصابة للفطريات القاطنة في التربة هو صنف بيتا ألفا. عند دراسة التأثير التضادي لعزلات من التريكودرما هارزيانم وفيردي واثنان من البكتريا النافعة هما باسيلس ستلس وسيدوموناس فلوريسنس على الريزوكتونيا سولاني تحت ظروف المعمل، أظهرت التريكودرما هارزيانم تأثيرا معنويا فى خفض النمو الميسليومي لفطر ريزوكتونيا سولاني وذلك مقارنة بمعاملة الكنترول. وفي تجربة الصوبة تم اختبار تأثير مستويات مختلفة من التسميد النيتروجيني والفوسفاتي والبوتاسي على مرض موت البادرات للخيار. وقد أوضحت النتائج أن البوتاسيوم والفوسفور عند استخدامهم بضعف الجرعة أعطيا أفضل تأثير في مقاومة المرض. أعطت نصف الجرعة من الفوسفور أقل تأثير في مقاومة المرض. تم اختبار كل من باسيلس ستلس، سيدوموناس فلوريسنس و تريكودرما هارزيانم وفيردي وخليط منهما و وكذلك من خليط فطريات الميكروهيزا في وجود العدوي الصناعية بالفطر الريزوكتونيا في الصوبة في وجود نصف الجرعة من العناصر الكبرى. وقد أوضحت نتائج تجارب الصوبة أن بكتريا سيدوموناس فلوريسنس يليها الميكروهيزا أظهرا أعلى تأثير في خفض نسبة الإصابة فضلا عن تحسين نمو المحصول وزيادة نشاط انزيمي البيروكسيديز والبولي فينول اوكسيديز.