

Efficacy of Seed Treatment with Microbial Agents And/or Waste Products for the Control of Cucumber Damping – off

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Abstract: The present study aims to suggest a safe method to control damping – off disease in cucumber seedlings caused by the fungus *Pythium ultimum*, as a biocontrol method by using the microbial isolates *Bacillus subtilis* and *Trichoderma harzianum*, and also to improve the efficacy of this method by using some waste products. Positive correlation between added *B. subtilis* or *T. harzianum* and percentage of healthy cucumber seedlings were detected. Farm wastes were more effective in controlling soilborne pathogen, when added to soil sown by bacterized seeds and *T. harzianum* treatments. Similarly a significant increase in photosynthetic pigments and a significant increase in element content in leaves.

Key words: Biocontrol, Damping- off, cucumber, *Bacillus subtilis*, *Trichoderma harzianum*, waste products.

INTRODUCTION

Root and seedling diseases are increasingly difficult to control because few resistant varieties are available and pesticides are receiving increased environmental scrutiny.

Pythium ultimum is a phytopathogenic fungus of a recognized importance in nurseries production. Once introduced, infection can reach a higher level because of pathogen development and spreading to the whole cultural system.

Biological control of soilborne plant pathogens can be achieved by seed treatment with antagonists. Liu and Vaughm (1965) succeeded in controlling *P. ultimum* in table beet seedlings by coating the seeds in a suspension of conidia. Similarly, Harman *et al.* (1980) reported the biocontrol of *R. solani* and *Pythium* spp. by coating radish and pea seeds with *T. harzianum*. Several microorganisms have been reported to be biocontrol agents for suppression of *Pythium* damping – off (Harman and Hader, 1983 and Hoitink and Boehm, 1999).

Trichoderma spp. have been known to be able to attack other fungi, to produce antibiotics that affect other microbes and to act as biocontrol microbes (Weindling, 1934 and Weindling and Fawcett, 1936). *Trichoderma* are important sources of commercial cellulase, natural agents of decomposition of plant material and inhibitors of several soilborne pathogenic fungi.

Bacillus spp. have been tested on a wide variety of plant species for their ability to control diseases (Cook and Baker, 1983 and Kildea *et al.*, 2008). Bacilli are appealing candidates for biocontrol because they produce endospores that are tolerant to heat and desiccation, which is an advantage over some of the root colonizing bacteria. *Bacillus subtilis* A13, isolated from lysed mycelium of *Sclerotium rolfsii*, was inhibitory *in vitro* to several plant pathogens and improved the growth of many plant species in steamed and natural soils (Broadbent *et al.*, 1977; Yuen *et al.*, 1985). Improved plant growth was attributed to suppression of major and minor pathogens and possibly also to direct stimulation of plant growth.

The use of organic amendments to suppress soilborne diseases has gained renewed attention by farmers and researchers (Abawi and Widmer, 2000; Aryantha *et al.*, 2000 and Stone *et al.*, 2003).

Composts prepared from heterogenous organic wastes showed the potential of biological control for several plant diseases, caused by soilborne pathogens (Hoitink *et al.*, 1991, 1997). Numerous reports of the suppressive effects of composts against damping - off, caused by *Pythium* sp., were also published (Chen *et al.*, 1988; Mandelbaum and Handar, 1990; Diab *et al.*, 2003 and others).

In the Philippines, some species are used to enhance compost making of industrial and agricultural waste materials like rice straw, coconut coir dust and weeds (Cuevas, 1987).

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In the present study we tested a bacterial strain, *B. subtilis* and a fungal strain, *T. harzianum* and two organic wastes: bean straw and chicken manure for their efficacy in controlling cucumber damping - off caused by *Pythium ultimum*.

MATERIAL AND METHODS

Strains and inoculum production

Pathogen:

P. ultimum, was isolated from potato tuber showing typical leak symptoms. This pathogen was cultured on PDA at 25°C for one week and stored at 4°C for preservation.

Trichoderma harzianum Rifai EMCC 540 was obtained from Cairo MIRCEN Faculty of Agriculture – ASU.

T. harzianum and *P. ultimum* were grown on PDA.

Bacillus subtilis was kindly submitted from Faculty of Agriculture, Zagazig University.

Cucumis sativus L was obtained from Wadi El Nil of Agriculture development, Egypt.

Inoculum Production of *Pythium Ultimum*:

Inoculum of *P. ultimum* was prepared by a method developed by M.G. Boosalis cited in Conway *et al.* (1997). A 33x23 cm cake pan was one third filled with vermiculite, covered with aluminum foil, and autoclaved (121°C, 1.05 kg/cm², 15 min). In a separate container, 400 to 500 ml of cornmeal was covered and autoclaved three times. The autoclaved cornmeal and vermiculite was mixed in the cake pan, and 550 ml of sterile tap water was added. Agar and mycelium in three to four Petri dishes of actively growing *P. ultimum* was cut into cubes and added to each pan, mixed thoroughly, and incubated 2 to 3 weeks at room temperature. The mixture was loosened from the pans, spread on a tray, covered with cheesecloth and allowed to dry overnight at room temperature. The mixture was further separated using a rolling pin. The mixture was placed in paper bags and stored at room temperature. The mixture was sieved for greater particle uniformity.

Preparation of the Inoculum Used to Infest the Potting Mix:

The *T. harzianum* inoculum was prepared by the method of Smith *et al.* (1990), with slight modification. Four or five discs (5 mm diameter) were cut from the edge of the actively growing *T. harzianum* colony and added to a glass Petri dish (90 mm diameter) containing a mixture of peat and wheat bran (3.1 v/v) that had been moistened with 15 ml of dist water and sterilized for 15 min at 121 °C on each of two consecutive days. 3.0 % of antagonistic *T. harzianum* was used when required.

Organic Manures Used:

1-Bean straw:

It was collected from a farm from the Faculty of Agriculture, Cairo University. It was thoroughly washed with water and left to dry at room temperature, then, it was ground and preserved for further use.

Chicken manure:

It was obtained from a farm in the Faculty of Agriculture, Cairo University. It was dried by exposing to direct sunlight, ground and then preserved in plastic containers.

Seed bacterization with *B. subtilis*:

Cucumber seeds were surface sterilized as will be mentioned later and bacterized with a strain of *B. subtilis*, grown on potato dextrose agar (PDA, pH 7.0) for 48 h at 30 °C. The bacterial cells were scrapped with sterile glass rod from two Petri plates (9 x 1.5 cm) and added to 25 ml of 0.5% sterilized carboxymethyl cellulose. Surface sterilized seeds were bacterized by immersion in this bacterial suspension for about 15 min. and then dried in flowing sterile air for 2 – 3 h.

Seed treatment with waste products. (Bardin *et al.*, 2004):

Homogenous seeds were soaked in 1% carboxymethyl cellulose solution for 15 min, then put in sterile plastic bags containing 2.5 g bean straw or chicken manure or a mixture of the two wastes per 100 seeds. The bags were shaken well, then seeds were left to dry.

Greenhouse experiments:

Experiments were set up in plastic sterilized pots 20 cm diameter. Before sowing, seeds were surface – disinfected by soaking in 1% sodium hypochlorite for 1 min then rinsed three times in sterile distilled water. The seeds were then soaked in sterilized distilled water for 3 – 4 hours, in the dark.

1.5 g bean straw or 2.0 g chicken manure per kg soil or a mixture of the two wastes, were added to the infested soil before sowing.

3.0% *T. harzianum* or bacterized seeds or a combination of the two organisms were added to the infested soil with or without the wastes. Three replicate pots were included per treatment, arranged in complete randomized design. Control pots were treated with an equal quantity of autoclaved cornmeal vermiculite preparation. Control treatments contained sterile soil and sterile seeds. Pots were left for two weeks at room temperature in a greenhouse, and watered by dist water every three days. Cucumber seeds (15 per pot) were surface disinfected as described above, then sown. Pots were watered every three days by knop's solution.

Analytical Methods:

After 3 weeks of sowing, samples from the seedlings of the different treatments were taken at random to record their morphological data as percentage of healthy seedlings, length, fresh and dry weights. Estimation of some metabolic aspects such as pigment content in leaves using Lichtenthaler (1987) method. The flame emission spectrophotometry (Stewart, 1972) was used to detect the test elements in the seedling leaves.

RESULTS AND DISCUSSION

The present study concerning the biological control of pythium damping – off disease and the improvement of biological control agents by the two tested waste products. Table 1, shows that, using the biocontrol agent *B. subtilis* by seed bacterization gave better results in infested soil. These results were also reflected on seedlings height, fresh and dry weights. An increase in seedling height was 1.67% in comparison with control treatments. In infested soil sown by bacterized seeds and 1.5% bean straw g/kg or chicken manure 2.0% g/kg, the percentage of healthy seedlings reached 88.89% with an increase of 40% in relation to control treatments. Podile *et al.* (1995) found that, seed bacterization with *B. subtilis* AF1 enhanced the levels of total phenols, phenylalanine ammonia lyase, peroxidase and lipoxygenase in the bacterized seedlings, indicating the possible involvement of induced host plant resistance in AF-1 – mediated disease control. In the context of biocontrol of plant diseases, the three families of *Bacillus* lipopeptides – surfactins, iturins and fengycins were mostly studied for their antagonistic activity for a wide range of potential phytopathogens, including bacteria, fungi and oomycetes (Ongena and Jacques, 2007).

Table 2 reveals that biological control of damping – off disease in cucumber seedlings using the antagonistic fungus *T. harzianum* increased the percentage of healthy seedlings in infested soil, there was also an increase in length, fresh and dry weights.

Hawell (2003) reported that *T. harzianum*, a biocontrol agent, produces enzymes such as chitinase, protease and cellulase. These enzymes have been proven to be involved in the antagonistic activity, they act by breaking down and dissolving the polysaccharides, responsible for the rigidity of fungal cell wall. He also noted that a higher enzyme activity of cellulase was detected in culture substrate supplemented with *T. viride*. Treatments of bacterized seeds by either bean straw or chicken manure with the addition of the straw or the manure to the infested soil improved the efficacy of *B. subtilis* in controlling the disease. It also increased the growth criteria of the seedlings (Table 1).

Better biocontrol was achieved when *T. harzianum* was added and seeds were coated with *B. subtilis* and soil was containing either bean straw or chicken manure (Table 1).

Boehm *et al.* (1993); Bowers and Lock (2000); Bardin and Huang (2003) and Bardin *et al.* (2004) found that plant wastes or animal manures contain high values of nitrogenous compounds that could be converted to simple nitrogenous compounds by the effect of enzymes produced by *B. subtilis* when grown within these wastes. They also showed that straw fermentation by microbes added, led to the accumulation of ammonia in great amounts by reduction of nitrates present in the straw. This ammonia caused toxic effect to the pathogens (Soltani and Lazarovits, 1999). Pavalou and Vakalounakis (2005), reported that, these wastes and its ingredients indicate the possible involvement of induced host-plant resistance.

Table 3, shows that, soil treatments with *P. ultimum* resulted in lower accumulation of chlorophyll a, b and carotenoids, as compared to control treatments without any treatments (sterile soil + sterile seeds). This result gives an indication that the pathogen interfered with the biotransformation of chl. a to chl. b, i.e. suppressed the photosynthetic activity of cucumber leaves. However, there was an increase in the photosynthetic pigments in seedling leaves using the bioagents *B. subtilis*, *T. harzianum* or a combination of

the two microbes. The best results obtained from this study were in case of mixing the two tested waste products in addition to the two bioagents (*T. harzianum* and *B. subtilis*) to the infested soil. These responses may be attributed to the hormones secreted by either *B. subtilis* or *T. harzianum* that was absorbed by the seedlings during their growth (Levenfors *et al.*, 2004). Straub and Lichtenthaler (1973) reported that addition of cytokinins to the plant maintains the natural level of both chlorophyllase and ribonuclease enzymes which lead to increase the photosynthetic product. Moreover, waste products used in this study, increased the organic matter in the soil which in turn increased nutrients and essential elements necessary for plant growth, leading to an increase in photosynthetic pigments in seedling leaves (Garica *et al.*, 2004 and Paul and Soliman, 2004). Table 4 shows that the presence of *P. ultimum* in the soil lowered the accumulation of the tested elements in seedling leaves. However the element content of seedling leaves increased as biocontrol agents were added to the infested soil. These results joined that obtained by Howell *et al.* (2000) and Yedidia *et al.* (2001) who reported that, the bioagents, *B. subtilis* and *T. harzianum* increased the lateral branches of the root surfaces resulting in the absorption of large amounts of elements, in addition of producing some metabolites that may dissolve amounts of elements to be absorbed by the plant. The combination of wastes and biocontrol agents established a direct role in mineral uptake by the plant. The decomposition of the wastes may supply the plant with some nutrients particularly microelements present in these wastes (Fuchs and Larbi, 2004).

Table 1: Impact of *B. subtilis* with bean straw or chicken manure with or without *T. harzianum* on the incidence of damping - off disease in *Cucumis sativus*, and the estimation of some growth parameters.

Treatment	Healthy seedlings %		Seedling height (cm)		Fresh seedling weight (g)		Dry seedling weight (g)	
	1	2	1	2	1	2	1	2
Sterile soil + sterile seeds	95.53 ± 2.22	95.53 ± 2.22	27.63 ± 0.67	27.63 ± 0.67	8.41 ± 0.26	8.41 ± 0.26	0.843 ± 0.02	0.843 ± 0.02
3.0% <i>P. ultimum</i> + sterile seeds	48.89 ± 4.44**	48.89 ± 4.44**	22.50 ± 0.32*	22.50 ± 0.32*	5.18 ± 0.59**	5.18 ± 0.59**	0.528 ± 0.01**	0.528 ± 0.01**
Sterile soil + bacterized seeds	97.78 ± 2.22	97.78 ± 2.22	28.09 ± 0.41*	28.09 ± 0.41*	8.65 ± 0.86	8.65 ± 0.86	0.855 ± 0.01	0.855 ± 0.01
3.0% <i>P. ultimum</i> + bacterized seeds	75.55 ± 2.22	75.55 ± 2.22*	26.30 ± 0.38	26.30 ± 0.38	7.89 ± 0.75*	7.89 ± 0.75*	0.768 ± 0.01*	0.768 ± 0.01*
Sterile soil + 1 or 2 + bacterized seeds	100.00 ± 0.00	95.55 ± 2.22	28.98 ± 0.25**	28.34 ± 0.85*	8.69 ± 0.70	8.44 ± 0.40*	0.856 ± 0.07*	0.849 ± 0.01*
3.0% <i>P. ultimum</i> + 1 or bacterized seeds	77.78 ± 2.22	82.22 ± 5.88**	27.92 ± 0.25*	26.95 ± 0.30	8.13 ± 0.60*	7.52 ± 0.25*	0.804 ± 0.02	0.723 ± 0.01
3.0% <i>P. ultimum</i> + bacterized seeds and either treated by bean straw or chicken manure	88.89 ± 4.44*	91.11 ± 2.22	28.67 ± 0.25	28.14 ± 0.60	8.57 ± 0.42	8.30 ± 0.44*	0.846 ± 0.04*	0.846 ± 0.01*
3.0% <i>P. ultimum</i> + bacterized seeds and either treated by bean straw or chicken manure + soil treated either by 1 or 2	95.55 ± 2.22*	86.67 ± 3.85*	28.78 ± 0.33	27.40 ± 0.20*	8.89 ± 0.75	8.04 ± 0.90*	0.853 ± 0.01*	0.824 ± 0.00
3.0% <i>P. ultimum</i> + 1 or 2 + <i>T. harzianum</i> + bacterized seeds	97.78 ± 2.22	97.78 ± 2.22	29.07 ± 0.36*	28.68 ± 0.20	8.96 ± 0.20	8.53 ± 0.70	0.882 ± 0.01*	0.848 ± 0.051

1: bean straw 1.5 g/kg soil
2 :chicken manure 2.0 g/kg soil
Values represent mean ± SE (* , ** significant differences at 0.001, 0.0001, respectively).

Table 2: Impact of *T. harzianum* with bean straw or chicken manure with or without seed bacterization on the incidence of damping - off disease in *Cucumis sativus*, and the estimation of some growth parameters.

Treatment	Healthy seedlings %		Seedlings height (cm)		Fresh seedling weight (g)		Dry seedling weight (g)	
	1	2	1	2	1	2	1	2
Sterile soil + sterile seeds	95.53 ± 2.22	95.53 ± 2.22	28.07 ± 0.58	28.07 ± 0.58	8.43 ± 0.50	8.43 ± 0.50	0.831 ± 0.08	0.831 ± 0.08
3.0% <i>P. ultimum</i> + sterile seeds	48.89 ± 5.88*	48.89 ± 5.88*	23.54 ± 0.37*	23.54 ± 0.37*	5.68 ± 0.16**	5.68 ± 0.16**	0.551 ± 0.02**	0.551 ± 0.02**
Sterile soil + 3.0% <i>T. harzianum</i> + sterile seeds	97.78 ± 2.22	97.78 ± 2.22	28.30 ± 0.67*	28.30 ± 0.67*	8.67 ± 0.64**	8.67 ± 0.64**	0.846 ± 0.07*	0.846 ± 0.07*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + sterile seeds	75.55 ± 2.22*	75.55 ± 2.22*	27.44 ± 0.23	27.44 ± 0.23	8.29 ± 0.12	8.29 ± 0.12	0.799 ± 0.00*	0.799 ± 0.00*
Sterile soil + 1 or 2 + 3.0% <i>T. harzianum</i>	97.78 ± 2.22	100.00 ± 0.00	29.67 ± 0.26*	29.20 ± 0.55*	9.13 ± 0.50*	8.77 ± 0.50*	0.876 ± 0.02*	0.854 ± 0.00
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 1 or 2 + sterile seeds	88.89 ± 2.22**	91.11 ± 2.22**	28.98 ± 0.07*	27.82 ± 0.32	8.22 ± 0.70*	8.43 ± 0.50	0.821 ± 0.00*	0.826 ± 0.01
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + seeds treated by bean straw or chicken manure	86.67 ± 3.85*	87.89 ± 2.85**	28.63 ± 0.70	27.56 ± 0.28*	8.11 ± 0.60	8.33 ± 0.50*	0.830 ± 0.01*	0.801 ± 0.50
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 1 or 2 + seeds treated by bean straw or chicken manure	95.56 ± 2.22	95.56 ± 2.22	29.12 ± 0.02*	28.83 ± 0.28*	8.77 ± 0.70**	8.64 ± 0.80	0.858 ± 0.01	0.872 ± 0.01*
3.0% <i>P. ultimum</i> + 1 or 2 + 3.0% <i>T. harzianum</i> + bacterized seeds	97.78 ± 2.22	97.78 ± 2.22	29.07 ± 0.36*	28.68 ± 0.20	8.96 ± 0.20	8.53 ± 0.70	0.882 ± 0.01*	0.848 ± 0.051

1 :bean straw 1.5 g/kg soil
2 :chicken manure 2.0 g/kg soil
Values represent mean ± SE (* , ** significant differences at 0.001, 0.0001, respectively).

Table 3: Effect of different treatments, for controlling, damping – off disease on the photosynthetic pigments in *Cucumis sativus* leaves (mg/g fresh weight, Mean of replicates ± SE).

Treatment	Chlorophyll A	Chlorophyll B	Carotenoids
Sterile soil + sterile seeds	15.46 ± 0.02	8.39 ± 0.02	6.92 ± 0.02
3.0% <i>P. ultimum</i> + sterile seeds	12.01 ± 0.08**	4.94 ± 0.08**	3.47 ± 0.08
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i>	15.38 ± 0.32*	7.88 ± 0.33	6.18 ± 0.02**
Sterile soil + bacterized seeds	15.64 ± 0.16	8.53 ± 0.15	6.96 ± 0.02
3.0% <i>P. ultimum</i> + bacterized seeds	14.74 ± 0.36*	7.67 ± 0.31**	6.03 ± 0.01**
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + bacterized seeds	15.12 ± 0.07*	8.05 ± 0.07*	5.82 ± 0.02**
3.0% <i>P. ultimum</i> + 1.5 g/Kg bean straw + bacterized seeds	14.85 ± 0.12**	7.68 ± 0.12**	6.62 ± 0.02*
3.0% <i>P. ultimum</i> + 2.0 g/Kg chicken manure + bacterized seeds	14.77 ± 0.34**	7.76 ± 0.34*	6.65 ± 0.02**
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 1.5 gm/Kg bean straw	15.41 ± 0.01**	8.09 ± 0.02	6.75 ± 0.02*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 2.0 gm/Kg chicken manure	15.43 ± 0.02**	8.11 ± 0.02**	6.84 ± 0.01**
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 1.5 g/Kg bean straw + bacterized seeds	15.43 ± 0.02	8.19 ± 0.02**	6.84 ± 0.02*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 2.0 g/Kg chicken manure + bacterized seeds	15.47 ± 0.02*	8.31 ± 0.01*	6.87 ± 0.02*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + the two waste products + bacterized seeds	15.52 ± 0.01**	8.92 ± 0.02**	6.97 ± 0.02**

Values represent mean ± SE (*, ** significant differences at 0.001, 0.0001, respectively).

Table 4: Effect of different treatments, for controlling damping – off disease, on some elements in *Cucumis sativus* seedling leaves mg or µg/g dry weight (Mean of replicates ± SE).

Treatment	Ca mg	K mg	Mg mg	Cu µg	Fe µg	Zn µg	Mn µg	Na µg
Sterile soil + sterile seeds	3.73± 0.05	14.15± 1.0	18.85 ± 0.50	26.59 ± 0.52	352.40 ± 14.57	292.33 ± 2.33	168.03 ± 2.00	0.90 ± 0.00
3.0% <i>P. ultimum</i> + sterile seeds	1.63 ± 0.08**	7.94± 0.3	9.34± 0.30**	15.95± 1.13**	268.37 ± 17.80*	160.77 ± 1.24**	77.12 ± 7.16**	0.70 ± 0.00**
3% <i>P. ultimum</i> + 3% <i>T. harzianum</i>	3.45 ± 0.18	12.55± 1.06**	16.28 ± 0.75	23.40 ± 2.10	301.33 ± 16.15	250.57 ± 10.08	133.67 ± 6.79	0.80 ± 0.01
Sterile soil + bacterized seeds	3.74± 0.03	14.97 ± 0.15**	19.88 ± 0.50**	30.92 ± 0.42*	498.97 ± 7.18**	284.07 ± 4.68	228.40 ± 3.68**	0.91 ± 0.00
3.0% <i>P. ultimum</i> + bacterized seeds	2.92 ± 0.14*	8.51 ± 0.05	13.03 ± 0.05**	18.24 ± 0.19**	288.17 ± 1.98**	227.07 ± 5.00*	130.10 ± 2.26	0.77 ± 0.02*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + bacterized seeds	3.78± 0.6	12.91 ± 0.12	19.11± 0.40	25.00 ± 0.60**	303.33 ± 32.77*	266.1 ± 5.60*	153.73 ± 1.09	0.81 ± 0.01*
3.0% <i>P. ultimum</i> + 1.5 g/Kg bean straw + bacterized seeds	3.34 ± 0.07	11.28 ± 0.02*	17.77 ± 0.60	23.18± 0.40*	318.07 ± 6.15	237.43 ± 1.23*	150.03 ± 2.28**	0.79 ± 0.03*
3.0% <i>P. ultimum</i> + 2.0 g/Kg chicken manure + bacterized seeds	3.47 ± 0.04*	11.32 ± 0.28	16.21 ± 0.20**	21.60 ± 0.93*	307.87 ± 4.19*	230.93 ± 1.42**	137.00 ± 2.91**	0.77 ± 0.0*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 1.5 gm/Kg bean straw	3.77 ± 0.09	12.56 ± 0.1	18.04 ± 0.10**	23.55 ± 0.08*	323.00 ± 1.15*	262.63 ± 0.92**	136.33 ± 1.65*	0.819±0.03**
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 2.0 g/Kg chicken manure	3.67 ± 0.01	12.55 ± 0.09*	17.64 ± 0.14**	23.43 ± 0.09*	318.00 ± 1.32*	257.10 ± 2.33**	132.20 ± 1.15*	0.800 ± .06**
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 1.5 g/Kg bean straw + bacterized seeds	3.82 ± 0.05	12.97 ± 0.06	19.22 ± 0.06**	24.63 ± 0.11	329.23 ± 1.15	280.0 ± 0.92*	157.67 ± 1.20*	0.866 ± 0.04*
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + 2.0 g/Kg chicken manure + bacterized seeds	3.79± 0.04	12.93± 0.09*	19.14 ± 0.29**	24.16 ± 0.12	320.33 ± 3.71*	273.43 ± 3.29*	154.67 ± 1.57**	0.848±0.05**
3.0% <i>P. ultimum</i> + 3.0% <i>T. harzianum</i> + the two waste products + bacterized seeds	3.93 ± 0.06	13.49 ± 0.07	20.35 ± 0.34	25.16 ± 0.03	341.63 ± 1.27	297.23 ± 2.89	169.47 ± 1.27*	0.906 ± 0.05

Values represent mean ± SE (*, ** significant differences at 0.001, 0.0001, respectively).

The current study showed that the tested biocontrol agents and the addition of waste products, were able of inhibiting the mycelial growth of *P. ultimum* and suppressing the cucumber damping off. Thus they could be a promising way for the biological control of plant diseases and could reduce the need of fungicides use and they are safe for the environment preservation.

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