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BIOLOGICAL CONTROL OF CUCUMBER DAMPING–OFF CAUSED BY *Pythium aphanidermatum*

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ABSTRACT

Seven treatments were applied to the soil in a greenhouse to control cucumber damping – off *Trichoderma harzianum* was used in the four of them. *T.harzianum* preparations applied to soils infested with *Pythium aphanidermatum* (natural and artificial) decreased disease incidence, severity and increased shoot dry weight. Mycelium and mycelial preparation appear to be more effective than conidia and conidial preparation. The mycelial preparation was the best in controlling the disease.

Key words: *cucumber, damping–off, Pythium aphanidermatum, Trichoderma harzianum.*

1. INTRODUCTION

Biological control of plant pathogens is becoming an important component of plant disease management practices (Cook and Baker, 1983). Different isolates of *Trichoderma* spp. appeared to be effective in controlling an array of soil-borne pathogenic fungi (Hadar *et al*., 1979; Elad *et al*., 1980; Lewis and Papavizas, 1985; Mohamed and Abu-Raya, 1993; Adm, 2000; and Al-Ameiri, 2001).

Trichoderma spp. have also been used to protect seeds of cucumber, tomato, okra and pea against *Pythium* damping–off in both green house and field trials (Hadar *et al*., 1984; Harmen and Tyler, 1988; Paulitz *et al*., 1990; Adm, 2000; and Al-Ameiri, 2001). This isolate reduced *Pythium* damping – off and colonization of cucumber root tips induced by *Pythium ultimum* (Ahmad and Baker, 1988; and Paulitz *et al*., 1990). A wide variety of *Pythium* spp. has been described from a greenhouse production systems (Van der Pleats-Nitterink, 1981). The genus of *Pythium* includes a number of readily recognized species with wide distribution and host range (McCarter and Littrell, 1970). *Pythium aphanidermatam* is one of the most serious pathogens of cucumber, tomato, and sugarbeets at the seedling stages causing both pre-and post – emergence damping – off (McCarter and Littrell, 1970; Patel and Patel, 1975; Adm, 2000; and Al-Ameiri, 2001).

P.aphanidermatum prefers temperatures between 27 and 34 °C, wet conditions and approximately 6.2 pH (Lumsden *et al*., 1976). Furthermore, the numbers of propagules are increased through August and September while decreased in March and April (Leach, 1947; and Dewan, 1994).

The objectives of this study were to assess the efficacy of biocontrol agent in controlling *Pythium* damping – off of cucumber in green house and to compare the effect of young mycelial preparation and conidial preparation of biocontrol agent in controlling *Pythium* damping – off of cucumber.

2. MATERIALS AND METHODS

Soil used in the experiment was taken from plastic house where the pathogens isolated. The soil had the following characteristics: a pH of 7.2, total N of 0.22%, P of 160 ppm, K of 350 ppm and 2.1% organic matter. The soil was divided into two parts, the first was without steaming (raw soil) natural infested (25-35 CFU / g) *Pythium aphanidermatum* and the other was steamed by autoclaving three times at 80 °C for one hour. The investigation was performed using plastic pots, 15 cm in diameter round, 500 g soil/pot.

Inoculum's isolate of *Pythium aphanidermatum* was isolated from diseased cucumber plants , grown in plastic house at Rabba Agriculture Station dead infected seedling at the stem base and rotting roots in post-emergence damping – off of cucumber seedlings rinsed in tap water for an hour, disinfected by 0.50% sodium hypochlorite, then rinsed in sterile water, dried on filter paper and then plating on PDA, identified according to Waterhouse (1968), maintained on potato

dextrose agar (PDA), the inocula were incubated under specific conditions at 22°C , 10x 3x2 cm glass tubes covered with aluminum foil.

Trichoderma harzianum was isolated from the same soil, by dilution method plated and identified according to Rifai (1969) mutagenized by benomyl tolerant as described by Ahmad and Baker (1987) and grown on (PDA) containing 10 µg a.i. benomyl /ml. Factorial arrangements (7x2) contained three replicates per treatment on the greenhouse bench in a completely randomized design. The seven treatments were; control (1) *Pythium aphanidermatum* alone, control (2) no pathogen and no biocontrol agent (3) autoclaved wheat bran 5g/ kg of soil and *Pythium aphanidermatum* (4) conidia suspension (*Trichoderma harzianum* grown on (PDA) at 25° C were harvested by scraping the surface of the colonies with a spatula and transferred to flash of water to give a spore concentration at 10^7—10^8 / ml and 40 ml/ pot added (5) conidial preparation; 150 g of wheat bran and 150 ml of water were autoclaved at 121°C for 1 h twice, cooled then 40 ml of the above conidia suspension were added directly to wheat bran and 5 g/kg of the soil were added as conidial preparation before incubation (6) mycelium; potato dextrose (PD) medium was autoclaved then cooled, seeded with 0.5 cm of *Trichoderma harzianum* disc and incubated for three days. The mycelium was filtered by filter papers type (Whatman) repeated three times, and washed to be free from nutrient before addition to the soil (5g / kg of the soil) and (7) mycelial preparation; a modification of the wheat bran culture (used as a carrier and food substance for *Trichoderma harzianum* to increase effectiveness) for the biocontrol was used (Hadar *et al*., 1979) by autoclaving wheat bran then inoculated by conidia suspension as the same concentration above, incubated for five days and then dried (at room temperature) for three days 5 g/kg of soil. Half of *Pythium aphanidermatum* dishes, 9 cm diameter, of two day old were added to pots in the sixth treatment (Saydam, *et al*., 1973). Two days later, the soils were inoculated with biocontrol agents. After one day, ten cucumber (*Cucumis sativus*) variety (beit alpha) seeds were planted in pots and watered daily. Seven days later, the number of seeds, which did not germinate, was calculated as a pre – emergence damping – off. The damping – off. Post – emergence damping – off were after two weeks (number of dead seeds (pre-emer.) or dead and infected seedling (post-emer.) / total number per pot x 100%), and the seedlings were thinned to one/pot. Damping – off seedlings were planted on (PDA) routinely to identify the pathogen. Disease severity, incidence and shoot dry weights were recorded. Disease severity was recorded on a scale of (0 – 3) where 0, healthy seedling, 1 diseased seedling (infected seedling and did not die during experiment time lesion appeared brown in color at lower stem near the soil line), 2 dead post - emergence and 3 dead pre-emergence.

3. RESULTS

Addition of *Trichoderma harzianum* to the soil with all treatments significantly reduced pre- and post – emergence damping–off of cucumber compared to *Pythium aphanidermatum* alone (Table 1) . In the steamed soil, pre- emergence damping – off with the pathogen reached 70% while in raw soil it was considerably less. Mycelium and mycelial preparation reduced disease incidence at pre -and post –emergence than 100% higher than conidia and conidial preparation. In the raw soil, pre-emergence disease pressures were lower than in the steamed soil. The combination of wheat bran and pathogen significantly increased disease incidence in pre-emergence damping – off at steamed soil if compared with raw soil (53.33% , 30%) while no significant differences were observed at post – emergence between the two soils (33.33% , 26.076%; respectively) (Table 1). Mycelial preparation significantly reduced pre– damping – off incidence than conidia and conidial preparation, with most of the reduction occurred in post –emergence (Table 1).

Disease severity significantly increased with *Pythium aphanidermatum* only or with wheat bran added in artificial infested and raw soils more than *Trichoderma harzianum* treatments except conidia treatment in raw soil (Table 2). There was no significant difference between *Trichoderma harzianum* added in raw and steamed soil but the severity increased at raw soil except conidia treatment. In the raw soil, severity becomes less significant at pathogen only or with wheat bran than steamed soil. Mycelial preparation was significant by reduced infection more than conidia and conidial preparation in decreasing disease severity while no significant difference with mycelium treatment was detected (Table 2).

Trichoderma harzianum increased shoots dry weight at all types of application. *T. harzianum* added to raw and steamed soil significantly increased dry weight than treatment of *Pythium*

Table (1): Effect of *Trichoderma harzianum* **preparation on pythium pre and post- emergence damping – off percentage of cucumber planted in soil infested with** *Pythium aphanidermatum***.**

Treatments	Steamed soil (Artificially infested)		Raw soil (Naturally infested)*	
	Pre-emergence damping-off	Post-emergence damping-off	Pre-emergence damping-off	Post-emergence damping-off
Pythium aphanidermatum	70.00 ** a	16.67 ghi	40.00 c	33.33 cde
Control	0.00 k	0.00 k	16.67 cd	26.67 defg
Wheat Bran	53.33 b	33.33 cde	30.00 cdef	26067 defg
Conidia	33.33 cde	23.33 igk	26.67 ghi	16.67 ghi
Conidial Preparation	20.00 fgh	6.667 igk	20.00 fgh	13.33 hij
Mycelium	16.67 ghi	6.667 igk	13.33 hij	6.667 ijk
Mycelial Preparation	6.667 ijk	3.333 jk	13.33 hij	6.667 ijk

* Naturally - infested with *Pythium aphanidermatum* (25-35 CFU/g)

** Each number is the mean of three replicates, numbers in the table followed by the same letter do not significantly differ from each other according to Duncan s multiple range test, p= 0.05.

Table (2): Effect of *Trichoderma harzianum* **preparation on disease severity of cucumber planted in soil infested with** *Pythium aphanidermatum.*

Treatments	Steamed Soil (Artificially Infested)	Raw Soil (Naturally Infested)*
Pythium aphanidermatum	0.8557** a	0.5443 b
Control	0.000 g	0.2337 def
Wheat Bran	0.8337 a	0.6223 b
Conidia	0.555 b	0.4777 bc
Conidial Preparation	0.323 cd	0.3777 cd
Mycelium	0.2147 def	0.289 de
Mycelial Preparation	0.0890 fg	0.1557 efg

* Naturally - infested with *Pythium aphanidermatum* (25-35 CFU/g)

** Each number is the mean of three replicates, numbers in the table followed by the same letter do not significantly differ from each other according to Duncan s multiple range test, p= 0.05.

Table (3): Effect of *Trichoderma harzianum* preparation on shoot dry weight of (g/ plant) Cucumber planted in soil infested with *Pythium aphanidermatum* **.**

Treatments	Steamed Soil (Artificial Infested)	Raw Soil (Natural Infested)*
Pythium aphanidermatum	0.280** e	0.283 e
Control	0.4033 cd	0.373 de
Wheat Bran	0.3167 de	0.3067 de
Conidia	0.3967 cd	0.4067 cd
Conidial Preparation	0.490 bc	0.5067 b
Mycelium	0.540 b	0.454 b
Mycelial Preparation	0.6707 a	0.673 a

* Naturally - infested with *Pythium aphanidermatum* (25-35 CFU/g)

** Each number is the mean of three replicates, numbers in the table followed by the same letter do not significantly differ from each other according to Duncan s multiple range test, p= 0.05.

aphanidermatum alone (Table 3).When the soils were amended with wheat bran, *T. harzianum* enhanced shoot biomass in both steamed and raw soils. T. *harzianum* application significant increased dry weight than treatment of *Pythium aphanidermatum* alone or with wheat bran except conidia treatment. Under the same treatment of *Trichoderma harzianum*, no significant differences were found between the two soils, and there were no significant differences between the control and wheat bran.

4. DISCUSSION

The results show that *Trichoderma harzianum* applied to the soil (steamed and raw) infested with *Pythium aphanidermatum* decreased disease incidence, severity and increase shoot dry weight (Tables 1, 2 and 3). These results are in agreement

with those of (Hadar *et al*., 1979; Harmen and Taylor, 1988; Paulitz *et al.*, 1990; Adm, 2000 and Al-Ameiri, 2001). *Trichoderma spp*. applied to the soil as mycelium and mycelial preparation decreased disease incidence and severity more than conidia and conidial preparation. This is attributed to that they are containing young active growing hyphae and abundant of chlamydospores, the hyphae already occupying the food base do not appear to be subjected to fungistases and that direct contact between mycelium and food base enable the antagonistic to grow through the soil (Lewis and Papavizas, 1983,1984 and 1985 ; Papavizas *et al*., 1984; and Al-Ameiri, 2001). Conidia of *Trichoderma* spp were used in disease control because they are abundantly produced on agar media and easy to handle. The results indicated that the conidia added to the soil from fresh culture with or without wheat bran were ineffective in decreasing the disease. They may need time to germinate to produce hyphae that are able to parasite and destroy the pathogen and leach by irrigation through the soil pores compared to mycelium .These results are in agreement with the results of Lewis and Papavizas, (1984) and Al-Ameiri, (2001). Suppression of the disease in raw soil occurred in absence of *Trichoderma harzianum* may be due to germination lyses of a known bacteria or fungal antagonist (Tsao and Oster, 1981; and Paulitz *et al*., 1990).

These results showed that the increase in disease incidence and severity occurred with the application of wheat bran to infested soil. This means that the pathogen utilized the food base (wheat bran) and increased its population because of the ability of *Pythium* spp.to live as saprophyte fungi and increase infections of hosts (Holmes *et al*., 1998; and Al-Amieri,2001).

The use of a bioagent also resulted in an increase of shoot dry weight in steamed and raw soils with or without wheat bran, this means that the bioagent is able to increase plant height and weight through protection from infection with the pathogens (Paulitz *et al*., 1990; Mohamed and Abo-Raya, 1993; and Adm, 2000).

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المقاومة الإحيائية لموت الخيار المفاجئ والمتسبب عن الفطر بيثيوم *Pythium aphanidermatum*

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ملخص

اجري هذا البحث بهدف دراسة كفاءة المقاوم الإحيائي تريكوديرما هريزانم في المقاومة و طرق إضافته إلى تربة ملوثة طبيعيا وصناعيا بالفطر بيثيوم افانيديرماتم المسبب للموت المفاجئ للخيار. وقد تمت إضافة المقاوم بشكل معلق أبواغ ومعلق أبواغ مع نخالة الحنطة مضافا إلى التربة بدون حضانة وغزل فطري وغزل فطري مربى على نخالة الحنطة . أظهر المقاوم الإحيائي فعالية عالية في مقاومة المرض. وأظهرت جميع طرق الاضاف' للمقاوم كفاءة في خفض كل من نسبة وشدة المرض معنويا وزيادة معنوية في الوزن الجاف. وان إضافة نخالة الحنطة زادت في كفائة المقاوم مقارنة مع عدم إضافتها .وأثبتت معاملة المقاوم المربى على نخالة الحنطة(غزل فطري) أفضل المعاملات في مقاومة المرض وزيادة الوزن الجاف للنبات.

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