Minufiya J. Agric. Res. Vol. 32 No.2: 359 - 378 (2007)

IMPROVEMENT OF ANTAGONISTIC ABILITY OF *Trichoderma hamatum* MOUNTED IN DIFFERENT CARRIERS TO CONTROL ROOT ROT OF FABA BEAN

G. A. Amer[(1)] and Rania Z. El-Shennawy[(2)]
(1)- Agric. Bot. Dept., Fac. of Agric., Minufiya Univ., Shibin El-Kom, Egypt.
(2)- Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt.

(Received: Feb. 18, 2007)

ABSTRACT: ***This study was conducted to improve the antagonistic ability of T. hamatum by inducing mutants and mounted in different carriers to control root rot disease of faba bean. All mutants of T. hamatum were able to suppress the radial growth of Rhizoctonia solani, Fusarium solani and Pythium ultimum compared with the wild type in vitro. The survivability of T. hamatum wild type or mutant (9) at room temperature differed in colony forming units (cfu) according to the different organic matters or carriers used. Using preparations of the antagonistic fungus mounted on wheat bran or sugar can bagasse as well as rice flour supported the maximum colony forming units (cfu) of T. hamatum wild type or mutant (9). Different organic matters or carriers used differed in their efficiency for control of damping-off and root rot of faba bean. Coating faba bean seeds with different powder formulation of T. hamatum wild type or mutant (9) were more effective than soil treatment and reduced damping-off and root rot severity index (DSI).***

Key words: ***Faba bean, root rot, biological control, T. hamatum, mutant, organic matter and carriers.***

INTRODUCTION

Biocontrol by implies application of a microbial preparation to either soil, seed or phyllosphere was used to prevent plant infection by a pathogen (Cook and Baker, 1983). Chemical pesticides have been used for pest control in agriculture. Recently, however due biologically based pest management has become a very important research area (Cook and Baker, 1983; Leathers *et al*, 1993). The control of phytopathogens by the introduction of selected microbial antagonists has been studied widely but has resulted in little commercial success.

The first requirement for successful biological control is that a highly effective strain be identified and employed (Pe'er and Chet, 1990; Harman and Stasz, 1991). Thus the uses of biocontrol agents become important to integrate with fungicides in plant disease control program in order to reduce the quantity of fungicide used. The most commonly used biocontrol agent is *Tricoderma* spp. (Papavizas and Lumsden, 1980; Papavizas, 1985). However, available sources for biocontrol are currently inadequate and need further

improvement. The efficiency of fungal biocontrol agents can be improved by inducing stable tolerant mutants to the third-generation fungicide that are routinely used in agriculture. Abd El-Moity *et al.* (1982) developed a *Trichoderma* strain resistant to iprodione. Similarly, *Trichoderma* strain resistant to benomyl for control of *Rhizoctonia solani* in cotton was produced by Papavizas *et al. (*1982). *Trichoderma* isolates tolerant to Metataxyl for controlling of *Pythium aphanidermatum* in sugarbeet, tobacco, tomato and brinjal have been developed by Mukhopadhyay *et al*. (1986). Ahmad and Baker (1987) developed a benomyl tolerant mutant of *T. harzianum* which was rhizosphere competent when benomyl was added at 10 µg a.i. / g of soil.

Thereafter, several attemps were made to improve the fungicide tolerance of fungal biocontrol agents by Mutaition. Ultraviolet (UV) radiation or alternative exposures to UV and Ethyl methanesulfonate (EMS) were used to mutagenize the biocontrol fungi, but failed to generate stable tolerant mutants (Papavizas *et al.,* 1990). Kay and Stewart (1994) isolate iprodione-tolerant mutants for *Chaetomium globosum*, *T. hariziamm*, *T. viride* and *Trichoderma* sp. by mediation of conidia with UV light. They failed to generate benomyl-tolerant mutants. Salama and Amer (1996) are able to induce stable mutants to *Trichoderma lignorum* tolerant to benemyl and iprodione.

The aim of the present study was to improve the antagonistic ability of *T. hamatum* by inducing mutants and mounting in different carriers to control root rot disease of faba bean.

MATERIALS AND METHODS

Induction of *T. hamatum* biotypes tolerant to Topsin-M fungicide:

Conidia were produced by growing the wild type strain of *T. hamatum* on PDA for 6 days; conidia were removed from the agar surface by pipetting 5 ml of sterile distilled water onto the surface and gently rubbing the surface with a sterile cotton-tipped applicator. Aqueous suspensions of conidia were centrifuged twice at high speed and washed in 0.067 M phosphate buffer (pH 7.0). After the second washing, the pellets were re-suspended in 4.0 ml phosphate buffer. One milliliter of condial suspension was added to a sterile 200 ml erlenmeyer flask and 41.8 ml, of 0.2 M ethyl methane sulfonate (EMS) in 1.0 ml of 0.067 M phosphate buffer was added to the spore suspension for a final condial concentration of 2 x 10^2/ml. The mixture was incubated at 25°C in a water-bath shaker (150 rpm). After 1 h of incubation, 2.0 ml of 0.3 M $Na_2S_2O_3$ was added to the conidial EMS mixtures to denature the mutagen. Then the procedure for obtaining mutants was used according to Papavizas *et al.* (1990).

Effect of selected mutants on the radial growth of the pathogens:

The procedure used for the antagonistic studies was used to evaluate the antagonistic studies between the selected mutants and the pathogens. Radial growth of the pathogens was recorded and the percentage of reduction in growth was pooled out using the formula:

Reduction% = (Contrl – Treatmen / Control) x 100.

1. Effect of some organic matters on the growth of *T. hamatum* wild type and mutant (9):

The most effective mutants *in vitro* M (9) and the wild type (W.T.) were used to study the effect of some organic matters on mass production of the antagonists.

Six different cheap substances of agriculture products namely rice husks, wheat bran, sugarcane bagasse, saw dust, Farm Yard Manure (FYM) and peat soil were used for growth and multiplication of the selected antagonists. Sugarcane bagasse was crushed into small pieces 0.5 - 1 cm long, the remaining materials were sift through 1mm^2 mesh. Moisture was adjusted to 50% (w/v) for all substrate. They were packed in polypropylene bags (500g each) that were sealed and autoclaved for 2 h on two successive days. Each bag was inoculated with two mycelial disks (9 mm) that were cut from the periphery of the antagonist's culture grown previously on PDA medium for three days. The bags were sealed aseptically and incubated at room temperature under alternating 12 h light and darkness.

One gram of each substrate for each of *T. hamatum* or Mutant (9) was drawn aseptically after through mixing 15, 30, 45 and 60 days after incubation, then successive dilutions were prepared in water and the number of colony forming units (cfu) were counted using the dilution plate technique on PDA medium.

2. Effect of different carriers of *T.hamatum* wild type and mutant (9) on their survival ability:

The formulations of biocontrol agents are the most important issue for modern agriculture to minimize the quantities of biocontrol agents required for field application, the study was conducted to formulate the wild types of *T. hamatum* and its mutant (9) to investigate the effect of formulates on their survival ability under field conditions.

The strains were cultured on PDA plates for 7 days at 25°C. Conidia along with mycelidl mates were removed from the agar surface by pipetting 5 ml of sterile distilled water onto the surface and gently rubbing the surface with sterile cotton tipped applicator, 20 plates were used for each strain and combined in sterile 250 ml flasks. Conidia of each strain were counted using haemocytometer and the suspensions were adjusted with distilled water to

provide the desired concentration of condia to contain about 2 x 1 0^9 condia /ml.

The conidial suspension for each strain was incorporated into the different carriers used namely Rice flour, Talc powder (commercial grade) and Vermiculite. The spore suspension was mixed with the carriers as follow:

1. **Talc powder (50 g) + 25 ml spore suspension + 0.5 gm carboxy methyl cellulose (CMC).**
2. **Rice flour (50 g) +40 ml spore suspension + 0.5 gm CMC.**
3. **Vermiculite (50 g) + 35 ml spore suspension + 0.5 gm CMC.**

The contents were homogenized under aseptic conditions in laminar flow; the contents of each material for each strain were packed in polythene bags (250 g each), sealed and stored under room temperature.

For studying the survival ability in the carrier, one gram of the carrier was drawn aseptically at 15, 30, 45 and 60 days after storage. The colony forming units (cfu) were assessed by serial dilution technique.

Sterilized bags were inoculated with mycelial disks of actively growing antagonists, 7 bags were inoculated by each particular antagonist. The inoculated bags were incubated for 15 days until the growth filled the medium. The multiplied inocula of the antagonist were applied at the rate of 10 g/kg of pot culture soil pre-inoculated with root rot pathogens in 25 cm diameter pots.

Pots inoculated with or without the pathogen only served as control. Surface sterilized faba bean seeds cv. G-716 was sown at the rate of 7 seeds /pot.

3. Effect of Topsin-M induced mutants of *T. hamatum* alone or combined with Topsin-M on the pathogens:

The effect of selected Topsin-M induced mutants of *T. hamatum* as compared to its wild type strain and the fungicide Topsin-M on disease severity index was carried out under greenhouse conditions.

The selected mutants and its wild type inoculum were prepared on (sand: wheat bran medium as indicated before with fungal antagonist's inoculum. The wild type and selected mutants were applied as follows:

1. **Applied to the soil at the rate of 10 g/kg soil and surface sterilized seeds were sown.**
2. **Applied to the soil at the same rate and the sown seeds were pre-dressed with the fungicide Topsin-M at the rate of 2.5 g / kg seeds.**
3. **Soil without any antagonists and the seeds were pre dressed with the fungicide Topsin-M at the same rate. Pots inoculated with or without pathogens alone or pathogens mixture served as control and sown with only surface sterilized seeds.**

4. Effect of soil treatment with *T. hamatum* mutant (9) and its wild type multiplied on organic matters on the pathogens:

The inoculum was multiplied as mentioned earlier on the effect of organic matters on the growth of *T. hamatum.* The soil was inoculated by each particular treatment at the rate of 10 g / kg soil of each particular organic matter. The inoculated pots were sown by 7 surface sterilized seeds, also pots inoculated with or without pathogens alone served as control.

5. Effect of seed dressing with T. *hamatum* and mutant (9) mounted on different carriers on the pathogens:

The inoculum was prepared as indicated before in studying the effect of different carriers on the survive ability of *T. hamatum* under laboratory conditions. The faba bean seeds were coated by each particular carrier at the rate of 4 g/kg seeds as slurry. The slurry was prepared by mixing 4 gm of each particular carrier-based formulation in 25 ml of water. The seeds were mixed with the slurries each alone, the seeds were then air dried in shadow for over night, the treated seed were sown (7 seeds per pot) in pathogen inoculated soil.

6. Effect of soil treatment with T. *hamatum* and Mutant (9) mounted on different carriers on the pathogens:

The powder formulations of *T. hamatum* wild type and mutant (9) were investigated under greenhouse conditions for their efficacy on root rot disease incidence of faba bean. The different formulations were incorporated into potting soil pre-inoculated with individual root rot pathogens. The particular formula was applied at the rate of 1% (w / w). Pots inoculated or uninoculated with the pathogen served as control. Surface sterilized faba bean seeds cultivar G-716 was sown at the rate of 7 seeds per pot. Three pots were used for each particular treatment.

Disease assessment:

Disease severity index was recorded according to Soleman *et al.* (1988).

RESULTS:

1. Effect of different mutants of *T. hamatum* on the radial growth of the pathogenic fungi:

Results showing the effect of different mutants of *T. hamatum* on the radial growth of the pathogenic fungi are presented in Table (1). It is clear that all these mutants are able to suppress the radial growth of *Rhizoctonia solani, Fusarium solani* and *Pythium* sp. more than the wild type of *T. hamatum.*

Table (1): Effect of different mutants of *T. hamatum* on the radial growth of *R. solani, F. solani* and *Pythium* sp., the causal organisms of root rot of faba bean.

Mutant	*Rhizoctonia solani*		*Fusarium solani*		*Pythium* sp.	
	R. growth (mm)	Reduction %	R. growth (mm)	Reduction %	R. growth (mm)	Reduction %
T.hamatum (W.T.)	25.0	71.5	28.7	67.5	25.3	71.5
Mutant (1)	23.2	73.5	22.7	74.3	25.0	71.8
Mutant (2)	25.7	70.7	25.3	71.3	27.7	68.8
Mutant (3)	30.7	64.9	24.7	72.0	27.3	69.2
Mutant (4)	26.3	70.0	25.0	71.7	28.7	67.6
Mutant (5)	25.4	71.1	29.0	67.2	27.3	69.2
Mutant (6)	18.7	78.8	24.7	72.0	27.7	68.8
Mutant (7)	31.5	64.1	25.3	71.3	32.0	63.9
Mutant (8)	28.2	67.8	26.7	69.8	31.0	65.1
Mutant (9)	18.1	79.4	23.3	73.6	25.3	71.5
Control	87.7		88.3		88.7	

W.T = wild type of *T. hamatum*
Mutant (1) = EMs - u v - F 10/11
Mutant (2) = EMs - u v - F 25/1
Mutant (3) = EMs - u v - F 25/6
Mutant (4) = EMs - u v - F 25/9
Mutant (5) = EMs - u v - F 25/10
Mutant (6) = EMs - u v - F 50/1
Mutant (7) = EMs - u v - F 50/2
Mutant (8) = EMs - u v - F 50/3
Mutant (9) = EMs - u v - F 50/5

All the 9 UV induced mutants gave more inhibition than the wild type of *T. hamatum.* Mutant (1) of *T. hamatum* showed greater inhibition of the growth of the three causal organisms (74.3% for Fusarium, 73.5% for Rhizoctonia and 71.8% for Pythium) than other mutants. Mutant (5) of *T. hamatum* came in the second rank for inhibiting the linear growth of Rhizoctonia, while mutant (9) came in the second rank for inhibiting the linear growth of Fusarium and Pythium.

2- Effect of organic matters on the survival of *T.hamatum*:

Results concerning the effect of different organic matters as a carrier for *T. hamatum* (W.T.) are presented in Table (2). It can be concluded that the survival ability of the antagonistic fungi differed according to the carrier. Data revealed also that wheat bran substrate supported the maximum colony forming units (cfu) of *T. hamatum* followed by sugar cane baggase. On the other hand, Peat soil followed by farm yard manure substrates inhibited the cfu of *T. hamatum.*

Table (2): Effect of different organic matters on the growth and survival ability of *T. hamatum* (W.T.) after 15-60 days from inoculations under room conditions.

Wild type of *T.hamatum* + Organic matter	cfu of *T. hamatum* (W.T) /g after inoculations			
	1 5 days	30 days	45 days	60 days
Farm yard manure (FYM)	3.0×10^7	2.3×10^6	2.0×10^6	11.2×10^5
Saw dust	4.0×10^7	3.0×10^6	2.2×10^6	13.7×10^5
Wheat bran	8.3×10^7	10.7×10^6	15.0×10^6	19.0×10^5
Sugar cane baggase	7.7×10^7	9.0×10^6	14.0×10^6	11.3×10^5
Rice husks	1.3×10^7	8.7×10^6	13.7×10^6	13.3×10^5
Peat soil	4.0×10^7	3.3×10^6	3.2×10^6	12.3×10^5

3. Effect of organic matters on the survival of Mutant (9) of *T.hamatum*:

Data in Table (3) show the effect of different organic matters on the growth of Mutant (9) of *T.hamatum* and its survival ability under room conditions. Results indicate that wheat bran substrate followed by sugar cane baggase supported the maximum cfu of Mutant (9) of *T. hamatum* up to 60 days after inoculation. On the contrary, Peat soil and farm yard manure inhibited the cfu of mutant (9) of *T. hamatum* up to 60 days after inoculation. On the contrary, peat soil and farm yard manure inhibited the cfu of mutant (9) of *T. hamatum.*

Out of results shown in Tables (2 and 3), it can be concluded that using a preparation of the antagonistic fungus (*T. hamatum*) contain wheat bran or

sugar cane baggase led to more reduction in the growth of root rot fungi of faba bean.

Table (3): Effect of different organic matters on the growth and survival ability of Mutant (9) of *T. hamatum* after 15-60 days from inoculation under room conditions.

Mutant (9) of *T.hamatum* + Organic matter	cfu of *T.hamatum* /g after inoculations			
	15 days	30 days	45 days	60 days
Farm yard manure (FYM)	1.3×10^7	2.3×10^6	4.0×10^6	16.3×10^5
Saw dust	6.0×10^7	3.3×10^6	3.1×10^6	10.3×10^5
Wheat bran	6.7×10^7	11.3×10^6	13.7×10^6	18.3×10^5
Sugar cane baggase	6.5×10^7	11×10^6	12.0×10^6	19.7×10^5
Rice husks	5.0×10^7	9.7×10^6	13.7×10^6	12.3×10^5
Peat soil	2.0×10^7	4.0×10^6	3.4×10^6	11.7×10^5

Mutant (9) of *T. hamatum* = EMs - U V - F 50/5.

4. Effect of different carriers on the survival ability of W.T of *T.hamatum*:

The effect of different carriers on the survival ability of the wild type of *T. hamatum* is presented in Table (4). Results show that the survival ability of the antagonist (wild type of *T. hamatum*) differed according to the carrier substrates. It is evident that using Rice flour as antagonist-carrier promotes the cfu of the antagonist up to 60 days from inoculation consequently will be more effective in controlling the pathogens. Talc powder takes the second rank in this respect. On the other hand, vermiculite as antagonist carrier inhibits the cfu of the antagonist.

Table (4): Effect of different carriers on the survival ability of *T. hamatum* after 15-60 days from inoculation under room conditions.

T. hamatum (WT) + carrier	cfu of *T. hamatum* /g after inoculation			
	15 days	30 days	45 days	60 days
Talc powder	6.3×10^7	10.3×10^6	10.0×10^6	6.5×10^5
Rice flour	8.0×10^7	11.0×10^6	12.3×10^6	9.0×10^s
Vermiculite	2.0×10^7	4.0×10^6	6.3×10^6	3.0×10^5

5. Effect of different carriers on the survival ability of Mutant (9) of *T.hamatum*:

The effect of different carriers on the survival ability of Mutant (9) of *T. hamatum* is presented in Table (5). It can be noticed that Rice flour promotes cfu of Mutant (9) of *T. hamatum* up to 60 days from inoculation; vice versa vermiculite substrate as fungal carrier inhibits these cfu.

Table (5): Effect of different carriers on the survival ability of *T. hamatum* after 15-60 days from inoculation under room conditions.

Mutant (9) of *T. hamatum* + carrier	cfu of *T. hamatum* /gm after inoculation			
	15 days	30 days	45 days	60 days
Talc powder	5.7×10^7	9.0×10^6	12.7×10^6	13.0×10^5
Rice flour	6.3×10^7	11.3×10^6	14.0×10^6	19.0×10^5
Vermiculite	2.0×10^7	2.7×10^6	9.0×10^6	7.2×10^5

Mutant (9) of T.hamatum = EMs - U V - F 50/5.

6. Effect of coating seeds with *T.hamatum* mounted on different carriers on the damping off of faba bean:

To study the effect of *T.hamatum* mounted on different carriers on damping off of faba bean plants, the seeds was coated with the powder formulation of the different carriers.

a. Wild type of *T. hamatum*:

Data presented in Table (6) indicate that in soil infested with *R. solani,* coating the seeds with rice flour decreased the damping off (12.3%) followed by talc powder (14.3%), compared to control (28.6%). In soil infested with *F. solani,* coating the seeds with rice flour and vermiculite formations decreased the damping off (14.3%) followed by talc powder formulation (18.6%) compared to control (28.6%). In soil infested with *Pythium* sp., coating the seeds with rice flour formulation reduced the clamping off (10.0%) followed by vermiculite (14.3%) compared to control treatment (67.1%).

Table (6): Effect of coating seeds with *T.hamatum* (W.T) mounted on different carriers on damping off of faba bean plants under greenhouse conditions.

W.T.+ carrier	*Rhizoctonia solani*		*Fusarium solani*		*Pythium* sp.	
	Damping-off%	Survival %	Damping-off%	Survival %	Damping-off%	Survival %
Talc powder	14.3	85.7	18.6	81.4	18.6	81.4
Rice flour	12.3	87.7	14.3	85.7	10.0	90.0
Vermiculite	18.6	81.4	14.3	85.7	14.3	85.7
Control (infested)	28.6	71.4	28.6	71.4	67.1	32.9
Control (uninfested)	4.3	95.7	10.0	90.0	0.0	100.0

W.T = wild type of *T.hamatum*.

b. Mutant (9) of *T.hamatum*:

Data presented in (Table 7) indicate that, in soil infested with *R. solani,* coating the seeds with rice flour was the most effective method against the damping off (8.9%) followed by talc powder and vermiculite (10.0%). In soil infested with *Fusarium solani,* coating seeds with rice flour formulation of Mutant (9) reduced the damping off (18.6%) followed by talc powder or vermiculite formulations (24.3% for both) compared to the control treatment (28.6%). In soil infested with *Pythium* sp., coating the seeds with talc powder on rice flour formulations of Mutant (9) of *T.hamatum* reduced the damping off of faba bean (14.3% for both carriers) followed by vermiculite (18.6%) compared to the control (67.1%).

Table (7): Effect of coating seeds with Mutant (9) of *T.hamatum* mounted on different carriers on damping off of faba bean plants under greenhouse conditions.

Mutant (9) + carrier	*Rhizoctonia solani*		*Fusarium solani*		*Pythium* sp.	
	Damping-off%	Survival %	Damping-off%	Survival %	Damping-off%	Survival %
Talc powder	10.0	90.0	24.3	75.7	14.3	85.7
Rice flour	8.9	91.1	18.6	81.4	14.3	85.7
Vermiculite	10.0	90.0	24.3	75.7	18.6	81.4
Control (infested)	28.6	71.4	28.6	71.4	67.1	32.9
Control (uninfested)	4.3	95.7	10.0	90.0	0.0	100.0

Mutant (9) = wild type of *T.hamatum*.

7. Effect of treating soil with *T.hamatum* mounted on different carriers on the damping off of faba bean:

To study the effect of powder formulations of *T. hamatum* on the damping off of faba bean, the soil was treated with the different powder formulations of *T. hamatum* on different carriers.

a. Wild type of *T.hamatum*:

Data presented in Table (8) indicate that, treating the soil infested with *R. solani,* by rice flour formulation reduced the damping off of faba bean plants (8.6%) and increased the percentage of survived plants (91.4%) followed by talc powder formulation (14.2% damping off and 85.8% survived plants) compared to the control Treating the soil infested with *Fusarium* sp., rice flour carrier reduced the damping off (12.9%) and increased the survived plants (87.1%) followed by talc powder formulation (17.1% damping off and 82.9% survived plants).

In soil infested with Pythium sp., treating the soil with rice flour formulation of T. hamatum reduced the damping off (20.9%) and increased the survived plants (79.1%) followed by talc powder (damping off 24.3% and 75.7% survived plants).

Table (8): Effect of treating soil with *T. hamatum* (W.T) mounted on different carriers on damping off of faba bean plants under greenhouse conditions.

T. hamatum (WT) + carrier	*Rhizoctonia solani*		*Fusarium solani*		*Pythium* sp.	
	Damping-off%	Survival %	Damping-off%	Survival %	Damping-off%	Survival %
Talc powder	14.2	85.8	17.1	82.9	243	75.7
Rice flour	8.6	91.4	12.9	87.1	20.9	79.1
Vermiculite	18.6	81.4	18.6	81.4	28.6	71.4
Control (infested)	28.6	71.4	28.6	71.4	67.1	32.9
Control (uninfested)	4.3	95.7	10.0	90.0	0.0	100.0

W.T = wild type of *T.hamatum*.

b. Mutant (9) of T.hamatum:

Data dealing with the effect of Mutant (9) of T.hamatum mounted on different carriers on the damping off of faba bean are presented in Table (9).

In soil infested with R. solani, the application of rice flour formulation decreased the damping off (14.3%) consequently increased the survived plant (85.7%) followed by talc powder formulation compared to the control. With F. solani infested soil, the most effective powder formulation in reducing the damping off was rice flour (12.6%) followed by vermiculite (14.3%).

Table (9): Effect of treating soil with Mutant (9) of *T.hamatum* mounted on different carriers on damping off of faba bean plants under greenhouse conditions.

Mutant (9) + carrier	*Rhizoctonia solani*		*Fusarium solani*		*Pythium* sp.	
	Damping-off%	Survival %	Damping-off%	Survival %	Damping -off%	Survival %
Talc powder	14.3	85.7	32.9	67.1	38.6	61.4
Rice flour	14.3	85.7	12.6	87.4	22.9	77.1
Vermiculite	18.6	81.4	14.3	85.7	24.3	75.7
Control (infested)	28.6	71.4	38.6	61.4	67.1	32.9
Control (uninfested)	4.3	95.7	10.0	90.0	0.0	100.0

Mutant (9) of *T. hamatum* = EMs - U V - F 50/5

In soil infested with Pythium sp., rice flour formulation was the most effective one (22.9%) followed by vermiculite (24.3%) compared to control (67.1%).

8. Effect of coating seeds with T. hamatum mounted on different carriers on the root rot of faba bean:

To study the effect of T.hamatum powder formulations on root rot severity, the seeds were coated with the different carrier's formulations and sown in pathogens infested soil.

a. Wild type of T. hamatum:

Data presented in Table (10) indicate that the seed coating with the different powder formulations of the biocontrol agent T.hamatum (W.T.) reduced the root rot severity in all pathogens infested soil.

Rice flour powder formulation was the most effective one in reducing root rot. It reduced the disease seventy indexes to 9.8% followed by vermiculite formulation which gave 15.1% DSI%. Talc powder formulation was the least effective one in reducing root rot severity (19.9%) compared to the infested control (66.4% disease severity).

Table (10): Effect of coating seeds with *T.hamatum* (W.T) mounted on different carriers on root-rot of faba bean under greenhouse conditions.

W.T+ carrier	Percentage of disease severity index (DSI %)			
	R. solani	*F.solani*	*Pythium* sp.	Pooled aver.
Talc powder	19.3	22.2	18.3	19.9
Rice flour	11.7	10.5	7.2	9.8
Vermiculite	16.7	17.1	11.4	15.1
Control (infested)	58.0	70.2	71.1	66.4
Control (uninfested)	42.0	41.7	41.1	41.6

W.T = wild type = *T. hamatum*.

b. Mutant (9) of T. hamatum:

The effect of seed coating with Mutant (9) of T. hamatum is shown in Table (11) resulted a clear reduction in the percentage of disease severity index. In

all pathogens infested the soil, the most effective one in reducing root rot was rice flour powder carrier (9.6%) followed by vermiculite (13.6%).

Regarding the pooled average, it is clear that rice flour formulation gave the lowest percentage of DSI (9.6%), followed by vermiculite carrier (13.6%). Talc powder carrier gave the highest percentage of DSI (18.4%) compared to the infested control (66.4%).

Table (11): Effect of coating seeds with mutant (9) of *T.hamatum* mounted on different carriers on root-rot of faba bean under greenhouse conditions.

Mutant (9)+ carrier	Percentage of disease severity index (DSI %)			
	R. solani	*F.solani*	*Pythium* sp.	Pooled aver.
Talc powder	18.4	22.0	14.8	18.4
Rice flour	9.2	10.3	9.3	9.6
Vermiculite	14.5	16.3	9.9	13.6
Control (infested)	58.0	70.2	71.1	66.4
Control (uninfested)	42.0	41.7	41.1	41.6

Mutant (9) of *T. hamatum* = EMs - U V - F 50/5

9. Effect of treating soil with T.hamatum mounted on different carriers on the root rot of faba bean:

The soil was treated with the different powder formulations of T.hamatum on different carriers.

a. Wild type of T.hamatum:

The results of soil treatment with T.hamatum (W.T.) on the severity of root rot of faba bean are presented in Table (12).

Data indicate that the application of biocontrol agent formula to the soil decreased the disease severity in all soil infested pathogens.

Regarding the pooled average of disease severity index of the three pathogenic fungi, rice flour formulation gave the lowest disease severity index (7.5%) followed by vermiculite (10.3%). Talc powder formulation came at the last rank (13.6%) in comparison with infested control treatment (66.4%).

Table (12): Effect of treating soil with T. hamatum (W.T) mounted on different carriers on root-rot of faba bean under greenhouse conditions.

W.T+ carrier	Percentage of disease severity index (DSI %)			
	R. solani	*F. solani*	*Pythium* sp.	Pooled aver.
Talc powder	12.5	12.5	15.7	13.6
Rice flour	7.2	8.3	7.1	7.5
Vermiculite	8.8	10.9	11.1	10.3
Control (infested)	58.0	70.2	71.1	66.4
Control (uninfested)	42.0	41.7	41.1	41.6

W.T.= wild type = *T. hamatum*.

b. Mutant (9) of T. hamatum:

Data concerning the effect of soil application with Mutant (9) of T.hamatum on root rot of faba bean are presented in Table (13).

Applying Mutant (9) of T.hamatum to the soil infested with the pathogens of root rot decreased the disease severity.

Treating the pathogens infested soil with Mutant (9) as biocontrol agent, decreased root rot severity on faba bean from 66.4 to 8.2% in case of applying rice flour formulation to the soil. Vermiculite formulation decreased the disease severity from 66.4 to 9.8%.

Table (13): Effect of treating soil with mutant (9) of *T.hamatum* mounted on different carriers on root-rot of faba bean under greenhouse conditions.

Mutant (9)+ carrier	Percentage of disease severity index (DSI %)			
	R. solani	*F. solani*	*Pythium* sp.	Pooled aver.
Talc powder	10.8	11.8	14.8	12.5
Rice flour	9.2	7.2	8.2	8.2
Vermiculite	7.3	9.9	12.2	9.8
Control (infested)	58.0	70.2	71.1	66.4
Control (uninfested)	42.0	41.7	41.1	41.6

Mutant (9) of *T. hamatum* = EMs - U V - F 50/5

DISCUSSION

In order to obtaining successful biocontrol agent, it may be necessary to adopt a much broader bases selection and development program that has been used in the past. Selection of antagonists must be related to inoculum production, formulation, storage and application procedures.

Biological control agents are known to be less effective and more inconsistent than chemical pesticides and these short-comings have been attributed to the failure of the inoculants to become established and / or to express antagonism in soil or phyllosphere (Powell, 1992; Hagedorn, *et al.,* 1993). Formulation may enhance the shelf-life of biocontrol agents by providing physical protection from adverse edaphic conditions. Commercial preparation of biocontrol agents must be stable, possess adequate shelf-life for at least one year but more practically 18-24 months, to be successfully integrated into existing agricultural products distribution systems, contain high levels of antagonist colony forming units, be easy to prepare and apply and be cost effective (Lewis, 1991; Amer and Utkhede, 2000). The formulation must also provide the effective performance of the biocontrol agent once applied to the field.

The biological control of plant pathogens is limited by problems associated which formulating biocontrol agents for particular crop system (Lumsden and Lewis, 1989). With field crops, seed treatment with biocontrol fungi or bacteria appears to be the most possible method for soilborne diseases .Root dips with suspension of bacteria or fungi offer protection of shrubs or seedling against soil borne pathogens.

Diatomaceous earth granules saturated with molasses was inoculated with *T. harzianum* (Backman and Rodriguez-Kabana, 1975) to improve survivability in soil. The spores adsorbed in the granules survived better due to the nutrients supplied by molasses. Chet (1987) used 1:1 mixture of wheat bran and peat to improve survivability of *Trichoderma* sp. propagules in the soil (Amer and El-Desouky, 1999).

Experimental powder formulations have been successfully prepared by diluting biomass of isolates of *Trichoderma* and *Gliocladium* spp. with commercially available pyrophylite clay (Pyrax) as a carrier (Papavizas *et al.* 1984; Papavizas and Lewis, 1989). Fungal spores and biomass preparations also have been formulated into pastes, tablets and fluid-drill gels. Other kinds of formulations, such as dusts, wettable powders, pellets or emulsifiable liquids have been developed as the end products from liquid fermentation.

Lewis *et al.* (1996) formulated *G. virens* and *Trichoderma* sp. in extruded granular formulations using rice flour, gluten Pyrex, vermiculite, canola oil, for control of *R. solani* in soil less mix under greenhouse conditions. Lewis and Larkin (1997) formulated *Cladorrhinum foecundissimum* in forms of alginate prill and extruded flour/clay granules to cotrol damping-off disease caused by *R. solani* and *P. ultimum.*

The most extensively and successfully used material is alginate. Lewis and Papavizas (1985) encapsulated *Trichoderma* and *Gliocladium* spp. in alginate-wheat bran mixture. Conidia, chlamydospores or fermentation biomass were mixed in 1% sodium alginate solution and added into a 2-5% calcium chloride solution to form pellets which collected and dried for use as biofungicide. The shelf-life of the product was not satisfactory showing low cell viability (10%) after 6 months at 25ºC. An alginate prill formulation of *G. virens* registered as Glio Gard was used to control *R. solani* and *P. ultimum* (Connik *et al.* 1990).

Formulation may be either liquid or dry (wettable powder, dry flowable, granular). Dry formulations generally utilize carriers in which the inoculums absorbed. The choice of carriers is critical to the performance of the inoculum as carriers are necessary to ensure adequate dispersal and protection to biocontrol agent.

REFERENCES

Abd El-Moity, T. H., G. C. Papavizas and M. N. Shatla (1982). Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. Phytopathology, 72: 396-400.

Ahmad, J. S. and R. Baker (1987). Rhizosphere competence of *Trichoderma harzianum.* Phytopathology, 77: 358-362.

Amer, G. A. and R. S. Utkhede (2000). Development of formulations of biocontrol agents for management of root rot of lettuce and cucumber. Can. J. Microbiol., 46: 809-816.

Amer, G. A. and S. M. El-Desouky (1999). Using cheap agricultural wastes for mass production of *Trichoderma* and *Gliocladium* species in biocontrol of white mould disease of cucumber. Minufiya J. Agric. Res., 24(6): 1881-1892.

Backman, P. A. and R. Rodriguez-Kabana (1975). A system for the growth and delivery of biological control agents in the soil. Phytopathol. 65: 819-821.

Chet, I. (1987). Trichoderma application, mode of action and potential as a biocontrol of soil-borne pathogenic fungi. In: Innovative Approaches to Plant Disease Control (Ed. By I.Chet), pp. 137-160.

Connick, W. J., J. A. Lewis and P. C. Quimby (1990). Formulation of biocontrol agents for use in plant pathology, in new directions in biological control: Alternatives for Suppressing Agricultural Pests and Diseases (Baker, R. and P.E.Dunn, Eds.) Alan R. Liss, Inc., New York, pp. 345-372.

Cook, R. J. and K. F. Baker (1983). The nature and practice of biological control of plant pathogens. American Phytopathological society, St. Paul, Minn., 539pp.

Hagedorn, C., G. W. Gould and T. R. Bardinelli (1993). Field evaluations of bacterial inoculants to control seedling disease pathogens on cotton. Plant Disease, 77: 278-282.

Harman, G. E. and T. E. Stasz (1991). Protoplast Fusion for the production of superior biocontrol fungi. In TeBeest Do (Ed): Microbial Control of Weeds. New York\. Chapman and Hall. pp. 171-186.

Kay, S. J. and A. Stewart (1994). Evaluation of fungal antagonists for control of onion white rot in soil box trials. Plant Patholohu,43 (2): 371-377.

Leathers, T. D., S. C. Gupta and N. J. Alexander (1993). Mycopesticides: status, challenges and potential. J. Ind. Microbial. 12: 69-75.

Lewis, J. A. (1991). Formulation and delivery systems of biocontrol agents with emphasis on fungi. In "The rhizosphere and plant growth" (D.L. Keister and P.B. Gregan, Eds.), pp. 279-287. Kluwer Academic, Dortdrecht, The Netherlands.

Lewis, J. A., R. D. Lumsden and J. C. Locke (1996). Damping-off diseases caused by *Rhizoctonia solani* and *Pythium ultimum* as affected by alginate prills with biomass of biocontrol fungi and various food bases. Biocontrol Science and Technology, 6: 163-173.

Lewis, J. A. and G. C. Papavizas (1985). Characteristics of alginate pellets formulated with *Trichoderma* and *Gliocladium* and Their effect on the proliferation of the fungi in soil. Plant Pathol., 34: 572-577.

Lewis, J. A. and R. B. Larkin (1997). Extruded granular formulation with biomass of biocontrol *Gliocladium virens* and *Trichoderma* sp. to reduce daping-off of eggplant caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in soil-less mix. Biocontrol Science and Technology, 7: 49-60.

Lumsden, R. D. and J. A. Lewis (1989). Problems and progress in the selection, production, formulation and commercial use of plant disease control fungi. In: Biotechnology of Fungi for Improved Plant Growth. Eds. J.M.Whipps and R.d. Lumsden. pp. 171-190.

Mukhopadhyay, A. N., A. Brahmbhatt and G. V. Patel (1986). *Trichoderma harzianum* a potential biocontrol agent for tobacco damping-off Tob. Res., 12: 26-35.

Papavizas, G. C. (1985). *Trichoderma* and *Gliocladium*: Biology, ecology and potential for biocontrol. Annu. Rev. Phytopathol. 23: 23-54.

Papavizas, G. C. and J. A. Lewis (1989). Effect of *Gliocladium* and *Trichoderma* on damping off and blight snapbean caused by *Sclerotium rolfsii*. Plant pathol., 38: 277-286.

Papavizas, G. C. and R. D. Lumsden (1980). Biological control of soil borne fungal pathogens Ann. Rev. Phytopathol., 18: 389- 413.

Papavizas, G. C., D. P. Roberts and K. K. Kim (1990). Development of mutants of *Gliocladium virens* tolerant to benomyl. Canadian Journal of Microbiology, 36: 484-489.

Papavizas, G. C., J. A. Lewis and T. H. Abd El-Moity (1982). Evaluation of new biotypes of *Trichoderma harzianum* of tolerance of benomyl and enhanced biocontrol capabilities. Phytopathology, 72: 126-132.

Papavizas, G. C., M. T. Dunn, J. A. Lewis and J. Beagle-Ristaino (1984). Liquid fermentation technology for experimental production of biocontrol fungi. Phytopathology, 74: 1171-1175.

Pe'er, S. and I. Chet (1990). Trichoderma protoplast fusion: a tool for improving biocontrol agents. Can. J. Microbial 36: 6-9.

Powell, K. (1992). Is biological control the answer to sustainable agriculture, Chemistry and Industry, 5: 168-170.

Salama, S. A. and G. A. Amer (1996). Induction of stable mutants in *Trichoderma* spp. *Tolerant* to some fungicides, Egyptian J. Gen & Cytol., 25: 39-49.

Soleman, N. K., M. S. Mikhail, R. K. Harb and E. M. Khalil (1988). Response of broad bean plants infected with *Rhizoctonia solani* to application of growth regulators and calcium. Egypt. J. Phytopathol., 20 (1): 1-11.

تحسين كفاءة التضاد اىوي ح ل للفطر تريكودرما هاماتم وتحميله على مواد مختلفة لاستخدامها فى المقاومة الحيوية لمرض عفن الجذور فى الفول البلدى

جمعه عبد العليم عامر[١] ، رانيا زكى الشناوى[٢]

(١) قسم النبات الزراعي – كلية الزراعة – جامعة المنوفية– شبين الكوم– مصر

(٢) معهد بحوث أمراض النباتات – مركز البحوث الزراعيه– الجيزه– مصر

الملخص العربي

هذه الدراسة توضح أهمية تحسين كفاءة الفطر تريكودرما هاماتم وذلك عن طريق استحداث طفرات لهذا الفطر وتحميله على مواد مختلفة كذلك استخدامه فى المقاومة الحيوية لمرض عفن الجذور فى الفول البلدى. جميع الطفرات الناتجة لفطر التضاد الحيوى تريكودرما هاماتم ثبطت من نمو الفطريات الممرضة مثل رايزوكتونيا سولانى،فيوزاريوم سولانى، بيثيم ألتيمم وذلك بالمقارنة بالعزلة الأصلية تحت ظروف المعمل. وقد اختلفت كل من العزلة الأصلية للفطر تريكودرما هاماتم والطفرة المحسنة رقم ٩ فى فترة بقائها حيه وفى إنتاجها للوحدات المكونة للجراثيم تبعا للمواد الحاملة المستخدمة لإكثار فطر التضاد الحيوى تحت الدراسة. أظهرت جميع التركيبات المجهزة التى احتوت على نخالة القمح أو مصاصة القصب وكذلك التى احتوت على دقيق الأرز أكبر فترة بقاء حيه وكذلك أعلى انتاج للوحدات التكاثريه لكل من العزله الأصليه أو الطفره رقم ٩ لفطر التضاد الحيوى تريكودرما هاماتم. اختلفت أيضا التركيبات المجهزة لكل من العزلة الأصلية للفطر تريكودرما هاماتم والطفرة المحسنة رقم ٩ فى كفاءتها فى مقاومة مرض موت البادرات وعفن الجذور فى الفول البلدى وكانت معاملة البذره أكفأ من معاملة التربة.