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EVALUATION OF SINGLE OR COMBINED ISOLATES OF *TRICHODERMA harzianum* IN DIFFERENT FORMULATIONS FOR CONTROLLING ROOT ROT DISEASES OF STRAWBERRY

[41]

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ABSTRACT

Five isolates of *Trichoderma harzianum* were tested, in preliminary study, for their efficiency in controlling root rot of strawberry. Three isolates proved higher efficiency in controlling Rhizoctonia root rot. The most effective isolates namely 1, 2 &3 in addition to their mixture were formulated at different forms *i.e.* suspension, powder and granules and tested at different concentrations in greenhouse and field experiments. Results of greenhouse studies indicated that the all isolates and their mixture were effective in controlling Rhzoctonia root rot disease and increasing percentage of survived plants. Moreover, results indicated that granules was the best form in controlling root rot diseases of strawberry followed by powder and suspension in decreasing order. Higher biocontrol efficiency was obtained when mixture of isolates was used and increasing the dose of application increased it. Results of field studies, also, showed that granules was the best formulation, however, there was no significant difference between suspension and powder forms. The highest biocontrol efficiency against soil borne pathogens of strawberry was obtained with mixture of isolates, and the efficiency increased by increasing the dose of the bioagent.

Keywords: Biological control, *Trichoderma harzianum*, Strawberry, Root rot, Formulation.

INTRODUCTION

Since the pioneering work of **Weindling (1932) and Weindling & Emerson (1936)** on the antagonistic effects of *Trichoderma* strains against *Rhizoctonia solani* Kuhn, considerable attention has been paid for biocontrol as a method for plant disease management **(Baker & Cook, 1974 and Liu & Baker, 1980).** Great efforts have been done to make biocontrol effective as pesticides in the suppression of fungal pathogens. Proposed mechanisms resulting in biocontrol are competition for the substrate, the ability to colonize the niche favored by the

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pathogen (**Elad *et al* 1980 and Prasad & Rongeshwaran, 1999**), antagonism by antibiotic (**Dennis & Webster, 1971a & b; Brewer *et al* 1987; Fravel, 1988 and Almassi *et al* 1991**) and the hyperparasitic potential of the bioagent (**Weindling, 1932; Elad, 1995; Elad *et al* 1983 and Sundheim & Tronsmo, 1988**). On the other hand, chitinolytic enzymes produced by *Trichoderma harzianum* Rifai have been suggested as being responsible for the observed antifungal activity (**Chet, 1987; Harman *et al* 1993 and Lorito *et al* 1993**) Isolates of *Trichoderma harzianum* varies in their antifungal activities (**Elad *et al* 1981; Abd-El-Moity, 1985 and Haran *et al* 1995**). The present study aimed to screen different isolates and different formulations of *Trichoderma harzianum* that may differ in the mechanisms by which affect the bioagent and make a mixture of them to magnify their effect against the pathogen.

MATERIAL AND METHODS

Plant material

Fresh transplants of strawberry (*Fragaria ananssa* Duch.) cv. sweet charly were obtained from Strawberry and Non-traditional Crops Research Station, Nobaria, Behaira governorate.

Preparation of pathogen propagules and soil infestation

Isolate of *Rhizoctonia solani* , previously isolated from diseased strawberry plants and its pathogenicity was proven, was kindly obtained from Department of Plant Pathology Faculty of Agriculture, Ain Shams University. Inoculum was prepared by growing the fungus on corn meal- sand medium supplement with 0.2% peptone solution (**Abd-El-Moity, 1985**). Bottles containing the medium were inoculated with equal disks 5mm of *R. solani* from three days old cultures. Inoculated bottles were incubated horizontally for 4 weeks at 25 °C. Loaded fungal growth was mixed with the soil at the rate of 10g / Kg soil. Infested pots were irrigated periodically and left for 10 days to adequate distribution of the inoculum. Pots containing non-infested soil, were supplied with the same amount of autoclaved corn meal- sand medium containing 0.2% peptone served as control.

Source of *Trichoderma harzianum* isolates

Plant samples of strawberry, date-palm, peppermint, garlic, faba-bean, wheat, cotton, basil, bean and pea representing different regions namely Demiatta, Fayoum, Giza, Ismallia, Nubaria, Sharkia, Sharm-Elshikh. According to their cultural characteristics isolates were classified into five identical groups. The five isolates representing these groups were identified according to their microscopic characteristics as *Trichoderma harzianum*. Identification was kindly carried out in Mycology and Plant Disease Survey Research Department, Plant Pathology Research Institute, ARC , according to **Rifai (1969)**.

Preparation of *Trichoderma harzianum* propagules

Propagules of the bioagents were prepared by growing each *T. harzianum* isolate in flasks (250 ml) each contained 100

ml of gliotoxin fermentation medium (**Brian and Hemming, 1945**). Flasks were inoculated with 5 mm fungal discs obtained from periphery three days old culture of each isolate. Inoculated flasks were incubated at 25°C for 10 days under complete darkness, to stimulate toxin production (**Abd-El-Moity *et al* 1982**).

Formulation of biocontrol agents

Different *T. harzianum* cultures were formulated in three different forms, granules, powder or as water suspension. Granules were prepared using the method developed by **Abd-El-Moity (1986)**, suspension containing $30x10^6$ CFU / g was used for the preparation. For preparation of powder, fungal growth and culture filtrate were homogenized using a blender and the homogenate was adjusted to be contain 30X 10^7 CFU /ml, then 10 ml were mixed thoroughly with 90 g talc powder. Suspension was prepared by homogenizing fungal growth and culture filtrate and the homogenate was adjusted to be contain 30X 10^6 CFU /ml for single isolates, or 10X 10^6 CFU /ml from each isolates in combined treatment (1, 2 and 3).

Transplants treatment

Powder or suspension were diluted in water to be containing $2x10^5$, $3x10^5$ and $6x10^5$ CFU, transplants were immersed in suspension for 5 min prior transplanting. Granule was carried out by added the granules to the soil directly at the rates of 1.0, 1.5, 2.0 g / pit of transplant which keeping gave the same previously mentioned concentrations as determined practically using plate counting technique.

Disease assessment

At the end of the experiment *i.e.* 90 day for pots trials and 150 day for field trials, plants were examined and percentage of survived plants was calculated as follows:

$$\% \text{ Survived plants} = \frac{\text{Number of survival}}{\text{Total number of planted transplants}}$$

Statistical Analysis

Complete randomly design was followed for both field and pots experiment. Data were analyzed using analysis of variance (ANOVA), and means are separated by Duncan's multiple range test at a significance level of P= 0.05. (**SAS Institute 1989**, SAS/STAT User's Guide, Version 6).

RESULTS

Evaluation of different isolates of *Trichoderma harzianum* as biocontrol agents for controlling Rhizoctonia root rot of strawberry

Five *Trichoderma harzianum* isolates were tested for their efficiency in controlling Rhizoctonia root rot disease of strawberry in greenhouse experiments. Results in Figure (1) indicated that *T. harzianum* isolates differed in their efficacy in controlling root rot disease caused by *R. solani*. Isolate number three was the most effective one followed by isolates one and two respectively, meanwhile isolates four and five were clearly less effective.

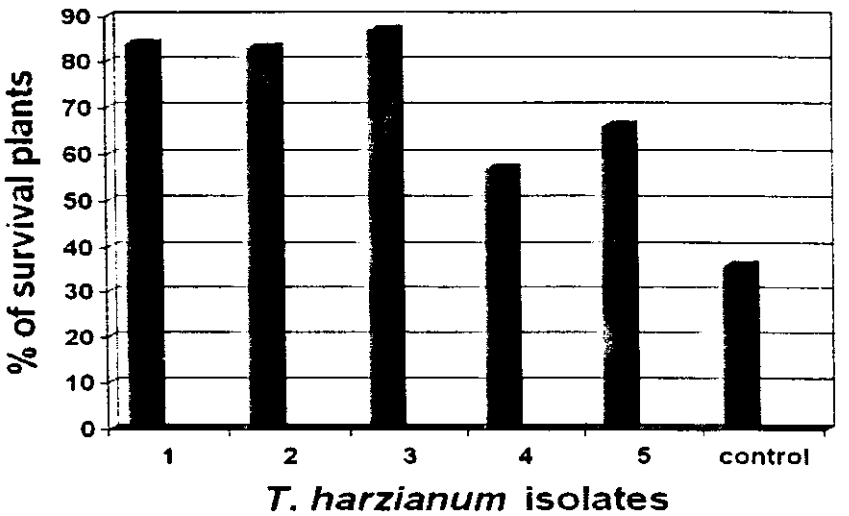

Figure 1. Evaluation of different *Trichoderma harzianum* isolates as biocontrol agent, for their reaction, in controlling *Rhizoctonia solani* of strawberry plants.
(1) Three replicates were specified for each treatment , each with 30 plants.
(2) The concentration used was $3x10^5$ CFU/ml.
(3) Control: treatment without adding the biocontrol agent into soil.

Evaluation of different forms of *Trichoderma harzianum* as biocontrol agents for controlling Rhizoctonia root rot of strawberry in greenhouse experiment

Different forms of *T. harzianum* propagules, i.e. suspension, powder, and granules containing $3x10^5$ CFU /ml or g were tested for their efficacy in controlling root rot disease of strawberry in greenhouse. The study was carried out using the most antagonistic and effective isolates i.e. one, two and three, in addition to their mixture. Results in Table (1) indicated that , in most cases, granules gave the best result for controlling Rhizoctonia root rot of strawberry followed by powder and suspension formulations.

Effect of different concentrations from different forms of *Trichoderma harzianum* isolates and their mixture for controlling Rhizoctonia root rot of strawberry in greenhouse experiment

The most effective isolates, i.e. 1, 2 & 3, and their mixture were prepared in three forms namely suspension, powder and granules, each of these was applied in a greenhouse experiment at three concentrations, i.e. $2x10^5$, $3x10^5$ and $6x10^5$ CFU/ml. Results in Table (2) indicated that all tested forms were effective in reducing disease incidence with all the tested isolates and all concentrations. Granules was the best tested form, followed by powder and suspension in decreasing order. Concerning the concentration of conidia/unit (g or ml, for powder, granules and

Table 1. Evaluation of different formulations of *Trichoderma harzianum* isolates and their mixture on controlling *Rhizoctonia solani* in strawberry plants.

Formulation	Percentage of survived plants [1]			
	Isolate 1	Isolate 2	Isolate 3	Mixture
Suspension	75.0a[2]	78.4b	80.0b	85.0b
Powder	80.0a	80.0b	85.0ab	90.0a
Granules	81.7a	86.7a	88.4a	90.0a
Control[3]	30.0b	30.3c	30.0c	30.0c

(1) Data recorded after 90 day
(2) Figures in each column with the same letter not significantly different according to Duncan's Multiple Range test (P=0.5)
(3) Control: treatment without adding the biocontrol agent into soil.
(4) Three replicates were specified for each treatment, each with 30 plants.

Table 2. Effect of different formulations and concentrations of different single isolates of *Trichoderma harzianum* and their mixture on percentage of survived strawberry plants grown in artificially infested soil with *Rhizoctonia solani.*

Formulation	Concentration CFU(10^5)	Percentage of survived plants[1]			
		Isolate1	Isolate 2	Isolate 3	Mixture
Suspension	2	70.0b[2]	70.0d	80.0c	81.6cd
	3	75.0ab	76.7bcd	83.0ab	83.0cd
	6	80.0ab	81.7abc	85.0b	85.0c
Powder	2	70.0b	75.0cd	80.0c	83.0cd
	3	78.4ab	80.0abc	83.0ab	85.0c
	6	80.0ab	85.0ab	85.0b	90.0b
Granules	2	80.0ab	83.0ab	83.0ab	88.0bc
	3	83.4a	84.0ab	84.0ab	90.0b
	6	85.0a	88.4a	90.0a	95.0a
Control[3]		40c	42e	41d	40e

(1) Data recorded after 90 day
(2) Figures in each column with the same letter not significantly different according to Duncan's Multiple Range test (P=0.5)
(3) Control: treatment without adding the biocontrol agent into soil.
(4) Three replicates were specified for each treatment, each with 30 plants.

suspension), it was found that with increase the concentration of bioagengt propagules give rise increasing in controlling the disease. As for the efficiency of different single isolates and their mixture in controlling the diseases, it was found generally, that mixture of isolates was the most effective.

Evaluation of different forms of different isolates of *Trichoderma harzianum* and their mixture for controlling root rot diseases of strawberry in field experiment

The three aforementioned formulations of most effective isolates and their mixture were applied in field experiment in three concentrations, i.e. $2x10^5$, $3x10^5$ and $6x10^5$.

Results in Table (3) indicated that all forms of *T. harzianum* were effective in reducing disease severity with all the tested isolates and all concentrations. Granules was the most effective form followed by powder and suspension in decreasing order. All forms achieved the higher percentage of survived plants with all single isolates and their mixture. Results, also confirmed that the increase of concentration, of different forms, give rise the increase in controlling the disease. As for the efficiency of different single isolates and their mixture in controlling the diseases, it was found generally, that mixture of isolates was the most effective.

Table 3. Effect of different formulations and concentrations of different single isolates of *Trichoderma harzianum* and their mixture on percentage of survived strawberry plants grown under field condition [1].

Formulation	Concentration CFU(10^5)	Percentage of survived plants [2]			
		Isolate 1	Isolate2	Isolate3	Mixture
Suspension	2	81.3e[3]	82.0f	83.0e	85.0f
	3	85.7d	86.0e	88.7cd	92.3d
	6	87.07cd	88.0d	89.70c	93.0d
Powder	2	82.7e	82. 7f	84.3e	88. 0f
	3	88.3c	90.3c	92.0b	93.0cd
	6	91.0 b	92.3b	93.0b	95.0bc
Granules	2	85.7d	87.0e	88.0d	90.00e
	3	92.3 ab	94.0a	94.7a	96. 0ab
	6	93.3a	95.0a	95. 7a	97.3 a
Control[4]		60.33f	60.67g	60.33f	60.97

(1) Three replicates were specified for each treatment, each has 100 plants.
(2) Data recorded after 150 days.
(3) Means in each column with the same letter not significantly different according to Duncan's Multiple Range test (P=0.5).
(4) Control: treatment without adding the biocontrol agent into soil.

DISCUSSION

Strawberry production is an important source of foreign currency income for Egypt. Root rot diseases are often associated with strawberry plants grown in non-fumigated soil. Several pathogens, including *Rhizoctonia* and *Pythium*, are causal agents of this disease (**Abad *et al* 2002 and Martin, 2000**). While not currently a widespread problem in properly fumigated commercial production fields, with the impending phase out of the use of methyl bromide and alteration of current fumigation practices, this disease may once again become a problem (**Martin, 2000**).

However, the degradation of chemical compounds that can be used to control soil borne diseases and alternatives to methyl bromide for strawberry production is very difficult and the concentration and/or accumulation of them in food chains are leading to higher toxicity levels in living organisms (**Chet, 1987 and Lynch, 1990**). Many *Trichoderma* spp isolates were previously found to control the development of *R. solani* diseases of strawberry under field conditions and, consequently, to control the disease. Maximizing the biocontrol to reach the level of chemical control is still required (**Elad *et al* 1981; Campbell, 1990 and Tronsmo & Hjeljord, 1998**). Substantial information provides support that the extraordinary capacity of *T. harzianum* to attack the structures of phytopathogenic fungi is related to the synergistic action of competition for key nutrients, antagonism by antibiotics, production of cell wall-degrading enzymes, hyperparasitic potential of the bioagent, stimulation of plant defense mechanisms, and a combination of these possibilities (**Neethling & Nevalainen, 1995 and Tronsmo & Hjeljord, 1998**).

Use of an isolate of *Trichoderma* that has more than one mechanism against the pathogen increase the potentiality of biocontrol (**Stasz *et al.*, 1988**). Moreover, using more than one isolate increase the possibility of occurring a synergistic effect of different mechanisms against the pathogen (**Schisler *et al* 1997**). In this respect, **Cook (1993)** stated that several strains of *Trichoderma* should be included in a formulation to widen the range of control. Combining effective strains of bioagents was also studied by **Harman *et al* (1989); Harman (1990); Janisiewicz (1996) and Schisler *et al* (1997)**.

This wide range of effect is very promising for the future of commercial biological control. In addition a correlation was reported between concentration of the bioagent propagules and efficacy of biocontrol **Janisiewicz and Roitman (1988)**. In this respect, it was found that with increase the concentration of conidia give rise increasing in controlling the disease. Moreover the formula of the bioagent that keeps its viability and its efficacy is of a greet importance, in this respect, it was found that granules was the best form for applying followed by powder and suspension form in decreasing root rot disease of strawberry. The ideal biocontrol strategy attempts to introduce or promote the activity of biocontrol agents only when and where they are needed or are most effective and minimizes wasteful application of inoculum to non-target habitats. Thus, a granule application to the infested soil directly may create disease-suppressive soils and possibly enhance plant vigor. *Rhizoctonia* is important soil-borne pathogens, and its

sclerotia in soil serve as primary inoculum. Consequently, suppression of the primary inoculum will be the first step in managing this disease of strawberry.

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مجلة حوليات العلوم الزراعية، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م(٥٠)، ع (٢)، ٦٠١-٦١١، ٢٠٠٥

تقييم اشكال مختلفة لعزلات فطر *Trichoderma harzianum* ومخاليطهم لمكافحة أمراض أعفان الجذور في الفراولة

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٢- كلية الزراعة- جامعة عين شمس-شبرا الخيمة- القاهرة- مصر

يهدف هذا البحث إلى رفع كفاءة المكافحة الحيوية لأمراض الجذور فى الفراولة إلى درجة تقارب الكفاءة التى تصل إليها المكافحة الكيماوية للمرض. أحد الوسائل التى يمكن بها تحقيق ذلك هو حشد عدة عزلات من فطر *Trichoderma harzianum* معا واستخدامها فى المكافحة، على اعتبار أن ذلك يتيح فرصة وجود عديد من آليات فعل العامل الحيوى تجاه الممرض. وقد تم عزل عديد من عزلات *Trichoderma* spp من مصادر ومناطق مختلفة صنفت إلى خمسة مجموعات، تبعا لصفاتها المزرعية، وعرفت جميعها على أنها *Trichoderma harzianum*. في دراسه مبدئية تم إختبار كفاءة العزلات الخمس فى مكافحة أعفان الجذور في الفراولة.وجد من النتائج أن ثلاثة منها كانت متميزة فى مكافحتها للمرض، وكانت أرقامها ١ و٢ و٣. جهزت مستحضرات لأفضل العزلات ١، ٢ و٣ ومخلوط منها في عدة صور هي معلق مسحوق وحبيبات وتم اختبارها بتركيزات مختلفة في كل من الصوب والحقل. وقد أوضحت النتائج أن كل العزلات بالإضافة الي مخلوطها كانت فعالة

في مكافحة المرض وزيادة النسبة المئوية للنباتات الحية. وقد أكدت دراسات الصوبة إن الحبيبات كانت أفضل المستحضرات في تقليل عفن الجذور في الفراولة المتسبب عن الفطر *Rhizoctonia solani* يليها كلا من المسحوق و المعلق. تم الحصول علي اعلي كفاءة للمكافحة الحيوية حينما إستخدم خليط من العزلات وقد زادت الكفاءة بزيادة الجرعة المستخدمة في التطبيق. أكدت الدراسات الحقلية أن الحبيبات كانت أفضل المستحضرات ومن ناحية اخري لم يوجد إختلاف معنوي بين كلا من مستحضرات المعلق والمسحوق. وقد تم الحصول علي أفضل كفاءة للمكافحة الحيوية حينما تم التطبيق بإستخدام خليط من العزلات وبخاصة عند إستخدامها بالتركيزات العالية.

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