

Combination of biocontrol agents and chemical fungicides in controlling *Fusarium oxysporum* and *Rhizoctonia solani* pathogenic fungi

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Abstract

The fungicidal properties of Rizolex-T, Moncut, Hesta, Rolex, Vitavax 200 and Tazolen against two pathogenic soil borne fungi (*Fusarium oxysporum* and *Rhizoctonia solani*) grown on glucose-mineral salts agar media as well as three non-pathogenic biocontrol agents (*Trichoderma harzianum*, *Tricodermaviride* and *Trichoderma virens*) were investigated and the EC₅₀ values were calculated. The results indicated that both pathogenic fungi were more sensitive than the biological control agents all tested fungicides. Although both Hesta and Rolex were more toxic against *R. solani* than Rizolex-T according to the EC₅₀ values, the sensitivity of *R. solani* toward Rizolex-T was obvious. However, all six tested compounds were more toxic of *R. solani* than *F. oxysporum*. The results exerted feeble toxic effect against the three fungal biological agents. Generally, the tested fungicides exerted higher fungicidal potency against both pathogenic fungi *F. oxysporum* and *R. solani* than the three biocontrol agents *Trichoderma* isolates.

The interactions between the three isolates of *Trichoderma* and both pathogenic fungi indicated that the antagonistic activity was greatly influenced by the presence of the tested fungicides in the culture media at 300 ppm for any of the tested fungicides that all *Trichoderma* isolates caused complete growth inhibition of both pathogenic fungi. Also combination of *Trichoderma* isolates in the presence of 200 ppm caused a very high inhibition degree with most cases of tested fungicides. *Trichoderma* isolates alone suppressed the growth of pathogenic fungi. The results clearly proved that increasing the concentration of all tested fungicides enhanced the antagonistic activity of *Trichoderma* isolates against the hyphal growth of both tested pathogenic fungi leading to lowering the EC₅₀ values of tested fungicides in the presence of *Trichoderma* isolates, that leads to decrease of conventional fungicide chemicals released in the environment in order to control these pathogenic fungi.

Key words: *Trichoderma* isolates; pathogenic fungi; fungicides; biocontrol agents.

Introduction

The new trend of integrated pest management (IPM) has been preferred and implemented in pest control program all over modern and most developed countries. The new (IPM) program has been replacing traditional methods that used to protect crops from diseases which largely based on the use of chemical pesticides. Applications of

fungicides and fumigants can have drastic effects on the environment and consumer, and are often applied in greater quantities than herbicides and insecticides in agricultural production. Chemical methods, are not economical in the long term because they pollute the atmosphere, harm the environment, leave harmful residues, and can lead to the development of resistant strains

among the target organisms with repeated use (Naseby *et al.*, 2000). A reduction or elimination of synthetic pesticide applications in agriculture is highly desirable. One of the most promising means to achieve this goal is by the use of new tools based on biocontrol agents (BCAs) for disease control alone, or in combination with reduced doses of chemicals in the control of plant pathogens resulting in minimal impact of the chemicals on the environment (Chet and Inbar, 1994; Harman and Kubicek, 1998 ; Ahmed and Hemada, 2005 and Vinale *et al.*, 2008). To date, a number of BCAs have been registered and are available as commercial products, including strains belonging to bacterial genera such as *Agrobacterium*, *Pseudomonas*, *Streptomyces* and *Bacillus*, and fungal genera such as *Gliricium*, *Trichoderma*, *Ampelomyces*, *Candida* and *Coniothyrium* (Vinale *et al.*, 2008).

Trichoderma spp. have attracted attention for controlling various soil borne fungi (Coskuntuna and Ozer, 2008). *Trichoderma* spp. are among the most frequently isolated soil fungi and present in plant root ecosystems (Harman *et al.*, 2004). These fungi are opportunistic, a virulent plant symbionts, and function as parasites and antagonists of many phytopathogenic fungi, thus protecting plants from disease. So far, *Trichoderma* spp. are among the most studied fungal BCAs and commercially marketed as biopesticides, biofertilizers and soil amendments (Estevez *et al.*, 2001; Harman, 2000; Harman *et al.*, 2004 and Lorito *et al.*, 2004). Depending on the strain, the use of *Trichoderma* in agriculture can provide numerous advantages: (i) colonization of the rhizosphere by the BCA

("rhizosphere competence") allowing rapid establishment within the stable microbial communities in the rhizosphere;(ii) control of pathogenic and competitive/deleterious microflora by using a variety of mechanisms; (iii) improvement of the plant health and (iv) stimulation of root growth (Harman *et al.*, 2004).

The aim of this research is to implement combination of both biological agents and fungicides in controlling pathogenic fungi as *F. oxysporum* and *R. solani*.

Materials and Methods

Tested fungicides

- 1- Rizolex-T: Tolclofos-methyl + Thiram
- 2- Moncut: Flutolanil
- 3- Hesta: Thio phanate-methyl
- 4- Rolex: Metalaxyl+Copperoxy chloride
- 5- Vitavax 200 : Carboxin+Thiram
- 6- Tazolen: Mancozeb+Metalaxyl

Fungi isolate

Two isolates of soil borne plant pathogenic fungi (*Fusarium oxysporum* and *Rhizoctonia solani*) in addition to three isolates of *Trichoderma* spp. (*T. harzianum*, *T. viride* and *T. virens*) were kindly obtained from plant pathology department, Faculty of Agriculture Alexandria university, were used in the present study.

Buffer solution

Citrate – phosphate buffer was used in the tests to maintain the pH value of the medium at 7.0. The buffer solution was prepared as indicated by Vogel (1961) and Sidney and Kaplan (1959).

Radial-Growth Tests

Radial-growth test was used according to the conventional methods reviewed by Torgeson (1967). This method of testing was carried out against

two tested fungi as follows: A definite volume of the Czapeck-Dox medium (12ml) containing the required amount of agar (1.5 gm/100 ml) as well as a calculated volume of distilled water were sterilized in conical flasks. The buffer solution was autoclaved separately in test tubes, and then three milliliters of the buffer solution was added to the conical flask. The calculated volume of the six tested compounds was finally added. The total volume in each flask was 36 ml. The contents of each flask were poured in three sterilized Petri-dishes and considered as one treatment. Three replicates without tested compounds were conducted and considered as an untreated (control). All the additions or mixings were accomplished under a septic condition. After solidification of the Czapeck-Dox medium, a disc inoculum (5mm On diameter) was located in the center of the medium in the Petri-dish. The results were recorded by measuring two vertical radii of the growth in each Petri-dish. The percentage of inhibition was calculated according to Topps and Wain equation (1957) that can be represented as follows:

$$\text{Toxicity \%} = [(A-B)/A] \times 100$$

Where, A is the diameter of untreated fungus (control), while B is the diameter of treated Fungus. When the growth of the untreated (control) completely covered the surface of the Petri-dish the experiment was ended. A regression line between 1% values and the concentration of each tested compound was drawn on Log-probit paper, from which the concentration causing 50% inhibition in the hyphal growth was determined for each tested compound.

Effect of Fungicides on antagonistic activity

The antagonistic activity of tested biological agents against fungi was determined in Fungicides-free. One disk (5mm) from 7-day old culture of *T. harzianum*, *T. viride* and *T. virens* were transferred to one side of a Czapeck-Dox Petri plates (9 cm diameter) containing different concentrations of the tested fungicides, i.e. 5, 10, 25, 50, 100, 200 and 300 Mg/ml. At the same time, one disk (5mm) of *R. solani* and *F. oxysporum* were placed on the other side of plate oppositely at 5 cm apart from the tested biological agents disk. Three replicates were used for each concentration, and plates with tested fungi or tested fungi with tested biological agents without fungicide were used as control treatments. The plates were incubated at 25 C° for 7 days and the inhibition area between tested fungi and tested biological agents were measured and the inhibition percentage was calculated based on control treatments.

Results and Discussion

Effect of tested fungicides against pathogenic and biocontrol fungi

The results of the fungicidal activity as EC₅₀ values (which is the concentration that caused 50% inhibition of the fungal hyphal growth) of six tested commonly fungicides being used as chemical control for soil-borne pathogenic fungi for both pre and post emergence damping off, wilt and/or sore shine diseases against two pathogenic fungi (*Fusarium oxysporum* and *Rhizoctonia solani*) grown on glucose-mineral salts agar media as well as three non-pathogenic soil borne fungi (*Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma virens*) are presented in Table 1.

The results indicated that both pathogenic fungi were more vulnerable towards tested compounds, contrary to the biological agents. The EC₅₀ values were 45 ppm and 26 ppm for Hesta against *F. oxysporum* and *R. solani* respectively, these values were the lowest among all others obtained EC₅₀ values indicating that Hesta fungicide (thiophenate-methyl) caused highest fungicidal activity against both pathogenic fungi. Vitavax 200 was the lowest fungitoxic compound against both tested pathogenic fungi (the EC₅₀ values were the highest ones obtained against *F. oxysporum* 230 ppm and *R. solani* 160 ppm). The other tested compounds could be arranged descendingly according to their fungitoxic action as follows: Moncut, Rolex, Tazolen and Rizolex-T that the EC₅₀ values were 100, 110, 140 and 170 ppm respectively. Although both Hesta and Rolex were more toxic against *R. solani* than Rizolex-T according to the EC₅₀ values, the sensitivity of *R. solani* toward Rizolex-T was obvious and rank of toxicity moved higher from 5th to 3rd for *F. oxysporum* and *R. solani* respectively. However, all six tested compounds were more toxic against growth of *R. solani* than *F. oxysporum*. The toxicity ratios were between 1.12 for Tazolen and 2.93 for Rolex against both tested pathogenic fungi. The fungitoxic effects of the six tested fungicides against the three *Trichoderma* species exhibited feeble toxic effect against the three fungal biological agents. The highest concentration used (1000 ppm) did not exceed the average of inhibition percentage more than 50% except in few

cases. The highest degree of inhibition was obtained due to Rizolex-T effect against *T. virens*, both Tazolen and Hesta were moderately effective against both *T. viride* and *T. virens*. The results also indicated that both concentrations of 100 and 200 ppm that were very close to the EC₅₀ values against both pathogenic fungi *F. oxysporum* and *R. solani* exerted very weak fungitoxic effect on the three *Trichoderma* isolates, the *T. harzianum* was most tolerant isolate to all tested fungicides than both *T. viride* and *T. virens*. The results were in complete agreements with (Ho *et al.*, 1992 ; Chase , 1992; Wahid *et al.*, 1995 and El-Mougy *et al.*, 2006). These findings encourage and enhance using these non-pathogenic isolates in the integrated plant disease control program.

Generally, the tested fungicides exerted high fungicidal potency against both pathogenic fungi *F. oxysporum* and *R. solani* than the three *Trichoderma* isolates. These results come in complete agreement with Vyas (1993) who suggested that *Trichoderma* species have greater tolerance for broad spectrum effective fungicides. In addition to several other investigators, that stated and proved the same findings of highly effectiveness of fungicides against pathogenic fungi while caused little effect on beneficial biocontrol agents as *Trichoderma* spp. (Montealegre and Henriques , 1990 ; Kay and Stewart, 1994; Figueras *et al.*, 1996 ; Mahmoud and Khalifa , 2003 and Ahmed and Hemada, 2005).

Table 1: Effect of tested fungicides on the growth of *F. oxysporum* and *R. solani* pathogenic fungi and *Trichoderma* biological control agents, shown as EC₅₀ µg/ml.

Fungicides	EC ₅₀ µg/ml				
	Pathogenic fungi		Biological control agents		
	<i>F.oxysporum</i>	<i>R.solani</i>	<i>T.harzianum</i>	<i>T.viride</i>	<i>T.virens</i>
Rizolex-T	170	64	831.77	676.5	244.30
Moncut	100	76	> 1000	985.97	994.17
Hesta	45	26	867.45	531.72	698.98
Rolex	110	58	> 1000	860.56	903.93
Vitavax 200	230	160	979.95	> 1000	> 1000
Tazolen	140	125	806.52	531.69	904.43

Antagonistic activity

The antagonistic activity of the three *Trichoderma* isolates on both pathogenic fungi *F. oxysporum* and *R. solani* in absence and presence of tested fungicide are tabulated in Tables 2 and 3. The data revealed that *Trichoderma* isolates were less inhibitory effective against *R. solani* hyphal growth than *F. oxysporum*. The degree of hyphal growth inhibition of *F. oxysporum* was higher than 50% in the case of the three *Trichoderma* isolates. The highest antagonistic activity was 55.5% of *F. oxysporum* growth due to *T. virens* isolate while the lowest inhibition value was 52.2% for *T. harzianum* effect. The interactions between the three isolates of *Trichoderma* and both pathogenic fungi indicated that the antagonistic activity was greatly influenced by the presence of the tested fungicides in the culture media. 300 ppm of any of the tested fungicides with all *Trichoderma* isolates caused complete growth inhibitions of both pathogenic fungi. Also combination of *Trichoderma* isolates in the presence of 200 ppm of the tested fungicides caused a very high degree of hyphal growth inhibition with most cases of tested fungicides. The combination of both *Trichoderma* isolates and tested fungicides used caused a remarkable fungitoxic effect especially

against the hyphal growth of *R. solani*. In spite of that hyphal growth inhibition percentage were less than 50% against *R. solani* in the absence of fungicides in the case of *T. viride* and *T. virens* (48.2% and 44.4% respectively). The inhibition of the hyphal growth was complete starting with 200 ppm with all tested fungicides except only very few cases even though, the inhibition were more than 90 even in these few cases. However at 5 ppm Moncut, Hesta and Rolex slightly reduced the antagonism of the three *Trichoderma* isolates against both pathogenic fungi *F. oxysporum* and *R. solani* while Rizolex-T and Tazolen reduced this effect of *T. harzianum* against both *F. oxysporum* and *R. solani*. The results clearly proved that increasing the concentration of all tested fungicides enhanced the antagonistic activity of *Trichoderma* isolates against the hyphal growth of both tested pathogenic fungi.

Several investigators have revealed the same finding that presence of chemical fungicides enhances the antagonistic effect of the biocontrol agents against pathogenic fungi (Roberti *et al.*, 1993; Yobo *et al.*, 2004; Ashrafizodeh *et al.*, 2005 and Arias *et al.*, 2006). The main advantage of implementation both chemicals as well as

biocontrol agents will lead to decrease the amount of chemicals released in the environment in order to control pathogenic fungi causing several loss of crop production.

Table 2: Antagonistic activity of biological control agents on the growth of *F. oxysporum* in the absence and presence of different concentrations of the tested fungicides, shown as pathogenic fungal growth inhibition percentage.

Biological control agents	Absence of fungicides	Fungicides conc. (ppm)	<i>F.oxysporum</i>					
			Rizolex-T	Moncut	Hesta	Rolex	Vitavax-200	Tazolen
<i>T. harzianum</i>	52.2	10	56.3	57.4	60.4	59.3	55.5	55.5
		25	60.7	64.1	66.66	67.4	56.66	60
		50	64.8	68.5	73	70.7	63	70
		100	75.5	78.5	81.1	80.4	80	88.8
		200	88.8	90	92.6	100	100	95.5
<i>T. viride</i>	53.7	10	56.66	56.3	61.1	59.3	64.1	60.4
		25	62.6	64.4	69.6	70.7	71.5	68.8
		50	70	73	78.2	79.3	79.3	81.1
		100	77.4	80.4	86.3	91.5	93.7	93
		200	90.4	94.4	96.3	100	100	100
<i>T. virens</i>	55.5	10	60.4	56.66	58.2	60.4	64.8	58.2
		25	70	60.4	64.1	70.7	68.8	75.2
		50	85.2	73	73.3	76	75.2	84.1
		100	100	81.8	91.1	88.5	84.1	91.1
		200	100	92.2	100	100	98.2	100

Table 3 : Antagonistic activity of biological control agents on the growth of *R. solani* in the absence and presence of different concentrations of the tested fungicides, shown as pathogenic fungal growth inhibition percentage.

Biological control agents	Absence of fungicides	Fungicides conc. (ppm)	<i>R.solani</i>					
			Rizolex-T	Moncut	Hesta	Rolex	Vitavax 200	Tazolen
<i>T. harzianum</i>	53.6	10	61.5	64.1	64.1	60.44	72.6	66.6
		25	66.6	68.5	70	68.2	78.8	74.1
		50	74.1	77.7	76	78.8	91.5	84.4
		100	86.3	91.5	88.2	89.6	100	94.8
		200	100	100	100	100	100	100
<i>T. viride</i>	48.2	10	57.7	60.4	72.2	58.8	71.1	62.2
		25	64.8	66.6	77.7	64.8	77.1	68.2
		50	70	79.6	82.22	77.1	87.4	77.4
		100	80	92.2	88.8	88.5	100	88.2
		200	100	100	96	100	100	97.4
<i>T. virens</i>	44.4	10	56.3	59.3	61.8	59.3	67.7	64.4
		25	60.7	67.1	69.6	68.2	80	72.6
		50	70.4	74.4	80	75.5	88.5	79.3
		100	78.2	84.1	89.6	84.8	100	89.6
		200	96	100	100	90.4	100	100

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أختبرت ستة من المبيدات الفطرية (ريزولكس-ت, مون كت, هيستا, رولكس, فيتافكس 200 والتازولين) ضد نوعين من الفطريات الممرضة وهي فيوزاريوم أكسبوريوم وريزوكتونيا سولاني بالإضافة إلى ثلاث أنواع من الفطريات الغير ممرضة والمستخدمه في مكافحة الحيوية وهي (تريكوديرما هاريزيانم, تريكوديرما فيردي و تريكوديرما فيرنس) و تم تقدير قيم ج ق 50 للمبيدات المختبرة. وقد أشارت النتائج إلى أن كلا من الفطريات الممرضة كانت أكثر حساسية بالمقارنة بالفطريات المستخدمة في مكافحة الحيوية ضد المبيدات الفطرية المختبرة وقد اظهرت النتائج ضعف سمية المبيدات المختبرة ضد الأنواع الثلاثة من الفطريات المستخدمة في مكافحة الحيوية عن الفطريات الممرضة. أعلى درجة تثبيط ترجع إلى تأثير الريزولكس-ت ضد فطر تريكوديرما فيرنس, وكلا من التازولين والهستا أكثر اعتدالاً من كلا من تريكوديرما فيردي و تريكوديرما فيرنس . كان فطر تريكوديرما هاريزيانم أكثر قدرة على الاحتمال ضد المبيدات الفطرية المستخدمة عن تريكوديرما فيردي و تريكوديرما فيرنس . على وجه العموم المبيدات الفطرية المختبرة أظهرت تأثير أبادي فطري فعال ضد كلا الفطرين الممرضين فيوزازيم أكسبوريوم و ريزوكتونيا سولاني بالمقارنة بالثلاث أنواع من التريكوديرما المعزولة. التأثير المثبط لعزلات التريكوديرما كان ضعيفاً ضد نمو هيفات الريزوكتونيا سولاني عنه في حالة فيوزازيم أكسبوريوم . درجة تثبيط نمو هيفات فيوزازيم أكسبوريوم كان أعلى من 50 % بأستخدام الثلاث عزلات من التريكوديرما.التداخل بين الثلاث عزلات من التريكوديرما وكلا الفطرين الممرضين أشارت إلى أن التضاد الحيوي كان أعلى تأثيراً بوجود المبيدات الفطرية المختبرة في بيئة الأجار عند 300 جزء في المليون لأي من المبيدات الفطرية المختبرة مع كل عزلات التريكوديرما والتي سببت تثبيط تام لنمو الفطريات الممرضة المختبرة . أيضاً التداخل بين عزلات التريكوديرما في وجود 200 جزء في المليون سبب درجة عالية من التثبيط في معظم حالات المبيدات الفطرية المختبرة. وقد أوقفت عزلات التريكوديرما نمو الفطريات الممرضة ومع ذلك تركيز 5 جزء في المليون بالنسبة للمبيد الفطري مون كت وهيستا وكذلك الرولكس كان هناك نقص طفيف في التضاد الحيوي للثلاث عزلات من التريكوديرما ضد كلا من الفطرين الممرضين الممرضين فيوزازيم أكسبوريوم و ريزوكتونيا سولاني بينما ريزولكس-ت والتازولين كانت أقل تأثير بالتريكوديرما هاريزيانم ضد كل من الممرضين فيوزازيم أكسبوريوم و ريزوكتونيا سولاني . وقد برهنت النتائج على أن زيادة التركيز في كل المبيدات الفطرية المختبرة زادت من التضاد الحيوي لعزلات التريكوديرما ضد نمو هيفات كلا من الفطريات الممرضة المختبرة.