

## ANTAGONISTIC EFFECT OF TRICHODERMA VIRIDE AND ITS COMPATIBILITY WITH FUNGICIDES ON RHIZOCTONIA SOLANI

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**ABSTRACT** This study was conducted to investigate the antagonistic action of *Trichoderma viride* on *Rhizoctonia solani* and its compatibility with three fungicides. The three fungicides were triticonazole (sterol biosynthesis inhibitor), azoxystrobin (inhibitor of mitochondrial respiration at complex III) and flutolanil (inhibitor of respiration complex II). The sensitivity of *R. solani* and *T. viride* grown on potato dextrose agar medium (PDA) to the tested fungicides was evaluated. It was found that triticonazole and flutolanil inhibited completely the growth of *R. solani* at concentration of 10 µg/ml, but azoxystrobin caused the same results at 100 µg/ml. In view of *T. viride*, the fungus was not affected by triticonazole or flutolanil, on contrary azoxystrobin affected the fungal growth. The antagonistic activity of *T. viride* on growth of *R. solani* was greatly influenced by the presence of the tested fungicides in culture media. The results revealed that the compatibility of triticonazole or flutolanil with *T. viride* increased the suppression of *R. solani* growth. On contrary, azoxystrobin did not increase the antagonistic activity of *T. viride* against growth of *R. solani*. In pot experiments, the application of the tested fungicides and *T. viride* controlled the incidence of pre-and post emergence damping off of maize caused by *R. solani*. Triticonazole and flutolanil were considered to be the most effective treatments for controlling pre and post emergence damping off at the rate of 3 or 5g/kg seed. *T. viride* at  $5 \times 10^6$  spores/ml greatly controlled pre-emergence damping off but it did not sufficiently control post-emergence damping off. The compatibility of *T. viride* with only triticonazole or flutolanil controlled pre-and post-emergence damping off on maize caused by *R. solani*. The results indicated that the efficiency of *T. viride* for controlling post-emergence damping off was improved by the addition of the two fungicides at low rates. Also, it could be concluded that the biocontrol efficiency of the *T. viride* for controlling *R. solani* was enhanced by its compatibility with low rates of Triticonazole and flutolanil.

## INTRODUCTION

One means of overcoming soil borne fungal pathogens is the use of an integrated pest management (IPM) system which would include biocontrol strategies. A component of such strategies might include the use of non-pathogenic soil borne fungi which secrete low molecular weight, diffusible antimycotic compounds which inhibit the growth of soil borne pathogenic fungi (Ahmed & Bakr, 1987; Fravel, 1988 and Handelsman & Parke, 1989).

One strategy is the use of isolates of soil borne rhizosphere-competent fungi *Trichoderma* spp. antagonistic to soil borne fungal pathogens such as *Rhizoctonia solani* Kuhu. (Elad *et al.*, 1983). It has been reported that *T. harzianum* isolates secrete several extracellular enzymes which are potentially antagonistic to *R. solani* (Bertagnolli *et al.*, 1996). It was found that *T. harzianum* secrete trichoderma (MW = 292) and small peptide (MW=876) in culture (Bertagnolli *et al.*, 1998). These compounds were antagonistic in culture to the mycelial growth of *R. solani*.

*Trichoderma* species are known to have greater tolerance for broad-spectrum fungicides than many other soil biota and to colonize the treated soil more rapidly than other soil competitors (Munnecke, *et al.*, 1981). There are very few reports available on the positive effects of fungicide use on the proliferation of *Trichoderma* species (Papavizas, 1985). It has been reported that thiram, as a seed treatment fungicide, had a selective effect on *Trichoderma* spp and *Penicillium* spp. consistently survived well and multiplied and controlled damping off of peas caused by *Pythium aphanidermatum* for a considerable time after the fungicide reached levels not toxic to the pathogen (Richardson, 1954). Interesting non-target effects on *T. harzianum* were also obtained with metalaxyl; the infusion of pea seed with this fungicide before coating it with conidia of *T. harzianum* improved the survival of conidia and even increased it in the rhizosphere compared with seeds that received conidia only (Papavizas, 1981). It was been reported that *T. koningii*, *T. harzianum* and *T. lignorum* were compatible with carboxin at 200 and 500 ppm for controlling *Ustilago segetum* var. tritici. (Mondal *et al.*, 1995).

The present investigation was conducted to test the effect of three fungicides belonging to different mode of actions against pathogenic fungi, i.e. triticonazole (sterol biosynthesis inhibitors), azoxystrobin (inhibitor of mitochondrial respiration at complex III) and flutolanil (inhibitor of respiration at complex II), for their compatibility with *T. viride* against *R. solani*.

## MATERIAL AND METHODS

### 1. *In vitro* sensitivity tests

Sensitivity of *Rhizoctonia solani* and *Trichoderma viride* grown on potato dextrose agar medium (PDA) to the tested fungicides was evaluated

according to Frisina and Benson (1988). The fungicides were; triticonazole (Premis 10% D.S.), azoxystrobin (Amstar 20% S.C.) and flutolanil (Moncut 25% W.P.). The fungicides were suspended in sterile distilled water then added to cooled (50°C) PDA medium at concentrations of 0.1, 0.5, 0.8, 1, 5, 10, 50, 100, 200 and 250 µg/ml for each fungicides. Disks (5mm in diameter) of *R.solani* and *T.viride* mycelial growth were taken from one week-old culture grown on PDA, and each one was placed into three replicates of PDA medium at each fungicide concentration. Radial growth of each replicate was measured after 7 days at 25°C.

## 2. Effect of Fungicides on Antagonistic Activity

The antagonistic activity of *T.viride* against *R. solani* was determined in fungicides -free or - amended media, according to the method described by Diab *et al.* (1990). On disk (5mm) from 7-day old culture of *T. viride* was transferred to PDA plates (9cm) containing different concentrations of the fungicides, i.e. 0.0, 0.1, 0.5, 1.0, 3.0, 5.0, 10.0, 50.0 and 100.0 µg/ml. At the same time, one disk (5mm) of *R. solani* was placed on the same plate oppositely at 5 cm apart from *T. viride* disk. Three replicates were used for each concentration and plates with *R. solani* alone or *R. solani* with *T. viride* without fungicide were used as control treatments. The plates were incubated at 25°C for 7 days and the inhibition area between *R. solani* and *T. viride* was measured and the inhibition percentage was calculated based on control treatments.

## 3. Pot Experiments

### A. Preparation of the fungal inoculum:

To obtain the inoculum of *R. solani*, the fungus was grown at 25°C for 15 days in sterile sand-corn meal medium (250 gm of dry sand, 14 g of corn meal and 100 ml of distilled water). Portions of this inoculum were mixed with the sterile clay loamy soil at the rate of 1% (w/w), the infested soil was distributed in pots (25 cm diameter). The pots were irrigated, using tap water and were left for 7 days before sowing to insure the establishment of the inoculum.

### B. Fungicidal seed and biocontrol treatments:

For seed dressing, 1, 1.5, 2, 3 and 5g fungicide/kg seed of maize (c.v. Giza hybrid 10) were applied as slurry in distilled water by agitating the seeds in a glass jar until the seeds were completely covered with the fungicides. Biological control treatments was carried out by adding spore suspension (10ml/pot) of *T. viride*, at concentrations of 1, 3, 5 x 10<sup>6</sup>/ml water on the surface of infested soil.

*The experiments were designed as follows:*

1. Fungicide-treated seeds in soil infested with *R. solani*.
2. Fungicide-free seeds in soil infested with *R. solani* and *T. viride*.
3. Fungicide-treated seeds in soil infested with *R. solani* and *T. viride*.
4. Fungicide-free seeds in soil infested with *R. solani*.
5. Fungicide-free seeds in unfested soil.

Each pot was planted with 15 seeds and each treatment was replicated three times. After 15 and 30 days from the sowing, the efficiency of each treatment against pre and post-emergence damping off were inspected, also shoot and root lengths (cm) were assessed. Percentage of control efficiency (PCE) was determined according to Samoucha and Cohen (1989) by the following equations:

$$\text{PCE} = 10 (1 - X/Y)$$

Where : X = Number of diseased plants in treatment.

Y = Number of diseased plants in control.

## RESULTS AND DISCUSSION

### *1. In vitro sensitivity*

Results in Table (1) show the efficiency of the tested fungicides against the growth of *R. solani* and *T. viride*. It was found that triticonazole and flutolanil were more potent against *R. solani* than azoxystrobin. Both triticonazole and flutolanil inhibited completely the growth of *R. solani* at 10 µg/ml, however, the same effect was achieved by azoxystrobin at 100 µg/ml. This indicated that inhibitors of sterol biosynthesis (triticonazole) and mitochondrial respiration at complex (II) were more fungitoxic to *R. solani* than inhibitor of respiration at complex III (azoxystrobin).

In view of *T. viride* (Table 1), the fungus was not affected by the tested fungicides, whereas the fungus could grow up to 250.0 µg/ml of the fungicides. The fungus could grow normally up to 1.0 and 50 µg/ml (9 cm) of triticonazole and flutolanil, respectively. The fungus seemed to be more affected by azoxystrobin than the other fungicides. At 250 µg/ml triticonazole, azoxystrobin and flutolanil at 250 µg/ml reduced growth of *T. viride* by 44.4, 51.1 and 18.18%, respectively. This indicated that flutolanil was the least effective fungicide against growth of *T. viride*.

Generally, the tested fungicides were more potent to growth of *R. solani* than *T. viride*. These results agreed with Vyas (1993), who suggested that Trichoderma species have greater tolerance for broad spectrum fungicides than many other soil biota.

## 2. Antagonistic activity :

Results presented in Table (2) showed that the antagonistic activity of *T. viride* on growth of *R. solani* was greatly influenced by the presence of the tested fungicides in culture media. *T. viride* suppressed the growth of *R. solani* by 52.2% in the absence of the fungicides. Triticonazole and flutolanil at 0.1 µg/ml slightly reduced the antagonism of *T. viride*. Interestingly, increasing the concentrations of the both fungicides enhanced the antagonistic activity. In this respect, it was found that triticonazole and flutolanil at 5 µg/ml gave 100 and 93.3% inhibition, respectively, compared to 92.2 and 84.4% inhibition of *R. solani* in the absence of *T. viride* (Table 1). This indicated that the compatibility of triticonazole or flutolanil with *T. viride* increased the suppression of *R. solani* growth. Alternatively, azoxystrobin did not increase the antagonistic activity of *T. viride* against growth of *R. solani*, versely, its low concentrations greatly suppressed the action of *T. viride*. Azoxystrobin alone at 50 µg/ml gave 81.1% inhibition, and at the same time *T. viride* alone inhibited *R. solani* by 52.2%, but their combination gave 51.1% inhibition of *R. solani*. Thus, it could be mentioned that compatibility of *T. viride* with azoxystrobin reduced the antagonistic action of *T. viride*.

## 3. Pot Experiments :

Results in Table (3) show the application of the tested fungicides at 1, 1.5, 2, 3 and 5 g/Kg seed and *T. viride* at  $1 \times 10^6$ ,  $3 \times 10^6$ ,  $5 \times 10^6$  and  $7 \times 10^6$  spores/ml to control the incidence of pre-and post emergence damping off of maize caused by *R. solani*. The incidence of pre-and post emergence of untreated maize seeds were 35.3 and 47.9%, respectively with shortage of the growth of shoot and root. It was found that increasing the rate of the tested fungicides resulted in enhancing their efficiencies against the pathogenic fungus with increasing the growing plants. Low rates of the fungicides and *T. viride* did not sufficiently control the diseases, azoxystrobin at 2 g/kg seed and *T. viride* at  $3 \times 10^6$  spores/ml did not effectively control the incidence of pre-emergence damping off. Triticonazole and flutolanil were considered to be the most effective treatments for controlling pre-and post emergence damping off caused by *R. solani*. At the rate of 5 g/kg seed they gave 85.72 and 91.15% PCE, respectively. All the fungicides at higher rates markedly controlled post emergence damping off. Although *T. viride* at 5 and  $7 \times 10^6$  spores/ml greatly controlled pre-emergence damping off, it exhibited low action against the incidence of post-emergence. This indicated that *T. viride* could antagonis *R. solani* in the early stages of the disease development.

Regarding the growth parameters, including shoot and root lengths of maize seedlings, the enhancement of all growth parameters by the fungicidal treatments was more pronounced at higher rates of fungicides. This increment was more pronounced using the rate of 5g/kg seed of triticonazole, azoxystrobin and flutolanil which they increased shoot length up to 22.33, 20.2 and 22.2 cm, respectively. Also, this rate of these fungicides (5 g/kg seed) gave longest root, being 10.8, 9.6 and 10.6 cm, respectively. Concerning *T.viride* gave longest shoot and root when applied at  $7 \times 10^6$  spore/ml (18.5 and 9.7 cm, respectively).

Results in Table (4) show the influence of the three fungicides, at different rates, on the bioprotectant activity of *T.viride* against the incidence of pre and post-emergence damping off of maize caused by *R. solani*. The results indicated that *T.viride* ( $5 \times 10^6$  spore/ml) + flutolanil (2.5 g/kg seed) gave a high PCE of pre-and post emergence damping off (95.5 and 95.57%, respectively). On the other hand, *T. viride* ( $3 \times 10^6$  spore/ml) + azoxystrobin (1.5 g/kg seed) gave the lowest PCE of the disease (28.56 and 33.43%, respectively). In the same trend growth parameters, including shoot and root lengths of maize seedlings, increased when *T. viride* at  $5 \times 10^6$  spore/ml + flutolanil at 2.5 g/kg seed were (22.5 and 10.4 cm, respectively). However shoot and root lengths were decreasing when used *T. viride* at  $3 \times 10^6$  spore/ml + azoxystrobin at 2.5 g/kg seed (9.6 and 6.3 cm, respectively). The results also indicated that addition of fungicides with *T.viride* improved the antagonistic action against post-emergence damping off. From all previous results it could be concluded that efficiency of *T.viride* for controlling *R. solani* and improving the growing plants was increased by the addition of the fungicides. Increasing the concentration of *T. viride* spore suspension increased the efficiency of the tested fungicides for improving growth parameters. Also, it could be concluded that the efficiency of the fungicides for controlling *R. solani* and improving the growth of the growing seedling was increased by the addition of *T. viride*. Thus this work shows that the biocontrol strategy which is using throughout integrated pest management (IPM) could be improved by using fungicides at low rates, especially flutolanil.

These results agreed with previous works, which indicated that control of chickpea wilt by *T. harzianum* was enhanced by combining it with carboxin at 200 ppm (Kaur and Mukhopadhyay, 1992). Also, combined effect of lower dose of PCNB and *T. harzianum* decreased the inoculum potential of *R. solani* and increased disease control of radish (Henis *et al.*, 1978).

Table (1) : Effect of different concentrations of the tested fungicides on the linear growth of *Rhizoctonia solani* and *Trichoderma viride* grown on PDA medium, after 7 days incubation at 25°C.

Concentrations (µg/ml)	<i>Rhizoctonia solani</i>						<i>Trichoderma viride</i>					
	Triticonazole		Azoxystrobin		Flutolanil		Triticonazole		Azoxystrobin		Flutolanil	
	Linear growth	Inhibition %	Linear growth	Inhibition %	Linear growth	Inhibition %	Linear growth	Inhibition %	Linear growth	Inhibition %	Linear growth	Inhibition %
0.0	9.0	—	9.0	—	9.0	—	9.0	—	9.0	—	9.0	—
0.1	5.4	40.0	6.7	25.5	6.4	28.8	9.0	—	8.3	7.7	9.0	—
0.5	3.6	60.0	6.0	33.3	4.5	50.0	9.0	—	8.0	11.1	9.0	—
0.8	2.8	68.8	5.2	42.2	3.0	66.6	9.0	—	8.0	11.1	9.0	—
1.0	1.5	83.3	4.8	46.6	2.3	74.4	9.0	—	7.5	16.6	9.0	—
5.0	0.7	92.2	4.1	54.4	1.4	84.4	8.3	7.7	7.3	18.8	9.0	—
10.0	0.0	100	2.8	68.8	0.0	100	7.9	12.2	6.8	24.4	9.0	—
50.0	0.0	100	1.7	81.1	0.0	100	7.0	22.2	6.4	28.8	9.0	—
100.0	0.0	100	0.0	100	0.0	100	6.5	27.7	6.0	33.3	8.5	5.5
200.0	0.0	100	0.0	100	0.0	100	5.7	36.6	5.3	41.1	8.0	11.1
250.0	0.0	100	0.0	100	0.0	100	5.0	44.4	4.4	51.1	7.3	18.8

## M.B. Mahmoud & H.M.S. Khalifa: Antagonistic Effect of *Trichoderma*

**Table (2) : Antagonistic activity of *Trichoderma viride* on the growth of *Rhizoctonia solani* in the presence of different concentrations of the tested fungicides.**

Concentrations (µg/ml)	% Antagonistic activity in the presence of fungicides*		
	Triticonazole	Azoxystrobin	Flutolanil
0.0	52.2	52.2	52.2
0.1	46.6	24.4	42.2
0.5	70.0	33.3	52.2
1.0	90.0	36.6	64.4
3.0	92.4	36.6	78.8
5.0	100.0	40.0	93.3
10.0	—	43.0	100.0
50.0	—	51.1	100.0
100.0	—	56.0	—

\* Antagonistic activity was measured as the inhibition activity of *T. viride* to growth of *R. solani*

**Table (3) : Efficiency of different rates of the tested fungicides and different concentrations of conidial suspension *Trichoderma viride* on the incidence of pre-and post-emergence damping off of maize caused by *Rhizoctonia solani*, 15 and 30 days after sowing, respectively.**

Treatments (g/kg seed)		Efficacy Against (%)		Shoot length (cm)	Root length (cm)
		Pre-emergence damping off	Post-emergence damping off		
Triticonazole	1.0	42.86	41.02	8.6	5.3
	1.5	46.42	48.98	15.4	6.6
	2.0	67.86	56.72	17.3	7.4
	3.0	73.56	86.43	20.0	9.3
	5.0	85.72	91.62	22.3	10.8
Azoxystrobin	1.0	28.56	32.45	9.3	6.3
	1.5	33.82	50.86	12.5	7.4
	2.0	50.86	67.86	16.6	9.1
	3.0	67.86	78.82	18.4	9.3
	5.0	78.57	83.42	20.2	9.6
Flutolanil	1.0	46.42	42.38	9.5	6.5
	1.5	57.15	56.46	13.2	6.8
	2.0	75.01	68.72	16.1	7.7
	3.0	85.72	83.72	20.3	9.8
	5.0	91.15	85.57	22.2	10.6
* <i>T. viride</i>	1x10 <sup>6</sup>	21.42	10.25	10.3	5.1
	3x10 <sup>6</sup>	46.42	15.38	13.4	6.5
	5x10 <sup>6</sup>	73.81	17.94	18.3	8.6
	7x10 <sup>6</sup>	75.01	38.46	18.5	9.7
L.S.D at 5%		10.6	8.5	3.2	2.4

\* spore per ml.

pre- and post emergence damping off of untreated maize were 35.3 and 47.9%, respectively. The shoot and root lengths were 7.2 and 4.8 cm, respectively.



**Table (4) : Effect of compatibility between *Trichoderma viride* and three fungicides for controlling pre and post-emergence damping off of maize caused by *Rhizoctonia solani*, 15 and 30 days after sowing, respectively.**

Treatments (g/kg seed)	Efficacy Against (%)		Shoot length (cm)	Root length (cm)
	Pre-emergence damping off	Post-emergence damping off		
T.V. (3x10 <sup>6</sup> ) + Triticonazole 1.5g	56.44	48.62	9.1	6.6
+ Triticonazole 2.0g	73.73	71.43	15.6	7.8
+ Triticonazole 2.5g	81.41	86.82	21.4	9.3
T.V. (5x10 <sup>6</sup> ) + Triticonazole 1.5g	64.42	50.86	10.3	7.1
+ Triticonazole 2.0g	75.01	76.28	15.8	7.4
+ Triticonazole 2.5g	93.44	91.44	22.4	10.2
T.V. (3x10 <sup>6</sup> ) + Azoxystrobin 1.5g	28.56	33.43	6.6	4.2
+ Azoxystrobin 2.0g	33.86	42.86	7.4	5.5
+ Azoxystrobin 2.5g	48.40	56.43	9.6	6.3
T.V. (5x10 <sup>6</sup> ) + Azoxystrobin 1.5g	33.82	41.02	6.6	4.4
+ Azoxystrobin 2.0g	67.86	48.48	8.2	5.5
+ Azoxystrobin 2.5g	73.56	56.43	10.4	7.8
T.V. (3x10 <sup>6</sup> ) + Flutolanil 1.5g	62.86	54.43	9.6	6.8
+ Flutolanil 2.0g	73.44	71.43	15.8	8.2
+ Flutolanil 2.5g	86.82	88.43	20.3	9.8
T.V. (5x10 <sup>6</sup> ) + Flutolanil 1.5g	73.43	61.43	10.4	7.7
+ Flutolanil 2.0g	84.43	77.53	16.3	8.6
+ Flutolanil 2.5g	95.05	95.57	22.5	10.4
L.S.D at 5%	9.7	8.3	3.6	2.5

\* Spore per ml.

pre- and post emergence damping off in untreated maize were 35.3 and 47.9%, respectively. The shoot and root lengths were 7.2 and 4.8 cm, respectively.

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## تأثير التضاد الحيوي لفطر ترايكودرما فيريدي وخلطه مع المبيدات الفطرية علي فطر ريزوكتونيا سولاني

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تمت هذه الدراسة لمعرفة فعل التضاد الحيوي لفطر ترايكودرما فيريدي المضادة لفطر ريزوكتونيا سولاني كذلك تأثير خلط فطر ترايكودرما فيريدي مع بعض المبيدات الفطرية المستخدمة، وهي تراي تيكونازول وأذوكس ستروبين وفلوتولانيل. وقد تم دراسة حساسية فطري ترايكودرما فيريدي وريزوكتونيا سولاني في بيئة PDA للمبيدات الفطرية. وقد وجد أن كلا من مبيدي تراي تيكونازول وفلوتولانيل قد ثبتا تماما نمو فطر ريزوكتونيا سولاني عند تركيز ١٠ ميكرو جرام / مللى في حين أن مبيد أذوكس ستروبين قد سبب نفس التثبيط للفطر عند تركيز ١٠٠ ميكرو جرام / مللى. أما بالنسبة لفطر ترايكودرما فيريدي فإنه لم يتأثر كثيراً بكل من المبيدين الفطرين تراي تيكونازول وفلوتولانيل بينما مبيد أذوكس ستروبين ذو تأثير مثبط أكبر من هذين المبيدين.

وعند دراسة فعالية التضاد الحيوي لفطر ترايكودرما فيريدي المضاد لفطر وريزوكتونيا سولاني في وجود المبيدات الفطرية في البيئة وجد أن خلط كل من المبيدين الفطرين تراي تيكونازول وفلوتولانيل مع فطر ترايكودرما فيريدي أدى إلى زيادة لتثبيط نمو فطر ريزوكتونيا سولاني وعلي العكس فإن المبيد الفطري أذوكس ستروبين لم يكن له هذا التأثير.

وفي تجارب الاخص قبل وبعد انبثاق بادرات الذرة المصابة بفطر ريزوكتونيا سولاني أوضحت النتائج أن كلا من المبيدين الفطرين تراي تيكونازول وفلوتولانيل كانا أكثر كفاءة في مكافحة المرض قبل وبعد انبثاق بادرات الذرة وذلك عند معدل ٣ ، ٥ جرام / كيلو جرام بذور وكذلك فإن فطر ترايكودرما فيريدي بمعدل  $7 \times 10^6$  جراثيم / مللى كانت لهم كفاءة عالية في مكافحة المرض قبل انبثاق بادرات الذرة ولكن هذا المعدل لم يكن كافياً في مكافحة المرض بعد الانبثاق. وبالنسبة لخلط المبيدات الفطرية مع فطر ترايكودرما فيريدي لمكافحة فطر ريزوكتونيا سولاني الممرض لبادرات الذرة قبل وبعد الانبثاق فإن كل من المبيدين الفطرين تراي تيكونازول وفلوتولانيل كافحا هذا المرض قبل وبعد انبثاق بارات الذرة. ومن ذلك يتضح أن الكفاءة الحيوية لفطر ترايكودرما فيريدي لمكافحة فطر ريزوكتونيا سولاني تزداد عند خلط لقاح الفطر مع التركيزات المنخفضة لكل من المبيدين الفطرين تراي تيكونازول وفلوتولانيل.