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EFFECT OF HERBICIDES APPLICATION ON BIOPROTECTANT ACTIVITY OF *TRICHODERMA HARZIANUM* AGAINST *RHIZOCTONIA SOLANI* INFECTING MAIZE SEEDLINGS.

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ABSTRACT: Effect of atrazine and oxadiargyl herbicides on the antagonistic activity of *Trichoderma harzianum* against *Rhizoctonia solani* was studied *in vitro* and *in vivo*. *Trichoderma harzianum* was able to grow well on PDA medium supplemented with atrazine up to 10µg/ml, where as 200µg/ml of this herbicide caused 52.2% reduction of colony growth of *T.harzianum*. At the same time, this concentration reduced the growth of *Rhizoctonia solani* by 28.8%. Both fungi were able to grow normally on PDA amended with oxadiargyl up to 100 µg/ml. Concentration of 200µg/ml of this herbicide caused 7.7 and 13.3% reduction of *T.harzianum* and *R.solani*, respectively. Results of antagonistic activity of *T.harzianum* in the presence of herbicides indicated that low concentrations of herbicides did not affect the antagonistic effect against *R.solani*; 10 µg/ml in PDA of any herbicide enhanced the antagonistic activity of *T.harzianum*. Gradual, increase the concentrations of the herbicides in PDA, gradually declined the antagonistic action of *T.harzianum* against *R.solani*. Oxadiargyl was more effective than atrazine, whereas 200µg/ml of atrazine or oxadiargyl decreased the antagonistic activity of *T.harzianum* by 20 and 10%, respectively. *In vivo* studies showed that using atrazine at 500, 750 and 1000g/fed., and oxadiargyl, at 66.4, 68.4 and 106.4 g/fed did not control *R.solani* on maize as shown by the percentage of emerged seedlings. Presence of *T.harzianum* in soil infested with *R.solani* controlled the pathogen and increased the emerged seedlings, and also increased the shoot and root growth of maize seedlings. Soil treated with low rates of the herbicides did not markedly affect the bioprotectant activity of *T.harzianum*. On contrary, the highest rate of the herbicides decreased the bioprotecant activity as indicated by the percentage of emerged seedlings and plant growth parameters.

INTRODUCTION

Biological agents have been used for controlling plant diseases and several authors suggested their beneficial effects in controlling various pathogens. Diab *et al.* (1990) reported that *Trichoderma harzianum* inhibited the growth of *Rhizoctonia solani* and *Fusarium solani* on PDA medium. They also found that seed treatment or soil infestation with *Trichoderm harzianum* caused a high significant reduction in pre-and post- emergence damping-off and increased the survival of pea plants. They added that *T.harzianum* in combination with root rot pathogens has a great effect on improving plant survival. Yieldz (1993) found that *Trichoderma* spp. may be applied either as seed coat or as wheat bran preparation by mixing inocula with the soil to control soilborne pathogens. Knudsen *et al.* (1995) reported that *Trichoderma* spp. were active as seed treatment to control the seed and soilborne fungi (*Fusarium culmorm and Cochliobolus sativus*). Ninq *et al.* (1998) reported that the seed borne pathogenic fungus, *Bipolaris* (Cochliobolus) supp, of Bermuda grass was controlled by coating seed with a conidial suspension (10^7 spore /ml) of *Trichoderma* sp. isolate and this resulted in better seed germination. However a spore concentration of *Trichoderma* sp. lower than (10^5 spore/ml) did not protect the seed from infection.

Effect of herbicides on plant pathogenic fungi may lead up to increase, decrease or no affect the incidence of plant diseases, depending on the fungus and the type of herbicide. Altman and Ross (1967) reported that the incidence of sugarbeet seedling damping - off caused by *Rhizoctonia solani* was increased in steamed and field soils treated with pebulate and PCA (Pyramin). Smiley and Wilkins (1992) found that wheat seedlings in chlorsulfuron - treated soil which was previously infested with *Rhizoctonai solani*, showed more severe root rot and reduced growth than those grown in untreated soil. Kawate *et al.* (1997) recorded that peas planted in soil where either downy brome or henbit had been treated with the herbicide glyphosate could be exposed to higher populations of *Fusarium solani f.*sp.*pisi* and *Pythium ultimum*.

Rodriguez-Kabana *et al.* (1966) found that the total mycelial dry weight of *Rhizoctonai solani* was considerably less in atrazine treatment than in the check. They found a positive relation between herbicidal concentration and the degree of growth inhibition. Cole and Baston (1975) reported that diphenamid reduced growth of *Rhizoctonia solani* and Pythium incidence of pre-emergence damping-off of tomato seedlings. Nowak and Michalcewicz (1995) studied the effects of two herbicides, i.e. Dicuran 80 WP and Dosmix on three fungal species, i.e. *Trichoderma viride* Pers ex Gray, *Trichocladium asperum* Harz and *Fusarium athrosporoides* Sherb. They observed that the rate of a colony diameter growth during incubation was limited by the two herbicides and the growth rate was decreased with increasing herbicidal dose in all the tested fungi.

The present study aims at investigating the effect of some herbicidal treatments on the bioprotectant activity of *Trichoderma harzianum*.

MATERIALS AND METHODS

The effect of two herbicides, i.e.: Gesaprim [80% atrazine, W.P.: 6-chloro-N^2- ethyl-N^4 isopropyl -1,3,5-triazine-2,4-diamine] and Topstar [80% oxadiargyl,W.G.: 5-tert-butyl-3- (2,4-dichloro-5-propargyloxy-phenyl)- 1,3,4-oxadiazol-2-(3H)-one] on two fungi used in this study, i.e.: *Trichoderma harzianum* and *Rhizoctonaia solani* was investigated in vitro and in vivo

In vitro test:

Aqueous stock solutions or suspensions of each herbicide were prepared, and 0.1 ml was added to autoclaved PDA medium cooled for 50°C to obtain various concentrations, i.e. 0.0, 1.0, 5.0, 10.0, 50.0, 100.0, 150.0 and 200.0 µg a.i/ml for each herbicide. The herbicide free treatments received the same quantity of water equivalent to the used in the herbicide treatments. After throughly mixing, herbicide - amended PDA was dispended into 9-cm petri plates. Discs of PDA bearing fungal growth 4 mm in diameter were cut from the growing edge of cultures representing each fungus (7 day - old culture) and were placed, each, in the center of each plate. Each fungus - herbicide combination was replicated three times. Radial growth of each replicate was measured after 7 days incubation at 25°C, and percent inhibition of radial growth for each fungus-herbicide combination was calculated.

Antagonistic activity:

The antagonistic activity of *Trichoderma harzianum* against *Rhizoctonia solani* was determined in herbicides amended media, according to the method described by Diab *et al.* (1990).One disc (5mm) from 7-days old culture of *T.harzianum* was transferred to PDA plates (9cm). At the same time, one disc (5mm) of *R.solani* was placed on the same plate oppositely at 5 cm apart from *T.harzianum* disc. Three replicates were used for each concentration and the mention before plates with *R.solani* alone or *R.solani* with *T.harzianum* without herbicide were used as control treatments. The plates were incubated at 25°C for 7 days and the inhibition area between *R.solani* and *T.harzianum* was measured and the inhibition percentage was calculated based on control treatments.

Pot experiment:

The experiment was designed as follows:

1- Soil treated with the herbicide 7 days after infestation with *Rhizoctonia solani*

2- Soil treated with the herbicide 7 days after infestation with *Trichoderma harzianum* and *R.solani.*

3- Herbicides - free soil infested with *R.solani* and *T.harzianum.*

4- Herbicides - free soil infested with *R.solani.*

5- Herbicides - free soil without fungal infestation .

A- Preparation of the fungal inoculum:

To obtain the inoculum of each of *R.solani* and *T.harzianum*, the fungi were grown at 25°C for 15 days in sterile sand - cornmeal medium (250gm of dry sand, 14gm of cornmeal, 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) for each fungus. The infested soil was distributed in pots (12 cm diameter). The pots were irrigated, using tap water and were left for 7 days to insure the establishment of inoculum.

B- Planting and soil treatments:

The pots were planted with maize seeds (*Zea mays* L., c.v. Giza hybrid 10), 10 seeds per each pot. The pots were sprayed with 10 ml containing the desired rate, i.e. 500, 750, 1000g/fed for atrazine and 66.4, 86.4 and 106.4 g/fed for oxadiargyl. Each treatment was replicated three times. After 21 days the emerged seedlings were recorded and the percentage of emergence was calculated based on control treatments and the means of shoots and roots lengths (cm) were taken into consideration

RESULTS and DISCUSSION

Results in Table (1) indicate that atrazine at 1 and 5µg a.i. /ml did not affect the growth of *R.solani*, but at 10µg a.i/, it reduced the fungal growth by 3.3%. The high reduction was observed at 200µg a.i./ml (28.8%). Although atrazine did not affect the growth of *T.harzianum* up to 10µg a.i./ml, it was potent at higher concentrations, at 200µg a.i./ml, it caused 52.2% inhibition. Thus, it could be mentioned that *T.harzianum* is more sensitive to atrazine than *R.solani*. On the other hand, oxadiargyl did not exhibit any fungitoxic effect to both fungi up to 100µg a.i./ml. At the higher concentration (200µg/ml) of this herbicide some reduction of the fungal growth was found.

Table (1): Effect of different concentrations (µg a.i./ml) of atrazine and oxadiargyl the growth of *Rhizoctonia solani* and *Trichoderma harzianum*.

Concentrations (µg a.i./ml)	% Growth inhibition			
	Atrazine		Oxadiargyl	
	R.solani	*T.harzianum*	*R.solani*	*T.harzianum*
1	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0
10	3.3	0.0	0.0	0.0
50	11.1	17.7	0.0	0.0
100	16.6	30.0	0.0	0.0
150	22.2	38.8	8.8	5.5
200	28.8	52.2	13.3	7.7

Effect of the herbicides on the antagonistic activity of T.harzianum

a) In vitro test:

Results presented in Table (2) show that the antagonistic activity of *T.harzianum* on the growth of *R.solani* was greatly influenced by the presence of atrazine and oxadiargyl in culture medium. *T.harzianum* suppressed the growth of *R. solani* 62.2% in the absence of the herbicides. At 1 µg a.i/ml, atrazine and oxadiargyl the antagonistic activity of *T.harzianum* was not greatly affect. However, both herbicides at 5 and 10µg a.i./ml increased the antagonistic activity. At 10µg a.i./ml the antagonistic activity reached 76.6 and 77.7% for atrazine and oxadiagrayl, respectively. Gradual, increasing the concentrations of the herbicides resulted in a great reduction in the antagonistic activity of *T.harzianum* against *R.solani*.

Table (2): Antagonistic activity of *Trichoderma harzianum* on the growth of *Rhizoctonia solani* in the presence of different concentrations of two herbicides.

Concentrations of herbicides (µg a.i./ml)	% Antagonistic activity in the presence of herbicides*	
	Atrazine	Oxadiargyl
1	64.4	60.0
5	73.3	75.5
10	76.6	77.7
50	51.1	42.2
100	30.0	32.2
150	22.2	16.6
200	20.0	10.0
Check**	62.2	62.2

* Antagonistic activity was measured as the inhibition activity of *Trichoderma harzianum* to growth of *Rhizoctonia solani*.

** Inhibition of *Rhizoctonia solani* by *Trichoderma harzianum* on herbicide-free PDA medium.

It was found that addition of 50µg a.i./ml of any herbicide in the medium declined the antagonistic activity of *T.harzianum* against *R.solani* 51.1 and 42.2% in the presence of atrazine and oxadiargyl, respectively. Moreover, addition of 200µg a.i./ml, of any herbicides reduced the antagonistic activity 20 and 10% for atrazine and oxadiargyl, respectively. These results demonstrated that low concentrations of the tested herbicides enhanced the antagonistic activity of *T.harzianum* against *R.solani*. Mean while, higher concentrations of atrazine and oxadiargyl reduced the antagonistic action. In this respects, oxadiargyl showed the lowest effect.

b. In vivo test:

Results in Table (3) summarize the influence of atrazine and oxadiargyl, at different rates, on the bioprotectant activity of *T.harzianum* against the infectivity of *R.solani* to maize. The results showed that atrazine and oxadiargyl were not sufficiently able to increase the emerged seedlings indicated by of maize in soil infested with *R.solani* as indicated by the shoot (18.4 cm) and root growth of emerged seedlings. Presence of *T.harzianum* in soil greatly controlled the infectivity of *R.solani* to maize seedlings as the percentage of emerged seedlings (70.8%) and the growth of shoot (18.4 cm) and root length (8.3cm). Atrazine on the basis of 500 g/fed. to somewhat reduced the bioprotectant activity of *T.harzianum* against *R.solani*. However, at 750 g/fed., it somewhat enhanced the *T.harzianum* bioactivity. Oxadiargyl, at 66.4 and 86.4g/fed, enhanced the activity of *T.harzianum* against *R.solani* as indicated by the percentage of emerged seedlings (75.0%) and the length of shoots and roots of emerged seedlings. On the other hand, both herbicides, at the high rates, markedly reduced the bioprotectant activity of *T.harzianum*. It was found that *T.harzianum* was not effectively able to control *R.solani* (37.0 and 33.3% emerged seedlings when the soil was treated with 1000 and 106.4 g/fed of atrazine and oxadiargyl, respectively). Also, these treatments showed the lowest values of shoots and roots of maize seedlings compared with herbicide- free soil. These results indicated that presence of atrazine and oxadiargyl at the highest rates suppressed the bioprotectant activity of *T.harzianum* against the *R.solani* and this caused reduction of the emerged seedlings and shortage maize seedlings.

It has been previously reported that *T.harzianum* can inhibit the growth of the most pathogenic fungi (Elad and Chet, 1995). The inhibitory action of *T.harzianum* was attributed to hyphal coils, hooks or appressoria formation of *T.harzianum* (Elad *et al.*, 1983). Micheal *et al.* (1988) reported that *T.harzianum* produced antibiotic against soil borne fungi. Recently, it has been reported that protease plays a role in biocontrol activity of *T.harzianum* against *Botrytis cinerea* (Elad and Kapat, 1999). Also, endo-1,3, β-glucanase and chitinase play a part in the antagonistic action of *T.harzianum* (Limon *et al.*, 1999).

Table (3) : Bioprotectant activity of *Trichoderma harzianum* against the pathogensis of *Rhizoctonai solani* on maize in the presence of different rates of the herbicides. Data were expressed as percentage of emerged seedlings and plant growth 21 days after planting.

Treatments (g/fed)	%, Emerged seedlings	Mean of plant growth (cm)	
		Shoot	Root
Atrazine 500 x R.S.	25.0	8.3	5.3
Atrazine 750 x R.S.	29.2	8.5	5.6
Atrazine 1000 x R.S.	33.3	9.4	6.1
Oxadiargyl 66.4 x R.S.	25.0	7.4	4.3
Oxadiargyl 86.4 x R.S.	33.3	8.1	4.6
Oxadiargyl 106.4 x R.S.	41.6	9.2	5.1
Atrazine 500 x T.H. x R.S	61.6	14.2	7.8
Atrazine 750 x T.H. x R.S	75.0	18.1	9.5
Atrazine 1000 x T.H. x R.S	37.0	6.1	5.7
Oxadiargyl 66.4 x T.H. x R.S	75.0	18.5	9.2
Oxadiargyl 86.4 x T.H. x R.S	75.0	16.8	9.8
Oxadiargyl 106.4 x T.H. x R.S	33.3	6.7	6.5
T.H. x R.S	70.8	18.4	8.3
Check 1	100.0	20.2	9.5
Check 2	28.3	6.9	4.6
L.S.D at 5%	--	2.8	1.6

T.H.: *Trichoderma harzianum* R.S.: *Rhizoctonia solani*

Check I: Healthy seeds in uninfested soil

Chedk II: Healthy seeds in soil infested with *R. Solani*

The present results indicated that low rates of the tested herbicides enhanced the bioprotectant activity of *T.harzianum* against the infectivity of *R.solani* to maize plants. Higher rates declined this bioprotecant activity. This may be explained by the effect of the herbicides on the growth of *T. harzianum* or by suppressing the antibiotic formations, protease, endo-1,3, β-glucanase and chitinase produced by *T.harzianum*, which consequently decrease its bioprotectant activity.

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مجلة الأزهر للبحوث الزراعية
العدد(٧) يونيو ٢٠٠٢

تأثير المعاملة بمبيدات الحشائش على فاعلية المكافحة الحيوية لفطر ترايكودرما هرزيانم المضاد لفطر ريزوكتونياسولاني الممرض لبادرات الذرة

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تم دراسة تأثير كل من مبيد الاترازين والاوكساداي أرجيل في المعمل والصوبة على فاعلية التضاد الحيوي لفطر ترايكودرماهرزيانم المضادة لفطر ريزوكتونياسولاني، وقد أوضحت النتائج أن فطر ترايكودرما هرزيانم يستطيع أن ينمو على بيئة PDA المعاملة بمبيد لاترازين بتركيز حتى ١٠ ميكروجرام/مللي في حين أن تركيز ٢٠٠ ميكروجرام/مللي من هذا المبيد قد أدى إلى انخفاض في معدل نمو الفطر بنسبة٥٢,٢٪ وكذلك فإن نفس هذا التركيز ادى إلى إنخفاض فى معدل نمو فطر ريزوكتونيا سولانى بنسبة ٢٨,٨٪ أما بالنسبة للبيئة المعاملة بمبيد الأوكساداي أرجيل فإن كلاً من الفطريـن قد نميا بشكل عادي حتى تركيز ١٠٠ ميكروجرام / مللي في حين أن تركيز ٢٠٠ ميكروجرام / مللي من هذا المبيد تسبب في انخفاض معدل نمو الفطر ترايكودرما هرزيانم بنسبة ٧,٧٪ ولفطر ريزوكتونيا سولاني بنسبة ١٣,٣٪ وقد أوضحت النتائج أن فاعلية التضاد الحيوي لفطر ترايكودرما هرزيانم في وجود التركيزات المنخفضة من كل من مبيدي الحشائش لم تؤثر على فاعلية التضاد الحيوي تجاه الفطر ريزوكتونيا سولاني، وعلى العكس فإن التركيزات المرتفعة تقلل بدرجة كبيرة على التأثير الحيوي لفطر ترايكودرما هرزيانم تجاه فطر ريزوكتونيا سولاني. كذلك فقد أوضحت النتائج أن مبيد الأوكساداي أرجيل كان أكثر تأثيراً من مبيد الاترازين في البيئة المعاملة بمبيد الأوكساداي أرجيل بتركيز ٢٠٠ ميكروجرام/مللي حيث سبب هذا التركيز نقص في التأثير الحيوي لفطر ترايكودرما هرزيانم بنسبة ١٠% في حين أن نفس هذا التركيز لمبيد الاترازين قد سبب نقص بنسبة ٢٠% للتأثير الحيوي المضاد لفطر ترايكودرما هرزيانم.

وأوضحت النتائج في تجارب الاصص على بادرات الذرة في أن المعدلات المستخدمة لمبيد الاترازين على أساس ٥٠٠ ، ٧٥٠ ، ١٠٠٠ جرام / فدان ومبيد الأوكساداي أرجيل بمعدل٤,٦٦ ، ٨٦,٤ ، ١٠٦,٤ جرام / فدان لم تكن فعاله ضد لفطر ريزوكتونيا سولاني. وكذلك فإن نفس المعدلات السابقة من مبيدي الحشائش قد سببا انخفاض الإصابة بالفطر ريزوكتونيا سولاني في وجود فطر ترايكودرما هرزيانم في التربة المعداة بفطر ريزوكتونيا سولاني مما أدى إلى زيادة في ظهور بادرات الذرة وزيادة في نمو المجموع الخضري والجذري للبادرات. وأخيراً فإن التربة المعاملة بمبيدي الحشائش بالمعدلات المنخفضة لم يكن لها تأثير على فاعلية المكافحة الحيوية للفطر ترايكودرما هرزيانم، وعلى العكس فإن المعدلات المرتفعة لمبيدي الحشائش قد سببا نقصاً في فاعلية المكافحة الحيوية للفطر وكان ذلك واضحاً من النسبة المئوية لإنبات البادرات وكذلك نمو النبات.