AJAB

Impact of pesticides on *Trichoderma harzianum* and on its possible antagonistic activity against *Fusarium oxysporum* under *In vitro* conditions

Nowara A. Mohamed¹, M. A. Radwan^{*2}

¹Department of Plant Protection, Faculty of Agriculture, Omar Al-Mukhtar University, El-Beida, Libya ²Department of Pesticide Chemistry and Technology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt

Received: July 26, 2017 Accepted: October 11, 2017 Published: December 30, 2017

Abstract

In vitro study was conducted to evaluate the effect of fourteen selected pesticides including six fungicides, four insecticides and four herbicides on the growth of a local strain of Trichoderma harzianum and on possible antagonistic activity of the treated fungus against Fusarium oxysporum. For compatibility study, each pesticide was tested at seven concentrations using poisoned food technique. While for the antagonistic activity study, treated T. harzianum was tested against F. oxysporium using dual culture technique under laboratory conditions. Significant differences were observed between the pesticides and the concentration used in the inhibition of mycelial growth and the inhibition increased with concentration increase. None of the concentrations tested of wettable sulphur, copper oxychloride, diazinon, cypermethrin, oxamyl, tribenuron-methyl and metribuzin suppressed the mycelial growth and the antagonistic potential of T. harzianum against F. oxysporium indicating the possibility of the integration between these pesticides and T. harzianum without any fear. However, the use of incompatible pesticides at all tested concentrations such as penconazole, iprodione, fenarimol and mancozeb and high concentrations of oxyfluorfen, glyphosate and imidacloprid may lead to inhibition of the growth and the antagonistic activity of *T. harzianum* as well as caution must be taken when using these pesticides in the disease management program. The obtained results will enable choice of combining T. harzianum with the selected pesticides within integrated disease management strategy.

*Corresponding author email: mohamedalymahmoud2020@gmail.com **Keywords:** Antagonistic activity, Compatibility, *Fusarium oxysporum*, Pesticides, *Trichoderma harzianum*

Introduction

Antagonistic microorganisms as biocontrol agents, offers environmentally safe, durable and cost effective alternative to chemical pesticides for disease management (Hallman et al., 2009). One of those organisms, *Trichoderma* spp., in particular *T. harzianum*, has been developed as a very promising

biocontrol agent to control a wide spectrum of soilborne plant pathogens (Hajieghrari et al., 2008) and it is also known to control root-knot nematodes (Radwan et al., 2012). *Trichoderma* type fungi have evolved different mechanisms of action, such as contest with the pathogen for space and nutrients, inhibition of pathogen multiplication by secreting antibiotics, antibiosis, production of fungal cell wall and



Nowara A. Mohamed et al.

nematode cuticle degrading enzymes, reduction of pathogen population through mycoparasitism, plant growth improvement and induction of the resistance in plants toward pathogens have been proposed as mechanisms for their biocontrol potential (Harman. 2006). However, biological control agent is often conflicting in its act especially in the field, scarcity of broad-spectrum disease suppression when compared with chemical pesticides (Roberts et al., 2005). Indeed, the bioagent alone will not be feasible to combat the diseases particularly in the case of severe incidence (Hildalgo-Diaz and Kerry, 2008). The combined use of biocontrol agents and chemical pesticides in integrated disease management (IDM) programs may be a promising solution to manage such plant diseases (Locke et al., 1985; Kredics et al., 2003). This integration may be synergistic or additive in disease suppression (Locke et al., 1985) and led to reduction of the amount of applied pesticides, minimizing environmental contamination hazards and plant pathogens resistance (Monte, 2001). However, the antagonistic activity of biocontrol agent may be influenced by the addition of pesticides and therefore these chemicals act as an important hindrance for increasing their role and effectiveness in IDM programs (Dłużniewska, 2003). There is scarce information in Libya about the response of the biocontrol efficacy of Trichoderma spp to commercial pesticides. Therefore, chemical laboratory investigations were conducted to evaluate the effects of some commercial pesticides on the mycelial growth of a local strain of T. harzianum and on its antagonistic ability against Fusarium oxysporum f. sp. lycopersici.

Materials and Methods

Fungal strains

A local strain of the bioagent, *Trichoderma harzianum* (TM-1) used in the present study was isolated from soil samples collected from a farm in El-Beida city, Al-Jabal Al-Khader Governorate, Libya, through serial dilution method on Potato Dextrose Agar (PDA) medium (Elad and Chet, 1983). The isolate was identified, based on phenotypic characters according to the references of Alexopoulos and Mime (1979) and Bisset (1991). Stock pure cultures of the test fungus were maintained on PDA slants and stored at 4°C until use. This strain (TM-1) has been shown antagonistic action against *Fusarium oxysporum* f. sp. *Lycopersici* (FM-1) which was isolated from greenhouse tomato

seedlings at El-Wesita region, El-Beida city, Libya (Mohamed and Idris, 2009).

Tested pesticides

Fourteen commercial pesticides which commonly used in Libya, were used in this study comprising six fungicides namely; copper oxychoride (Cupravit® 50% WP), fenarimol (Rubigan® 12% EC), iprodione (Rovral® 50% WP), mancozeb (Diathane® M-45 80% WP) penconazole (Topaz® 10% EC) and sulfur (Microvit® 80% WP); four insecticides namely; cypermethrin (Cyperkill® 25% EC), diazinon (Diazonox® 60% EC) imidacloprid (Confidor® 20% EC) and oxamyl (Vydate® 24% L) as well as four herbicides namely; glyphosate (Phomac® 48% SL), metribuzin (Vapcor® 70% WP), oxyflurofen (Platinor® 24% EC) and tribenuron-methyl (Granstar® 75% DF).

In vitro evaluation of some pesticides on the growth of *T. harzianum*

Fourteen pesticides were used to evaluate their effect on the growth of T. harzianum (TM-1). The pesticides were tested against the bioagent based on active ingredient using poisoned food technique (Dhingra and Sinclair, 1995). All pesticides were used at seven concentrations (1, 5, 10, 50, 100, 500 and 1000 µg/ml). The pesticide solutions were added to sterilize PDA medium (150 ml) from stock solution (2000 μ g/ml) to obtain the required concentrations and were totally mixed. Four Petri plates of each concentration of the pesticides were prepared by pouring 15 ml PDA aliquot in each sterilized plate of 90 mm diameter. After solidification of PDA, 5 mm discs of four days old PDA cultures of T. harzianum were centered placed in the plates for inoculation. The control was inoculated with the fungus without any addition of pesticide. All plates were incubated at 25 ± 2 °C till the mycelial growth of the bioagent completely covered the PDA in control plates (3 days). The radial growth of the colonies on PDA with and without pesticides was measured in two directions at right angles to each other. The percentage of inhibition in mycelial growth of the bioagent over control was calculated using the following formula

Mycelia growth inhibition (%) = $(C-T/C) \times 100$

Where C = average diameter of fungal colony in the control, and T = average diameter of fungal colony in treatment group.

Effect of pesticides on the antagonistic action of *T*. *harzianum* against *F*. *oxysporum*

To understand if the actual effect of pesticides is permanent or temporary, the pesticides treated fungus was grew on pesticide free medium for the recovery (72 h), after this period the possible antagonistic activity of the fungus against Fusarium oxysporum f. sp. Lycopersici was investigated. The antagonistic activity of the treated T. harzianum with fungicides at concentrations of 1, 5 and 10 µg/ml as well as with insecticides or herbicides at 100, 500 and 1000 µg/ml, was evaluated against the pathogenic fungus, F. oxysporum by dual culture technique (Morton and Stroube, 1955). Discs of 5 mm of seven days old culture of the F. oxysporum were placed on PDA plates on one side, 2.7 cm away from the edge of the plate. The discs of the recovered bioagent were placed in the same petri plate opposite side 6 cm apart. Inoculum of the pathogen and inoculum of the bioagent for control combination were sampled from cultures grown on the medium with no pesticide added. Plates having only pathogen served as control. The experiment was designed as a completely randomized with four replication for each treatment and the experiment was repeated twice. All plates were incubated at 25 ± 2 °C for 7 days after inoculation. The radial growth of the pathogen from the disc towards the center of the plate was recorded when the control plates were completely covered with mycelial growth of the pathogen. The percentage of inhibition in growth of the pathogen in the presence of bioagent was calculated over control as follows:

 $I = (C-T/C) \times 100$

Where I is inhibition of radial mycelial growth; C is radial growth measurement of the pathogenic fungus in control; T is radial growth of the pathogenic fungus in the presence of treated *T. harzianum*.

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) test except data of the radial growth of bioagent were arc sin transformed before being subjected to analysis. The least significant differences (LSD) at the 5% level of probability were used for comparison between means (Gomez and Gomez, 1984). Probit analysis outlined by Finney (1971) was made to determine the 50% and 90% inhibitory concentration (IC₅₀ and IC₉₀, respectively) of each pesticide.

Results

In vitro effect of some pesticides on mycelial growth of *T. harzianum*

As shown in Table 1, all the tested pesticides exerted varying degrees of inhibition in mycelial growth of the local strain T. harzianum. Out of six fungicides tested, wettable sulfur and copper oxychloride proved most compatible with T. harzianum. Compared to the control, these fungicides did not exert any lethal effect on the bioagent up to the concentration of 500 µg/ml and even $>1000 \mu g/ml$ concentration seems under safe tolerance limit (IC₅₀) which exhibited least growth inhibition of 4.03 % and 31.94%, respectively. Conversely, the bioagent was found most sensitive to penconazole, iprodione, fenarimol and mancozeb. Tolerance limits (IC₅₀) of these fungicides were < 1, 2.9, 3.6 and 17 μ g/ml, respectively. This indicates that these tested fungicides were lethal to the tested fungus and proved completely incompatible and are not suitable for integration with the bioagent (Table 1). Among the insecticides tested, the bioagent also showed good compatibility with cypermethrin, diazinon and oxamyl. No inhibition of bioagent growth was recorded up to the concentration of 1000 µg/ml of these insecticides while tolerance limits (IC₅₀) of these chemicals were $>1000 \mu g/ml$. Only cypermethrin inhibited just 41.67% growth of T. harzianum at 1000 µg/ml concentration (Table 1). For the herbicides tested, metribuzin, tribenuron-methyl and glyphosate proved good compatible with the local T. harzianum strain and reduced maximum growth (IC₉₀) at the concentration of $>1000 \mu g/ml$, while IC₅₀ of these herbicides were >1000, >1000 and $940 \mu g/ml$, respectively. No inhibition of bioagent growth was recorded up to the concentration of 1000 ppm tribenuron-methyl, whereas metribuzin and glyphosate inhibited 40.28% and 53.47% growth of T. harzianum at 1000 µg/ml, respectively comparable to the control. On the contrary, oxyfluorfen was less compatible with bioagent which exhibited toxicity towards its growth, with inhibition percent of 83.33 at the highest concentration of 1000 μ g/ml. The IC₅₀ and IC₉₀ values of this herbicide were 54 and $>1000 \mu g/ml$ (Table 1).

Nowara A. Mohamed et al.

Pesticidal groups	% Inhibition in mycelial growth over control								IC ₅₀	IC ₉₀
	1 μg/ml	$5 \mu g/ml$	$10 \mu g/ml$	50µg/ml	$100 \ \mu g/ml$	$500 \ \mu g/ml$	$1000 \ \mu g/ml$	Mean	(µg/ml)	
Fungicides	01973/0	35 65 65 7 8	20030-000	the second second	19262.0006	0.545.62595	1-040-040-040	A440682915	89	01201014
Penconazole	83.61	88.61	100.00	100.00	100.00	100.00	100.00	96.03	< 1	9.5
	(66.12)	(70.28)	(90.00)	(90.00)	(90.00)	(90.00)	(90.00)	(83.77)		
Mancozeb	16.94	20.69	27.78	79.58	100.00	100.00	100.00	63.57	17	110
	(24.30)	(26.87)	(31.81)	(63.27)	(90.00)	(90.00)	(90.00)	(59.46)		
Iprodione	17.22	74.31	83.33	100.00	100.00	100.00	100.00	82.12	2.9	11
	(23.91)	(59.59)	(65.90)	(90.00)	(90.00)	(90.00)	(90.00)	(72.77)		
Fenarimol	20.83	61.11	75.00	100.00	100.00	100.00	100.00	79.56	3.6	22
	(27.11)	(51.47)	(60.03)	(90.00)	(90.00)	(90.00)	(90.00)	(71.23)		
Wettable sulphur	0.00	0.00	0.00	0.00	0.00	0.00	4.03	0.57	>1000	>1000
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(11.38)	(5.09)		
Copper oxychloride	0.00	0.00	0.00	0.00	0.00	0.00	31.94	4.56	>1000	>1000
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(34.26)	(8.36)		
Insecticides										
Diazinon	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	>1000	>1000
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)		
Cypermethrin	0.00	0.00	0.00	0.00	0.00	0.00	41.67	5.95	>1000	>100
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(40.16)	(9.20)	0.7.7.7.7.7	0.0249.03
Imidacloprid	0.00	0.00	0.00	12.22	44.72	53.33	64.44	24.95	470	>1000
	(4.05)	(4.05)	(4.05)	(18.63)	(41.79)	(46.99)	(53.47)	(24.71)		
Oxamyl	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	>1000	>1000
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)		
Herbicides	()	()	()	((((()		
Oxyfluorfen	33.33	40.28	41.67	47.22	52.78	64.44	83.33	51.86	54	>1000
	(35.10)	(39.38)	(40.16)	(43.40)	(46.59)	(53.41)	(65.90)	(46.27)	1	
Tribenuron-methyl	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	>1000	>1000
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	- 1000	- 1000
	(1.02)	(1.05)	(1.52)	(1.05)	(1.05)	(1.02)	((1.05)		
Metribuzin	12.50	14.72	15.56	23.75	26.94	31.94	40.28	23.67	>1000	>1000
	(20.63)	(21.72)	(23.20)	(28.79)	(31.26)	(34.26)	(39.35)	(28.45)		
Glyphosate	0.00	0.00	0.00	0.00	0.00	47.22	53.47	14.38	940	>1000
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(43.39)	(47.01)	(15.80)		
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)	(4.05)		
Mean	12.29	19.98	22.88	30.85	34.96	39.79	47.94	()		
	(15.57)	(20.38)	(23.17)	(30.43)	(34.13)	(37.76)	(44.51)			
SD 0.05 Pest	(15.57) (25.50) (25.17) (56.45)				Concentrations (C) = 3.51			$P \ge C = 1.75$		

Table - 1: In vitro compatibility of Trichoderma harzianum with different concentrations of some commonly used pesticides

Average of four replications ; Figures in parentheses indicate arc sine transformed values

Effect of tested pesticides on the antagonistic activity of the fungus *T. harzianum* against *F. oxysporum*

Results from the dual culture assay showed that T. harzianum as antagonistic microorganism inhibited the mycelial growth of F. oxysporum, with varying efficiencies according to the pesticide concentrations exposed to the fungus. The local isolate of the bioagent, Τ. harzianum showed reasonable antagonistic activity against the pathogenic fungus, F. oxysporum, where the percent inhibition in growth of F. oxysporum in the presence of the bioagent was about 70% (Figures 1, 2 and 3). Compared to the control, none of either copper oxychloride or wettable sulfur concentrations used resulted a significant effect on the antagonistic ability of T. harzianum towards F. oxysporum. Fenarimol and penconazole significantly reduced growth inhibition of F. oxysporum and this means reduced the antagonistic ability of T. harzianum when treated with the concentration of 10 µg/ml. While its antagonistic activity significantly reduced when treated with mancozeb at the concentrations of 5 and 10 µg/ml. On the other hand, all tested concentrations of iprodione were highly effective as they significantly reduced the antagonistic ability of T.

harzianum compared to the control (Fig. 1). Regarding the insecticides, the results given in Fig. 2 show that cypermethrin, oxamyl or diazinon at the concentrations tested (100, 500 and 1000 µg/ml) did not significantly affect the antagonistic activity of T. harzianum against F. oxysporum compared to the control. A significantly reduced of the antagonistic ability of T. harzianum was observed only after the application of high imidacloprid concentrations (500 and 1000 µg/ml). As shown in Fig. (3), tribenuronmethyl at the concentrations tested (100, 500 and 1000 µg/ml) did not significantly affect the antagonistic activity of T. harzianum against F. oxysporum compared to the control. Either oxyfluorfen or metribuzin at the concentrations of 100 and 500 µg/ml did not significantly affect the antagonistic ability of T. harzianum compared to the control. Whereas the antagonistic ability of the bioagent was not affected by glyphosate at the concentration of 100 µg/ml. In general, oxyfluorfen and metribuzin have lower effect in reducing the antagonistic activity of T. harzianum than glyphosate.



Fig – 1: Effect of some fungicides on the antagonistic activity of *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *lycopersici:* (A) Fenarimol: (B) Penconazole; (C) Copper oxychloride; (D) Mancozeb: (E) Iprodione and (F) Wettable sulfur. Bars represent means (n=4) and vertical lines the standard deviation (SD). * Significantly different at p < 0.05 over their respective controls.



Fig - 2: Effect of some insecticides on the antagonistic activity of *Trichodema harzianum* against *Fusarium* oxysporum f. sp. lycopersici. (A) Imidacloprid (B) Cypermethrin; (C) Diazinon and (D) Oxamvl. Bars represent means (n = 4) and vertical lines the standard delation (SD). * Significantly different at p < 0.05 over their respective controls.



Fig – 3: Effect of some herbicides on the antagonistic activity of *Trichoderma harzianum* against *Fusarium oxysporum* f. sp. *lycopersici:* (A) Metribuzin; (B) Oxyfhiorfen; (C) Tribenuron- methyl and (D) Glyphosate. Bars represent means (n = 4) and vertical lines the standard deviation (SD). * Significandy different at p < 0.05 over their respective controls.

Discussion

A biocontrol agent *T. harzianum*, in addition to being as a key component of IDM program, must also be adaptable to modern crop agricultural practices such as pesticides. Therefore, it has become essential to verify the compatibility of the bioagent with chemical pesticides. Different concentrations, lower or higher that of recommended field dosage revealed varying effects of chemical pesticides on the growth of the strain of *T. harzianum* and on its antagonistic activity against *F. oxysporum* f. sp. *lycopersici*. In the present study, the fungicides; copper oxychloride and wettable sulphur were found to be non-lethal to *T. harzianum* and highly compatible with it (Likewise, Gaur and Sharma, 2010; Sarkar et al., 2010 and Tapwal et al., 2012). These findings may be due to this native fungus is able to degrade the two contact fungicides (Bhosale et al., 2015). In our study, however, penconazole a member of triazole fungicides, has more deleterious effect which completely inhibited the mycelial growth of *T. harzianum*. Similarly, penconazole was found to be incompatible with the bioagent with 87.41% growth inhibition was recorded at 25 μ g/ml (Sushir et al., 2015). The obtained results of iprodione are consistent with the findings of Saxena et al., (2014) who reported that iprodione was not found to be compatible with *T. harzianum* even at 25 μ g/ml, as it adversely inhibited the growth of the test antagonist by 83.1 %. Fenarimol at 800 μ g/ml was toxic to both

spores and mycelium of T. viride (Baicu, 1982) concur the present findings. The obtained results regarding to the fungicide, mancozeb supported by a study of Mclean et al., (2001) who stated that *T. harzianum* was extremely sensitive to this compound in terms of the inhibition. complete percent However, Ranganathswamy et al., (2012) showed that mancozeb was moderately compatible with Trichoderma Tapwal et al. (2012) also reported isolates. compatibility of Trichoderma sp with Dithane M-45 up to 300 µg/ml. Lower concentrations of mancozeb did not affect the radial growth of T. harzianum, whereas concentration above 8000 µg/ml was significantly reduced its growth (Bhale and Rajkonda, 2015). Cypermethrin and diazinon were found to be compatible with T. harzianum in the present study, which is supported by Baicu (1982) who reported that these pesticides were non-toxic to both the spores and the mycelium of T. viride. Imidacloprid has no adverse effect on T. harzianum at the low concentrations up to 100 μ g/ml, while it was found to be detrimental at the two higher concentrations (500 and 1000 µg/ml). It was moderately compatible with the bioagent and reduced maximum growth (IC_{90}) at the concentration of >1000 μ g/ml, while IC₅₀ value of this insecticide was 470 µg/ml (Table 1). Our findings are in harmony with those of Kumar et al., (2008) who reported that the radial growth of T. viride was less at higher concentrations of imidacloprid up to 72 h. Furthermore, Madhavi et al., (2008) reported that both T. harzianum and T. viride showed high compatibility with imidacloprid at concentration of 250 µg/ml. The low impact of imidacloprid on the fungus may be due to the ability of the fungus to degrade the pesticide especially in the case of low concentrations. This result is consistent with the findings of Tamilselvan et al., (2008) who found that the degradation rate of imidacloprid by the fungus T. viride was faster in lower concentrations than in higher concentrations. Soil fungus, Trichoderma spp. degraded diazinon to water soluble metabolites (Matsumura and Boush, 1968), degraded cypermethrin up to 100 µg/ml (Bhosle et al., 2013) and also degraded oxamyl when the fungus exposed to low dose of gamma radiation (Afify et al., 2013). The T. harzianum in the present study may be used the degradation products of these chemical pesticides as carbon and nitrogen source for their growth and activity. In our study, metribuzin was found to be safer up to 1000 μ g/ml where the growth inhibition of T. harzianum was 39.35%. These results are in agreement with that reported by Shrivastava

(2015) who found that metribuzin at the half recommended dose, recommended dose and double recommended dose, was not found to have any inhibitory effect against Trichoderma sp. Tribenuronmethyl is one of the sulfonylurea based herbicides used in the present study with no adverse effect against the bioagent. Similarly, Ciraj (1996) concluded that this group of herbicides had no significant deleterious effect on Trichoderma spp., and in some cases they stimulated growth of the fungi. Indeed, Trichoderma spp. were the most frequently isolated fungi from sulfonylurea herbicide treated soils (He et al., 2006). In our study, glyphosate had only a slight effect, in which the mycelial growth inhibitions were 47.22 and 53.47 % for 500 and 1000 µg/ml, respectively. This finding confirmed the published reports in which the radial growth of T. atroviride was not affected by glyphosate compared to control (Santoro et al., 2014). Also, Bhosale et al., (2015) proved that T. harzianum was able to degrade glyphosate at 300 µg/ml. In contrast, the obtained results did not agree with those reported by Chattannavar et al., (2006) who found that glyphosate suppressed the mycelial growth of T. harzianum by 73.81%. In the present study, oxyfluorfen had an inhibitory effect on the mycelial growth of T. harzianum, with percent inhibition increasing with the increase of its concentration. Also, oxyfluorfen had toxicity towards T. harzianum where the growth inhibition was 36.66% at 500 µg/ml (Sushir, 2001). Higher doses of oxyfluorfen have reduced the radial growth of the bioagent, whereas no adverse effect was observed at lower doses of the herbicide (Shrivastava, 2015).

of Several mechanisms direct and indirect antagonistic properties have been reported for Trichoderma spp. against pathogens (Harman, 2006). In our study, the insecticides; cypermethrin, oxamyl and diazinon and the fungicides; copper oxycloride and wettable sulphur as well as with some extent the herbicides; metribuzin, oxyfluorfen and tribenuron-methyl showed no deleterious effect on the antagonistic potential of T. harzianum against F. oxysporum. These results are in agreement with the results of Baicu (1982) who reported no common action of cypermethrin and diazinon against F. graminearum when associated with T. viride. In contrary, Dłużniewska (2003) found that copper oxvchloride (Miedzian 50 WP) showed most unfavorable to the antagonistic activity of Trichoderma spp. towards Botrytis cinerea, F. solani and Rhizoctonia solani. Anderson (1978) declared

Nowara A. Mohamed et al.

that soil fungi are less sensitive to herbicides and insecticides than fungicides. Similarly, in our investigation the local isolate of *T. harzianum* showed greater adverse effect with systemic fungicides than with herbicides and insecticides because of these systemic fungicides were not only safe to mycelial growth of the fungus but also to its antagonistic ability against the pathogenic fungus, *F. oxysporium*.

Conclusion

The results demonstrated that the tested pesticides caused different levels of growth inhibition of T. harzianum and of the antagonistic activity the treated bioagent against the pathogenic fungus, F. oxysporium. These differences may be due to the type of pesticide used and their concentrations as well as varies according to the sensitivity of the local strain fungus to the pesticide. None of the concentrations tested of wettable sulphur, copper oxychloride, diazinon, cypermethrin, oxamyl, tribenuron-methyl and metribuzin inhibited either T. harzianum growth or the antagonistic potential of this bioagent against F. oxysporium indicating the possibility of the integration between these pesticides and T. harzianum without any fear. However, the use of incompatible pesticides at all tested concentrations such as penconazole, iprodione, fenarimol and mancozeb and high concentrations of oxyfluorfen, glyphosate and imidacloprid severely affected the growth and the antagonistic activity of T. harzianum and caution must be taken when using these pesticides in IDM program. Under the experimental conditions, the current findings will supply the literature with more information in this context and the obtained results will enable choice of combining T. harzianum with the pesticides within IDM strategy. To confirm these results, further research under greenhouses and field conditions need to be investigated.

Conflict of interest statement

The authors declare that there is no conflict of interest in this research.

References

Afify AMR, Abo-El-Seoud MA, Ibrahim GM and Kassem BW, 2013. Stimulating of biodegradation

of oxamyl pesticide by low dose gamma irradiated fungi. J. Pl. Pathol. Microb. 4: 201.

- Alexopoulos CJ and Mime CW, 1979. Introduction of Mycology. 3th Edition. John Wiley and Sons.
- Anderson JR, 1978. Pesticide effect on non-target soil microorganisms; in Pesticide Microbiology, Eds., Hill IR, Wright SJL. London: Academic Press. pp. 313-533.
- Baicu T, 1982. Toxicity of some pesticides to *Trichoderma viride* Pers. Crop Prot. 1: 349-358.
- Bhale UN and Rajkonda JN, 2015. Compatibility of fungicides and antagonistic activity of *Trichoderma* spp against plant pathogens. Biosci. Methods. 6: 1-9.
- Bhosale AM, Borade SV and Raut RR, 2015. *In vitro* effect of certain fungicides, insecticides and herbicides on *Trichoderma harzianum*. Int. J. Inform. Futur. Res. 3:271-273.
- Bhosle NP, Khan ZS and Nasreen S, 2013. *In-vitro* degradation of cypermethrin through microorganisms by scale up technique. Int. J. Adv. Res. 1: 229-238.
- Bisset J, 1991. A revision of the genus *Trichoderma*. II. Infragernic classification. Canadian J. Bot. 69: 2357-2372.
- Chattannavar SN, Srikant K and Khadi BM, 2006. Effect of herbicides on *Trichoderma harzianum*. J. Cotton Res. Dev. 20: 296-297.
- Ciraj M, 1996. Impact of some sulfonylurea herbicides upon selected soil fungi. Sodobno kmetijstvo 29: 99-108.
- Dhingra OD and Sinclair JB, 1995. Basic plant pathology methods. CRC Press, Inc. Boca Raton, Florida. pp. 435.
- Dłużniewska J, 2003. Reaction of fungi of *Trichoderma* genus to selected abiotic factors. Electronic Journal of Polish Agriculture University (EJPAU) 6(2), Available Online at http://www.ejpau.media.pl/volume6/issue2/agron omy/art-04.html.
- Elad Y and Chet I, 1983. Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp.Phytoparasitica 11: 55-58.
- Finney DJ, 1971. Probit analysis, 3rd ed., Cambridge University Press, London pp.318.
- Gaur RB and Sharma RN, 2010. Biocontrol of root-rot in cotton and compatibility of potential bioagents with fungicides. Indian J. Pl. Prot. 38: 176-182.

- Gomez KA and Gomez AA, 1984. Statistical Procedure for Agricultural Research. 2nd Edition John Wiley and Sons, Inc. p. 680.
- Hajieghrari B, Torabi-Giglou M, Mohammadi MR and Davari M, 2008. Biological potential of some Iranian *Trichoderma* isolates in the control of soil borne plant pathogenic fungi. Afr. J. Biotechnol. 7: 967-972.
- Hallman J, Davies KG and Sikora R, 2009. Biological control using microbial pathogens, endophytes and antagonists. In: Perry, R.N., Moens, M., Starr, J.L. (Eds.), *Root-knot Nematodes*. CAB International, Wallingford, UK, pp. 380- 411.
- Harman GE, 2006. Overview of mechanisms and uses of *Trichoderma* spp. Phytopathol. 96: 190-194.
- He YH, Shen DS, Fang CR and Zhu YM, 2006. Rapid biodegradation of metsulfuron-methyl by a soil fungus in pure cultures and soil. World J. Microbiol. Biotechnol. 22: 1095-1104.
- Hildalgo-Diaz I and Kerry BR, 2008. Integration of biological control with other methods of nematode management. Pp. 29-49. *In*: Integrated Management and Biocontrol of Vegetable and Grain Crops: Nematodes (Integrated Management of Plant Pests and Diseases Vol. 2) (Ciancio A., Mukerji K.G. eds). Springer, Dordrecht, The Netherlands.
- Kumar K, Santharam G and Sridhar R, 2008. Compatibility of imidacloprid with plant pathogenic antagonistic microorganisms, *Trichoderma viride* (Pers) and *Pseudomonas fluorescens* (Migula) in cotton. Asian J. Bio Sci. 3: 171-175.
- Kredics L, Antal Z, Manczinger L, Szekeres A, Kevei F and Nagy E, 2003. Influence of environmental parameters on *Trichoderma* strains with biocontrol potential. Food Technol. Biotechnol. 41: 37- 42.
- Locke JC, Marois JJ and Papavizas GC, 1985. Biological control of *Fusarium* wilts of greenhouse-grown *Chrysanthemums*. Plant Dis. 69: 167-169.
- Madhavi M, Kumar C, Chandra P, Reddy DR and Singh TVK, 2008. Compatibility of mutant isolates of *Trichoderma* spp. with agrochemicals. J. Biol. Cont. 22: 51-55.
- Matsumura F and Boush GM, 1968. Degradation of insecticides by a soil fungus *Trichoderma viride*. J. Econ. Entomol. 61: 610-612.
- Mclean KL, Hunt J and Stewrat A, 2001. Compatibility of *Trichoderma harzianum* with

selected fungicides. New Zealand Plant Prot. 54: 84-88.

- Mohamed N and Idris JA, 2009. Evaluation the effective of some plant extracts on mycelium growth and spore germination of *Fusarium oxysporum* and *Alternaria alternate*. J. Agric. Sci., Mansoura Univ. 34: 2297-2308.
- Monte E, 2001. Understanding *Trichoderma*: between biotechnology and microbial ecology. Int. Microbiol. 4: 1- 4.
- Morton DT and Stroube NH, 1955. Antagonistic and stimulatory effects of microorganism upon *Sclerotium rolfsii*. Phytopathol. 45: 419- 420.
- Radwan MA, Farrag SAA, Abu-ELamayem MM and Ahmed NS, 2012. Biological control of the rootknot nematode, *Meloidogyne incognita* on tomato using bioproducts of microbial origin. Appl. Soil Ecol. 56: 58-62.
- Ranganathswamy M, Patibanda AK, Chandrashekar GS, Masuthi D, Dangi S and Kumar HBH, 2012. Compatibility of *Trichoderma* isolates to selected fungicides *in vitro*. Int. J. Plant Prot. 5:12-15.
- Roberts DP, Lohrke SM, Meyer SLF, Buyer JS, Bowers JH, Baker CJ, Lie W, de Souzaf JT, Lewis JA and Chung S, 2005. Biocontrol agents applied individually and in combination for suppression of soil borne diseases of cucumber. Crop Prot. 24: 141–155.
- Santoro PH, Cavaguchi SA, Alexandre TM, Zorzetti J, Oliveira Neves PM, 2014. *In vitro* sensitivity of antagonistic *Trichoderma atroviride* to herbicides. Brazilian Arch. Biol. Technol. 57: 238-243.
- Sarkar S, Narayanan P, Divakaran A, Balamurugan A and Premkumar R, 2010. The *in vitro* effect of certain fungicides, insecticides and biopesticides on mycelium growth in the biocontrol fungus *Trichoderma harzianum*. Turkish J. Biol. 34: 339 – 403.
- Saxena D, Tewari AK and Rai D, 2014. The *in vitro* effect of some commonly used fungicides, insecticides and herbicides for their compatibility with *Trichoderma harzianum* PBT23. World Appl. Sci. J. 31: 444-448.
- Shrivastava S, 2015. Non-target effects of various herbicides on biocontrol agent *Trichoderma spp.* and pathogen *Sclerotium rolfsii*. Indian J. Appl. Pure Biol. 30:11-19.
- Sushir MA, 2001. Testing the tolerance of *Trichoderma harzianum* (Rifai) to weedicides. J. Mycol. Plant Pathol. 31: Abstract.



- Sushir MA, Suryawanshi KK and Patole SP, 2015. Sensitivity of *Trichoderma harzianum* Rifai against systemic fungicides. Int. J. Appl. Res. 1: 403-405.
- Tamilselvan C, Pramila B, Hemananthan E, Hariharan B and Devarajan N, 2008. Aerobic degradation of the insecticide, imidacloprid by the antagonistic organisms, *Pseudomonas fluorescens* and

Trichoderma viride under *in-vitro* conditions. Pestol. 32: 16-19.

Tapwal A, Kumar R, Gautam N and Pandey S, 2012. Compatibility of *Trichoderma viride* for selected fungicides and botanicals. Int. J. Plant Pathol. 3: 89-94.