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Compatibility of *Trichoderma* spp. with Seven Chemical Fungicides Used in the Control of Soil Borne Plant Pathogens.

Ibrahim E. Elshahawy[a*], Karima H. E. Haggag[b], and Hassan Abd-El-Khair[a]

[a]Plant Pathology Department, Agricultural and Biological Research Division, National Research Centre, Dokki, Giza, Egypt.
[b]Pest Rearing Department, Central Agricultural Pesticides Laboratory, Agricultural Research Centre, Dokki, Giza, Egypt.

ABSTRACT

Compatibility of ten *Trichoderma* spp. isolates (three of *T. harzianum*, three of *T. viride*, one of *T. virens* and three of *Trichoderma* spp.) with seven fungicides *viz*., carbendazim, flutolanil, mancozeb, metalaxyl M + mancozeb, pencycuron, thiram + tolclofos-methyl and thiophanate-methyl was tested, under different concentrations ranged from 50 to 800 ppm, *in vitro* using poisoned food technique. Results reveal that *Trichoderma* spp. isolates were compatible with thiophanate-methyl, mancozeb, metalalaxyl M + mancozeb, pencycuron and flutolanil. While it was incompatible with carbendazim and thiram + tolclofos-methyl. All *Trichoderma* spp. isolates had antagonistic effect against *Fusarium solani, F. oxysporum, Rhizoctonia solani, Macrophomina phaseolina* and *Sclerotinia sclerotiorum* by reducing the growth in agar assays. Results also show that significant differences were observed among pathogenic fungi in their response to fungicides. Carbendazim completely inhibited the radial growth of all pathogenic fungi even at 100 ppm. While other fungicides show that with increase the concentrations, the efficacy was increased. Each of flutolanil, pencycuron and thiophanate-methyl when separately combined with *Trichoderma* spp. isolates reduced the growth of the tested soil borne pathogens in the ranges of 22.2 - 100%, 43.7 - 100% and 50.4 - 100%, compared to the reduction of 0.0 - 21.1% , 0.0 - 18.9% and 15.6 - 18.9% resulted by the same fungicides when used alone, respectively. Results suggested that the fungicides-*Trichoderma* spp. isolates combination may be effective in controlling soil borne pathogenic fungi than individual treatment and reduced the amount of fungicide used.

Keywords: Compatibility of fungicides with *Trichoderma* spp. isolates, biocontrol *in vitro*, soil borne pathogens.

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INTRODUCTION

Trichoderma spp. is one of the most common soil inhabitants and extensively studied as biocontrol agent in the management of soil borne plant pathogens such as *Fusarium, Phytophthora, Pythium, Rhizoctonia* and *Sclerotium* [1, 2]. On the other side, use of chemical fungicides for the control of soil borne diseases is costly in addition to fungicide residue is a major problem where causes soil & environmental pollution, human health hazards and adversely affects on the beneficial microorganisms in soil [3]. The combined use of biocontrol agents and chemical pesticides has attracted much attention in order to obtain synergistic or additive effects in the control of soil borne diseases [4]. Bardia and Rai [5] reported that *Trichoderma harzianum* Rifai combined with carbendazim were the best treatments in inhibiting the growth of *Fusarium oxysporum* Schlecht. emend. Snyd. *et* Hans f.sp. *cumini* Prasad et Patel *in vitro*. Khan and Shahzad [6] found that Topsin-M and carbendazim were the most effective fungicides that inhibit the growth of *T. harzianum*, *T. pseudokoningii* Rifai*, T.longibrachiatum* Rifai and *T. viride* Pers even at low concentration. Topsin-M completely suppressed the growth of *T. harzianum* at 10 ppm. Hameed [7] reported that tolclofos-methyl was strongly inhibited the growth of *R. solani* (100%) at all concentrations of 0.025, 0.05, 0.25 and 0.5 ppm, while it was slightly inhibited the growth of *T. harzianum* (65.60% - 79.66%), compared with *R. solani*.

Sarkar *et al*. [8] found that among the systemic fungicides, hexaconazole was the most toxic to mycelial growth of *T. harzianum*, followed by propiconazole and triflumizole. Toxicity of the contact fungicides was lower than the systemic fungicides. Ranganathswamy *et al*. [2] also found that carbendazim, benomyl, carboxin, propiconazole, hexaconazole, tricyclozole, tridemorph, chlorothalonil were incompatible with *T. harzianum* and *T. virens* J. H. Miller, Giddens &A.A. Foster) Arx showing 100 % inhibition of radial growth at field concentration. Dinocap, copper oxychloride, Fosetyl-Al, Captan, thiram and metalaxyl were least compatible showing more than 70 % inhibition of radial growth. Bordeaux mixture, azoxystrobin and mancozeb were moderately compatible with radial growth inhibition in the range of 20 - 45 %. Only watable sulphur was highly compatible with least inhibition of radial growth (2.2%) of tested *Trichoderma* [2]. Tapwal *et al*. [9] reported that Captan and Blue copper were compatible with *T. viride*. Archana *et al*. [10] showed that *T. viride* was inhibited by azoxystrobin 23 SC at a concentration above 15 ppm. *T viride* also was inhibited by Copper hydroxide (Kocide 3000) at a concentration above 2500 ppm [11]. Mohiddin and Khan [12] studied the compatibility of *T. harzianum* and *T. virens* with carbendazim, mancozeb, metalaxyl, captan, thiram, and Nemacur. They indicated that the safe tolerance concentration were 60, 1050, 160, 225, 25, and 980 µg/ml for *T. harzianum,* whereas the corresponding values for *T. virens* were 40, 1000, 125, 177, 9, and 700 µg/ml, respectively.

This work is aimed to study the compatibility of ten *Trichoderma* spp. isolates (three of *T. harzianum*, three of *T. viride*, one of *T. virens* and three of *Trichoderma* spp.) with seven selected chemical fungicides *viz.,* carbendazim, flutolanil, mancozeb, metalaxyl M + mancozeb, pencycuron, thiram + tolclofos-methyl and thiophanate-methyl and study the combined effect of *Trichoderma* spp. isolates with fungicides for controlling soil borne pathogenic fungi of *Fusarium solani* (Mart.) Appel *et* Wollenw. emend. Snyd. *et* Has*, Fusarium oxysporium* Schlecht. emend. Snyd. *et* Hans *, Rhizoctonia solani*, *Macrophomina phaseolina* (Tassi.) Goid and *Sclerotinia sclerotiorum* (Lib.) de Bary *in vitro*.

MATERIALS AND METHODS

Trichoderma spp. Isolates

Ten of *Trichoderma* spp. isolates *viz.,* three of *T.harzianum* (Th_1, Th_2 and Th_3), three of *T. viride* (Tv_1, Tv_2 and Tv_3), one of *T. virens* (Tvr) and three of *Trichoderma* spp. (Tsp_1, Tsp_2 and Tsp_3), which was isolated from Egyptian soil, were used in this study.

Chemical fungicides

Seven commercial fungicides *viz.,* carbendazim (Kemazed 50% WP), flutolanil (Moncut 25% WP), mancozeb (Tridex 80%), metalaxyl M + mancozeb (Ridomil Glod 68%), pencycuron (Monceren 25% WP), thiram + tolclofos-methyl (Rizolex T 50% WP) and thiophanate-methyl (Topsin–M 70% WG) were obtained from the Central Agricultural Pesticides Laboratory, Dokki, Giza, Egypt and used in this study.

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Soil borne plant pathogens

Five soil borne plant pathogens *viz.*, *F. solani* , *F. oxysporum, R. solani, M. phaseolina* and *S. sclerotiorum*, were obtained from Plant Pathology Department, National Research Centre (NRC). All soil borne plant pathogens were incubated at 25±2°C in all experiments in the present study, except *S. sclerotiorum* that was incubated at 20±2°C.

Compatibility of *Trichoderma* spp. isolates with fungicides

Compatibility of *Trichoderma* spp. isolates *viz.*, Th_1, Th_2 , Th_3 ,Tv_1, Tv_2 ,Tv_3 ,Tvr ,Tsp_1, Tsp_2 and Tsp_3 with the tested fungicides was tested via the method of poisoned food technique described by Schmitz [13]and Borum & Sinclair [14].The experiment was conducted in Completely Randomized Design. The autoclaved potato dextrose agar (PDA) medium was used. Fungicides concentrations were prepared based on the active ingredient and then was added to autoclaved PDA medium before its solidification to obtain the final concentrations of 0, 50, 100, 200, 300, 400, 500, 600, 700 and 800 ppm. About 0.1ml of Tween 80 (Sigma) was mixed gently with the medium to enhance its solubility. Then, 15 ml of fungicide amended PDA medium was poured in sterilized Petri plates (9 cm - diameter). The poisoned medium was allowed to solidify. The Tween 80 amended PDA medium without fungicide was kept as control. Then, 0.5 cm fungal mycelial disc of each *Trichoderma* spp. isolate was picked from 7-days-old purified culture with the help of a sterilized cork borer and then the disc was inoculated in the center of each plate. Three Petri plates were used as replicates for each treatment as well as untreated control. The diameter (cm) of *Trichoderma* spp. isolates growth was measured when the *Trichoderma* spp. isolates growth reached to the Petri plate edge in the control. The percent inhibition in the radial mycelial growth of the *Trichoderma* spp. isolates was calculated using the formula given by **Vincent [15]** as follow:

$$\textit{Trichoderma}\text{ growth inhibition (\%)} = [(dc-dt)/dc] \times 100$$

Where: dc = Average diameter of *Trichoderma* growth in control.
dt = Average diameter of *Trichoderma* growth in fungicide treatment.

Effect of fungicides on the growth of soil borne plant pathogens

Effect of all tested fungicides at the concentrations of 0, 100, 200, 400 and 600 ppm on the radial growth of *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. sclerotiorum* were evaluated via the poisoned food technique as mentioned before. The percent inhibition in radial growth of the pathogenic fungi caused by fungicides over the control was calculated using the formula given by Vincent [15] as mentioned before.

Antagonistic activity of *Trichoderma* spp. isolates against soil borne plant pathogens

The potential antagonistic activity of *Trichoderma* spp. isolates, *i.e.* Th_1, Th_2, Th_3, Tv_1, Tv_2, Tv_3, Tvr, Tsp_1, Tsp_2 and Tsp_3 toward the radial growth of pathogenic fungi were determined according to the method described by Bell *et al.*, [16]. The method consists of placing an active mycelial disc (5-mm in diameter) of 7-days-old purified culture of the pathogen at 1cm from the edge of a Petri plate (9-cm-diameter) containing freshly sterilized prepared PDA medium. A disc (5-mm in diameter) of 7- days-old purified culture of each *Trichoderma* spp. isolates was deposited in a diametrically opposed position at 1cm from the other set of the Petri plate. As a reference control, each pathogen as well as *Trichoderma* spp. isolates was separately grown on PDA Petri plate. Three Petri plates were used as replicates for each treatment as well as the control. Pathogen growth reduction (%) was calculated when the pathogen growth reached to the edge of Petri plate in control according the following formula:

$$\text{Pathogen growth reduction \%} = C - T / C \times 100$$

Where: C = Pathogen growth in the control plate.
T = Pathogen growth in the treatment plate.

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Effect of fungicides -*Trichoderma* spp. isolates combination against soil borne plant pathogens

The combined treatment of each of flutolanil, pencycuron and thiophanate-methyl at the concentration of 100 ppm with *Trichoderma* spp. isolates *viz.*, Th_1, Th_2, Th_3, Tv_1, Tv_2, Tv_3, Tvr, Tsp1, Tsp_2, and Tsp_3 against the radial growth of *F. solani*, *F. oxysporum*, *R. solani*, *M. phaseolina* and *S. sclerotiorum* was assessed by poisoned food technique and dual culture technique using PDA medium [13, 14, 17]. The experiment was conducted in Completely Randomized Design. Each concentration of flutolanil, pencycuron and thiophanate-methyl was separately prepared based on the active ingredient and then added to autoclaved PDA medium before its solidification to obtain the final concentration of 100 ppm. About 0.1 ml of Tween 80 (Sigma) was mixed gently with the medium to enhance its solubility. Then 15 ml of fungicide amended PDA medium was poured in sterilized 9 cm Petri plates. The poisoned medium was allowed to solidify. Mycelial discs (5-mm in diameter) of 7- days-old purified culture of each *Trichoderma* spp. isolate as well as pathogen were placed in Petri plate as mentioned before. Inhibition of pathogenic fungal growth (%) was calculated as mentioned before.

Statistical analysis

All experiments were designed Complete Randomized Design and data analyzed by using least squares analysis of variance (ANOVA), Least Significant Difference (LSD) test at P = 0.05 level of significance [18].

RESULTS

Compatibility of *Trichoderma* spp. isolates with tested fungicides

Results reveal that the all tested *Trichoderma* spp. isolates were tolerance to thiophanate-methyl at all tested concentrations, where no growth inhibition was observed, except Th_3 and Tsp_2, which showing 3.3 and 4.4% inhibition at 800 ppm (Fig. 1-10). As shown in Fig. 1-10, all tested *Trichoderma* spp. isolates were tolerance to mancozeb up to 600 ppm. The maximum growth inhibition of *Trichoderma* spp. isolates by mancozeb was obtained with Th_1 (by 8.9 and 38.9 %), followed by Th_2 (by 6.7 and 12.2%) at 700 and 800 ppm, respectively. Results also reveal that mancozeb caused the minimum growth inhibition by 4.4% with Tv_1 and Tsp_2 at 800 ppm, while it was not inhibited other species even at 800 ppm (Fig. 1-10). *Trichoderma* spp. isolates were tolerance to Metalalaxyl M + mancozeb up to 600 ppm. Metalalaxyl M + mancozeb showed the maximum growth inhibition to Th_1, followed by Th_2 and Tvr by 45.7, 31.9 and 17.4% at 800ppm, while the corresponding values for 700 ppm were 15.2, 20.0 and 13.3%, respectively. The same fungicide was inhibited the growth of Tv_1 and Tsp_2 by 4.4 and 5.5 % at 800 ppm, respectively as shown in Fig. 1-10.

The maximum growth inhibition of *Trichoderma* spp. isolates was obtained by pencycuron at 800 ppm, where the corresponding values were 26.6, 23.4, 22.2, 21.1, 20.0, 18.1, 15.6, 15.6 and 5.6% for Tvr, Tsp_3, Tv_3, Th_1, Tv_1, Tsp_2, Th_2, Th_3 and Tsp_1, respectively (Fig. 1-10). The pencycuron at 700 ppm was inhibited the growth of Tvr, Th_1, Tv_3, Tsp_2 and Th_2 by 25.6, 17.8, 15.6, 8.9 and 4.4%, while at 600 ppm it was inhibited the growth of Th_1 and Th_2 by 6.7 and 2.2%, respectively. The isolate Tv_2 was not inhibited by pencycuron up to 800 ppm (Fig. 1-10). Flutolanil gave the maximum growth inhibition to Tv_1 (by 45.0, 30.0 & 14.1 %), followed by Th_1 (by 25.6, 15.6 and 11.1%) at 800, 700 and 600 ppm, respectively. At 700 ppm, the fungicide was inhibited the growth of Tv_3, Th_3, Tvr and Tsp_2 by 16.7, 11.1, 9.6 and 7.8 %, respectively (Fig. 1-10).

On other hand, *Trichoderma* spp. isolates were incompatible with fungicides of carbendazim and thiram + tolelofos –methyl (Fig. 1-10). Carbendazim completely inhibited the growth of all tested *Trichoderma* spp. isolates even at 50 ppm. *Trichoderma* spp. isolates were sensitive to thiram + tolelofos–methyl at all tested concentrations, where the fungicide reduced the growth of *Trichoderma* spp. in the ranges of 52.2 – 67.0% at 50 ppm, 65.2 - 86.7% at 100 ppm, 70.4 – 89.3% at 200 ppm, 82.2-100.0 % at 300 ppm and 100.0% at ≥400 ppm, respectively (Fig. 1-10).

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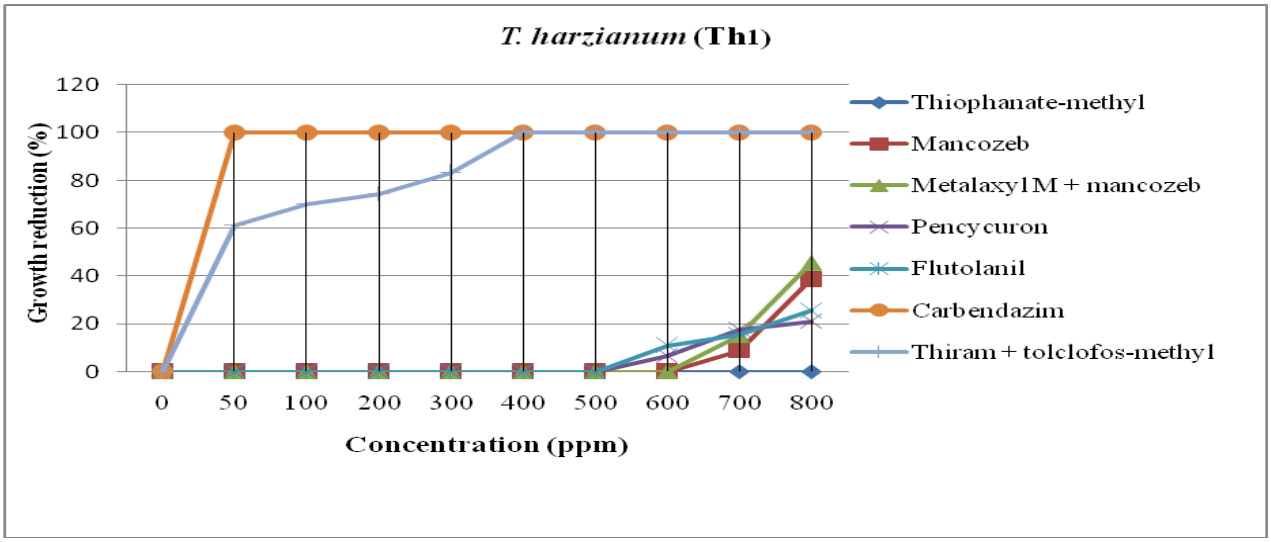

Figure 1: Compatibility of *T.harzianum* (Th_1) isolate with seven commercial fungicides *in vitro*.

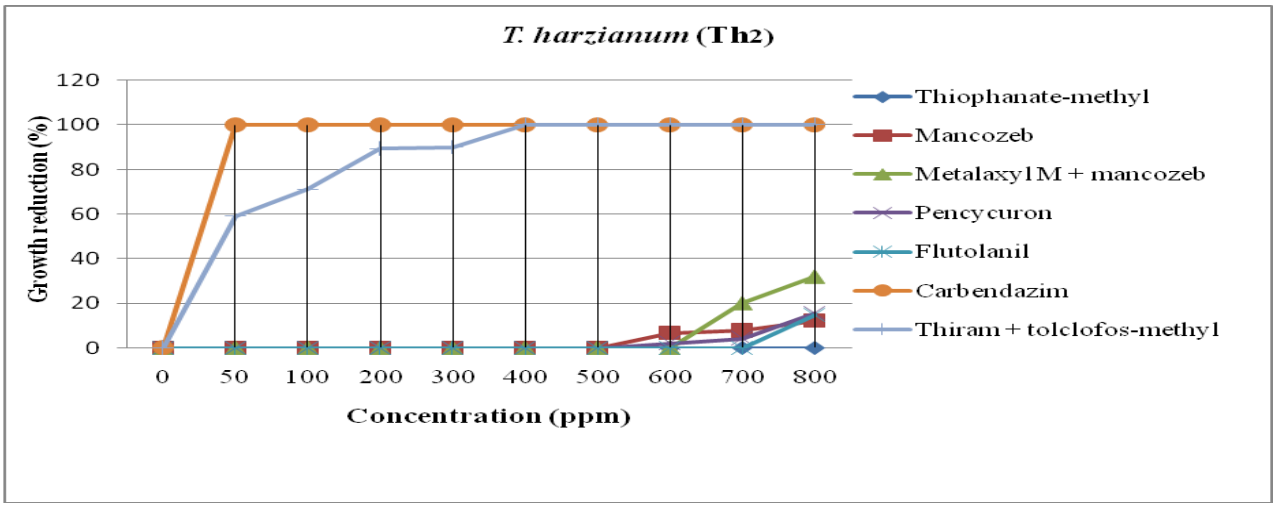

Figure 2: Compatibility of *T.harzianum* (Th_2) isolate with seven commercial fungicides *in vitro*.

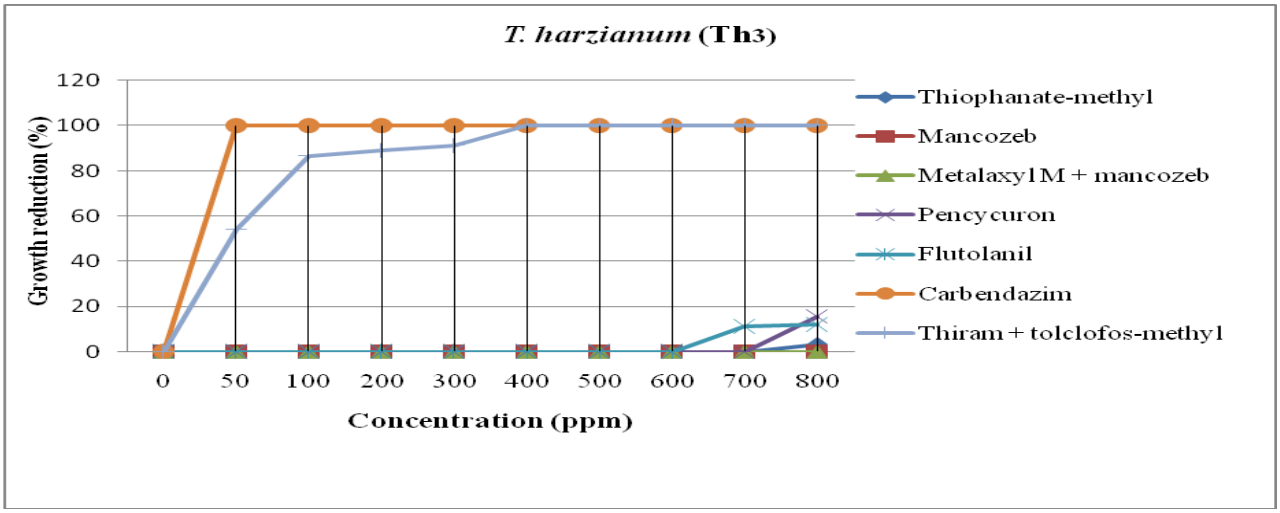

Figure 3: Compatibility of *T.harzianum* (Th_3) isolate with seven commercial fungicides *in vitro*.

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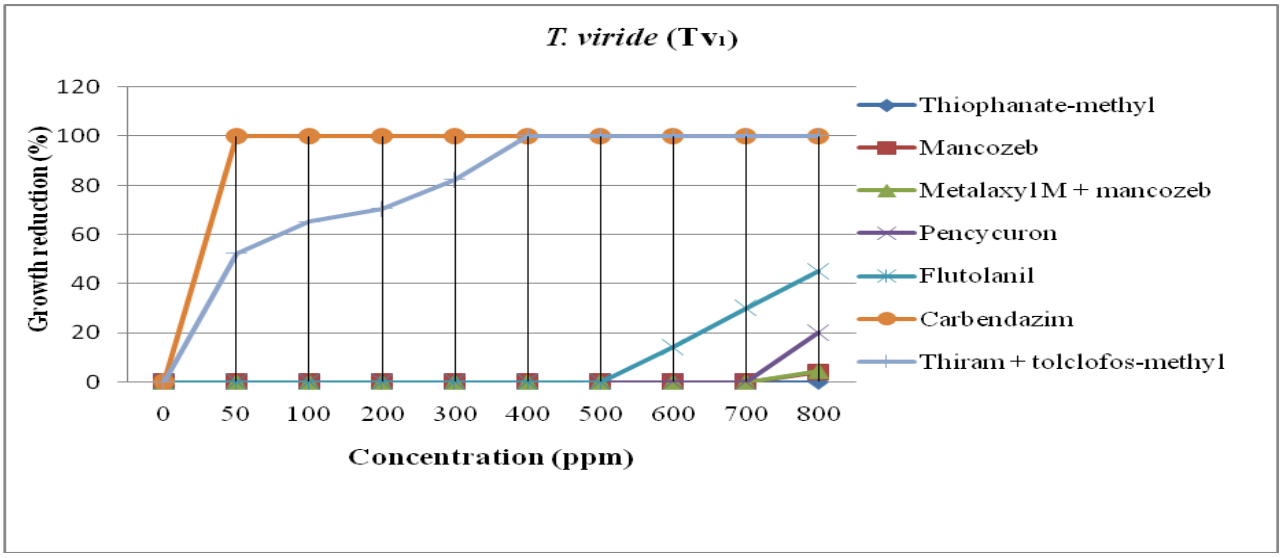

Figure 4: Compatibility of *T.viride* (Tv_1) isolate with seven fungicides *in vitro*.

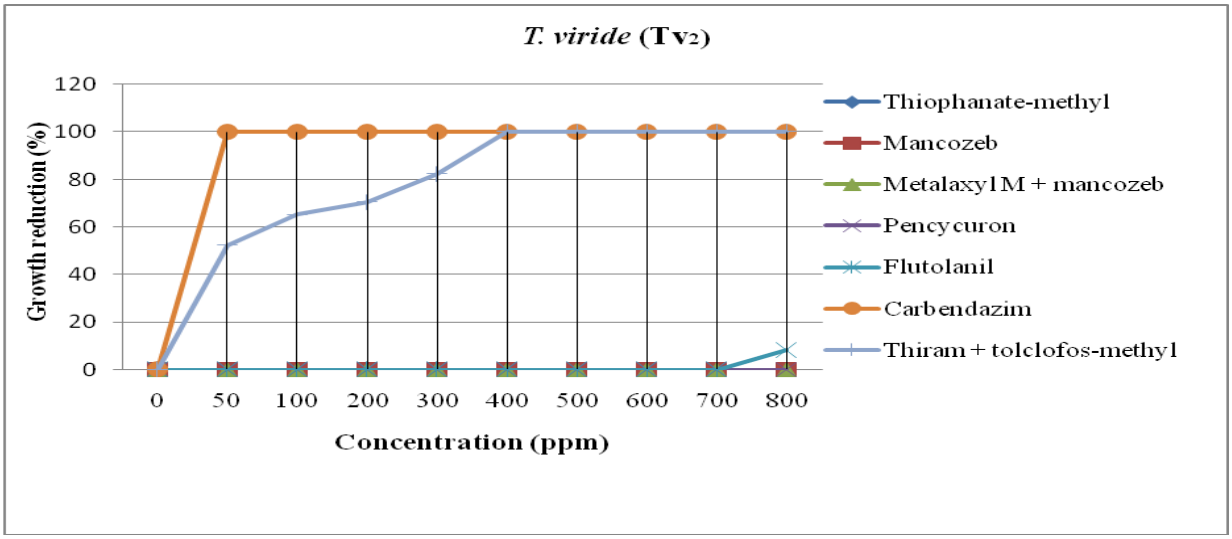

Figure 5: Compatibility of *T.viride* (Tv_2) isolate with seven fungicides *in vitro*.

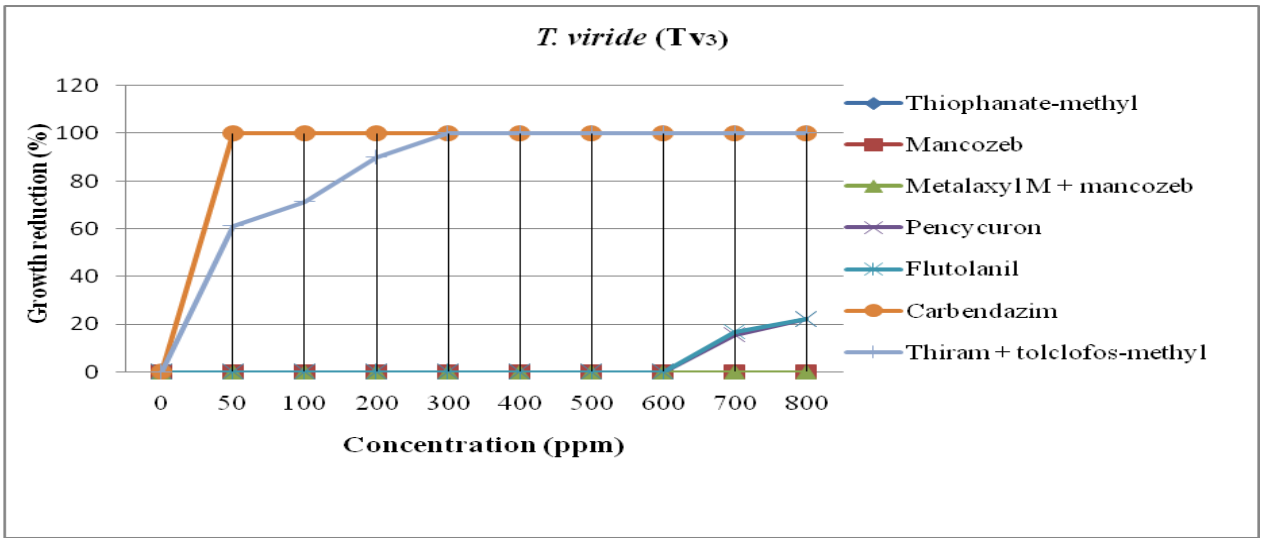

Figure 6: Compatibility of *T.viride* (Tv_3) isolate with seven fungicides *in vitro*.

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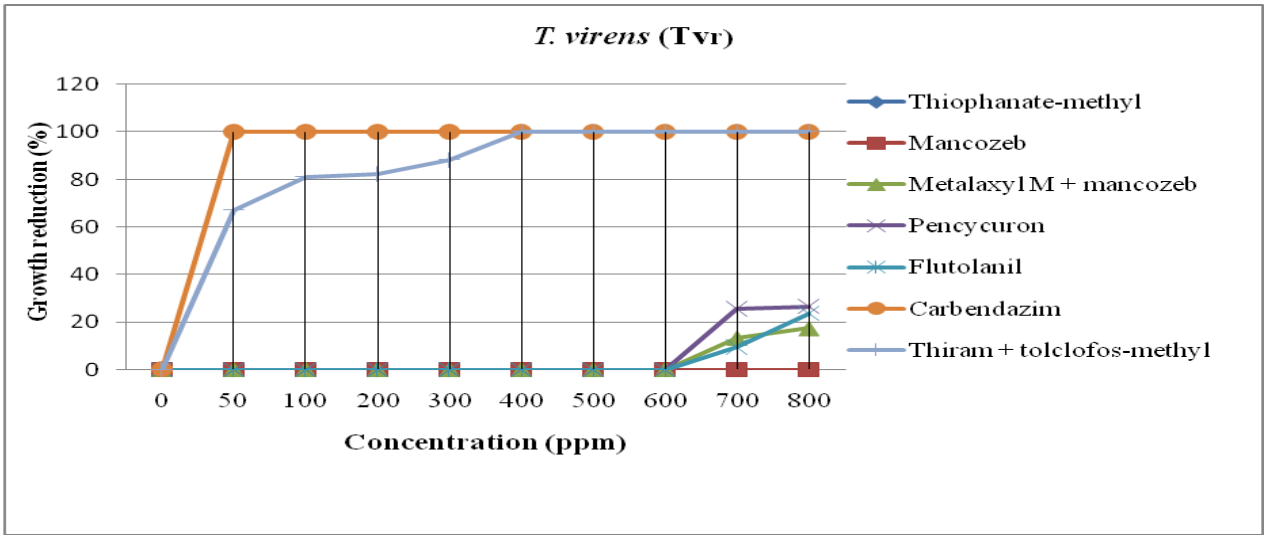

Figure 7: Compatibility of *T.virens* (Tvr) isolate with seven fungicides *in vitro*.

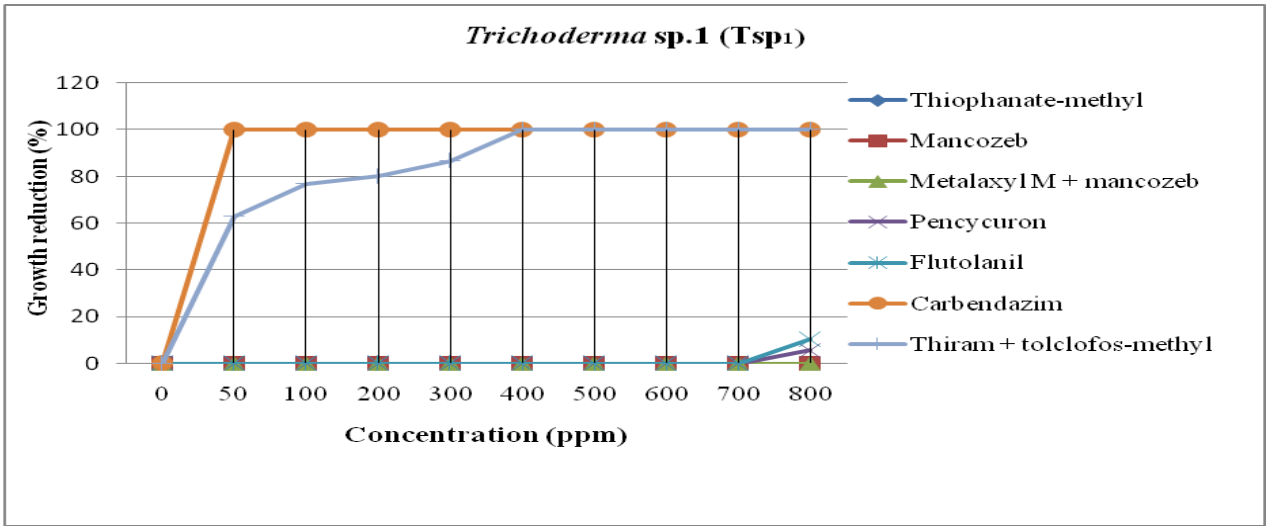

Figure 8: Compatibility of *Trichoderma* sp.1 (Tsp1) isolate with seven fungicides *in vitro*.

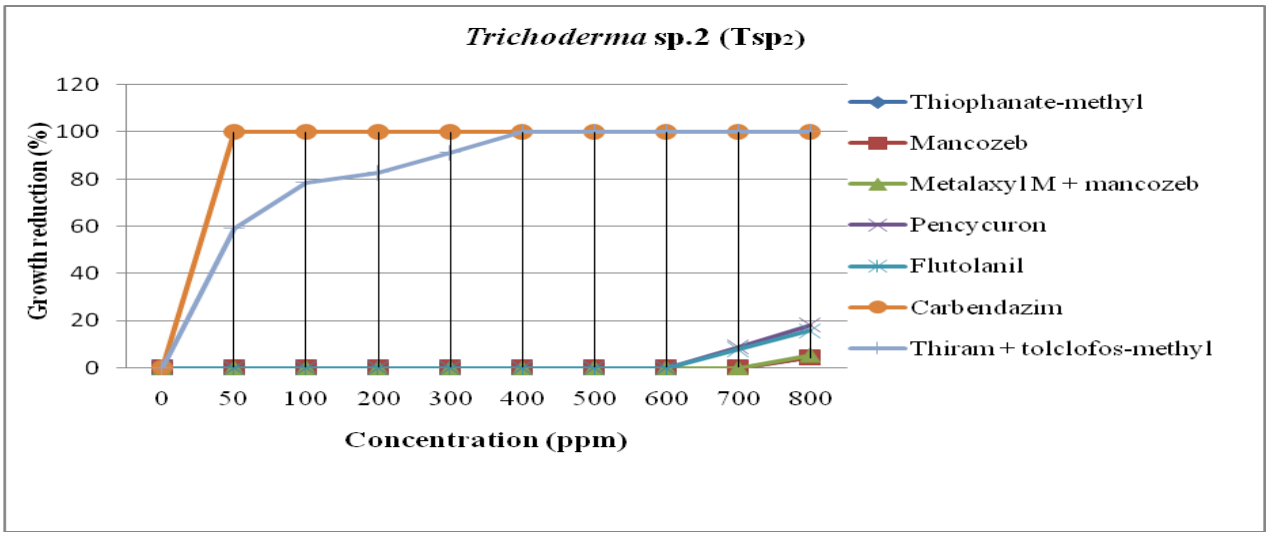

Figure 9: Compatibility of *Trichoderma* sp.2 (Tsp2) isolate with seven fungicides *in vitro*.

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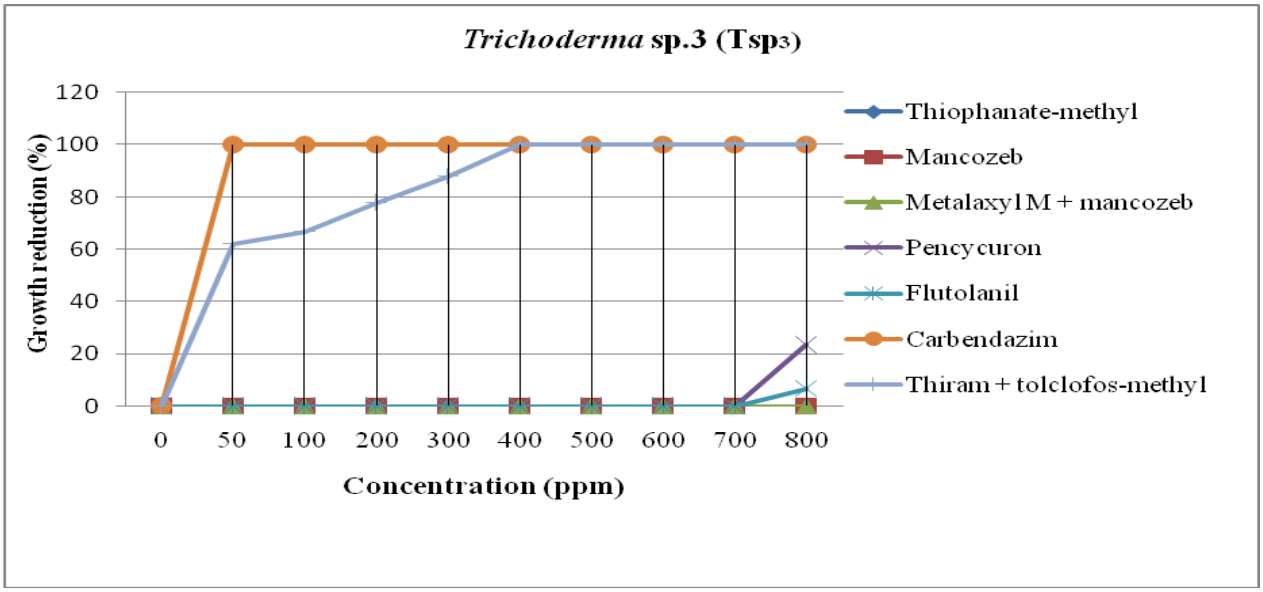

Figure 10: Compatibility of *Trichoderma* sp.3 (Tsp3) isolate with seven fungicides *in vitro*.

Inhibitor effect of the tested fungicides against soil borne plant pathogens

The inhibitory effect of the tested fungicides against the radial growth of soil borne plant pathogens at concentrations of 100, 200, 400 and 600 ppm are listed in Table 1. There were significant differences among pathogenic fungi in response to the tested fungicides. With increase the concentrations, the efficacy of fungicide was increased. Carbendazim was completely inhibited the growth of tested soil borne pathogens at all tested concentrations, except *F. oxysporum* which was inhibited by 60.2 and 88.2% at 100 and 200 ppm, respectively . Flutolanil also was completely inhibited the growth of *R. solani* at all concentrations, while it was inhibited the growth of *F. solani*, *F. oxysporum*, *M. phaseolina* and *S. scleratiorum* in the ranges of 17.7 - 61.5%, 21.1- 85.6%, 53.3 - 100.0% and 14.4 - 100.0%, respectively. Mancozeb was completely inhibited the growth of *S. scleratiorum*, while it was not showing any suppressive effect against *M. phaseolina* at all concentrations tested. The growth inhibition of *F. solani*, *F. oxysporum* and *R. solani* caused by mancozeb was in the ranges of 1.1 - 24.8%, 14.8 - 70.7% and 60.0 - 75.6%, respectively.

Metalaxyl M + mancozeb was completely inhibited the growth of *S. scleratiorun* at all concentrations, while it was slightly inhibited the growth of *M. phaseolina* by 10.4 and 16.7% at the concentrations of 400 and 600 ppm, respectively. Metalaxyl M + mancozeb was inhibited the growth of *F. solani*, *F. oxysporum* and *R. solani* in the ranges 4.4 - 70.0%, 7.8 - 70.4 % and 70.7 -100.0%, respectively (Table 1). Pencycuren was slightly inhibited the growth of *M. phaseolina* by 7.8% at 600 ppm only, while the other concentrations were not effective. The fungicide was inhibited the growth of *S. scleratiorun* (in the range of 18.9 - 72.2%), *R. solani* (in the range of 12.2 - 74.4%), *F. solani* (in the range of 4.4 – 51.1%) and *F. oxysporum* (in the range of 3.7 – 49.3%) at tested concentrations, respectively. Thiophanate-methyl was completely inhibited the growth of *S. scleratiorum* and *M. phaseolina* at 400 and 600 ppm, while the same fungi were inhibited by about 46.7 & 71.1% and 15.6 & 16.7% at 200 and 100 ppm, respectively. Thiophanate -methyl at all tested concentrations inhibited the growth of *R. solani*, *F. oxysporum* and *F. solani* by the range of 18.9 - 100.0 %, 18.2 - 71.5% and 17.1 - 64.8%, respectively. Thiram + tolelofos – methyl was completely inhibited the growth of *R. solani* at all tested concentrations ,while the fungicide was completely inhibited the growth of *F. solani*, *F. oxysporum*, *M. phaseolina* and *S. scleratiorum* at 400 and 600 ppm (Table 1).

Antagonistic effects of *Trichoderma* spp. isolates against soil borne plant pathogens

Trichoderma spp. isolates were significantly reduced the radial growth of *F. solani, F. oxysporum*, *R. solani, M. phaseolina* and *S. sclerotiorum* in the ranges of 38.1 - 66.7%, 49.2 - 58.2%, 35.5 - 51.1%, 25.6 - 41.5% and 34.4 - 88.9% , respectively. Among *Trichoderma* spp. isolates, Th_2 caused the maximum growth reduction of *F. solani*, followed by Tsp_3, Tsp_2, Tsp_1, Th_3, Tvr, Th_1, Tv_1, Tv_3 and Tv_2, where the reduction values were 66.7, 65.9, 63.3, 62.6, 61.5, 60.0, 59.2, 59.6, 51.1 and 38.1%, respectively. Significant differences were

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recorded between Tv_2 and Tv_3. For *F. oxysporum*, Th_1 highly reduced the fungus growth, followed by Tv_2, Tv_3, Tsp_3, Tsp_1, Th_3, Tsp_2, Tvr, Th_2 and Tv_1, where the corresponding reduction values were 58.2, 57.8, 57.4, 57.1, 57.0, 56.3, 56.3, 52.9, 52.2 and 49.2%. No significant differences were recorded among all *Trichoderma* species (Table 2).

Table 1: Inhibitor effect of seven commercial fungicides against the radial growth of soil borne plant pathogens *in vitro*.

Fungicide	Conc. (ppm)	Linear mycelial growth (cm) and growth reduction (%) of pathogenic fungi									
		F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. sclerotiorum*	
		Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
Carbendazim	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	0.00	100	3.53	60.2	0.00	100	0.00	100	0.00	100
	200	0.00	100	1.07	88.2	0.00	100	0.00	100	0.00	100
	400	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
	600	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
Flutolanil	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	7.43	17.7	7.10	21.1	0.00	100	9.00	0.0	7.70	14.4
	200	6.77	24.8	6.30	31.1	0.00	100	4.20	53.3	2.10	76.7
	400	5.40	29.0	5.47	39.3	0.00	100	1.90	80.0	0.00	100
	600	3.47	61.5	1.30	85.6	0.00	100	0.00	100	0.00	100
Mancozeb	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	8.90	1.1	7.67	14.8	3.60	60.0	9.00	0.0	0.00	100
	200	7.40	17.8	6.73	25.2	2.97	66.7	9.00	0.0	0.00	100
	400	7.20	20.2	4.10	54.4	2.30	74.4	9.00	0.0	0.00	100
	600	6.77	24.8	2.63	70.7	2.17	75.6	9.00	0.0	0.00	100
Metalaxyl M - Mancozeb	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	8.60	4.4	8.30	7.8	2.63	70.7	9.00	0.0	0.00	100
	200	6.27	30.4	6.17	31.5	1.43	83.3	9.00	0.0	0.00	100
	400	4.47	41.2	3.50	61.1	0.00	100	8.07	10.4	0.00	100
	600	2.70	70.0	2.67	70.4	0.00	100	7.50	16.7	0.00	100
Pencycuren	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	8.60	4.4	8.67	3.7	7.90	12.2	9.00	0.0	7.30	18.9
	200	6.40	28.9	5.90	34.4	3.83	55.6	9.00	0.0	4.30	52.2
	400	5.17	32.0	5.70	36.7	3.57	60.4	9.00	0.0	2.70	70.0
	600	4.40	51.1	4.57	49.3	2.30	74.4	8.30	7.8	2.50	72.2
Thiophanate - methyl	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	7.47	17.1	7.37	18.2	7.30	18.9	7.50	16.7	7.60	15.6
	200	5.00	44.4	4.97	44.8	1.47	83.3	2.60	71.1	4.77	46.7
	400	3.03	60.1	3.50	61.1	1.00	88.9	0.00	100	0.00	100
	600	3.17	64.8	2.57	71.5	0.00	100	0.00	100	0.00	100
Thiram + Tolelofos-methyl	0.0	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
	100	4.20	53.3	7.27	19.2	0.00	100	1.27	85.6	1.00	88.9
	200	0.80	91.1	1.97	78.2	0.00	100	0.00	100	0.00	100
	400	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100
	600	0.00	100	0.00	100	0.00	100	0.00	100	0.00	100

L.S.D. $_{0.05}$							
	Fungicide (F)	Pathogen (P)	F x P	Concentration (C)	F x C	P x C	F x P x C
Mycelial growth (cm)	0.10	0.08	0.22	0.07	0.19	0.16	0.43
Mycelial reduction (%)	0.9	0.7	1.9	0.7	1.7	1.5	3.8

The maximum growth reduction of *R. solani* was obtained by Tvr, followed by Tsp_3, Th_3, Tsp_2, Th_2, Tv_1, Th_1, Tv_2, Tsp_1 and Tv_3, where the growth reduction values were 51.1, 48.9, 48.2, 44.4, 42.6, 41.9, 38.2, 38.1, 36.7 and 35.5%, respectively. In case of *M. phaseolina*, the growth reduction by 41.5, 40.4, 39.6, 35.9, 35.9, 35.6, 35.6, 34.1, 25.6 and 25.6% was obtained with Th_1, Tvr, Tsp_3, Th_3, Tv_2, Th_2, Tsp_1, Tv_1, Tv_3 and Tsp_2, respectively. Tv_1 was highly reduced the growth of *S. sclerotiorum*, followed by Tv_1, Th_3, Th_2, Tsp_3, Tv_2, Th_1, Tv_3, Tvr, Tsp_1 and Tsp_2 where the inhibition values were 88.9, 79.6, 68.2, 54.8, 53.3, 50.0, 50.0, 49.6, 46.3 and 34.4%, respectively as shown in Table 2.

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Table 2: Biocontrol potential of *Trichoderma* spp. isolates against the radial growth of soil borne plant pathogens *in vitro*.

Trichoderma spp.	Linear mycelial growth (cm) and growth reduction (%) of pathogenic fungi									
	F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. sclerotiorum*	
	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
***T. harzianum* (Th_1)**	3.67	59.2	3.77	58.2	5.57	38.2	5.27	41.5	4.50	50.0
***T. harzianum* (Th_2)**	3.03	66.7	4.30	52.2	5.17	42.6	5.80	35.6	2.87	68.2
***T. harzianum* (Th_3)**	3.47	61.5	3.93	56.3	4.67	48.2	5.77	35.9	1.83	79.6
***T. viride* (Tv_1)**	3.63	59.6	4.57	49.2	5.23	41.9	5.93	34.1	1.00	88.9
***T. viride* (Tv_2)**	5.57	38.1	3.80	57.8	5.57	38.1	5.43	35.9	4.20	53.3
***T. viride* (Tv_3)**	4.40	51.1	3.83	57.4	5.80	35.5	6.70	25.6	4.50	50.0
***T virens* (Tvr)**	3.60	60.0	4.23	52.9	4.40	51.1	5.37	40.4	4.53	49.6
***Trichoderma* sp. (Tsp_1)**	3.37	62.6	3.87	57.0	5.70	36.7	5.80	35.6	4.83	46.3
***Trichoderma* sp. (Tsp_2)**	3.30	63.3	3.93	56.3	5.00	44.4	6.70	25.6	5.90	34.4
***Trichoderma* sp. (Tsp_3)**	3.07	65.9	3.87	57.1	4.60	48.9	5.43	39.6	4.07	54.8
Control	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
$L.S.D._{0.05}$										
	Bioagent (B)		Pathogen (P)		B x P					
Mycelial growth (cm)	0.47		0.32		1.06					
Mycelial reduction (%)	5.5		3.9		12.4					

Effects of fungicides -*Trichoderma* spp. combinations against soil borne plant pathogens: Flutolanil - *Trichoderma* spp. isolates combination

The combination of flutolanil with *Trichoderma* spp. isolates were reduced the *R. solani* growth by 100.0% as well as flutolanil alone (Table 3). Flutolanil in integration with *Trichoderma* spp. isolates reduced the growth of *F. solani* in the range of 55.9 to 65.6%, compared to 17.4% with flutolanil alone. Flutolanil combined with Th_3 or Tv_2 was highly reduced the growth of *F. solani*, followed by Tsp_3, Th_1, Tv_3, Tsp_1, Tsp_2, Tv_1, Tvr and Th_2, where the reduction values were 65.6, 65.6, 64.4, 62.6, 62.6, 62.2, 58.9, 58.2, 58.2 and 55.9%, respectively. Flutolanil - *Trichoderma* spp. isolates treatments were reduced the growth of *F. oxysporum* by the range of 38.9 to 64.4%, compared to 21.1% with flutolanil alone. Futolanil combined with Tv_2 showed the greater inhibition against *F. oxysporum* growth, followed by the combination of flutolanil with each of Tsp_1, Tv_1, Th_2, Tv_3, Tsp_3, Tvr, Th_3, Tsp_2 and Th_1, where the reduction values were 64.4, 63.3, 61.1, 60.4, 59.2, 58.2, 55.6, 54.0, 53.7 and 39.8, respectively. Significant differences were recorded between Th_1 and Tsp_1 as well as among other *Trichoderma* spp. isolates.

Table 3: Interaction effect of *Trichoderma* spp. isolates with Flutolanil (Fl) at the concentration of 100 ppm against the radial growth of soil borne plant pathogens *in vitro*.

Treatment	Linear mycelial growth (cm) and growth reduction (%)of pathogenic fungi									
	F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. sclerotiorum*	
	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
Control	9.00	-	9.00	-	9.0	-	9.00	-	9.00	-
Flutolanil (Fl) at 100 ppm	7.43	17.4	7.10	21.1	0.0	100	9.00	0.0	7.70	14.4
T. harzianum (Th_1) + Fl	3.37	62.6	5.50	38.9	0.0	100	6.67	25.9	2.27	74.8
T. harzianum (Th_2) + Fl	3.97	55.9	3.57	60.4	0.0	100	6.80	24.4	2.17	76.7
T. harzianum (Th_3) + Fl	3.10	65.6	4.13	54.0	0.0	100	6.70	25.6	0.97	89.3
T. viride (Tv_1) + Fl	3.77	58.2	3.50	61.1	0.0	100	6.90	23.3	1.77	80.4
T. viride (Tv_2) + Fl	3.10	65.6	3.20	64.4	0.0	100	6.40	28.9	1.00	88.9
T. viride (Tv_3) + Fl	3.37	62.6	3.67	59.2	0.0	100	5.00	44.4	1.13	87.4
T. virens(Tv_r) + Fl	3.77	58.2	4.00	55.6	0.0	100	6.67	25.9	1.20	86.7
Trichoderma sp. (Tsp_1) + Fl	3.40	62.2	3.30	63.3	0.0	100	6.57	27.0	2.23	75.2
Trichoderma sp. (Tsp_2) + Fl	3.70	58.9	4.17	53.7	0.0	100	7.00	22.2	1.27	80.7
Trichoderma sp. (Tsp_3) + Fl	3.20	64.4	3.83	58.2	0.0	100	6.77	24.8	1.43	84.1
$L.S.D._{0.05}$										
	Bioagents (B)		Pathogens (P)				B x P			
Mycelial growth (cm)	0.31		0.20				0.70			
Mycelial reduction (%)	4.5		2.7				9.0			

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Flutolanil - *Trichoderma* spp. isolates combination also was reduced the growth of *M. phaseolina* in the range of 22.2 to 44.4%, compared to zero reduction with flutolanil alone. Flutolanil - Tv_3 combination was significantly reduced the growth of *M. phaseolina* by 44.4%. The treatment reduced the growth of *S. sclerotiorum* in the range of 74.8 to 89.3%, compared to 14.4% with flutolanil alone. Flutolanil when separately combined with each of Th_3, Tv_2, Tv_3, Tvr, Tsp_3, Tsp_2, Tv_1, Th_2, Tsp_1 and Th_1 showed the inhibitory effect by 89.3, 88.9, 87.4, 86.7, 84.1, 80.7, 80.4, 76.7, 75.2 and 74.8% against the growth of *S. sclerotiorum*, respectively as shown in Table 3.

Pencycuron - *Trichoderma* spp. isolates combination

The combinations of pencycuron (100 ppm) with *Trichoderma* spp. isolates were significantly reduced the growth of *F. solani* in the range of 46.0 to 100.0%, compared to 4.4% in pencycuron alone. Pencycuron when combined with Th_3, Tv_3, Tsp_2 and Tsp_3 were completely inhibited the growth of *F. solani* by 100.0%. The combined of pencycuron with each of Tv_2, Tvr, Tv_1, Th_2, Tsp_1 and Th_1 were reduced the growth of *F. solani* by 72.2, 64.8, 54.4, 50.4, 49.3 and 46.0%, respectively. Pencycuron in integration with *Trichoderma* spp. isolates were reduced the growth of *F. oxysporum* by the range of 43.7 to 67.1%, compared to 3.7% in pencycuron alone. Tv_3 or Tvr when combined with pencycuron highly reduced *F. oxysporum* growth, followed by Th_3, Tv_1, Tsp_1, Tv_2, Tsp_2, Tsp_3, Th_2 and Th_1, where the growth reduction values were 67.1, 67.1, 65.9, 65.6, 64.4, 57.8, 57.1, 54.8, 49.3 and 43.7%, respectively. Significant differences were recorded between Th_1 and Th_2 as well as among other *Trichoderma* spp. isolates (Table 4).

Table 4: Interaction effect of *Trichoderma* spp. isolates with Pencyceron (Pe) at the concentration of 100 ppm against the radial mycelial growth of soil borne plant pathogens *in vitro*.

Treatment	Linear mycelial growth (cm) and growth reduction (%) of pathogenic fungi									
	F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. sclerotiorum*	
	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
Control	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
Pencycuron (Pe at 100 ppm	8.60	4.4	8.67	3.7	7.90	12.2	9.00	0.0	7.30	18.9
T. harzianum (Th_1) + Pe	4.87	46.0	5.07	43.7	1.10	87.8	4.57	49.3	2.57	71.5
T. harzianum (Th_2) + Pe	4.47	50.4	4.57	49.3	0.00	100	3.70	58.9	2.47	72.6
T. harzianum (Th_3) + Pe	0.00	100	3.07	65.9	0.00	100	4.30	52.2	2.90	67.8
T. viride (Tv_1) + Pe	4.10	54.4	3.10	65.6	0.00	100	3.47	61.5	2.40	73.3
T. viride (Tv_2) + Pe	2.50	72.2	3.80	57.8	0.00	100	3.67	59.3	2.37	73.7
T. viride (Tv_3) + Pe	0.00	100	2.97	67.1	0.00	100	3.73	54.4	2.40	73.3
T. virens (Tvr) + Pe	3.17	64.8	2.97	67.1	0.00	100	3.53	60.8	2.80	68.9
Trichoderma sp. (Tsp_1) + Pe	4.57	49.3	3.20	64.4	0.00	100	4.00	55.6	2.30	74.8
Trichoderma sp. (Tsp_2) + Pe	0.00	100	3.87	57.1	0.00	100	3.20	64.4	2.77	69.3
Trichoderma sp. (Tsp_3) + Pe	0.00	100	4.07	54.8	0.00	100	3.60	60.0	2.10	76.7
L.S.D. $_{0.05}$										
	Bioagents (B)		Pathogens (P)		B x P					
Mycelial growth (cm)	0.26		0.17		0.59					
Mycelial reduction (%)	3.3		2.3		7.5					

The radial growth of *R. solani* was reduced by the range of 87.8 to 100% with pencycuron - *Trichoderma* treatment, compared to 12.2% in pencycuron alone. All combinations were completely inhibited the *R. solani* growth, except Th_1 (87.8% reduction). The combinations also were reduced the growth of *M. phaseolina* in the range of 49.3 to 64.4%, compared to zero reduction with pencycuron alone. Results showed that the reduction values were 64.4, 61.5, 60.8, 60.0, 59.3, 58.9, 55.6, 54.4, 52.2 and 49.3% with pencycuron when combined with each of Tsp_2, Tv_1, Tvr, Tsp_3, Tv_2, Th_2, Tsp_1, Tv_3, Th_3 and Th_1, respectively. The radial growth inhibition of *S. sclerotiorum* was in the range of 67.8 to 76.7% by pencycuron - *Trichoderma* spp. isolates combinations, compared to 18.9% in pencycuron alone. Pencycuron -Tsp_3 treatment was highly reduced the *S. sclerotiorum* growth, followed by pencycuron when combined with each of Tsp_1,Tv_2, Tv_1, Tv_3, Th_2, Th_1, Tsp_2, Th_3 and Tvr, showing the reduction values of 76.7, 74.8, 73.7, 73.3, 73.3, 72.6, 71.5, 69.3, 67.8 and 68.9%, respectively. Significant differences were recorded between Tsp_1 and Tsp_3 as well as other *Trichoderma* spp. isolates (Table 4).

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Thiophanate-methyl - *Trichoderma* spp. isolates combination

Thiophanate-methyl combined with *Trichoderma* spp. isolates were reduced the growth of *F. solani* in the range of 50.4 - 86.7%, compared to 17.1% with fungicide alone. Thiophanate-methyl with Tv_3 showed the greater inhibitory effect against *F. solani*, followed by the combination of thiophanate-methyl with each of Tvr, Tsp_3, Tsp_2, Tsp_1, Tv_2, Tv_1, Th_1, Th_2 and Th_3, where the growth reduction values were 86.7, 77.8, 65.9, 60.0, 57.0, 55.6, 54.4, 51.1, 50.4 and 50.4%, respectively. The same treatments reduced the growth of *F. oxysporum* in the range of 56.7 to 100%, compared to 18.2 with fungicide alone. Thiophanate-methyl combined with each of Th_1, Tv_3, Tsp_3, Tsp_2, Tvr, Th_2, Tsp_1, Th_3, Tv_1 and Tv_3 was reduced the growth of *F. oxysporum* by 100.0, 100.0, 72.2, 70.0, 65.9, 62.2, 62.2, 60.4, 59.3 and 56.7% , respectively (Table 5).

Table 5: Interaction effect of *Trichoderma* spp. isolates with Thiophanate- methyl (Tm) at the concentration of 100 ppm against the radial mycelial growth of soil borne plant pathogens *in vitro*.

Treatment	Linear mycelial growth (cm) and growth reduction (%) of pathogenic fungi									
	F. solani		*F. oxysporum*		*R. solani*		*M. phaseolina*		*S. sclerotiorum*	
	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)	Growth (cm)	Red. (%)
Control	9.00	-	9.00	-	9.00	-	9.00	-	9.00	-
Thiophanate- methyl (Tm) at 100 ppm)	7.47	17.1	7.37	18.2	7.30	18.9	7.50	16.7	7.60	15.6
T. harzianum (Th_1)+ Tm	4.40	51.1	0.00	100.0	1.10	87.8	0.66	92.6	0.00	100
T. harzianum (Th_2) + Tm	4.47	50.4	3.40	62.2	1.27	86.0	1.30	85.6	0.00	100
T. harzianum (Th_3) + Tm	4.47	50.4	3.57	60.4	1.00	88.9	3.60	60.0	0.00	100
T. viride (Tv_1) + Tm	4.10	54.4	3.67	59.3	1.10	87.8	0.00	100	0.00	100
T. viride (Tv_2) + Tm	4.00	55.6	3.90	56.7	1.80	80.0	0.00	100	0.00	100
T. viride (Tv_3) + Tm	1.20	86.7	0.00	100	1.07	88.2	1.50	83.3	0.00	100
T. virens(Tvr)+ Tm	2.00	77.8	3.07	65.9	1.20	86.7	3.60	60.0	0.00	100
Trichoderma sp. (Tsp_1) + Tm	3.67	57.0	3.40	62.2	1.47	83.7	3.20	64.4	0.00	100
Trichoderma sp. (Tsp_2) + Tm	3.60	60.0	2.70	70.0	1.33	85.2	1.60	82.2	0.00	100
Trichoderma sp. (Tsp_3) + Tm	3.07	65.9	2.50	72.2	1.37	84.8	3.00	66.7	0.00	100
L.S.D. $_{0.05}$										
	Bioagents (B)		Pathogens (P)		B x P					
Mycelial growth (cm)	0.39		0.25		0.87					
Mycelial reduction %	4.5		3.0		10.1					

Thiophanate-methyl in integration with *Trichoderma* spp. isolates were reduced the growth of *R. solani* in the range of 80.0 to 88.9%, compared to 18.9% with thiophanate-methyl alone. The highest reduction was obtained with Thiophanate-methyl when combined with Th_3, followed by each of Tv_3, Th_1, Tv_1, Tvr, Th_2, Tsp_2, Tsp_3, Tsp_1 and Tv_2, where the corresponding values were 88.9, 88.2, 87.8, 87.8, 86.7, 86.0, 85.2, 84.8, 83.7 and 80.0%, respectively. No significant differences were recorded among Th_1, Th_2, Tv_1, Tv_3, Tsp_1, Tsp_2, Tsp_3 and Tvr. The same treatments were inhibited the growth of *M. phaseolina* in the range of 60.0 to 100%, compared to 16.7% in fungicide alone. Both of Tv_1 and Tv_2 combined with Thiophanate-methyl were completely reduced the growth of *M. phaseolina* by 100.0%, while Th_1, Th_2, Tv_3, Tsp_2, Tsp_3, Tsp_1, Tvr and Th_3 combined with the same fungicide reduced the growth of *M. phaseolina* by 92.6, 85.6, 83.3, 82.2, 66.7, 64.4, 60.0 and 60.0%, respectively. The combinations were reduced the growth of *S. sclerotiorum* by 100%, compared to 15.6% in fungicide alone, where no significant differences were noticed among treatments (Table 5).

DISCUSSION

Our results reveal that ten *Trichoderma* spp. isolates showing difference degrees of compatibility with tested fungicides. *Trichoderma* spp. isolates were highly compatible with thiophanate-methyl, where two species only slightly affected by fungicide at 800 ppm. Followed by mancozeb and metalaxyl M + mancozeb, where some *Trichoderma* spp. isolates also were affected at high concentrations (800ppm), respectively. *Trichoderma* species were moderate compatible with pencycuron and flutolanil, where all tested *Trichoderma* affected at 800 ppm. On other hand, our finding revealed that all *Trichoderma* species were not compatible with carbendazim and thiram + tolelofos – methyl, where carbendazim was completely inhibited the growth of *Trichoderma* species even at 50 ppm, while thiram + tolelofos – methyl inhibited the growth of *Trichoderma* species at all tested concentrations. Results show that the growth of *Trichoderma* spp. isolates affected by

flutolanil (Moncut 25% WP), metalaxyl M + mancozeb (Ridomil Glod 68%), pencycuron (Monceren 25% WP) and thiophanate-methyl (Topsin–M 70% WG) at the highest concentration were continued and full the Petri plate until after the growth of *Trichoderma* full the control plate. It is clear that the tested fungicides may be lasted the growth of *Trichodema* spp. isolates. These results are in agreement with those recorded by Gampala and Pinnamaneni [19]. They reported that *T. viride* was more compatible with fertilizers and pesticides. *T. harzianum* also showed more tolerance to metalaxyl, compared to carbendazim [20]. Gaur and Sharma [21] showed that copper oxychloride, mancozeb, fosetyl- AL and cymoxanil 8% + mancozeb 64% mixture showed moderate to good compatibility with *T. viride*.

Results reveal that considerable variation in efficacy of tested fungicides against soil borne pathogens. Carbendazim was completely inhibited the redial growth of all soil borne pathogens. Fulutolanil and thiarm-tolelofos methyl were completely inhibited the growth of *R. solani*, while mancozeb and metalaxyl M + mancozeb were completely inhibited the growth of *S. sclerotiorum,* respectively. Increase in concentrations was increased the efficacy of all tested fungicides. Most of tested fungicides were effective at high concentrations *in vitro*. It is clear that we need high concentrations for filed application. These results are in agreement with those recorded by Dar *et al.* [22]. They reported that among systemic fungicides maximum inhibition in mycelial growth and spore germination was observed in the carbendazim, followed by other fungicides. Manu *et al.* [23] mentioned that hexaconazole, propiconazole, difenconazole and combi products *viz*., Avatar (hexaconazole 4% + Zined 68%), Nativo (tebuconazole 50% + trifloxystrobin 25%) and Vitavax (thiram 37.5% + carboxin 37.5%) showed complete inhibition of *Sclerotium rolfsii* , while the contact fungicides mancozeb was found to be effective at highly concentrations. Diathane M 45, Benlate and Ridomil significantly affected the growth of *F. solani* [24]. On other hand, our results reveal that *Tricoderma* spp. had antagonistic effect against tested soil borne pathogens, where the reduction in linear growth was in the range of 25.6 – 88.9%. The degrees of inhibition observed in the present study are in agreement with Rudresh *et al*. [25]. They observed that 86.3 and 69.2% of mycelial growth inhibition of *F. oysporum* and *R. solani* by *T. harzianum,* respectively. Several species of *Trichoderma* suppress soil borne causing disease including *Fusarium* sp. [26].

According the above results, we studied the effect of fungicide-*Trichoderma* combination for control soil borne pathogens. Results show that combinations among *Trichoderma* spp. and fungicides of pencycuron, mancozeb and thiophanate-methyl, separately, highly reduced the growth of tested pathogens, being 100.0% inhibition with *R.solani* (pencycuron) and *S. sclerotiorum* (thiophanate-methyl), compared to the effects of each fungicide and ⁄ or *Trichoderma* species alone. Some combinations gave less growth reduction than fungicide and ⁄ or *Trichoderma* spp. only. It is may be due to the fungicide late the fungal growth in opposite the growth of pathogen. These results are in agreement with those recorded by Srinivas and Ramakrishnan [27]. They reported that the integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection, compared to fungicide and the fungal antagonists individually. Raziq and Ishtiaq [28] reported that the integration of *Trichoderma* with the lower doses of the fungicides might offer a promising control of *A. solani*. This will reduce reliance on the indiscriminate use of fungicides, leading to more cost-effective and environment friendly control of the disease [29].

In conclusion, the present study demonstrated that *Trichoderma* spp. isolates were uncompatible with carbendazim (Kemazed 50% WP) and thiram + tolclofos-methyl (Rizolex T 50% WP). While it was absolutely compatible with flutolanil (Moncut 25% WP), mancozeb (Tridex 80%), metalaxyl M + mancozeb (Ridomil Glod 68%), pencycuron (Monceren 25% WP) and thiophanate-methyl (Topsin–M 70% WG) at the concentration of 50, 100, 200, 300, 400 and 500 ppm and slightly at 600, 700 and 800 ppm. Each of flutolanil, pencycuron and thiophanate-methyl when separately combined with *Trichoderma* spp. isolates reduced the growth of the tested soil borne pathogens more strongly than did in individual treatment by each fungicide at 100 ppm. Our results suggest that the fungicide-*Trichoderma* combination may reduce the amount of fungicide application and can weaken the pathogen and render its propagules more susceptible to attack by the antagonist. Therefore, *Trichoderma* can be used also in an integrated pest management programs (IPM) with fungicides to control soil borne plant pathogens.

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