

Annual Research & Review in Biology

26(4): 1-11, 2018; Article no.ARRB.41295
ISSN: 2347-565X, NLM ID: 101632869

Effect of Six Fungicides against *Fusarium oxysporum* and *F. solani* Associated with Peach Seedlings Decline in Tunisian Nurseries

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Authors' contributions

This work was carried out in collaboration between all authors. Author SM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NHR and NBM managed the analyses of the study. Author NBM managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/41295

Editor(s):

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Complete Peer review History: <http://www.sciencedomain.org/review-history/24565>

Original Research Article

Received 21st February 2018

Accepted 4th May 2018

Published 11th May 2018

ABSTRACT

Aims: This study investigated the efficiency of six fungicides against *Fusarium* spp. associated to peach seedling decline in Tunisian nurseries.

Place and Duration of Study: Department of Biological Sciences and Plant Protection, Higher Institute of Agronomy of Chott Mariem, 4042, Sousse, Tunisia, between March 2016 and June 2016.

Methodology: The activity of six fungicides against *Fusarium oxysporum* and *F. solani* associated with peach seedling decline in Tunisian nurseries was evaluated *in vitro* and *in vivo*.

Results: The *in vitro* test showed that Carbendazim was the most effective at a low dose (10 ppm) against *F. solani* with 84.39% of hyphal growth inhibition, while it gives 60.55% of growth inhibition against *F. oxysporum* at 100 ppm. The percent of growth inhibition generated by Mancozeb was between 59.02 and 90.21% at 50 and 100 ppm, respectively. The efficacy of fosetyl-Al was not important *in vitro* at 10, 25, 50 and 100 ppm against the two tested pathogens. Hymexazol revealed

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to be effective against *F. oxysporum* but without effect on *F. solani* for all used doses. The Chinosol was effective at different doses against *F. oxysporum*, with 88% of growth inhibition at 50 ppm for the two tested species. Thus, the fosetyl-Al, hymexazol and chinosol are the most effective on *F. oxysporum* and *F. solani*. Carbendazim was moderately effective against *F. oxysporum* (40.15%), whereas it was the most effective against *F. solani* (98.02%). The mancozeb and metalaxyl-M+mancozeb were the lowest effective against the two pathogens tested with percents of inhibition of 27.41% and 28.96% for *F. oxysporum* respectively, and 48.02% and 42.46% for *F. solani*, respectively.

Results of *in vivo* test indicated that disease severity parameters of peach seedlings recorded after three months of inoculation by *Fusarium* spp. showed that the fosetyl-Al and metalaxyl-M + mancozeb reduced significantly root browning induced by *Fusarium oxysporum* by 62.55%. The mancozeb and carbendazim reduced also the root browning without significant effect. Whereas, all products didn't reduce the severity of root browning or the vegetative sanitary states index of peach seedlings inoculated by *F. solani*. For the growth parameters, plants inoculated by *F. oxysporum* and treated by fosetyl-Al and metalaxyl-M+mancozeb increased the root weight by 34% and 24.89%, respectively. The metalaxyl-M+mancozeb improved also the plant height by 8.05% compared to the control inoculated by *F. oxysporum*. However, chinosol was the only product that improved plant height by 10.31% and root weight by 9.78% of plants inoculated by *F. solani*.

Conclusion: The fungicides Alliette express (fosetyl-Al), Ridomil Gold (mancozeb+ metalaxyl-M), Dithane-M45 (mancozeb) and Prodam (carbendazim) were the most effective *in vivo* against *F. oxysporum* associated with a peach seedling decline in Tunisian nurseries, whereas Beltanol (chinosol) is the only product that improved the seedling growth inoculated by *F. solani*. Thus, it will be important to test them in the future against the other genera associated with this disease like *Pythium* and *Phytophthora* species. Then, it is necessary to test them against the combination of these pathogens because the causal agent of this disease is a complex.

Keywords: Peach decline; nurseries; fungicides; control; *in vitro*; *in vivo*.

1. INTRODUCTION

The peach decline in nurseries is one of the most destructive diseases [1,2]. It is a soil-borne disease, which has been reported in fruit trees-growing areas, throughout the world such as in Europe [3,4], North America [5,6], Australia [7] and North and South Africa [8,9]. Symptoms of this disease include general growth reduction, browning of roots, and stunting of the shoot of trees [3,9].

Many studies showed that *Fusarium* species are frequently isolated from peach orchards and nurseries showing decline symptoms [9, 10,11,12,13]. *Fusarium solani* and *F. oxysporum* were the most predominant species in the stem and root lesions and decline of young peach seedlings [14]. In addition, Nyczepir and Pusey [13] reported that these species are responsible of necrosis of peach feeder roots in greenhouse tests. They decreased shoot growth and plant height [13].

A recent study, showed the presence of peach decline in Tunisian nurseries [9]. Infected peach seedlings showed symptoms of drying and browning of the apical part of the scion and/ or

browning at the collar which will eventually result in a complete decline and death of the plant [9]. The uprooting showed root browning which was observed in all nurseries and on the two rootstocks, Garnem (*Prunus dulcis x hybride clonal of Prunus persica*) and bitter almond (*Prunus dulcis*) used in Tunisian nurseries [9]. This finding showed that *Fusarium oxysporum* and *F. solani* were the most dominant and virulent species.

Knowledge of the breadth of interactions and the species associated with peach may provide pure pathogenic opportunities for the management and control of pathogenic *Fusarium* spp. Management practices have a significant impact on quantitative and qualitative attributes of soil microbial communities in agricultural ecosystems [15].

In several countries, the fumigation with methyl bromide, which was effective in reducing soil-borne inoculum of numerous *Fusarium*, will be totally removed because of its ozone-depleting effect [16]. Several systemic fungicides such as Bavistin D.F (Carbendazim), Topsin M (Thiophanate-methyl), Ridomil gold (Mefenoxam) and Alliette (Fosetyl-aluminum) and contact like

Dithane M-45 (Mancozeb) have significantly reduced *Fusarium* spp. growth *in vitro* and *in vivo* on different crops [17,18].

Immediate research investigation is required to conduct on control measures of serious peach decline problem in Tunisian nurseries. The present research was conducted in order to evaluate the effectiveness, *in vitro* and *in vivo*, of six fungicides in the management of *F. oxysporum* and *F. solani* associated with peach seedling decline.

2. MATERIALS AND METHODS

2.1 Pathogen Inoculums

Two pathogenic *Fusarium* species were used in the present study. They were isolates of *Fusarium oxysporum* (MF993097) and *F. solani* (MF993094). These isolates were obtained from infected roots of peach seedlings in Tunisian nurseries. For their long term preservation, pathogen isolates were stored at -20°C in a 20% glycerol solution. The characterization and the pathogenicity test of these isolates were done in a previous study [9].

2.2 The Fungicides Tested (Dosage & Active Ingredient)

Five systemic fungicides and one contact fungicide were tested against the causal agents of peach seedlings decline in Tunisia. They represented different chemical groups. Each fungicide had one only active ingredient except Ridomil Gold that had two active ingredients (Table 1). For *in vitro* essay, 5 doses of active ingredient were used (10, 25, 50, 100 ppm and the registered dose) for each product except Ridomil Gold for which only the registered dose was used (120 ppm of Metalaxyl-M). For *in vivo* essay, the six products were tested using the registered dose for each except the chinisol for which the most effective dose *in vitro* (100 ppm) was used.

2.3 *In vitro* Studies of Mycelial Growth Inhibition

The tested fungicides were suspended in sterile water and added to the PDA medium (Potato Dextrose Agar) to obtain final concentrations (10, 25, 50, 100 ppm and the registered dose) except for the chinisol (10, 25, 50 and 100 ppm). This dose was approved against *Fusarium* spp. in *Cucurbitaceae*. Each fungicide was incorporated in the medium still in fusion and the mixture was

placed in Petri dishes. After solidification of the medium, a disk of 6mm diameter of 7-days old culture of *F. oxysporum* or *F. solani* grown on a PDA medium was placed in the center of Petri dish (90 mm in diameter). Control did not receive any treatments of fungicide. The plates were incubated at 25°C and in the dark for 6 days. Three replicates were performed for each concentration and each isolate. Statistical design used for this experiment was a completely randomized factorial model with two factors. The percent growth inhibition of each pathogen was calculated using the following formula:

$$\text{Inhibition (\%)} = \{(C - T)/C\} \times 100.$$

With: T: mean diameter of the colonies in the presence of the fungicide, C: average diameter of the control colonies.

2.4 *In vivo* Studies of Disease Severity

Four weeks-old peach seedlings of the variety 'Garnem' were used in this study. These seedlings were grown in pots (23 cm diameter x 23 cm deep) in a greenhouse. The determination of the *in vivo* efficacy of six fungicides on the inhibition of *Fusarium* species was conducted according to the method of Utkhede and Smith [19]. This method consists of placing ten mycelial discs (8 mm diam.) of each *Fusarium* isolate grown on PDA medium in an Erlenmeyer flask (250 ml) containing 150 ml of PDB (Potato-Dextrose-Browth). Then, the Erlenmeyer was agitated at 120rpm for one week at 25°C and a photoperiod of 12 h. The resulting conidial suspension was adjusted to 10^7 spores/mL, using Malassez blade. Then, the adjusted inoculum at 10^7 spores/mL was applied at 50 ml/bush and was mixed with potting mix (sterilized peat and sand in 2:1 v/v) around the seedling. Sterile distilled water was used as control. After plantation, each seedling was treated using the registered dose for each fungicide (50 ml per plant) [19] except for the chinisol for which the dose of 100 ppm was used. Pots were arranged in a completely randomized design and watered when needed and peach seedlings were examined after 3 months of inoculation.

The disease severity was noted, according to the sanitary state of the vegetative part of plants. This parameter is scored based on 0-5 scale, where: **0**=no obvious symptoms of peach decline; **1**=moderate discoloration of plant leaves ($\leq 25\%$); **2**= moderate discoloration and falling leaves ($\leq 50\%$); **3**= moderate discoloration of plant collar, stem and leaves ($\leq 75\%$);

Table 1. Characteristics of the fungicides used to control *Fusarium* spp. associated with the decline of peach seedlings in Tunisian nurseries

Trade Name (active substance)	Available form	Active substance concentration	Registered dose	Chemical group	Mode of action
Prodazim 50 wp (Carbendazim)	Wettable powder	50%	50 g/hl (250 ppm)	Carbamates	Systemic
Dithane M-45 (Mancozeb)	Wettable powder	80%	250 g/hl (2000 ppm)	Dithiocarbamate	Contact
Alliette Express (Fosetyl-AI)	Wettable powder	80%	250 g/hl (2000 ppm)	Ethyl phosphanates	Systemic
Tachigazol 300 (Hymexazol)	Solution	30%	20 ml/hl (60 ppm)	Isoxazoles	Systemic
Beltanol-L50% (Chinosol)	Solution	50%	400 ml/hl (2000 ppm)	Quinoleines	Systemic
Ridomil Gold MZ (Metalaxyl-M+Mancozeb)	Wettable powder	4% 64%	300 g/hl (120 ppm Metalaxyl)	Acylalanines	Systemic

4= extensive discoloration of plant collar and stem with falling leaves (>75%); and 5= dead plants. Then, peach seedlings were removed from the potting bags and washed under running water to remove excess potting mix adhering to the roots. For each seedling the height, root weight and root rot were noted. Root rot was rated onto a 0–5 scale (0=no obvious symptoms; 1=moderate discoloration of root tissue; 2=moderate discoloration of tissue with some lesion; 3=extensive discoloration of tissue; 4= extensive discoloration of tissue with girdling lesions; and 5= dead plants) [8]. Re-isolation was made from roots of seedlings to confirm the pathogenicity of the tested isolates and the efficacy of fungicides tested.

2.5 Statistical Analysis

Data were subjected to a one-way analysis of variance (ANOVA) by using statistical package for social sciences (SPSS) software, version 20.0. The *lab experiment* was analyzed according to a completely randomized factorial model with two factors (Fungicides tested and doses used). Three replicates were used per individual treatment. The greenhouse experiment was analyzed in a completely randomized model. Each individual treatment was replicated three times. Each experiment was conducted for one time. For all the tests mean variation were separated using Student-Newman-Keuls (SNK) test ($P \leq 0.05$).

3. RESULTS

3.1 *In vitro* Effect of Fungicides against *Fusarium solani* Mycelial Growth

The analysis of the variance relative to the inhibition percent of the mycelium growth of *F. solani* induced by Carbendazim showed that there is no significant difference observed between treatments. The percent inhibition was observed among the treatments ranging from 84.39 to 85.87 ($p=0.189$).

The statistical analysis of the variance relative to the inhibition percentage of the mycelium growth of *F. solani* induced by Mancozeb showed a significant difference between the four doses ($P \leq 0.05$). The percents of growth inhibition of this product were between 11.52 (10 ppm) and 44.61% (100 ppm) (Table 2).

For fosetyl-Al, the comparison between the efficacy of different doses *in vitro*, against *F. solani* gave a significant difference. The efficacy

of this active substance was unimportant. Indeed, the highest percent of growth inhibition was 14.87% at 100 ppm (Table 2).

The analysis of the variance relative to the inhibition percentage of the mycelium growth induced by Hymexazol showed a significant difference between the four doses ($P \leq 0.05$) for *Fusarium solani*. All doses were ineffective against this pathogen. Indeed, the percent of growth inhibition at 100 ppm, was 25.28%.

The analysis of the variance relative to the inhibition percent of the mycelium growth induced by Chinosol showed a significant difference between the four tested doses ($P \leq 0.05$).

It was the second effective active substance after the carbendazim against *F. solani*. The hyphal growth inhibition percent varied from 32.34% at 10 ppm to 88.10% at 100 ppm (Table 2).

For the test of registered doses, the statistical analysis of the hyphal growth inhibition percent of *F. solani* showed a significant difference ($P \leq 0.05$) in the efficiency of the registered dose of the active substances. Indeed, Carbendazim (250 ppm), Fosetyl-Al (200 ppm), Hymexazol (60 ppm) and chinosol (100 ppm) were the most effective against *F. solani* mycelium growth with inhibition percents growth 98.02%, 85.71%, 67.06% and 88.10%, respectively (Table 3).

The mancozeb (2000 ppm) and Metalaxyl-M (120 ppm) + Mancozeb (1920 ppm) were the lowest effective against *F. solani*. In fact, the percents of inhibition were 48.02% and 42.46%, respectively (Table 3).

3.2 *In vitro* Effect of Fungicides against *Fusarium oxysporum* Mycelial Growth

The analysis of the variance relative to the inhibition percent of the mycelium growth of *F. oxysporum* induced by each active ingredient showed a significant difference between the four doses ($P \leq 0.05$). The highest inhibition percent of hyphal growth induced by Carbendazim was 60.55% at the concentration 100 ppm.

The Mancozeb was more effective against *F. oxysporum* at 50 and 100 ppm with inhibition percent of 59.02 and 90.21%, respectively (Table 4).

In vitro, the fosetyl-Al revealed to be inefficient against *F. oxysporum*. The percent of growth inhibition at 100 ppm was 0.61 (Table 4).

All doses of hymexazol were effective against *F. oxysporum* with a percent of growth inhibition of 72% at 100 ppm.

The test of Chinosol showed its effectiveness at different doses against *F. oxysporum*, with a percent of growth inhibition varied from 52.29% at 10 ppm to 90.21% at 100 ppm (Table 4).

For the *in vitro* test of registered doses, the statistical analysis of the inhibition percent of hyphal growth of *F. oxysporum* showed a

significant difference ($P \leq 0.05$). Indeed, Fosetyl-Al (200 ppm), Hymexazol (60 ppm) and chinisol (100 ppm) were the most effective against *F. oxysporum* with inhibition percents of 94.59%, 94.98% and 90.21%, respectively (Table 5). The Carbendazim was less effective than Fosetyl-Al, Hymexazol and chinisol with a percent inhibition (40.15%). The mancozeb (2000 ppm) and Metalaxyl-M (120 ppm) + Mancozeb (1920 ppm) were the lowest most effective against *F. oxysporum*. In fact, the percent of inhibition was 27.41% and 28.96% respectively (Table 5).

Table 2. Percent of growth inhibition of *F. solani* by the different doses of the active ingredients of each tested fungicide, registered after six day of incubation at 25°C in the dark

Products	Active substance	Doses (ppm)	<i>F. solani</i>
Prodazim	Carbendazim	10	84.39±1.49 ^a
		25	84.01±1.42 ^a
		50	84.76±0.74 ^a
		100	85.87±0.86 ^a
P value			0.189
Dithane	Mancozeb	10	11.52±3.54 ^a
		25	29.74±2.54 ^b
		50	31.60±0.00 ^b
		100	44.61±4.44 ^c
P value			0.000
Alliette	Fosetyl-Al	10	4.46±0.74 ^a
		25	6.32±2.71 ^a
		50	8.18±4.27 ^a
		100	14.87±2.23 ^b
P value			0.001
Tachigazol	Hymexazol	10	14.13±3.91 ^{ab}
		25	10.41±3.07 ^a
		50	16.36±1.87 ^b
		100	25.28±1.42 ^c
P value			0.000
Beltanol	Chinosol	10	32.34±2.58 ^a
		25	57.99±8.18 ^b
		50	88.10±0.00 ^c
		100	88.10±0.00 ^c
P value			0.000

(*) Means ± standard error in the column followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$

Table 3. Percent inhibition of mycelial growth of *F. solani* by the different fungicides tested using the registered dose

Fungicides	Active ingredients	<i>Fusarium solani</i>
Prodazim	Carbendazim	98.02±1.52 ^a
Dithane M45	Mancozeb	48.02±1.52 ^d
Alliette Express	Fosetyl-Al	85.71±0.00 ^b
Tachigazol	Hymexazol	67.06±4.74 ^c
Beltanol	Chinosol	88.10±0.00 ^b
Ridomil Gold	Metalaxyl-M + Mancozeb	42.46±6.90 ^e

(*) Means ± standard error in the column followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$

Table 4. Percent of growth inhibition of *F. oxysporum* by the different doses of the active ingredients of each tested fungicide registered after six day of incubation at 25°C in the dark

Products	Active substance	Doses (ppm)	<i>F. oxysporum</i>
Prodazim	Carbendazim	10	36.09±4.04 ^a
		25	36.09±4.04 ^a
		50	46.79±1.58 ^b
		100	60.55±3.22 ^c
P value			0.000
Dithane	Mancozeb	10	18.35±2.89 ^a
		25	32.42±1.17 ^b
		50	59.02±3.08 ^c
		100	90.21±0.00 ^d
P value			0.000
Alliette Express	Fosetyl-AI	10	7.03±1.73 ^a
		25	3.06±1.17 ^{ab}
		50	3.06±1.54 ^{ab}
		100	0.61±2.35 ^b
P value			0.016
Tachigazol	Hymexazol	10	52.29±0.00 ^a
		25	59.33±1.54 ^b
		50	61.47±1.58 ^c
		100	72.17±1.54 ^d
P value			0.000
Beltanol	Chinosol	10	52.29±1.00 ^a
		25	75.23±0.61 ^b
		50	88.69±1.83 ^c
		100	90.21±0.00 ^c
P value			0.000

(*) Means ± standard error in the column followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$

Table 5. Percent inhibition of mycelial growth of *F. oxysporum* by the different fungicides tested using the registered dose

Fungicides	Active ingredients	<i>Fusarium oxysporum</i>
Prodazim	Carbendazim	40.15±10.58 ^b
Dithane M45	Mancozeb	27.41±2.18 ^c
Alliette Express	Fosetyl-AI	94.59±2.67 ^a
Tachigazol	Hymexazol	94.98±1.48 ^a
Beltanol	Chinosol	90.21±0.00 ^a
Ridomil Gold	Metalaxyl-M + Mancozeb	28.96±3.34 ^c

(*) Means ± standard error in the column followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$

3.3 In vivo Effect of Fungicides against *Fusarium* spp.

The variance analysis of disease severity parameters recorded after three months of inoculation by *Fusarium* species showed that all products improved the sanitary states of the plant aerial part, while this improvement was not significant. Nevertheless, the fosetyl-AI and metalaxyl-M+mancozeb significantly reduced root browning induced by *Fusarium oxysporum* by 62.55% (Table 6 and Fig. 1). The mancozeb

and carbendazim reduced also the root browning without significant effect. Concerning the growth parameters, the fosetyl-AI and metalaxyl-M+mancozeb increased the root weight by 34% and 24.89%, respectively compared to the control inoculated by *F. oxysporum*, but this effect was not significant. However, no effect was found for all products on the height of the plants inoculated by *Fusarium oxysporum* except the metalaxyl-M+mancozeb that had improved this parameter by 8.05% compared to the inoculated control (Table 6).

Table 6. Effect of fungicides tested on the aggressiveness of *Fusarium* spp. and the growth of peach plants 'Garnem' 3 months after inoculation and treatment

	<i>Fusarium oxysporum</i>				<i>Fusarium solani</i>			
	Plant height (cm)	Root rot	Root weight (g)	Sanitary state	Plant height (cm)	Root rot	Root weight (g)	Sanitary state
NIC	62.40±2.26 ^a	1.33±0.58 ^{cd}	4.79±0.37 ^{ab}	1.67±0.58 ^a	62.40±2.26 ^a	1.33±0.58 ^b	4.79±0.37 ^a	1.67±0.58 ^b
IC	55.00±10.11 ^{ab}	2.67±0.58 ^{bc}	4.50±0.55 ^{ab}	2.00±1.00 ^a	55.00±10.11 ^{ab}	2.67±0.58 ^{ab}	4.50±0.55 ^a	2.00±1.00 ^{ab}
Prodazim	48.00±2.00 ^{ab}	1.67±0.58 ^{cd}	3.47±0.95 ^{ab}	1.67±0.58 ^a	32.20±4.01 ^d	2.67±0.58 ^{ab}	2.89±1.26 ^a	3.67±0.58 ^{ab}
Dithane M45	46.67±3.51 ^{ab}	1.33±0.58 ^{cd}	4.12±0.72 ^{ab}	1.67±1.15 ^a	41.73±3.16 ^c	2.67±0.58 ^{ab}	2.64±1.79 ^a	3.00±1.00 ^{ab}
Alliette express	49.00±6.93 ^{ab}	1.00±0.00 ^d	6.03±0.98 ^a	1.33±0.58 ^a	47.33±4.16 ^{bc}	2.67±0.58 ^{ab}	3.59±1.40 ^a	3.00±1.00 ^{ab}
Ridomil Gold	59.43±7.81 ^a	1.00±0.00 ^d	5.62±1.53 ^a	1.00±0.00 ^a	45.83±5.48 ^{bc}	3.00±1.00 ^{ab}	2.91±0.84 ^a	3.33±0.58 ^{ab}
Tachigazol	38.60±14.95 ^b	4.00±1.00 ^a	2.20±2.12 ^b	2.67±2.08 ^a	30.00±1.73 ^d	3.33±0.58 ^a	2.19±0.85 ^a	4.00±0.00 ^a
Beltanol	48.00±2.00 ^{ab}	3.33±0.58 ^{ab}	3.90±0.37 ^{ab}	2.67±0.58 ^a	60.67±2.08 ^a	2.67±0.58 ^{ab}	4.94±0.40 ^a	2.33±0.58 ^{ab}

(*) Means ± standard error in the column followed by the same letter are not significantly different according to SNK test at $P \leq 0.05$.

NIC: uninoculated control; IC: inoculated control

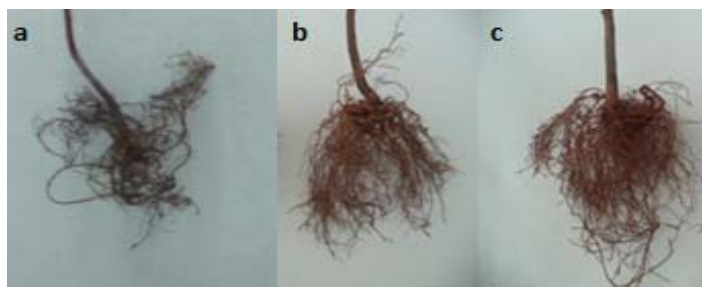


Fig. 1. Effect of fungicides on the root browning of peach plants variety 'Garnem' after 3 months of inoculation by *F. oxysporum* and treatment

a: Inoculated control, b: peach root inoculated and treated by fosetyl-Al, c: root inoculated and treated by metalaxyl-M+mancozeb

The results of the *in vivo* test of these six fungicides against *F. solani* showed that all products revealed with no effect on the severity of root browning and the sanitary states indexes of peach seedlings aerial parts (Table 6). However, chinisol was the only product that improved plant height by 10.31% and the root weight by 9.78% without significant effects compared to the inoculated untreated control (Table 6).

4. DISCUSSION

In our study, the *in vitro* and *in vivo* assays of each fungicide against *Fusarium solani* and *F. oxysporum* responsables of peach decline in Tunisia revealed a difference in the efficiency between different doses used. The efficacy of fungicides depended to the pathogen too.

The carbendazim was effective at a low dose (10 ppm) against *F. solani*, while it was effective against *F. oxysporum* at 100 ppm. Similar finding was found by Iqbal et al. [20] who demonstrated that carbendazim was highly effective fungicide in *in vitro* tests against *Fusarium* spp. isolated from malformed mango tissues. In addition, Gaur and Chakrabarti [21] showed that carbendazim was the most effective in reducing the hyphal growth of *F. mangiferae*.

Results of our study affirm also that the mancozeb was effective against *F. oxysporum* and moderately effective against *F. solani* *in vitro*. Shah et al. [17] worked on the effectiveness of different fungicides and reported that mancozeb was the best among the tested fungicides for *in vitro* effect on *Fusarium* spp.

The efficacy of fosetyl-Al was low *in vitro* at 10, 25, 50 and 100 ppm against the two tested pathogens, while, the test of registered dose (2000 ppm) showed that this active ingredient is

among the most effective products on both pathogen species. Thus, this product is effective against *F. oxysporum* and *F. solani* at a high concentration. Similar results were proved by Utkhede and Smith [18] who showed that fosetyl-Al was fungicidal to *Phytophthora cactorum*, *P. cambivora* and fungistatic to *Pythium ultimum* at high concentrations but, it was phytotoxic to apple seedlings when tested at 2.5 g l⁻¹ under greenhouse conditions.

For hymexazol, all tested doses were effective against *F. oxysporum*. Also, the test of registered dose showed that this fungicide is among the most effective products on the two tested pathogens species. Previous studies revealed the efficacy of hymexazol *in vitro* and *in vivo* in reducing the mycelium growth and *Fusarium* wilt of tomato and potato caused by *F. oxysporum* f. sp. *radicis-lycopersici* and *Fusarium oxysporum* f. sp. *tuberosae*, respectively [22,23].

In addition, the chinisol is among the most effective products tested *in vitro* against *Fusarium* species. It was effective at different doses. Previous studies found that chinisol effectively inhibits the growth of *Fusarium* spp. *in vitro* [24].

The test of metalaxyl-M+mancozeb *in vitro* showed a low inhibition of hyphal growth of the two pathogens tested. There is no study about the efficacy of this fungicide on *Fusarium* species. Contrarily, Thomidis and Tsiouridis [25] reported that metalaxyl significantly reduced the growth of *Phytophthora cactorum* compared to fosetyl-Al, cymoxanil and dimethomorph.

The *in vivo* screening of the ability of the six fungicides to suppress peach seedling decline severity induced by *F. oxysporum* and *F. solani* revealed the efficacy of some products. The fosetyl-Al significantly reduced root browning

induced by *Fusarium oxysporum* and increased the root weight. Similarly, Utkhede and Smith [19] showed that this product is effective against the three genera *Pythium*, *Phytophthora* and *Fusarium* associated with the root rot of apple young seedling. The metalaxyl-M + mancozeb significantly reduced root browning induced by *Fusarium oxysporum* and increased the root weight and the plant height. This product was more effective than mancozeb. These findings are also in agreement with previous studies reporting the capacity of metalaxyl to control peach root rot. In fact, Thomidis and Tsipouridis [25] reported that the application of metalaxyl as a soil drench of 2 g per 500 ml per tree, provide effective control of the artificial stem cankers of two-year-old peach trees. They deduced that metalaxyl is effective against *P. cactorum* and *P. citrophthora* on peach. However, the only other fungicide with some inhibitory effect was fosetyl-Al, but it was not as effective as metalaxyl [25]. In addition, Thomidis and Elena [26] found that the application of metalaxyl following the two methods of trunk painting or soil treatment is effective in combating root rot of peach trees caused by *Phytophthora cactorum*.

Besides, the mancozeb reduced the root browning without significant effect. The carbendazim reduced the root browning of plants inoculated by *F. oxysporum*. It was shown less effective than mancozeb, mancozeb+metalaxyl and fosetyl-AL. In a similar study, Pandey and Chakrabarti [18] applied Carbendazim against *F. moniliforme* and noted that Carbendazim inhibited the growth of the germ tubes produced by conidia and also reduced the infection rate.

Chinosol was the only product that improved plant height and root weight of plants inoculated by *F. solani*. This same fungicide was previously tested. It was shown able to control *Fusarium* wilt of different crops through its applications to the soil [24,27,28]. This compound may act by rendering heavy metals unavailable to the toxins from the pathogens [29,30].

5. CONCLUSION

The fungicides Alliette express (fosetyl-Al), Ridomil Gold (mancozeb+ metalaxyl-M), Dithane-M45 (mancozeb) and Prodam (carbendazim) were the most effective *in vivo* against *F. oxysporum* associated with a peach seedling decline in Tunisian nurseries, whereas Beltanol (chinosol) is the only product that improved the seedling growth inoculated by *F. solani*. Thus, it will be important to use them in the future against

Fusarium spp. that is serious for Tunisian nurseries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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