

## Research Article

# Evaluation of six fungicides for the management of nurseries apple seedling decline in Tunisia

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**Abstract:** Apple decline, responsible for seedlings root and collar rot in nurseries, is an important disease. Some Oomycetes species were associated with this disease. This study aimed to control this severe decline disease. The effect of six chemical products against *Pythium ultimum* and *Phytophthora mercuriale* associated with apple seedling decline was evaluated using *in vitro* poisoned food technique and *in vivo* greenhouse assays. The carbendazim was effective at 10 ppm against *P. mercuriale* (89.3%), while at 100 and 250 ppm the values were 70.06% and 75.30% for *P. ultimum*, respectively. The dose 2000 ppm of mancozeb completely inhibited the mycelial growth of *P. mercuriale* and *P. ultimum*. However, fosetyl-Al applied at 2000 ppm revealed an inhibition percent of 51 and 100% for *P. ultimum* and *P. mercuriale*, respectively. Regarding Hymexazol, the highest inhibition rates were against *P. mercuriale* (100%) and *P. ultimum* (90.55%) for 60 ppm. Chinosol revealed to be effective against *P. mercuriale* for all doses. It was effective against *P. ultimum* at 50 ppm (90.96%). The highest inhibition rate (86.59%) exhibited by metalaxyl-M was for *P. ultimum* at 120 ppm. *In vivo* test showed the efficacy of fosetyl-Al, metalaxyl-M + mancozeb, and chinosol to reduce root browning due to *P. ultimum* by 55.67%, 44.33%, and 55.67%, respectively, and improved the aerial part sanitary states of seedlings inoculated by *P. mercuriale* by 60.06, 49.85 and 49.85%, respectively. The chinosol improved the sanitary states of the aerial part of seedlings inoculated by *P. ultimum*.

**Keywords:** chemical control, apple decline, nurseries, *Pythium ultimum*, *Phytophthora mercuriale*

## Introduction

Several soil-borne biological agents are associated with apple tree roots in different producing countries. Some pathogenic agents incite a reduction in tree growth by damaging the roots of the tree (Mazzola, 1998; Manici *et al.*, 2003; Souli *et al.*, 2011a; 2011b; Tewoldemedhin

*et al.*, 2011; Mannai *et al.*, 2018a). Oomycetes species of genus *Pythium* contain facultative plant pathogens, causing a severe decline in crops acting either individually or in complexes with other organisms (Lévesque and De Cock, 2004).

*Pythium* species such as *P. indigoferae*, *P. irregulare*, *P. rostratiformis*, *P. sterilum*, *P.*

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*undulatum*, *P. indigoferae* are associated with apple trees decline in Tunisian orchards (Souli *et al.*, 2011a; 2011b; 2014). In contrast, there is no information about the role played by *Pythium* and *Phytophthora* species in the development of apple seedling decline in Tunisian nurseries. A recent investigation conducted in South Africa in apple nurseries revealed that apple seedling roots were infested by several *Pythium* species, such as *P. irregular* and *P. ultimum* (Moein *et al.*, 2019). Apple nurseries plants could be considered a potential inoculum source of apple decline disease in orchards (Mannai *et al.*, 2018a; Moein *et al.*, 2019).

Some chemical fungicides have effectively controlled oomycetes species in different host crops under different conditions. In fact, many studies have reported that fosetyl-Al, metalaxyl, and Chinisol were able to reduce the pathogens development of *P. ultimum*, *P. aristosporum*, *P. heterothallicum*, *Phytophthora cactorum* and *P. cambivora* (Vaartaja, 1964; Cook and Zhang, 1985; Menge, 1986; Utkhede and Smith, 1991; Brantner and Windels, 1998; Mazzola *et al.*, 2002; Boughalleb *et al.*, 2006).

In Tunisia, six chemical fungicides were evaluated for their *in vitro* and *in vivo* inhibitory potentials against *Pythium ultimum* associated with the peach seedling decline in nurseries. The *in vitro* poisoned food technique assay showed that the carbendazim, mancozeb, fosetyl-Al, hymexazol, chinisol, and metalaxyl-M effectively inhibit the mycelial growth of *P. ultimum* at different doses tested. The *in vivo* greenhouse assay demonstrated the efficacy of the Fosetyl-Al and Metalaxyl-M in reducing the peach seedling root browning induced by *Pythium ultimum* by 62.55% (Mannai and Boughalleb-M'Hamdi, 2021).

Despite the effectiveness and importance of chemical fungicides in the management of Oomycetes species in different host crops, there is little information on chemical control measures against oomycetes associated with apple decline in nurseries of Tunisia. Thus,

this study evaluates the efficacy of some fungicides *in vitro* and *in vivo* for managing this disease.

## Materials and Methods

### Characteristics of the pathogens isolates

Two Oomycetes species, *Pythium ultimum*, and *Phytophthora mercuriale* were used in this study. These isolates were obtained from declined apple seedlings in Tunisia and proved as the causative agents of this disease (Table 1).

**Table 1** Characteristic of pathogens isolates used in the present study.

Species	Isolates	Origins	Regions	GenBank accession number
<i>Pythium ultimum</i>	Po2	Apple roots	Kairouan	MH260594
<i>Phytophthora mercuriale</i>	Po26	Apple roots	Zaghuan	MF993112

### Characteristics of used chemical products

Six fungicides were tested against the oomycetes species *P. ultimum* and *P. mercuriale*. The main characteristics of these products are reported (Table 2).

**Table 2** Characteristics of the fungicides used to control Pythiaceae associated with the decline of apple seedlings in Tunisian nurseries.

Chemical name	Active substance concentration (%)	Registered dose
Carbendazim (Prodazim 50 wp)	50	50 g/hl (250 ppm)
Mancozeb (Dithane M-45)	80	250 g/hl (2000 ppm)
Fosetyl-Al (Alliette Express)	80	250 g/hl (2000 ppm)
Hymexazol (Tachigazol 300)	30	20 ml/hl (60 ppm)
Chinisol (Beltanol-L50%)	50	400 ml/hl (2000 ppm)
Metalaxyl-M + Mancozeb (Ridomil Gold MZ)	4	300 g/hl (120 ppm metalaxyl)

### Evaluation of *in vitro* mycelial growth inhibition

For *in vitro* assay, five doses of active ingredients were tested (10, 25, 50, 100 ppm, and registered dose) for each product. The fungicides suspended in sterile distilled water were added to the PDA medium (Potato Dextrose Agar) to obtain final concentrations. The medium was placed in Petri dishes. After solidification, a plug of 6 mm diameter of a

six-day-old culture of each Pythiaceae species isolate grown on PDA medium was placed in the center of the Petri dish (90 mm in diameter). Then, the plates were incubated at 25 °C and in the dark for three days. Control Petri dishes were challenged with pathogen plugs without fungicide treatments. Three replicates were used for each treatment. The statistical design used for this experiment was a completely randomized factorial model with two factors.

The growth inhibition percent (I) of each pathogen was calculated using the following formula:

$$I(\%) = \frac{C - T}{C} \times 100$$

With:

**T:** Diameter of the colonies in the presence of the fungicide,

**C:** Diameter of the control colonies.

#### Evaluation of *in vivo* disease severity

Four weeks-old apple seedlings of the rootstock 'MM106' (Mabrouka society) were used in this study. These seedlings were grown in a greenhouse in pots (23 cm diameter × 23 cm deep). The efficiency of the six fungicides against mentioned two *Pythiaceae* species was evaluated *in vivo* using the methodology of Utkhede and Smith (1991). For this, ten agar plugs of each isolate were incorporated in 500 ml flasks containing 200 g of sand, 20 g of oat, and 30 ml of distilled water previously autoclaved at 120 °C for 20 min for two consecutive days, under aseptic conditions. Then the flasks were mixed and incubated for seven days at 25 °C and shaken every two days to ensure thorough colonization. The sand-oat inoculum was added to a potting mix (peat and sand in 2:1 v/v) at a rate of 1% (v/v) which was then placed in 10 cm diameter plastic pots. For controls, an un-infested potting mix was used.

After plantation, each seedling was treated using the registered dose for each fungicide (50 ml/plant) except the chinisol for which the dose of 100 ppm was used. Pots were arranged in a completely randomized design and

watered when needed, and apple seedlings were examined after three months of inoculation. The disease severity was noted according to the vegetative part, sanitary state (health), and root browning indexes of plants. The sanitary state was rated on a 0-5 scale, where: 0 = no obvious symptoms; 1 = moderate discoloration of plant leaves ( $\leq 25\%$ ); 2 = moderate discoloration of plant and leaf drop ( $\leq 50\%$ ); 3 = moderate discoloration of plant collar, stem, and leaves ( $\leq 75\%$ ); 4 = extensive discoloration of plant collar and stem with falling leaves ( $> 75\%$ ); and 5 = dead plant (Santini *et al.*, 2006). Root rot was rated onto 0 g leaves ( $> 75\%$ ) 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 plant (Tewoldemedhin *et al.*, 2011).

For each seedling, the height and the root weight were noted.

#### Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) using Statistical Package for the Social Sciences software (SPSS), version 20.0. For all tests, means were separated using Student-Newman-Keul's (SNK) test ( $P \leq 0.05$ ).

#### Results

##### *In vitro* evaluation of the effectiveness of the chemical products against causative agents of apple decline

Results of *in vitro* assays exhibited highly significant differences ( $p \leq 0.001$ ) in the effect of the doses and the fungicides against the various pathogens targeted (Table 3). The results showed that the carbendazim is effective at ten ppm against *P. mercuriale* with a growth inhibition of 89.3%. Applied at 100 ppm and homologue dose (250 ppm), carbendazim generated an inhibition percent of 70.06% and 75.30%, respectively, for *P. ultimum* (Table 3 and Fig. 1).

Table 3 Percentage of mycelial growth inhibition by different doses of the different chemical products, recorded after three days of incubation at 25 °C, against *Pythium ultimum* and *Phytophthium mercuriale* responsible for apple decline.

Commercial name	Actives substances	Doses (ppm)	Inhibition (%)	
			<i>Phytophthium mercuriale</i>	<i>Pythium ultimum</i>
Prodazim	Carbendazim	10	89.30 ± 0.00	10.45 ± 1.93 <sup>a*</sup>
		25	89.30 ± 0.00	45.76 ± 2.92 <sup>b</sup>
		50	89.30 ± 0.00	67.80 ± 2.35 <sup>c</sup>
		100	89.30 ± 0.00	70.06 ± 2.16 <sup>c</sup>
		250 <sup>**</sup>	100.00 ± 0.00	75.30 ± 1.17 <sup>d</sup>
Dithane M45	Mancozeb	10	0.00 ± 0.00 <sup>a</sup>	0.80 ± 0.00 <sup>a</sup>
		25	1.00 ± 3.83 <sup>a</sup>	5.65 ± 0.65 <sup>b</sup>
		50	14.38 ± 3.94 <sup>b</sup>	24.01 ± 2.67 <sup>c</sup>
		100	11.71 ± 2.89 <sup>b</sup>	43.79 ± 1.69 <sup>d</sup>
		2000 <sup>**</sup>	100.00 ± 0.00 <sup>c</sup>	100.00 ± 0.00 <sup>e</sup>
Alliette express	Fosetyl-Al	10	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>
		25	16.39 ± 1.73 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>
		50	16.05 ± 4.55 <sup>b</sup>	0.00 ± 0.00 <sup>a</sup>
		100	29.43 ± 3.68 <sup>c</sup>	0.00 ± 0.00 <sup>a</sup>
		2000 <sup>**</sup>	100.00 ± 0.00 <sup>d</sup>	51.83 ± 11.37 <sup>b</sup>
Tachigazol	Hymexazol	10	35.12 ± 2.32 <sup>a</sup>	8.19 ± 2.32 <sup>a</sup>
		25	50.50 ± 3.28 <sup>b</sup>	13.56 ± 3.28 <sup>b</sup>
		50	62.88 ± 1.28 <sup>c</sup>	22.03 ± 1.28 <sup>c</sup>
		100	67.22 ± 0.77 <sup>d</sup>	32.20 ± 0.77 <sup>d</sup>
		60 <sup>**</sup>	100.00 ± 0.00 <sup>e</sup>	90.55 ± 6.71 <sup>e</sup>
Beltanol-L	Chinosol	10	73.91 ± 1.34 <sup>a</sup>	13.84 ± 6.35 <sup>a</sup>
		25	89.30 ± 0.00 <sup>b</sup>	27.68 ± 2.06 <sup>b</sup>
		50	89.30 ± 0.00 <sup>b</sup>	90.96 ± 0.00 <sup>c</sup>
		100	89.30 ± 0.00 <sup>b</sup>	90.96 ± 0.00 <sup>c</sup>
		2000 <sup>**</sup>	89.30 ± 0.00 <sup>b</sup>	90.96 ± 0.00 <sup>c</sup>
Ridomil Gold MZ68	Metalaxyl-M	10	4.78 ± 0.36 <sup>a</sup>	8.66 ± 0.26 <sup>a</sup>
		25	11.61 ± 1.05 <sup>b</sup>	21.65 ± 0.66 <sup>b</sup>
		50	23.22 ± 2.10 <sup>c</sup>	43.29 ± 1.32 <sup>c</sup>
		100	42.95 ± 4.02 <sup>d</sup>	79.70 ± 1.91 <sup>d</sup>
		120 <sup>**</sup>	46.45 ± 4.20 <sup>d</sup>	86.59 ± 2.63 <sup>e</sup>

(\*) The means ± standard deviation, in the same column followed by the same letter, are not significantly different according to the SNK test at P ≤ 0.05.

(\*\*) Approved dose.

Percent inhibition = (1 - T / C) × 100

T: Average diameter of colonies in the presence of the fungicide; C: Average diameter of the control colonies.

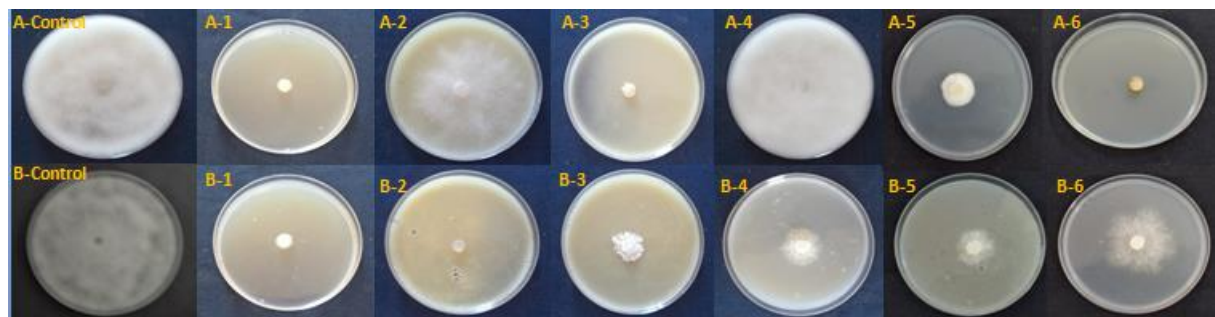


Figure 1 Effect of the homologue dose of hymexazol (1), mancozeb (2), metalaxyl-M (3), carbendazim (4), chinosol (5) and fosetyl-Al (6) on mycelial growth of *Pythium ultimum* (A) and *Phytophthium mercuriale* (B) recorded after three days of incubation at 25 °C.

The mancozeb was ineffective for the 2 pathogens tested at 10 and 25 ppm. At 2000 ppm, mancozeb appeared as the most effective against *P. mercuriale* and *P. ultimum* and could completely inhibit the mycelia growth of these pathogens (Table 3 and Fig. 1).

A negligible inhibition was found with the different doses of fosetyl-Al for these pathogens at the first four doses tested (10, 25, 50, and 100 ppm) with a percent of inhibition of 0% for *P. ultimum* and less than 29.43% for *P. mercuriale*. The dose of 2000 ppm induced a high percent of inhibition of *P. ultimum* and *P. mercuriale* development with respective values of 51% and 100% (Table 3 and Fig. 1).

Regarding hymexazol, the highest percent of inhibition was noted against *P. mercuriale* (100%) and *P. ultimum* (90.55%) at a 60 ppm dose (Fig. 1). However, the lowest level of inhibition was registered for *P. ultimum* at ten ppm (8.19%) (Table 3).

The effect of Chinosol against *P. mercuriale* generated an inhibition of mycelia growth with values of more than 70% for all tested doses. The highest inhibition percent was achieved at 25 ppm for *P. mercuriale* (89.30%) and 50 ppm for *P. ultimum* (90.96%) (Table 3 and Fig. 1).

Regarding metalaxyl-M, the highest inhibition level (86.59%) was noted for *P. ultimum*, at 120 ppm dose (Table 3 and Fig. 1).

#### **In vivo evaluation of the effect of chemical products on disease severity of apple decline in nurseries**

The variance analysis of disease severity parameters recorded three months post-inoculation by oomycetes species showed the positive effect of fosetyl-Al and metalaxyl-M + mancozeb and chinosol. Indeed, the fosetyl-Al and metalaxyl-M + mancozeb significantly reduced the root browning induced by *P. ultimum* by 55.67% and 44.33%, respectively (Table 4). The chinosol reduced the root browning by 55.67% and improved the sanitary state of aerial parts of seedlings inoculated by *P. ultimum* at 60.06%. However, chemical products tested on apple seedlings inoculated with *P. ultimum* did not improve either plant height or root weight (Table 4 and Fig. 2).

Nevertheless, fosetyl-Al, metalaxyl-M + mancozeb, and chinosol improved the sanitary state of the aerial parts of seedlings inoculated by *P. mercuriale* by 60.06, 49.85 and 49.85%, respectively. However, the fungicides tested on apple seedlings inoculated by *P. mercuriale* did not affect plant height and root weight (Table 4).

**Table 4** Effect of the fungicides tested on the severity of symptoms induced by *Pythium ultimum* and *Phytophthora mercuriale* and the growth of apple plants 'MM106' registered three months post-inoculation and treatments.

Fungicides	<i>Pythium ultimum</i>				<i>Phytophthora mercuriale</i>			
	Plant height (cm)	Root weight (g)	Root rot	Sanitary state	Plant height (cm)	Root weight (g)	Root rot	Sanitary state
TNI	91.17 ± 4.25 <sup>a</sup>	24.07 ± 4.52 <sup>a</sup>	0.67 ± 0.58 <sup>c</sup>	1.33 ± 0.58 <sup>b</sup>	90.33 ± 11.02 <sup>a</sup>	11.81 ± 1.32 <sup>a</sup>	1.33 ± 0.58 <sup>a</sup>	1.33 ± 0.58 <sup>b</sup>
TI	93.83 ± 23.26 <sup>a</sup>	11.84 ± 2.67 <sup>b</sup>	3.00 ± 0.00 <sup>a</sup>	3.33 ± 0.58 <sup>a</sup>	80.67 ± 2.08 <sup>a</sup>	11.64 ± 0.45 <sup>a</sup>	2.67 ± 0.58 <sup>a</sup>	3.33 ± 0.58 <sup>a</sup>
Carbendazim	36.43 ± 20.36 <sup>b</sup>	5.28 ± 3.82 <sup>b</sup>	2.00 ± 0.00 <sup>ab</sup>	2.67 ± 0.58 <sup>ab</sup>	36.00 ± 8.41 <sup>b</sup>	10.68 ± 2.23 <sup>a</sup>	2.33 ± 0.58 <sup>a</sup>	2.33 ± 0.58 <sup>ab</sup>
Mancozeb	50.93 ± 19.87 <sup>b</sup>	8.61 ± 3.38 <sup>b</sup>	2.33 ± 0.58 <sup>ab</sup>	2.67 ± 1.15 <sup>ab</sup>	45.83 ± 3.27 <sup>b</sup>	11.83 ± 0.62 <sup>a</sup>	1.67 ± 0.58 <sup>a</sup>	2.33 ± 0.58 <sup>ab</sup>
Fosetyl-AL	41.33 ± 13.19 <sup>b</sup>	5.30 ± 1.18 <sup>b</sup>	1.33 ± 0.58 <sup>bc</sup>	2.33 ± 0.58 <sup>ab</sup>	50.23 ± 1.66 <sup>b</sup>	12.37 ± 0.82 <sup>a</sup>	2.00 ± 0.00 <sup>a</sup>	1.33 ± 0.58 <sup>b</sup>
Metalaxyl-M + mancozeb	44.17 ± 4.07 <sup>b</sup>	6.01 ± 2.83 <sup>b</sup>	1.67 ± 0.58 <sup>bc</sup>	2.00 ± 0.00 <sup>ab</sup>	54.50 ± 17.84 <sup>b</sup>	7.90 ± 4.25 <sup>a</sup>	1.33 ± 0.58 <sup>a</sup>	1.67 ± 0.58 <sup>b</sup>
Hymexazol	38.50 ± 5.77 <sup>b</sup>	7.18 ± 5.26 <sup>b</sup>	3.00 ± 0.00 <sup>a</sup>	2.33 ± 0.58 <sup>ab</sup>	48.50 ± 24.39 <sup>b</sup>	8.06 ± 6.99 <sup>a</sup>	2.33 ± 0.58 <sup>a</sup>	2.00 ± 1.00 <sup>ab</sup>
Chinosol	70.83 ± 3.75 <sup>ab</sup>	9.31 ± 0.80 <sup>b</sup>	1.33 ± 0.58 <sup>bc</sup>	1.33 ± 0.58 <sup>b</sup>	59.00 ± 2.00 <sup>b</sup>	10.43 ± 0.80 <sup>a</sup>	2.00 ± 0.00 <sup>a</sup>	1.67 ± 0.58 <sup>b</sup>

(\*) For each pathogen, the means ± standard deviation in the same column followed by the same letter are significantly comparable according to the SNK test at  $P \leq 0.05$ .

TNI: Uninoculated control, TI: Inoculated control.



**Figure 2** Effect of fungicides on the root browning of apple plant variety ‘MM106’ after 3 months of inoculation by *Pythium ultimum* and treatment A: Inoculated control, B: apple root inoculated and treated by fosetyl-AI, C: root inoculated and treated by chinisol, D: root inoculated and treated by metalaxyl-M + mancozeb.

## Discussion

The aims of the present investigation were to evaluate the potential efficacy of six fungicides in the management of apple seedling decline in Tunisian nurseries. The results revealed a difference in the effectiveness of the active ingredients and the used doses against pathogens associated with apple decline.

It appears that carbendazim acts at a low dose (10 ppm) against *P.mercuriale*, while it was effective against *P.ultimum* at 50 ppm. The approved dose of this product (250 ppm) inhibited the development of *P.ultimum* by 75.3% and *P.mercuriale* by 100%. Whereas it did not improve the seedling development in the *in vivo* test. In the same sense, previous studies have found that carbendazim is a very effective fungicide against *Fusarium* spp. (Gaur and Chakrabarti, 2009; Iqbal *et al.*, 2010; Mannai *et al.*, 2018b) and *P. ultimum*. In addition, this fungicide was effective against some oomycetes species associated with peach seedling decline in nurseries. Carbendazim inhibited *P. ultimum* and *P. citrophthora* by 75.30 % and 100 % at 250

µg/l (Mannai and Boughalleb-M’Hamdi, 2021). The different doses of Mancozeb did not show any effect for all tested doses, while the approved dose (2000 ppm) was effective against these two pathogens. Previous studies have reported that Mancozeb is among the best fungicides tested *in vitro* against *Fusarium* spp. (Ahmad *et al.*, 2012; Shah *et al.*, 2006).

For Hymexazol, all doses were ineffective against *P.ultimum*. The efficacy of this product against *P. mercuriale* was reported at a low dose (10 ppm) and increased at a 100 ppm dose to 67.22%. The approved dose of this product was shown to be effective against the two pathogens tested. Previous studies have shown the efficacy of hymexazol *in vitro* and *in vivo* in reducing mycelial development and wilt of tomato and potato caused by *F. oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *tuberosis*, respectively (Hibar *et al.*, 2007; Ayed *et al.*, 2006). In addition, Daami-Remadi (2001) also reported its effectiveness in inhibiting potato leak syndrome caused by *P. aphanidermatum*.

The different doses of fosetyl-AI gave low efficacy *in vitro* assay against both pathogens,



while the registered dose (2000 ppm) was effective against these pathogens. *In vivo*, the six fungicides were able to reduce the severity of apple seedling decline, confirming the efficacy of fosetyl-Al. Indeed, this product reduced the root browning induced by *P. ultimum* and improved the sanitary state of aerial parts of seedlings inoculated with *P. mercuriale*. In a similar result, Utkhede and Smith (1991) showed that this product is effective against the three genera *Pythium*, *Phytophthora*, and *Fusarium* associated with root rot of apple seedlings. Fosetyl-Al was effective against *P. ultimum* at high concentrations, but it was phytotoxic to apple seedlings when tested at 2.5 g l<sup>-1</sup> under greenhouse conditions (Menge, 1986; Utkhede and Smith, 1991).

The Metalaxyl-M induced mycelial growth reduction of *P. ultimum*. Moreover, previous works have shown that several species of *Pythium*, such as *P. ultimum* are sensitive to this product (Cook and Zhang, 1985; Brantner and Windels, 1998; Mazzola *et al.*, 2002). Indeed, Mazzola *et al.* (2002) found that *Pythium* species associated with apple trees are sensitive to metalaxyl-M with a species-specific sensitivity. Brantner and Windels, (1998) proved that *P. aphanidermatum* isolated from sugar beet was less sensitive to metalaxyl than isolates of *P. ultimum* var. *sporangiferum* from the same host. Likewise, sensitivity to metalaxyl among isolates of *Pythium* spp. recovered from wheat in the Pacific Northwest was observed to be species dependent; *P. irregular* and *P. torulosum* were insensitive, whereas *P. aristosporum*, *P. heterothallicum*, and *P. ultimum* were sensitive to this fungicide (Cook and Zhang, 1985).

In 1986, Menge reported that both fosetyl-Al and metalaxyl could reduce the development of the pathogen without eradicating them and recommended using these two chemicals as preventative rather than curative treatments in nurseries.

Our investigation demonstrated that Chinosol was effective *in vitro* conditions at different doses against *P. mercuriale*. The registered dose (2000 ppm) reduced the mycelial growth of *P. ultimum* and *P. mercuriale*. The *in vivo* assays

also proved the efficacy of Chinosol. This product reduced the severity of apple seedlings decline induced by *P. ultimum* and *P. mercuriale* (the root browning and sanitary state of aerial seedling part). Our funding is in concordance with those of Vaartaja (1964), who mentioned the efficacy potential of Chinosol in managing different oomycetes and fungal species in different crops. Indeed, Chinosol has controlled *Pythium* damping-off of turf, pines, and some garden plants through its application to the soil. In addition, the Chinosol has effectively inhibited the *in vitro* growth of *R. solani*, *P. ultimum*, and *F. avenaceum*.

In conclusion, the fosetyl-Al, mancozeb, metalaxyl, and chinosol are the most effective *in vivo* against the two pathogens associated with apple seedling decline in Tunisian nurseries. Thus, it will be important to test them in the future against the combination of these pathogens because the causal agent of this disease is a complex of different pathogens.

### Conflict of Interests

We have no conflict of interest.

### References

- Ahmad, Z., Saifullah, S., Raziq, F., Khan, H. and Idrees, M. 2012. Chemical and biological control of *Fusarium* root rot of okra. Pakistan Journal of Botany, 44(1): 453-457.
- Ayed, F., Daami-Remadi, M., Jabnoun-Khiareddine, H., Hibar, K. and El Mahjoub, M. 2006. Evaluation of fungicides for control of *Fusarium* wilt of potato. Plant Pathology Journal, 5: 239-243.
- Boughalleb, N., Moulahi, A. and El Mahjoub, M. 2006. Effect of four fungicides on development and control of *Phytophthora* on apple trees *in vitro* and *in vivo*. International Journal of Agricultural Research, 1(6): 582-589.
- Brantner, J. R. and Windels, C. E. 1998. Variability in sensitivity to metalaxyl in vitro, pathogenicity and control of *Pythium* spp. on sugar beet. Plant Disease, 82: 896-899.
- Cook, R. J. and Zhang, B.X. 1985. Degrees of sensitivity to metalaxyl within the *Pythium*

- spp. pathogenic to wheat in the Pacific Northwest. *Plant Disease*, 69: 686-688.
- Daami-Remadi, M. 2001. Lutte chimique contre la pourriture aqueuse des tubercules de pomme de terre. *Annales de l'INRAT*, 74: 151-165.
- Gaur, V. P. and Chakrabarti, D. K. 2009. Incidence of malformation in mango (*Mangifera indica*) nurseries in eastern Uttar Pradesh. *Indian Journal of Agricultural Sciences*, 79(2): 160-162.
- Hibar, K., Daami-Remadi, M. and El Mahjoub, M. 2007. Effets de certains fongicides de synthèse et biologiques sur la croissance mycélienne et l'agressivité de *Fusarium oxysporum* f. sp. *radicis-lycopersici*. *Tropicultura*, 25(3): 146-152.
- Iqbal, Z., Pervez, M. A., Ahmad, S., Iftikhar, Y., Yasin, M., Nawaz, A., Ghazanfar, M. U., Dasti, A. A. and Saleem, A. 2010. Determination of minimum inhibitory concentrations of fungicides against fungus *Fusarium Mangiferae*. *Pakistan Journal of Botany*, 42(5): 3525-3532.
- Lévesque, C. A. and De Cock, A. W. A. M. 2004. Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycological Research*, 108: 1363-1388.
- Manici, L. M., Ciavatta, C., Kelderer, M. and Erschbaumer, G. 2003. Replant problems in South Tyrol: role of fungal pathogens and microbial populations in conventional and organic apple orchards. *Plant and Soil*, 256: 315324. <https://doi.org/10.1023/A:1026103001592>.
- Mannai, S. and Boughalleb-M'Hamdi, N. 2021. *In vitro* and *in vivo* effects of some chemical fungicides against *Pythium ultimum* and *Phytophthora citrophthora* associated with peach seedlings decline. *Novel Research in Microbiology Journal*, 5(6): 1431-1446 DOI: 10.21608/nrmj.2021.207166.
- Mannai, S., Horrigue-Raouani, N. and Boughalleb-M'Hamdi, N. 2018a. Characterization of *Fusarium* species associated with apple decline in Tunisian nurseries. *Journal of Biological Studies*, 1 (2): 14-34.
- Mannai, S., Horrigue-Raouani, N. and Boughalleb-M'Hamdi, N. 2018b. Effect of Six Fungicides against *Fusarium oxysporum* and *F. solani* Associated with Peach Seedlings Decline in Tunisian Nurseries. *Annual Research & Review in Biology*, 26 (4): 1-11.
- Mazzola, M. 1998. Elucidation of the microbial complex having a causal role in the development of apple replant disease in Washington. *Phytopathology*, 88: 930-938.
- Mazzola, M., Andrews, P. K., Reganold, J. P. and Lévesque, C. A. 2002. Frequency, virulence, and metalaxyl sensitivity of *Pythium* spp. isolated from apple roots under conventional and organic production systems. *Plant Disease*, 86: 669-675. <https://doi.org/10.1007/s10658-018-01631-9>
- Santini, A., Biancalani, F., Biancalani, F., Barzanti, G. P. and Capretti, P. 2006. Pathogenicity of four *Phytophthora* species on wild cherry and Italian alder seedlings. *Journal of Phytopathology*, 154: 163-167.
- Menge J. A. 1986. Use of new systemic fungicides on citrus. *Citrograph*, 71: 245-250.
- Moein, S., Mazzola, M., Ntushelo, N. S. and McLeod A. 2019. Apple nursery trees and irrigation water as potential external inoculum sources of apple replant disease in South Africa. *European Journal of Plant Pathology*, 153: 1131-1147 DOI: [doi.org/10.1007/s10658-018-01631-9](https://doi.org/10.1007/s10658-018-01631-9).
- Shah, M. I., Sultan, P. Nasir, A. Williams, P. Jan, A. Sajad, M. Rehman, S. and Shawal, A. S. 2006. *In vitro* study on effect of some fungicides viz., carbendazim, mancozeb, conjoint carbendazim, mancozeb and sulphur against *F. oxysporum*. *Research Journal of Microbiology*, 1(4): 360-365.
- Souli, M., Abad-Campos, P., Pérez-Sierra, A., Fatouch, S., Armengol, J. and Boughalleb, N. 2014. Etiology of apple dieback in Tunisia and abiotic factors associated with the disease. *African Journal of Microbiology Research*, 8: 2272-2281.
- Souli, M., Boughalleb, N., Abad-Campos, P., Álvarez, L. A., Pérez-Sierra, A., Armengol, J. and García-Jiménez, J. 2011a. First Report of *Pythium indigoferae* and *P. irregulare* Associated to Apple Trees Decline in Tunisia. *Journal of Phytopathology*, 159: 352-357.



- Souli, M., Boughalleb, N., Abad-Campos, P., Álvarez, L. A., Pérez-Sierra, A., Armengol, J., García-Jiménez, J. and Romdhani, M. S. 2011b. Diversity of the *Pythium* community infecting crown and roots apple in Tunisia. *Research in Plant Biology*, 1(4): 16-22.
- Tewoldemedhin, Y. T., Mazzola, M., Botha, W. J., Spies, C. F. J. and McLeod, A. 2011. Characterization of fungi (*Fusarium* and *Rhizoctonia*) and oomycetes (*Phytophthora* and *Pythium*) associated with apple orchards in South Africa. *European Journal of Plant Pathology*, 130: 215-229. <https://doi.org/10.1007/s10658-011-9747-9>.
- Utkhede, R. S. and Smith, E. M. 1991. *Phytophthora* and *Pythium* species associated with root rot of young apple trees and their control. *Soil Biology and Biochemistry*, 23: 1059-1063.
- Vaartaja, O. 1964. Chemical treatment of seedlings to control nursery diseases! Introduction Seed Treatments Soil Acidifying Soil Fumigation. *The Botanical Review*, 30 (1): 20-22.

## ارزیابی شش قارچکش برای مدیریت زوال نهال‌های سیب در تونس

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**چکیده:** زوال سیب، عامل پوسیدگی ریشه و طوقه نهال در نهالستان‌ها بیماری مهمی است. برخی از گونه‌های اوومیست با این بیماری مرتبط هستند. این مطالعه با هدف کنترل بیماری زوال انجام شد. اثر شش آفتکش شیمیایی روی *Pythium ultimum* و *Phytophthora mercuriale* مرتبط با زوال نهال سیب در شرایط آزمایشگاهی و سنجش‌های گلخانه‌ای مورد ارزیابی قرار گرفت. کاربندازیم در ۱۰ پی‌پی‌ام در برابر *P. mercuriale* ۸۹/۳ درصد مؤثر بود، درحالی‌که در ۱۰۰ و ۲۵۰ پی‌پی‌ام مقادیر به-ترتیب ۰/۶، ۷۰ و ۷۵/۳۰ درصد برای *P. ultimum* بود. دز ۲۰۰۰ پی‌پی‌ام مانکوزب به‌طور کامل رشد میسلیم *P. mercuriale* و *P. ultimum* را مهار کرد. با این حال، فوستیل آلومینوم اعمال شده در ۲۰۰۰ پی‌پی‌ام مهار ۵۱ و ۱۰۰ درصدی را برای *P. ultimum* و *P. mercuriale* نشان داد. در مورد هایمکسازول، بالاترین میزان مهار در برابر *P. mercuriale* ۱۰۰ درصد و *P. ultimum* ۹۰/۵۵ درصد برای ۶۰ پی‌پی‌ام بود. اما چینوسول در تمام دزها بر ضد *P. mercuriale* مؤثر است. در برابر *P. ultimum* در ۵۰ پی‌پی‌ام ۹۰/۹۶ درصد مؤثر بود. بالاترین میزان بازدارندگی ۸۶/۵۹ درصد در متالاکسیل-ام برای *P. ultimum* با ۱۲۰ پی‌پی‌ام بود. آزمایش *In vivo* کارایی فوستیل آل، متالاکسیل-ام، مانکوزب و چینوسول را در کاهش قهوه‌ای شدن ریشه ناشی از *P. ultimum* به‌ترتیب به میزان ۵۵/۶۷، ۴۴/۳۳ و ۵۵/۶۷ درصد و وضعیت اندام هوایی نهال‌های تلقیح شده توسط *P. mercuriale* به‌ترتیب ۶۰/۰۶، ۴۹/۸۵ و ۴۹/۸۵ درصد بهبود بخشید. به‌طورکلی چینوسول وضعیت اندام‌های هوایی نهال‌های تلقیح شده توسط *P. ultimum* را بهبود بخشید.

**واژگان کلیدی:** کنترل شیمیایی، زوال سیب، نهالستان، *Phytophthora mercuriale*، *Pythium ultimum*