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Fusarium Crown and Root Rot of Tomato and its Chemical Control

[1]Khaled Hibar, [2]Mejda Daami-Remadi, [1]Fakher Ayed and [1]Mohamed El Mahjoub
[1]Department of Phytopathology,
Agronomic Higher Institute of Chott-Mariem 4042 Sousse, Tunisia
[2]National Institute of Agronomic Research, Tunis,
PRRDA-CE Chott-Mariem 4042 Sousse, Tunisia

Abstract: *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) is a new emerging pathogen in Tunisia. It causes Fusarium crown and root rot (FCRR) of tomato. Being a new disease, no control methods are available. Therefore, looking for a solution to this pathogen is required. In this study, the efficacy of some chemical fungicides to suppress FORL was evaluated *in vitro*, in growth chamber as well as under greenhouse conditions. In *in vitro* tests, all fungicides inhibited mycelial growth of FORL at 75 to 90% with the exception of maneb which entailed the lowest growth inhibition estimated at 40%. Under growth chamber trials, with the exception of benomyl, the other tested fungicides (Hymexazol, Hydroxyquinolin, Sodium Tetraborohydrate Decahydrate, Oxyquinolin and Flutriafol+Thiabendazole) have significantly reduced disease incidence. Under greenhouse conditions, results were more encouraging. Indeed, the use of Hymexazol reduces the percentage of dead plants at 6.4%. This study demonstrated the efficacy of some chemical fungicides in controlling FORL especially when they were incorporated to the culture substrate.

Key words: *Fusarium*, fungicides, inhibition, disease incidence, greenhouse conditions

INTRODUCTION

Soilless cultivation systems of plant production are used worldwide to grow flower, foliage, bedding and vegetable crops (Song *et al*., 2004). Tomatoes can be cultivated quite well in a soilless system as can vegetables or ornamental species. The main diseases in tomato aerial parts are gray mold (*Botrytis cinerea*) and Cercospora leaf mold (*Cercospora fuliginea*).

These diseases can be controlled by spraying fungicides as well as using biocontrol agents such as *Trichoderma harzianum* (Moyano *et al.* 2003). The main soil-borne systemic diseases are Fusarium crown and root rot (*Fusarium oxysporum* f. sp. *radici-lycopersici*) (FORL), Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*), late blight (*Phytophthora infestans*) and Pythium damping-off (*Pythium aphanidermatum*) (Schwarz and Grosch, 2003). Of the soil-borne disease, Fusarium Crown and Root Rot (FCRR) is the most serious especially in soilless cultivation system. This disease newly recorded in Tunisia, during 2000-2001 crop season (Hajlaoui *et al*., 2001; Hibar, 2002), caused heavy losses reaching 90% of plants in some geothermal greenhouses. Although some cultivars with single dominant genes for resistance have been developed, control of FCRR is mainly restricted to eliminating the pathogen in soil by steaming or fumigating with chemicals and by planting pathogen-free transplants (Sivan *et al*., 1987).

While in several other countries, fumigation with methyl bromide, which was effective in reducing soilborne inoculum of numerous *Fusarium* species and other soil borne pathogens, will be totally removed from the agricultural markets, because of its ozone-depleting (Ma *et al*., 2001).

Corresponding Author: Khaled Hibar, Department of Phytopathology, Agronomic Higher Institute of Chott-Mariem 4042 Sousse, Tunisia Tel: +216 73 348 544 Fax: +216 733 486 91

In Tunisia, FORL is considered as a new emergent pathogen, so no control strategy is available to remedy to this problem although several chemical fungicides were used in the world to control FORL such as benomyl (Mihuta-Grimm, 1990) and Hymexasol (Veschambre, 1995). In an attempt to look for a solution to FORL, several chemical fungicides were tested *in vitro*, on mycelial growth, *in vivo*, on disease incidence and under greenhouses on percentage of wilted plants.

MATERIALS AND METHODS

Fungal Isolates

Four FORL isolates were used in this study. They were recovered from greenhouses tomato plants showing typical crown and root rot symptoms at 5ème saison exploitation in Hammet Gabès in South Tunisia where tomato culture, heated with geothermal water, is practised.

Fungal pathogen was isolated by planting plant tissues (surface-disinfected with 1% sodium hypochlorite for 2 min) on PDA (Potato Dextrose Agar) and incubating them at 25°C for 5 days (Katan *et al.*, 1991). Isolates were identified as *F. oxysporum* morphologically based on characteristics of the macroconidia, phialids, microconidia, chlamydospores and colony growth traits (Nelson *et al.*, 1983). The *Formae specialis* of this pathogen was identified using pathogenicity tests (Hibar, 2002). Based on these tests, the more aggressive isolates were selected for this study (Hibar *et al.*, 2005, 2007). The four isolates used in *in vitro* and *in vivo* tests are presented in Table 1.

Plant Material

FORL susceptible tomato seeds (*Lycopersicon esculentum* Mill. cv. Riogrande and Bochra) were selected to study the effect of chemical fungicides on disease incidence. Tomato seeds were sterilized by immersion in absolute ethanol for 7 min, followed by extensive rinsing in sterile distilled water (Benhamou and Chet, 1997). Seeds were sown in alveolus plates filled with previously sterilized peat. Seedlings were grown in a growth chamber at 24 to 26°C with 12 h photoperiod and 70% humidity. They were watered daily and fertilized twice a week with a standard nutrient solution according to Pharand *et al.* (2002).

Tests were performed with 5-week-old tomato plants carrying five or six fully expanded leaves (Benhamou and Bélanger, 1998).

Chemical Fungicides

On an attempt to control FCRR, seven chemical fungicides were evaluated on mycelial growth and on disease incidence (Table 2).

Table 1: FORL isolates used for study

Isolates	Host plant (Cultivar)	Date of isolation
Fo2-01	Durintha	2001
Fo4-02	Bochra	2002
Fo1-03	Samia	2003
Fo1-04	Olivette	2004

Table 2: Chemical fungicides used in this study

Active ingredient	Commercial name	Concentration of the active ingredient	Tested dose	Action mode
Hymexazol	Tachigaren 360	360 g L^{-1}	1 mL L^{-1}	Systemic
Benomyl	Benlate 50	50%	60 g hL^{-1}	Systemic
Oxyquinolin	Cryptonol	140 g L^{-1}	3.5 l hL^{-1}	Systemic
Hydroxyquinolin	Beltanol	500 g L^{-1}	1 mL L^{-1}	Systemic
Flutriafol+Thiabendazole	Vincit F	25 g L^{-1} +25 g L^{-1}	300 mL hL^{-1}	Systemic
Maneb	Manèbe 80	80%	250 g hL^{-1}	Contact
Sodium Tetraborohydrate Decahydrate	Prev-Am	9.9 g L^{-1}	10 mL L^{-1} (1%)	Contact

***In vitro* Inhibition of Fungicides on the Pathogen**

The inhibitory activities of the fungicides on mycelial radial growth of FORL were determined by growing the fungus on PDA media containing fungicides in Petri plates. Each fungicide was added at recommended label rates (Table 2) to 100 mL sterilized PDA media at 60°C and then poured equally into five Petri plates. In control plates, the quantity of fungicides was replaced by the same quantity of sterile distilled water. A disc (6 mm diameter) of 6-day-old pathogen mycelial culture was aseptically transferred to the center of the solidified PDA media in plates. The plates were subsequently incubated for 6 days at 25°C (Hibar *et al*., 2006). Mycelial growth of FORL was measured on each plate and the growth in PDA media containing fungicides was compared with the growth of the pathogen in control plates. The experiment was replicated twice for each treatment and the mean values taken.

Fungitoxicity was recorded in terms of percentage colony inhibition and calculated according to Hmouni *et al.* (1996). Percentage growth inhibition was determined as (1- Cn/Co)×100, where Cn is the average diameter increase of fungal colony with treatment and Co is the average diameter increase of fungal colony with control.

Effect of Fungicides on Disease Incidence

Based on their efficacy *in vitro*, the effect of fungicides on disease incidence was carried out with the same fungicides presented in Table 2 excepting the maneb.

Studying the effect of these fungicides on the aggressiveness of FORL has needed the following steps:

Inoculum Preparation

After the pathogen was cultured in the PDB (Potato Dextrose Broth), a spore suspension of 10^7 spores mL^{-1}, determined using a Malassez Blade, was obtained. Ten milliters of this conidial suspension was served to inoculate 600 cm^3 of perlite (enriched with 200 mL of PDB) prepared in Roux boxes. Infested perlite, prepared in these boxes and incubated for four weeks at 25°C was served to inoculate tomato plants.

Plant Treatments

Tomato plants of 5 week-old were transplanted into plastic pots (6.5 cm in diameter) containing a mixture of inoculated perlite and a previously sterilized peat to which 10 mL of fungicide was added at recommended label rates (Table 2). Indeed, each fungicide was dissolved in 1 L of sterile distilled water; this prepared solution was divided onto 10 plants (number of repetition per elementary treatment). Fungicides were applied, just after plant transplantation as a drench at the crown level. Once transplanted and treated, plants were grown in a growth chamber with 12 h photoperiod under 20-25°C.

Plants transplanted in a mixture of inoculated perlite and disinfected peat, without adding fungicides or in a mixture of a previously sterilized peat and perlite served as negative and positive control, respectively.

Disease assessment performed 30 days after treatment and the disease severity was recorded on 0 to 3 visual scale, in which:

0 = No symptoms;
1 = Light yellowing of leaves, light or moderate rot on taproot and secondary roots and crown rot;
2 = Moderate or severe yellowing of leaves with or without wilting, stunting, severe rot on taproot and secondary roots, crown rot with or without hypocotyls rot and vascular discoloration in the stem;
3 = Dead seedlings (Vakalounakis and Fragkiadakis, 1999).

Disease incidence percentage was determined using the following formula (Song *et al*., 2004):

$$\text{Diseas incidence}(\%)\left[\frac{\Sigma\text{scale}\times\text{number of plants inf ected}}{(\text{Highest scale}\times\text{total number of plants})}\right]\times 100$$

Ten plants per elementary treatment were used and variance analysis of the treatment effect on measured data was performed by using the general linear model procedure of SPSS (SPSS 10.0). Experiments were analyzed using standard analysis of variance (ANOVA) with factorial treatment structure and interactions. When F-values were significant at $p>0.05$, differences among the treatments were determined by S-N-K (Student-Newman-Keuls) test.

Greenhouse Experiment

Greenhouse experiment for controlling FCRR was performed using only one Fungicide (Hymexazol).

The greenhouse experiment was carried out in 2002-03 crop season at the 5ème Saison exploitation, situated in Hammet Gabès in Southern Tunisia. Hymexazol was applied once before transplanting in the breeding-ground to tomato plants cv. Bochra. These latest, with 3-4 true leaves, were transferred from breeding-ground to the greenhouse, heated with geothermal water. Soilless culture was adopted in sausage bags filled with perlite naturally infested with FORL and using a drip irrigation system.

In the greenhouse, fungicide was applied once a month from the date of transplantation (the end of August 2002) to the end of the crop season (the end of May 2003). This experiment was performed with a total number of 500 plants and the number of dead plants along the crop season was recorded.

The same number of plants, transplanted in another greenhouse, on sterilized perlite served as a positive control; whereas, negative control was constituted by the same number of plants transplanted on naturally infested perlite.

RESULTS

***In vitro* Inhibition of Forl Isolates by Fungicides**

Adding chemical fungicides to the PDA media has inhibited mycelial growth of FORL isolates. With the exception of maneb which entailed the lowest growth inhibition (about 40%), the other fungicides have significantly inhibited mycelial growth of FORL and the growth reduction recorded was more than 75% (Fig. 1). Indeed, the more important inhibition percentage was obtained with fungicides Hydroxyquinolin and Oxyquinolin with which the inhibition percentage has exceeded 90% and this for all tested isolates.

An important inhibition percentage was also obtained with fungicides Hymexazol and Sodium Tetraborohydrate Decahydrate with values exceeding 85%. Flutriafol+Thiabendazole and benomyl have also significantly halted mycelial growth of FORL.

Effect of Fungicides on Disease Incidence under Growth Chamber Experiments

In vitro efficacy of fungicides has served as a criterion for selecting fungicides used *in vivo*. Incorporation of fungicides to the culture substrate and the measurement of the disease incidence 30 days after inoculation, have revealed a high efficacy of Hymexazol, Hydroxyquinolen, Sodium Tetraborohydrate Decahydrate, Oxyquinolin and Flutriafol+Thiabendazole compared to benomyl. Indeed, the mean disease incidence for tomato plants treated with Hymexazol was about 23.33% (Table 3). Moreover, for plants treated with this fungicide and inoculated with Fo4-02 isolate, disease incidence has slightly exceeded 13%. We also note that in the plot of plants treated with Hemexazol, some of them appeared healthy and there are no wilting symptoms (Fig. 2).

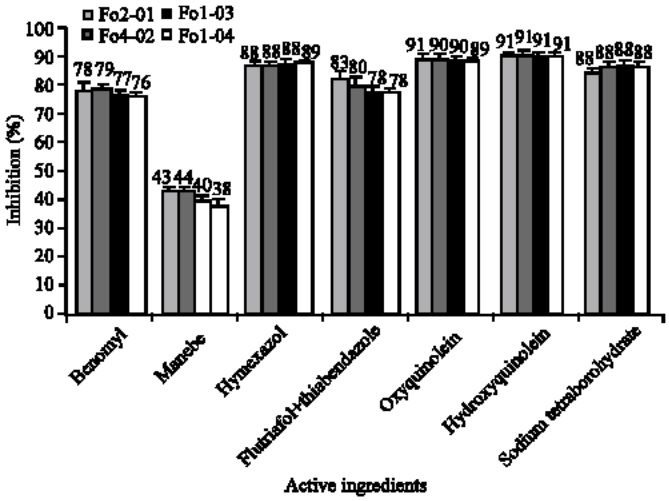

Fig. 1: Effect of various chemical fungicides on mycelial growth inhibition of *F. oxysporum* f. sp. *radicis-lycopersici* after an incubation period of 6 days at 25°C

Table 3: Disease incidence of Fusarium crown and root rot of tomato for the different treatments on tomato plants (cv. Riogrande), 30 days after inoculation

	Disease incidence (%)[x]				
Active ingredient	Fo2-01	Fo4-02	Fo1-03	Fo1-04	means
Hydroxyquinolin	23.33b	13.33a	20a	40b	24.16
Oxyquinolin	26.67b	16.67a	20a	43.33b	26.66
Flutriafol+Thiabendazole	30 b	20a	20a	56.67bc	31.66
Benomyl	56.67c	26.67a	46.67b	73.33c	50.81
Hymexazol	26.67b	13.33a	16.67a	36.67b	23.33
Sodium Tetraborohydrate Decahydrate	30b	16.67a	16.67a	40b	25.83
Inoculated and untreated control	86.67d	53.33b	83.33c	96.67d	80
Uninoculated control	0 a	0 a	0 a	0 a	0

[x]Within columns, means followed by the same letter(s) are not significantly different (p = 0.05) according to SNK-test

Fig. 2: Comparison between an inoculated and untreated control plant (A) and an inoculated plant treated with Hymexazol (B), 30 days after inoculation with *F. oxysporum* f. sp. *radicis-lycopersici* at 25°C

Table 4: Number and percentage of wilting plants obtained among 500 tomato plants (cv. Bochra) during 2002-2003 crop season in the 5ème Saison" exploitation

Active ingredient	No. of wilted plants	Percentage of wilted plants
Greenhouse treated with Hymexazol	32	6.4
Inoculated and untreated greenhouse	391	78.2
Untreated greenhouse	360	72

Good results were also obtained when treating with Hydroxyquinolin of which disease incidence was about 24%.

Disease incidence of FCRR was also low (about 26%) when treating tomato with fungicides Sodium Tetraborohydrate Decahydrate or Oxyquinolin. However, with benomyl disease incidence was more than 50% (Table 3).

Control of *Fusarium* Crown and Root Rot under Greenhouse Conditions

Based on its efficacy *in vitro* and *in vivo* and on its availability, only one fungicide (Hymexazol) was used to control FORL under greenhouse conditions.

From the end of August 2003 to the end of May 2003, only 32 plants were totally wilted on a total number of 500 plants representing 6.4%. However, in the non-treated greenhouse, where tomato plants were transplanted on infested perlite, the number of wilting plants was about 391 representing 78.2% (Table 4).

We also note that percentage of wilting plants, in the greenhouse where tomato plants were transplanted on sterilized perlite (positive control), was also important (72%). This high level of wilting plants should be explained by the ability of FORL to disseminate from greenhouse to another.

DISCUSSION

Fusarium crown and root rot of tomato caused by FORL is a new damaging disease of greenhouse crops in Tunisia. While causing heavy losses on tomato production, no or some effective disease control methods are available. In Tunisia, there are no approved fungicides to control FORL.

Screening fungicides for controlling this pathogen demonstrated that with the exception of the maneb, which entailed the lowest inhibition percentage of mycelial growth; the other tested fungicides (Hydroxyquinolin, Oxyquinolin, Hymexazol and Sodium Tetraborohydrate Decahydrate, Flutriafol + Thiabendazole and benomyl) have significantly limited mycelial growth of the studied pathogen.

The *in vitro* inhibitory activity of benomyl has previously studied. In fact, Daami-Remadi *et al.* (2006a) have reported that this fungicide has limited mycelial growth of several Fusarium species (*Fusarium solani*, *F. graminearum*, *F. oxysporum* and *F. sambucinum*) causing potato dry rot. These authors demonstrated that benomyl has inhibited mycelial growth of *F. solani* and *F. graminearum* by more than 90%.

Similarly, Mclean and Lawrence *et al.* (1994) demonstrated that benomyl has inhibited mycelial growth of *F. solani*, the causal agent of sudden death syndrome of soybean, by more than 66%.

While benomyl has significantly reduced mycelial growth of FORL, a more important inhibition percentage was obtained with Hymexazol. Tested against *F. oxysporum* f. sp *tuberosi*, the causal agent of Fusarium wilt of potato, this fungicide has entailed mycelial growth of this fungus by more than 77% (Ayed *et al*., 2006).

These authors also showed that with Oxyquinolin, inhibition percentage of *F. oxysporum* f. sp *tuberosi* was only about 30 to 40%; however in this study this fungicide has caused the highestmycelial growth inhibition of FORL.

Added to the PDA media, the Hydroxyquinolin has completely inhibited mycelial growth of *F. sambucnum* the causal agent of potato dry rot (Daami-Remadi *et al*., 2006b).

Several other fungicides have been reported to limit mycelial growth of some soil-borne fungi. In this case, Song *et al.* (2004) demonstrated that Prochloraz and Carbendazim were more efficient in reducing mycelial growth of *F. oxysporum* f. sp. *lycopersici*, causing tomato Fusarium wilt, compared to Thiram, Toclofos-methyl, Hymexazol, Azoxystrobin and Carboxin.

The efficacy of these fungicides has served as a criterion to use them *in vivo* to control FCRR. In our study and with the exception of benomyl with which disease incidence was more than 50%, the other tested fungicides have significantly reduced disease incidence of FORL.

Present results showing the inefficacy of benomyl in reducing disease incidence of FORL join those of Reid *et al.* (2002). These others showed that the use of benomyl to suppress Fusarium crown and root rot of *Asparagus* under greenhouse and growth chamber condition has entailed 65% of dead plants compared to Fludioxonil, with which percentage of dead plants was only about 20%. However, Mihuta-Grimm (1990) found that application of benomyl at 0.09 g L^{-1}, 21 days before planting, has significantly reduced disease incidence of Fusarium wilt of tomato.

In addition to benomyl, several other fungicides were tested to control soil borne disease. Indeed, Song *et al.* (2004) demonstrated that Prochloraz and Carbendazim were efficient in controlling tomato Fusarium wilt, entailing thus a reduction of disease incidence of about 69.6 and 87%, respectively.

Chemical control of Fusarium crown and root rot of tomato was the subject of several studies. In this case, Veschambre (1995) find that two application of Hymexazol, one when transplanting and the second 15 days later at 15l ha^{-1} has limited disease incidence of FORL, moreover this fungicide has stimulate root growth of treated plants. Similarly, Benhamou and Bélanger (1998) founded that tomato plants treated with Benzothiadiazole, a synthetic chemical, were resistant to FCRR of tomato. In the same way, Ishikawa *et al.* (2005) demonstrated that a foliar spry of tomato plants with Validamycine A or with Validoxylamin, a derivative of Probenazole and Benzothiadazole, has induced systemic resistance in these plants and this by activating PR genes responsible of the systemic acquired resistance.

Activating systemic resistance was also shown by Bubici *et al.* (2006). These others demonstrated that treating tomato plants with l'Acibenzolar-S-methyl has significantly controlled Corky root caused by *Pyrenochaeta lycopersici* and Verticillium wilt of eggplant caused by *Verticillium dahliae*.

CONCLUSION

Various chemical fungicides were tested for control of FCRR. Results obtained demonstrated that Hymexazol, Hydroxyquinolin, Sodium Tetraborohydrate Decahydrate, Oxyquinoléine, Flutriafol+Thiabendazole and benomyl have inhibited mycelial growth of FORL. Addition of these fungicides to the culture media has inhibited mycelial growth by more than 75%.

With the exception of benomyl, *in vivo* application of these fungicides, has limited FCRR development. The disease incidence reduction was greater whit Hymexazol; indeed, by treating plants with this fungicide, disease incidence did not exceed 24% compared to untreated plants for which the mean disease incidence was about 80% and in some cases, it exceeded 96%.

More promising results were obtained under greenhouse conditions by using Hymexazol to control FCRR. By treating tomato plants once in the seed bed before transplanting and once a month during the crop season, percentage of completely wilted plants was about 6.4%.

This study illustrates well the efficacy of some chemical fungicides, in particular Hymexazol against FCRR grown under growth chamber and greenhouse conditions. To be more affirmative, the other fungicides (Hydroxyquinolin, Sodium Tetraborohydrate Decahydrate, Oxyquinolin and Flutriafol+Thiabendazole) must be tested under greenhouse and field conditions to be used as alternatives to control FCRR and other soil-borne pathogens.

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