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In vitro effect of two fungicides on pathogenic fungi causing root rot on tomato in Algeria

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Tomato (*Lycopersicon esculentum*) is one of the most widely grown vegetables in the world. There is a growing concern in recent years, both in developed and developing countries, about the use of hazardous fungicides for controlling plant diseases. The *in vitro* effect of two fungicides commonly used in Algeria namely Trifidan and Antracol (propineb) on the growth of three pathogenic fungi of tomato, *Fusarium oxysporum* f.sp. *radicis-lycopersici*, *Fusarium commune* and *Fusarium redolens* was investigated to determine the effectiveness of the fungicides in reducing fungi growth. Each fungicide was assayed at 0, 100, 200, 400 and 500 mg L^{-1} rate in Potato Dextrose Broth (PDB) and incubated at 28°C for seven days. Data were collected on the mycelia weights of the fungi under each treatment. Mycelia weights of the three fungi were significantly reduced at 100 mg L^{-1} by the two fungicides under antracol treatment *F. redolens* and *F. commune* and a significant reduction was observed at 400 and 500 mgL^{-1}. Application of Trifidan, significantly decrease in mycelia weight of *F. oxysporum* f. sp. *radicis-lycopersici* and *F. commune* irrespective of the rate applied. *F. redolens* was completely inhibited by Trifidan, and its inhibition started at 100 mg L^{-1}.

Key words: *Fusarium* species, mycelium growth, inhibition, trifidan, antracol, tomato diseases, control, fungicides.

INTRODUCTION

Fusarium oxysporum f. sp. *radicis-lycopersici* (Forl) leading to Fusarium crown and root rot is one of the most destructive soil borne diseases of tomatoes occurring in greenhouse and field crop (Szczechura et al., 2013) *F. oxysporum* f.sp. *radicis-lycopersici* has a greater host range than *F. oxysporum* f. sp. *lycopersici.*

Trifidan is a Triazole groups, its active ingredient is Triadimenol at 25%. The chemical name is [beta-(4-chlorophenoxy)- alpha- (1.1-dimethylethyl)-1H-1.2.4 triazole-1-ethanol]. Trifidan is used for seed treatment to control loose, smut and smut covered, bunt of wheat, typhula blight of powdery mildew, barley, leaf spot, rust and take-all and common root and foot rot of wheat and barley. While the Antracol belongs to dithiocarbamates groups; its active ingredient is probineb at 70%. It is aprotectant foliar fungicide with long residual activity and it is used across the world as a protective treatment on several crops for the control of various fungi, especially

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Oomycetes, Ascomycetes, Basidiomycetes and imperfect Fungi for the control of blue mould of tobacco, target spot (early blight) and late blight (Irish blight) of potatoes and tomatoes, grey leaf spot of tomatoes, downy mildew of cucurbits and onions, black spot of citrus and certain other diseases (Anonymous, 2013).

F. commune and *F. redolens*is closely related, but phylogenetically independent of *F. oxysporum. F.commune* and *F. redolens* are species newly described in Algeria on tomato *F. commune* was described as a distinct species in 2003 based on DNA sequences (Skovgaard et al., 2003; Stewart et al., 2006), and it has been described as an pathogen on different crops caused internal root discoloration and root rot on tomato (Hamini-Kadar et al., 2010), cause damping-off and root rot of conifer seedlings in forest nurseries (Mee-Sook et al., 2012; Stewart et al., 2012; Dumroese et al., 2012) and horse radish (Yu and Babadoost, 2013*). F. redolens* was also referred as an important pathogen on different crops including asparagus (Wong and Jeffries, 2006), soybean (Bienapfl et al., 2010), lentil (Riccioni et al., 2008), chickpea (Jiménez-Fernàdez et al., 2010), pea (Taheri et al., 2011) and recently as a causal agent of allepo pine damping-off in Algeria (Lazreg et al., 2013).

Methods for complete control of diseases tomato are yet to be developed. Management strategies for these diseases include use of presumed disease free seeds, resistant cultivars and fungicides treatments. A considerable work has been done in controlling diseases of many crops caused by *F. oxysporum* both *in vitro* and pot culture experiments by using fungicides (Jahanshir et al., 2010; Taskeen et al., 2011; Muhammad et al., 2011; Ndikumana, 2013). Several studies on this disease were performed, among which investigations on fungicide treatments represent an important research area. Although this is the first time such a study is been conducted on *F. redolens* and *F. commune* in Algeria. The aim of this study was to investigate the antifungal activity of some fungicides (Trifidan and Antracol) against mycelial growth of *Forl, F. commune* and *F. redolens.*

MATERIALS AND METHODS

Fungi and cultures

The three fungi investigated were *F. oxysporum* f.sp *radicis lycopersici*, *F. commune* and *F. redolens* were obtained from the fungal collection of the Laboratory of Applied Microbiology, Faculty of Nature and Life Science, Oran University, Algeria. All strains were selected for their aggressiveness among tomatoes. The three pathogens were molecularly identified by their EF-1α sequences. All pathogenic fungus were cultured and purified on potato dextrose agar medium (PDA): extract of boiled potatoes, 200 ml; dextrose, 20 g; agar, 20 g and distilled water, 800 ml) at 28°C for 7 days.

Fungi proliferation

The method used is that described by Olajire and Oluyemisi (2009). Potato dextrose broth (PDB) was the media used for the growth experiment. The medium was prepared by mixing infusion of 200 g peeled potato; 20 g dextrose in 1000 ml distilled water. After cooking the medium was poured into 125 ml conical flasks at the rate of 50 ml per flask and were sterilized by autoclaving at 121°C, for 20 min. When the media was cool down 1 ml of chloramphenicol (1%) was aseptically added. Trifidan and Antracol were introduced separately into the flasks containing PDB at concentrations of 100, 200, 400 and 500 mg L^{-1}. Flasks without fungicides used as control. The flasks were inoculated with mycelial discs of 5 days old and incubated at 28°C for seven days. Three replicated flasks were used for each concentration of the two fungicides. At the end of incubation, the cultures in all flasks were filtered separately through pre-weighted filter paper. Dry weight of mycelium was obtained by subtracting weight of filter paper from weight of filter paper and mycelium. Inhibition of mycelia dry weight was determined by comparing the growth in control flasks following the formula given by Nikam et al. (2007).

$$\text{Percent inhibition } I\% = \frac{C-T}{C} \times 100$$

Where, C represents the weight of fungi growth on untreated PDB and T represents the weight of fungi on treated PDB.

Statistical analysis

Data collected were subjected to statistical analysis using simple ANOVA for least significant ($P<0.05$) by the Microsoft Excel software.

RESULTS AND DISCUSSION

The effect of Trifidan and Antracol at 100, 200, 400 and 500 ppm were tested *in vitro*. The later fungicides tested gave appreciable inhibition in mycelial growth. Differences in sensitivity to the fungicides *in vitro* were observed among the isolates. All the isolates were sensitive to the tested fungicides, but the sensitivity varied between the isolates and species. Results obtained with Trifidan were presented in (Table 1). In general adding Trifidan to PDB media has inhibited mycelium growth for the three fungi tested: *F. redolens*, *F. oxysporum* f.sp *radicis lycopersici* and *F. commune* compared to the control treatment without Trifidan.

There is a significant decrease in mycelium weight of the fungus with an increased in fungicidal concentration ($P<0.05$). But statistically showed no significant differences between the different concentrations used. The higher inhibition of growth was observed in *F. redolens* (96.89%) at 400 $mgL^{-1}$and this inhibition was the same at 500 mgL^{-1}.The high sensitivity of the *F. redolens*to Trifidan (95.14% at 100 mgL^{-1}) was an indication that lowerrates of Trifidan application in our study effectively inhibit mycelia growth. Trifidan is a triazole fungicide that inhibits ergosterol biosynthesis. Triazoles are sterol inhibiting fungicides. These fungicides have no immediate effect on the respiratory mechanism; therefore, they do not inhibit spore germination (Siegel, 1981) but are effective at preventing mycelial growth (Burlakoti et al., 2010). It provide the

Table 1. Effect of Trifidan® on the mycelium growth of *F. redolens*, *Forl*, *F.commune.*

Rate mg L^{-1}	Mycelium weight (g)					
	Fusarium redolens*	**I%**	***Forl****	**I%**	***Fusarium commune****	**I%**
0	0.515[d]		1.182[e]		0.356 [c]	
0100	0.025[a]	95.14	0.378[c]	68.02	0.202[b]	43.25
200	0.022[a]	95.72	0.367[c]	68.95	0.190[b]	46.62
400	0.016[a]	96.89	0.366[c]	69.03	0.189[b]	46.91
500	0.016[a]	96.89	0.331[c]	71.99	0.181[b]	49.15

* = Average of three replications, Mean values in columns differ significantly (P<0.05), if they are not marked with the same letter.

Table 2. Effect of Antracol® on the mycelium growth of *F. redolens*, *Forl*, *F. commune.*

Rate mg L^{-1}	Mycelium weight (g)					
	Fusarium redolens*	***I%***	***Forl ** **	***I%***	***Fusarium commune****	***I%***
0	0.520[a]		1.155[c]		0.339[b]	
100	0.514[a]	1.15	0.874[d]	24.32	0.150[f]	55.75
200	0.479[a]	7.88	0.781[e]	32.38	0.114[f]	66.37
400	0.449[a]	13.65	0.759[e]	34.28	0.063[g]	81.41
500	0.365[b]	29.80	0.666[e]	42.33	0.039[g]	88.49

* = Average of three replications. Mean values in columns differ significantly (P<0.05), if they are not marked with the same letter.

Table 3. Mean mycelium growth of tested fungi under effect of all fungicides.

Species	Mean mycelium growth
Fusarium redolens	0.341[a]
Fusarium oxysporum	0.466[a]
Fusarium commune	0.846[b]

Mean values in columns differ significantly (P<0.05), if they are not marked with the same letter.

Table 4. Mean concentrations of tested fungicides at all concentrations.

Concentration	Mean of inhibition
[C] 0 (Témoin)	1.01[b]
[C] 100	0.513[a]
[C] 200	0.457[a]
[C] 400	0.423[a]
[C] 500	0.356[a]

Mean values in columns differ significantly (P<0.05), if they are not marked with the same letter.

most effective control of *Fusarium* species which has been reported by other studies (Gareis and Ceynowa, 1994; Mesterhazy et al., 2003; Burlakoti et al., 2010; Ruan et al., 2011). Active ingredients of Trifidan is triadimenol, (Hall, 1987) showed that triadimenol reduced neither the incidence of infection of crowns by *Fusarium* nor the incidence of crown rot and Frank and Ayers (1986) and also Everts and Leath (1993) showed that triadimenol provides control of a foliar pathogen which may cause severe yield loss.

Antracol reduced the growth of *F. oxysporum f.s pradicis lycopersici* and *F. commune.* There is no significant difference in the mycelia growth of *F. oxysporum* and *F. redolens* between 100 and 500 mg L^{-1}. The antracol has an inhibitory effect on the growth of *F. commune* with a concentration between 400 and 500 mg L^{-1} (Table 2). The best inhibition rates were observed at 500 mg L^{-1}, 29.80, 42.33 and 88.49% for *F. redolens*, *F. oxysporum* f.sp. *radicis lycopersici* and *F. commune* respectively. In our study the mycelia weigh of the tree *Fusarium* reduced at higher concentration of antracol, the similar results were observed by Pathan et al. (2005). Other works were tested six fungicides against *Botryodiplodia theobromae* but the antracol revealed lowest response. Such observations were also reported by Uzma and Nusrat (2011) and Rajput et al. (2012).

Statistical analysis indicated the significant differences in susceptibility to fungicides between the tested species. It appears that *F. redolens* and *F. oxysporum* were the most sensitive fungus to the application of fungicides following by *F. commune* (Table 3). The two fungicides (Antracol and Trifidan) can be used at the lowest concentration, in order to decrease the residue of fungicides, because there is no significant difference between the four concentrations tested (Table 4).

Conflict of Interest

The authors have not declared any conflict of interest.

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