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Evaluation of Fungicides for Control of *Fusarium* Wilt of Potato

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Abstract: Four fungicides, which active ingredients are hymexazol, azoxystrobin, fludioxonil and quinoline, are tested *in vitro* and *in vivo* against five isolates of *Fusarium oxysporum* f. sp. *tuberosi*, causal agent of potato *Fusarium* wilt. Pathogen radial growth noted on PDA amended with fungicides after incubation for four days at 25°C varied upon pathogen isolates. Mycelial growth of all pathogen isolates was significantly inhibited by fungicide PDA amendment; inhibition obtained by hymexazol treatment reached 77% comparatively to untreated control. All chemical fungicides reduced disease incidence compared to the untreated control. Hymexazol and azoxystrobin are the most active during all the bioassay period. Whereas, fludioxonil and quinoline showed a limited effect in controlling *Fusarium* wilt development.

Key words: *Solanum tuberosum* L., interaction, chemical control, vascular wilt

INTRODUCTION

Fusarium oxysporum (Schlecht.) Snyd. et Hans. is frequent in warm areas. It causes vascular wilt in several plants (Theron, 1991; Meulemans, 1996; Norguès *et al*., 2002). In Tunisia, *Fusarium oxysporum* f. sp. *tuberosi* causes dry rot of tubers and vascular wilts of potatoe plants (Daami-Remadi and El Mahjoub, 2004).

This soilborne fungus infected plants through roots and colonized xylem vessels of stems. Potato plants infected by *Fusarium oxysporum* f. sp. *tuberosi* showed a characteristic discoloration in their vascular tissue due to obstruction of the water and nutrient-conducting tissue of the plant resulting in stunting, chlorosis, unilateral wilt, and eventual plant death (Hwang and Evans, 1985; Kucharek *et al*., 2000). This pathogen caused economic losses estimated of 10 to 53% of potato yield (Thanassoulopoulos and Kitsos, 1985).

As it is a soil-borne pathogen, control of *F. oxysporum* f. sp. *tuberosi* has generally been restricted to the use of long-term rotations (3-5 years) and solarization. However, in Tunisia these techniques are not generalised due to limited surfaces and high cost of solarization (Katan, 1980; Monnet, 2001; Triki *et al*., 2001). While in several other countries, fumigation with methyl bromide, which was effective in reducing soilborne inoculum of numerous *Fusarium*, will be totally removed from the agricultural markets, because of its ozone-depleting effect (Watson *et al*., 1992). As alternatives to these control measures, several reports demonstrated successful use of biological control agents (mostly bacteria and fungi) and fungicides for control of this disease (Larkin *et al*., 1998; Reid *et al*., 2002; Elmer and McGovern, 2004). In fact, some fungicides are reported to be rated with reduced risk toxicology (Errampalli, 2004).

Since *Fusarium* wilt is a serious threat a strategic crop in Tunisia, control measures integrating with cropping practices are searched. The present research was conducted in order to evaluate the effectiveness, *in vitro* and *in vivo*, of four fungicides in controlling *F. oxysporum* f. sp. *tuberosi*.

MATERIALS AND METHODS

Fungal cultures and fungicides: Isolates of *Fusarium oxysporum* f. sp. *tuberosi* used in this study (Fot_1, Fot_2, Fot_3, Fot_4 and Fot_5) were obtained from potato tubers showing dry rot symptoms collected from traditional potato-stores (Hammam Ghezaz, Hawaria and Korba).

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Table 1: Fungicides tested against *F. oxysporum* f. sp. *tuberosi* development

Commercial products	Active ingredient	Formulation	Action
Tachigaren 360	Hymexazol	360 $g.L^{-1}$	Systemic
Ortiva	Azoxystrobin	250 $g.L^{-1}$	Systemic
Beltanol-L	Quinoline	500 $g.hL^{-1}$	Systemic
Scholar	Fludioxonil	50 %	Contact

Table 2: Fungicide relative rates used in *in vitro* experiments against *F. oxysporum* f. sp. *tuberosi* (Fot)

Active ingredient (a.i.)	Rate of a.i.
Hymexazol	0.36 $g.L^{-1}$ H_2O
Azoxystrobin	0.25 $g.L^{-1}$ H_2O
Quinoline	0.5 $g.hL^{-1}$ H_2O
Fludioxonil	1 ppm

They were cultured on Potato Dextrose Agar (PDA) and incubated at 25°C in the dark. Single spore cultures were maintained on glycerol at -80°C for long-term storage.

For plant inoculation, mycelium taken from the edge colony of each isolate was transferred to 150 mL of Potato Dextrose Broth (PDL) and incubated at 25°C for 5 days in a rotary incubator (120 rpm). The liquid culture was filtered and the conidial suspension was adjusted to 10^7 spores.mL^{-1} by means of a Malassez cystometer.

The characteristics of the four fungicides tested were listed in Table 1.

***In vitro* experiments:** Five isolates of *F. oxysporum* f. sp. *tuberosi* was grown on Potato Dextrose Agar (PDA) at 25°C for 7 days. Agar disks (6 mm) bearing the fungus were taken from freshly growing colony and transferred on PDA amended with various concentrations of fungicides (Table 2). The fungal radial growth of colonies was recorded after incubation for four days at 25°C. Eight replicates were used per elementary treatment.

Data are arranged as a complete randomized factorial design where treatments and isolates are both fixed factors. They were analyzed using SPSS and subjected to analysis of variance and Fisher's least significant difference test LSD (at $p<5\%$).

***In vivo* experiments:** An isolate of *F. oxysporum* f. sp. *tuberosi* (Fot_3), being the most aggressive following pathogenicity tests (Ayed *et al*., 2006), was used for plant inoculation. Potato tubers, cv. Spunta, were planted in plastic pots (6.74 l) containing an autoclaved mixture of perlite and peat (1:3) and kept at 8-32°C (minimum and maximum temperatures, respectively). Two weeks after their emergence, plants were inoculated by irrigation with 100 mL^{-1} of conidial suspension (10^7 spores mL^{-1}). Ten non-inoculated control plants were treated similarly with 100 mL^{-1} of sterile distilled water. Ten days after inoculation, fungicides are applied at their relative rates (Table 3) using a drip spray method. A second fungicide treatment was realized 20 days after. Potato plants were irrigated regularly and fertilized with a nutrient solution ($20\,N{:}20\,K_2O{:}20\,P_2O_5$) following Manici and Cerato (1994) method.

Table 3: Fungicide rates used in *in vivo* experiments for suppression of potato *Fusarium* wilt

Active ingredient (a.i.)	Rate of a.i.
Hymexazol	0.27 g/plant
Azoxystrobine	0.25 $g.L^{-1}$ H_2O
Quinoline	0.5 $g.hL^{-1}$ H_2O
Fludioxonil	0.24 $g.L^{-1}$ 1 H_2O

Effect of the tested fungicides on *Fusarium* wilt development was assessed via disease severity index. A scale of 0-4 was used to assess disease severity: 0 = asymptomatic leaf, 1 = leaf wilted, 2 = Leaf with hemiplegic yellowing, 3 = leaf with necrosis, 4 = dead leaf. Incidence of *F. oxysporum* f. sp. *tuberosi* were estimated weekly via an Index of Leaf Damage (ILD) calculated per potato plant following formula (Béye and Lafay, 1985) where:

$$ILD = \sum notes / max$$

ILD : Index of Leaf Damage.
$\sum$ notes : Total notes.
Max : 4 times of developed-leaves number.

Data are arranged by completely randomized design where treatments (plants treated by each of five fungicides, inoculated and non-inoculated) are the only fixed factor. Ten plants per treatment were assessed. Mean comparisons were done following the LSD method ($p<5\%$).

RESULTS

***In vitro* experiments:** Colony diameters of *F. oxysporum* f. sp. *tuberosi* on PDA, amended with relative fungicide concentrations, noted after four days of incubation at 25°C, varied among fungicides and pathogen isolates. An interaction was observed between both fixed factors (Table 4). All fungicides reduced significantly mycelial growth of *F. oxysporum* f. sp. *tuberosi* isolates (Fig. 1 and Table 4).

Hymexazol showed the highest efficiency in reducing mycelial growth for more than 77%, compared to pathogen growth on unamended PDA. At 2 ppm, fludioxonil significantly limited mycelial development by 73% for Fot_3. Azoxystrobin also showed fungicidal activity against *F. oxysporum* f. sp. *tuberosi* isolates. Quinoline showed lesser efficiency; mycelial growth was reduced by 30 to 43% for all tested isolates.

Table 4: Mean diameters of *F. oxysporum* f. sp. *tuberosi* colonies noted in presence of several fungicide treatments comparatively with the untreated control (PDA, after six days of incubation at 25°C)

	Mean colony diameter of *F. oxysporum* f. sp. *tuberosi* colonies (cm)				
Isolates	Untreated control	Hymexazol	Fludioxonil	Azoxystrobin	Quinoline
Fot_1	5.9	1.387	1.612	2.612	4.069
Fot_2	5.9	1.394	3.194	3.087	3.637
Fot_3	6	1.269	1.575	2.55	3.931
Fot_4	6.5	3.025	3.275	2.781	3.725
Fot_5	6	1.487	2.787	2.344	3.862

Fot_1, Fot_2, Fot_3, Fot_4 and Fot_5: Isolates of *F. oxysporum* f. sp. *tuberosi* LSD at p<5% (Treatments × Isolates) = 0.273 cm

Table 5: Weekly evolution of mean Index of Leaf Damage (ILD) of potato plants, cv. Spunta, inoculated by *F. oxysporum* f. sp. *tuberosi* and treated by four fungicides comparatively with healthy (NI) and untreated-inoculated (I) plants

	Treatments						
Days after planting	NI	I	Hymexazol	Azoxystrobine	Fludioxonil	Quinoline	LSD at 5%
45	0	0.14	0	0	0	0	0.0636
52	0	0.2169	0.0743	0.1016	0.1052	0.1237	0.0609
59	0.0275	0.3279	0.1385	0.2237	0.28	0.2079	0.0864
66	0.0562	0.3974	0.2625	0.3127	0.3534	0.3701	0.0536
73	0.1016	2.362	1.28265	1.4575	1.7816	2.1646	0.358

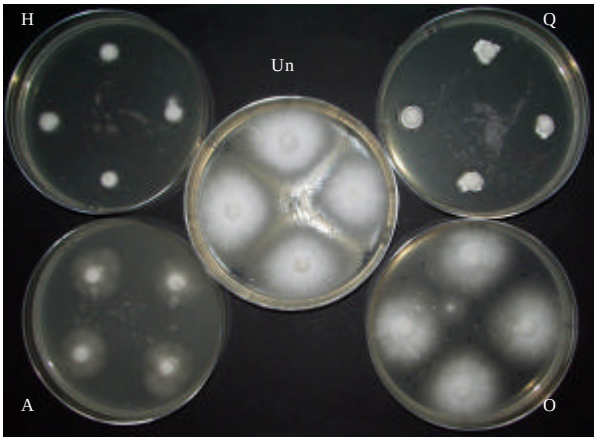

Fig. 1: Mycelial growth of a *F. oxysporum* f. sp. *tuberosi* isolate (Fot_3) observed in presence of several treatments (PDA, after 4 days of incubation at 25°C). (Un: Untreated; H: Hymexazol; A: Azoxystrobin; F: Fludioxonil; Q: Quinoline)

Fig. 2: Comparison between Non-inoculated (NI) and inoculated (I+U) potato plants and inoculated plants treated 10 and 30 days after inoculation by four fungicides (H: Hymexazol; A: Azoxystrobin; F: Fludioxonil; Q: Quinoline). (cv. Spunta, 73 days after inoculation)

Fot_4 had the least important inhibition, whereas Fot_3 was the most sensitive isolate to most tested fungicides.

***In vivo* experiments:** Weekly evolution of Leaf Damage Index (ILD) of potato plants, cv. Spunta, inoculated by a virulent *F. oxysporum* f. sp. *tuberosi* isolate and treated by several fungicides was assessed as soon as the first typical *Fusarium* wilt symptom appeared (30 days after inoculation).

All tested fungicides tested significantly reduced the *Fusarium* wilt incidence compared to untreated-inoculated plants during all this bioassay (Table 5 and Fig. 2).

The ILD of potato plants treated with hymexazol didn't exceed 1.28 at 73 days after planting in comparison to 2.36 for untreated-inoculated plants. Moreover, Azoxystrobin showed a remarkable efficiency in reducing severity of this disease. Fludioxonil reduced significantly *Fusarium* wilt but its efficiency was lesser than both previous fungicides. Quinoline was effective during the first three weeks after first symptom observation where the ILD increased from 0.37 to 2.16 at the end of the bioassay (Table 5). Furthermore, disease incidence increased suddenly in the last week of the bioassay especially for treated and untreated-inoculated plants.

DISCUSSION

Vascular infections by *F. oxysporum* f. sp. *tuberosi* and pathogen soilborne origin make disease control difficult. Chemical compounds tested in the present study (hymexazol, azoxystrobin, fludioxonil and quinoline) showed *in vitro* fungicidal effects on PDA of five isolates of *F. oxysporum* f. sp. *tuberosi*. Moreover, the *in vivo* experiment revealed their efficacy in controlling *Fusarium* wilt development. Hymexazol showed, *in vitro* as *in vivo*, the highest activity against this pathogen. Similar results were obtained with the same dosage and application procedures in reducing *Fusarium* wilt of tomato caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Hibar, 2002). Daami-Remadi (2001) signalled its effectiveness in inhibiting by 43 to 50% potato leak syndrome caused by *Pythium aphanidermatum*. Moreover, azoxystrobin provided efficacy in the control of potato *Fusarium* wilt, as reported in carnation-*F. oxysporum* f. sp. *dianthi* pathosystem (Gullino *et al*., 2000). However, Elmer and McGoven (2004) reported that this strobilurin has poor curative properties in reducing *Fusarium* wilt of Cyclamen caused by *F. oxysporum* f. sp. *cyclaminis* and suggested their preventive application for this disease suppression. Bertelsen *et al*. (2001) explained fungicidal action of azoxystrobin by blocking electron transport in the fungal mitochondrial respiratory chain. Fludioxonil and quinoleine showed limited efficiency against *F. oxysporum* f. sp. *tuberosi* in *in vivo* experiments, in spite of their good activity against *Botrytis cinerea*, *Monilinia* spp. and *Sclerotinia* spp. reported by Gullino *et al*. (2000). In the same way, Reid *et al*. (2002) found that fludioxonil limited plant death caused by *F. oxysporum* f. sp. *asparagi* and *F. proliferatum* at high inoculum level. However, Elmer and McGovern (2004) confirmed that fludioxonil has poor curative properties against *Fusarium* wilt of Cyclamen. Therefore, an increase in dose and/or soil incorporation should be tested especially where Errampalli (2004) signalled that any chemicals that significantly inhibit germination and mycelial growth should reduce the ability of pathogen to cause disease. Furthermore, Bains *et al*. (2001) found that mixture of fludioxonil with other compounds such as mancozeb or difenoconazole provided tuber protection from seed piece decay caused by *F. sambucinum*. The number, the frequency and timing of applications also play an important role in efficacy of fungicides in different pathosystems (Bubici *et al*., 2005).

Suddenly increase of disease incidence at the end of the bioassay can be caused by obstruction of the water and nutrient-conducting tissue of inoculated plants as a result of inoculum level increase as by loss of activity of fungicides (Kucharek *et al*., 2000).

The present study showed that all fungicides limited *F. oxysporum* f. sp. *tuberosi in vitro*, but hymexazol and azoxystrobin are the most efficient in the *in vivo* experiment probably due to rates used in plant treatments. Integrating chemicals with biological agents proven effective and consisted alternative in several pathosystems and it has not been completely explored for potato *Fusarium* wilt.

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