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In vitro and *in vivo* Interaction of Four Fungicides with the *Fusarium* Species Complex Causing Tuber Dry Rot in Tunisia

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Abstract: Several fungicides were tested against some isolates belonging to four *Fusarium* species causing potato tuber dry rot in Tunisia. Incorporated into the culture media PDA, the tested fungicides significantly inhibited the mycelial growth, observed after incubation at 25°C for 4 days, of all *Fusarium* isolates including those of *F. sambucinum* resistant to benzimidazoles. A significant interaction ($p \leq 0.05$) was observed between both fixed factors where inhibition percentage varied depending on tested pathogens and fungicides. Applied on potato tubers (tuber immersion for 10 min) prior inoculation, certain tested fungicides such as azoxystrobin and fludioxonil significantly reduced by more than 50%, comparatively to the untreated controls, the development of dry rot occasioned by *F. graminearum* and *F. sambucinum* observed after 21 days of incubation at 25-27°C. A significant interaction ($p \leq 0.05$) was noted between the treatments and the *Fusarium* species traduced by a variable inhibition percentage depending on tested pathogen and fungicides.

Key words: Benzimidazole-resistant isolates, mycelial growth, inoculation, chemical control

INTRODUCTION

In Tunisia, a complex of *Fusarium* species is responsible of potato tuber dry rot. *F. solani*, *F. oxysporum* f.sp. *tuberosi* and at a lesser frequency *F. sambucinum* and *F. graminearum* are present as mixed infections on tubers showing dry rot symptoms (Daami-Remadi and El Mahjoub, 1996, 2004; 2006; Priou and El Mahjoub, 1999; Chérif *et al*., 2001; Daami-Remadi *et al*., 2006a).

Benzimidazoles and conazoles fungicides were used since 1970 for the control of dry rot and other potato diseases (Leach, 1971; Murdock and Wood, 1972; Tisdale and Lord, 1973; Leach and Nielsen, 1975; Tivoli *et al*., 1986; Carnegie *et al*., 1990; Bang, 1992; Kawchuk *et al*., 1994; Mérida and Loria, 1994; Carnegie *et al*., 1998; Errampalli and Johnston, 2001). Thiophanate-methyl, carbendazim, iprodione, metalaxyl, procymidone and prochloraz inhibited dry rot development on tubers inoculated by *F. sambucinum* and other *Fusarium* species (Choiseul, 1996; Triki *et al*., 1996; Daami-Remadi and El Mahjoub,1997; Chérif *et al*., 2001). However, a recent *in vitro* screening of some tunisian *Fusarium* spp. isolates for their resistance to some benzimidazoles showed that *F. solani, F. oxysoprum* f.sp. *tuberosi* and *F. graminearum* isolates are susceptible to these fungicides whereas *F. sambucinum* isolates are resistant (Daami-Remadi and El Mahjoub, 2006). These chemicals having a single-site mode of action are more likely to lead to development of resistance (Kawchuck *et al*., 2002). It is to note that since emergence of thiabendazole resistance, chemical combinations of 8-hydroxyquinolin and thiabendazole increased and they are largely used in France as an anti-thiabendazole resistance strategy (Tivoli *et al*., 1986). Recently, we have shown that *in vitro* and *in vivo* inhibition of benzimidazole resistant isolates was reached by several mixtures of fungicides tested individually or in dual combination (Daami-Remadi *et al*., 2006b). Furthermore, Beresford (1994) reported that fungicides with mode of action different to that of benzimidazoles could optimise dry rot control and minimize the incidence of the mentioned fungicide resistance. Leadbeater and Kirk (1992) found that fenpiclonil, a phenylpyrrole fungicide is more efficient as a pre-planting treatment than thiabendaole or imazalil. Furthermore, fludioxonil, added of mancozeb or difenoconazol, applied at pre-plantation of potato seeds inoculated by *F. sambucinum,* are shown to be efficient in inhibiting pathogen dissemination around progeny tubers (Bains *et al*., 2001).

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Registered fungicides for potato dry rot control are lacking in the tunisian phytosanitary index (Anonymous, 2003). As some fungicides tested in previous studies against some isolates of *F. oxysporum* f.sp. *tuberosi* have shown efficacy in reducing potato vascular wilt (Ayed *et al*., 2006) and are reported to be of reduced risk for the environment (Errampalli, 2004), the present study focused on extend of their *in vitro* and *in vivo* efficacy evaluation against the development of the entire *Fusarium* complex causing potato tuber dry rot in Tunisia.

MATERIALS AND METHODS

Pathogens: *F. solani, F. graminearum, F. sambucinum* and *F. oxysporum* f.sp. *tuberosi* are isolated (on 2001, 2002, 2003 and 2004) from tubers of cv. Spunta showing typical symptoms of dry rot. Isolates of *F. sambucinum* (FRS1, FRS2, F.3/2.02, F.6.02, F.20.02, F.44.03, F.48.02 and F.17.04) implicated in this study are shown to be resistant to benomyl, carbendazim and thiophanate-methyl and all isolates of *F. graminearum* (F.10/2.02, F.21.02 and F. 45.03), *F. oxysporum* f.sp. *tuberosi* (F.33.02) and *F. solani* (F.12.03) are susceptible to these fungicides (Daami-Remadi and El Mahjoub, 2006).

Fusarium spp. are grown at 25°C on PDA for one week. They are stored at -20°C in 20% glycerol solution for long term preservation.

Potato cultivars: Tubers cv. Spunta, the most cultivated in Tunisia, are used in this current study. They are obtained, on 2004, from the Technical Centre of Potato of Tunisia. For laboratory experiments, tubers are stored in the darkness at 6°C and brought to room temperature three hours before use.

Fungicides: Active ingredient components of tested fungicides are shown to be efficient against several isolates of *F. oxysporum* f.sp. *tuberosi* causing potato vascular wilt in previous studies (Ayed *et al*., 2006). Main characteristics of the tested fungicides are presented in Table 1.

***In vitro* activity of tested fungicides against *Fusarium* spp:** Fungicides are dissolved in sterile distilled water before their incorporation (1%v/v), following chosen doses (Table 1), in PDA in surfusion. A culture media added with a same quantity of sterile distilled water serves as untreated control. After solidification, agar discs (of 6 mm in diameter) colonized by the tested pathogen are placed at four equidistant emplacements in the centre of the petri dish (four agar discs per petri dish).

Table 1: Characteristics and applied doses of fungicides tested against the four *Fusarium* species causing potato tuber dry rot

Active ingredients (a.i)	Trade names (tn)	Concentrations of a.i	Tested doses (tn)
Chlorothalonil	Daconil	75%	5 g L^{-1}
Azoxystrobin	Ortiva SC	250 g L^{-1}	2 mL L^{-1}
Hydroxyquinolin-sulfate	Beltanol	500 g.hL^{-1}	2 mL hL^{-1}
Fludioxonil	Scholar	50%	2 mg L^{-1}

Inhibitory activity of fungicides is evaluated on mycelial growth of tested *Fusarium* spp. estimated via mean colony diameter formed after 4 days of incubation at 25°C.

Statistical analyses (ANOVA) are performed following a completely randomised factorial design where treatments (fungicides and untreated control) and *Fusarium* isolates are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

***In vivo* activity of tested fungicides against *Fusarium* spp:** Efficacy of fungicides previously tested *in vitro* was estimated via development of dry rot on inoculated and treated tubers. Tubers (cv. Spunta) are superficially disinfected with a solution of 10% sodium hypochlorite, for 5 min and then rinsed abundantly with sterile distilled water. Container and alveolus plaques used for inoculated tubers incubation, are washed before use, dipped for 24 h in sodium hypochlorite solution then rinsed with sterile distilled water.

Fungicides are suspended in water according to tested doses and tuber treatment was realized by dipping tubers, during 10 min, in a fungicidal suspension prior inoculation. Inoculation technique consists of deposing an agar disc (6 mm diameter) colonized by pathogen at occasioned wounds (6 mm diameter and depth). Tuber incubation is realized at 25-27°C for 21 days at high relative humidity. Every elementary treatment is repeated twenty times (ten tubers x two wounds). After incubation period, tubers were cut longitudinally via sites of inoculation. Parameters of dry rot induced (maximal width (w) and depth (d)) are noted. The pathogen penetration within tubers is calculated following formula proposed by Lapwood *et al*. (1984) where:

$$\text{Penetration (mm)} = (w/2 + (d - 6))/2$$

Statistical analyses (ANOVA) are performed following a completely randomised factorial design where treatments (fungicides and untreated control) and *Fusarium* isolates are both fixed factors. Means are separated using Fisher's protected LSD test ($p \leq 0.05$).

RESULTS

Effects of fungicides on mycelial growth of *Fusarium* spp: The effect of some fungicides, incorporated in the

Table 2: Effect of some fungicides incorporated into culture media PDA, on mycelial growth of several isolates, belonging to four *Fusarium* species, as measured by the mean colony diameter (cm) noted after 4 days of incubation at 25°C

Treatments	Control	Chlorothalonil	Azoxystrobin	Hydroxyquinolin-sulfate	Fludioxonil
F.10/2.02	4.2	1.9	2.3	0.9	0
F.21.02	4.3	2.4	2.8	1.5	0
F.45.03	4.4	2.7	2.4	1.5	0
FRS1	3.6	0.8	0.7	0	0
FRS2	3.4	0.9	0.7	0.9	0
F.3/2.02	3.5	0.8	0.7	0	0
F.6.02	3.9	0.6	0.7	0	0
F.20.02	3.1	1.4	1.5	0.5	0
F.44.03	2.5	0.8	0.5	0.4	0
F.48.03	3.2	0.8	0	0	0
F.17.04	3.2	0.7	0.8	0	0
F.33.03	3.2	0.9	1.4	1.3	0
F.12.03	1.5	0.9	1.4	1.5	0

LSD (Treatments x Isolates of *Fusarium* sp.) = 0.26 cm ($p\leq0.05$). F.10/2.02, F.21.02 and F.45.03: isolates of *F. graminearum*, FRS1, FRS2, F.3/2.02, F.6.02, F.20.02, F.44.03, F.48.03 and F.17.04: isolates of *F. sambucinum*, F.33.03: *F. oxysporum* f.sp. *tuberosi* and F.12.03: *F. solani*

Table 3: Effect of some fungicides on dry rot development occasioned by *Fusarium* species as measured by the mean pathogen penetration (mm) into inoculated tubers noted after 21 days of incubation at 25-27°C

Treatments	Control	Chlorothalonil	Azoxystrobin.	Hydroxyquinolin sulfate	Fludioxonil
F.45.03	24.2	22.8	8.2	7.8	11.9
F.17.04	14.5	8.8	5.7	9.9	8.6
F.33.03	14.1	7.1	5.7	13.2	13.5
F.12.03	12.6	4.8	12.4	15.8	9.9

LSD (Treatments x *Fusarium* sp.) = 3.4 mm ($p\leq0.05$), F.45.03: *F. graminearum*, F.17.04: *F. sambucinum*, F.33.03: *F. oxysporum* f.sp. *tuberosi* and F.12.03: *F. solani*

Control +Azoxystrobin Control + Azoxystrobin

Fig. 1: Effect of azoxystrobin on incidence of dry rot occasioned by *F. graminearum* (left) and *F. solani* (right), in comparison to inoculated untreated controls, on potato tubers cv. Spunta noted after 21 days of incubation at 25-27°C

culture media, are tested against *in vitro* development of *Fusarium* spp. Table 2 showed that mean colony diameter, formed after 4 days of incubation at 25°C, varied upon tested *Fusarium* isolates and treatments revealing existence of a significant interaction (at $p\leq0.05$) between both fixed factors.

All tested *Fusarium* isolates (three of *F. graminearum*, eight of *F. sambucinum*, one of *F. oxysporum* f.sp. *tuberosi* and one of *F. solani*) showed differential susceptibility to fungicides which significantly reduced pathogen mycelial growth comparatively to the untreated controls.

Fludioxonil showed higher efficacy and totally inhibited the mycelial growth of all tested *Fusarium* isolates, even those of *F. sambucinum* which are shown to be resistant to benzimidazoles in previous studies (Daami-Remadi and El Mahjoub, 2006). Table 2 revealed that chlorothalonil, azoxystrobin and hydroxyquinolin-sulfate have inhibited, by more than 70%, the mycelial growth of the majority of tested *F. sambucinum* isolates comparatively to untreated controls. Their interaction with *F. solani*, *F. graminearum* and *F. oxysporum* f.sp. *tuberosi* was different; noted inhibition varied from 0 to 79% depending on *Fusarium* species and different isolates within the same specie. In fact, *F. graminearum* inhibition varied from 37% to 55% among tested isolates in the case of chlorothalonil. However, lesser growth reduction was noted in *F. solani* (F.12.03) where the maximum reached inhibition was of about 33%.

Effects of fungicides on *Fusarium* sp. agressivity on potato tubers: Table 3 showed that mean pathogen penetration noted after 21 days of incubation at 25-27°C varied upon *Fusarium* sp. used for tuber inoculation and

Control + Fludioxonil Control + Fludioxonil

Fig. 2: Effect of fludioxonil on incidence of dry rot induced by *F. graminearum* (left) and *F. sambucinum* (right), in comparison to inoculated untreated controls, on potato tubers cv. Spunta observed after 21 days of incubation at 25-27°C

Control + Hydroxyquinolin-sulfate

Fig. 3: Effect of hydroxyquinolin-sulfate on incidence of dry rot induced by *F. graminearum,* in comparison to the inoculated untreated control, on potato tubers cv. Spunta, observed after 21 days of incubation at 25-27°C

different tested treatments; a significant interaction was noted between both fixed factors ($p \leq 0.05$). *In vivo* development of *F. graminearum* (F.45.03), the most aggressive *Fusarium* species at these incubation conditions, was inhibited by more than 50% by azoxystrobin (Fig. 1), hydroxyquinolin-sulfate (Fig. 3) and fludioxonil (Fig. 2). However, the interaction of the other *Fusarium* species with these fungicides was slightly different (Fig. 1 and 2); the development of *F. sambucinum* (F.17.04) and *F. oxysporum* f.sp. *tuberosi* (F.33.03) regressed by 40 to 60% by an azoxystrobin treatment.

DISCUSSION

Potato tuber dry rot is a post harvest disease with an increasingly importance in Tunisia. The complex of *Fusarium* species involved in disease development, its agressivity on tubers and potato plants also as wilting agents (Daami-Remadi and El Mahjoub, 2004) and its survival in fields justified necessity of a tuber treatment in addition to respect of prophylactic methods. The appearance of benzimidazole resistant isolates of *F. sambucinum* incited us to search for other alternatives.

The present study revealed efficacy of fludioxonil, azoxystrobin and hydroxyquinolin-sulfate against major tested *Fusarium* isolates. These fungicides have never been tested for *F. graminearum*, *F. sambucinum*, *F. solani* control in Tunisia; they are previously tested only on *F. oxysporum* f.sp. *tuberosi* isolates and have shown to be efficient in reducing the *Fusarium* wilt incidence on inoculated treated plants. Our finding concerning efficacy of fludioxonil joins other studies showing induced mycelial growth inhibition of certain Deuteromycetes fungi achieved by this fungicide (Rosslenbroich and Stuebler, 2000). Fludioxonil, as fenpiclonil, belongs to the phenylpyrrole group and was also shown to have an inhibitory activity against *Botrytis cinerea*, *Monilia* sp. and *Sclerotinia* sp. (Gullino *et al*., 2000). Leadbeater and Kirk (1992) found that fenpiclonil, is more efficient as a pre-planting treatment than thiabendaole or imazalil. Furthermore, strains showing reduced sensitivity to carbendazim, diethofencarb or vinchlozolin did not show cross resistance with fludioxonil (Forster and Staub, 1996) and resistance to phenylpyrroles has never been observed in the field (Baroffio *et al*., 2003). In the current study, fludioxonil showed higher efficacy by totally inhibiting the mycelial growth of all tested *Fusarium* spp. isolates, including those of *F. sambucinum* resistant to benzimidazoles. When applied at 2 ppm, fludioxonil has completely inhibited the *in vitro* development of *F. sambucinum* isolates resistant to thiabendazole.

Similar results were obtained by Bains *et al.* (2001) when tubers are treated by 50 ppm of imazalil. These authors also found that fludioxonil, additionned by mancozeb or difenoconazol, applied as pre-plantation treatment of tuber seeds inoculated by *F. sambucinum,* was efficient. In the same way, fludioxonil, tested against *Penicillium expansum,* a post-harvest apple pathogen, has inhibited the mycelial growth of isolates sensitive and thiabendzole resistant (Errampalli and Crnko, 2004; Errampalli *et al*., 2004).

Chlorothalonil, azoxystrobin and hydroxyquinolin-sulfate have inhibited by more than 70% the mycelial growth of the majority of *F. sambucinum* isolates comparatively to the untreated control. Their interaction with the isolates of *F. solani*, *F. graminearum* and *F. oxysporum* f.sp. *tuberosi* was different; noted inhibition varied from 0 to about 80% depending of *Fusarium* species and different isolates within same specie. This result joins in part that of Gullino *et al.* (2000) and D'Mello *et al.* (2001) who reported that azoxystrobin showed higher efficacy and selectivity for the control of the *Fusarium* wilt of several plants. Activity of azoxystrobin against benzimidazole resistant isolates of *F. sambucinum* joins, in part, findings of Schutte *et al.* (2003) concerning the application of this fungicide for the control of benomyl-resistant *Guignardia citricarpa*.

The development of *F. graminearum*, the most aggressive pathogen at inoculation and incubation conditions, and at a lesser degree the other *Fusarium* species was inhibited *in vivo* by more than 50% by azoxystrobin, hydroxyquinolin-sulfate and fludioxonil treatments. This is the first report in Tunisia of reduced dry rot development on inoculated potato tubers due to the fludioxonil, azoxystrobin and hydroxyquinolin-sulfate inhibitory activity. Furthermore, as these fungicides are shown to inhibit mycelial growth of benzimidazole resistant isolates of *F. sambucinum*, they may be implicated in an anti-resistance strategy for dry rot control.

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