Evaluation of some fungicides for the control of Fusarium dry rot of potato

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Abstract: Five fungicides, with active ingredients azoxystrobin, imazalil, thiabendazole, azoxystrobin + difenoconazole and fludioxonil + difenoconazole, were tested against two isolates of Fusarium solani and two isolates of F. oxysporum, causing potato dry rot in Mashhad region. PDA media amended with the fungicides significantly inhibited the mycelia growth of all Fusarium isolates incubated at 25 °C for 7 days; however only Imazalil and Thiabendazole completely stopped the mycelia growth of all fungal isolates even at their lower concentration (40 and 5 ppm respectively). The mean penetration of F. solani FPO-67 and F. oxysporum FPO-39, the more virulent of the four isolates, after 21 days of incubation at 25-27 °C indicated that imazalil and thiabendazole at concentrations of 1.5 and 2/1000, completely inhibited the penetration of F. oxysporum FPO-39 into potato tubers, but in the case of F. solani FPO-67 all treatments (1, 1.5 and 2/1000) significantly reduced the development of dry rot compared to untreated control. In natural condition, tuber treatment with Imazalil and Thiabendazole (2/1000), prior storage, reduced F. solani FPO-67 development by 68 and 71.69% respectively. According to the results, these fungicides could play a role in integrated pest management against tuber-borne fungal pathogens.

Keywords: Azoxystrobin, Difenoconazole, Fludioxonil, F. oxysporum, F. solani, Imazalil, Mashhad, Thiabendazole

Introduction

Fusarium dry rot of potato is a devastating post-harvest disease affecting both seed potatoes and potatoes for human consumption. In Iran, potato dry rot is caused by a complex of Fusarium species, the incidence and frequency of which depends on the area of cultivation. F. culmorum, F. equiseti, F. semitectum, F. solani, F. sulphureum, F. oxysporum and F. trichoteciodes are the most frequently isolated species from local tubers showing dry rot symptoms (Eskandari, 1997; Falahati Rastegar et al., 2000; Karimi, 1970; Ghalamfarsa and Banihashemi, 2000; Nasr-Esfahani, 1998; Scharif and Ershad, 1966), however, F. solani has been reported as the most pathogenic species causing potato dry rot (Sharifi et al., 2009).

Benzimidazoles and Conazoles fungicides have been used since 1970 (Leach, 1971). Benzimidazoles such as Benomyl, Carbendazim, Thiophenate-methyl and Thiabendazole are used against Fusarium, Phoma exigua, Helminthosporium solani and Rhizoctonia solani (Errampalli and Johnston, 2001). Tecnazene and Dichloran had little effect on germination of spores or growth of F. solani (Murdoch and
Fungicides for the control of Fusarium dry rot species (Daami-Remadi and El Mahjoub, 2006). The chemicals with a single-site mode of action such as benzimidazoles are more likely to lead to development of resistance (Kawchak et al., 2002). In Tunisia, in vitro and in vivo inhibition of benzimidazoles resistant isolates was achieved by several mixtures of fungicides tested individually or in dual combination (Daami-Remadi et al., 2006b). As some Fusarium species are resistant to Thiabendazole and Funginoxonil, estimating the sensitivity level of different Fusarium species to fungicides, for proper management of dry rot, would be essential. According to Esther et al. (2011) Thiabendazole can still be used to control Fusarium species causing dry rot except for F. sambucinum. The present study was conducted to evaluate the efficacy of some fungicides in suppressing Fusarium dry rot of potato under in vitro, in vivo and in situ conditions.

Materials and Methods

Pathogens

Fungal isolates (two isolates of F. solani and two isolates of F. oxysporum) were obtained from the Plant Protection Research Department, Agricultural and Natural Resources Center of Khorasan-Razavi. Pathogenic fungi were maintained on sterile sand at 4°C to be used in further studies.

Source of potato tubers

The potato cultivar Agria, the most cultivated in Iran, was used in current study. They were obtained from the Plant Protection Research Department, Agricultural and Natural Resources Center of Khorasan-Razavi, stored in a traditional store and brought to room temperature a couple of hours before use.

Fungicides

The characteristics of the five fungicides tested is listed in Table 1.

In vitro activity of tested fungicides against Fusarium spp.

Four isolates of F. solani and F. oxysporum were grown on Potato Dextrose Agar (PDA) at 25°C...
for 7 days. Agar disks (5mm) bearing the fungus were taken from freshly growing colony and transferred on PDA amended with various concentrations of fungicides (Table 1). Culture media plus a same quantity of sterile distilled water served as untreated control. The radial growth of fungal colonies was recorded after incubation for 7 days at 25 °C. Each treatment was repeated four times. The data were collected as colony diameter values in mm per replication, and evaluated by one-way analysis of variance (ANOVA) using SPSS 16.0 for Windows.

**In vivo activity of tested fungicides against Fusarium spp.**

Efficacy of fungicides (Imazalil and Thiabendazole) previously tested in vitro was estimated via development of dry rot on inoculated and treated tubers. The selected potato tubers of Agria were washed in running water, dipped in sodium hypochlorite (3%) for 10 min, rinsed abundantly with sterile distilled water (10 min) and air-dried. An isolate of *F. solani* (FPO-67) and one of *F. oxysporum* (FPO-39), being the most aggressive following pathogenicity tests, were used for tuber inoculation. Fungicides were suspended in water according to tested doses (1/1000, 1.5/1000 and 2/1000) and tuber treatment was realized by dipping tubers, for 10 min, in a fungicidal suspension prior to inoculation. Inoculation technique consisted of depositing an agar disc (5mm) colonized by pathogen in wounds (5 × 5mm). Tubers were incubated in a growth chamber, at 25°C for 21 days and at high relative humidity. All treatments consisted of four replicates with two tubers per replicate, and experiments were repeated two times. After incubation period, tubers were cut longitudinally through sites of inoculation, and after that parameters of dry rot induced (maximal width (w) and depth (d)) were recorded. The pathogen invasion into tubers was calculated using formula of Lapwood et al. (1984) as follows:

\[ \text{Penetration} = \frac{\text{w} + (d - 6)}{2} \]

The invasion was evaluated by one-way analysis of variance (ANOVA) using SPSS 16.0 for Windows.

**Table 1 Characteristics of fungicides tested against Fusarium spp.**

<table>
<thead>
<tr>
<th>Active ingredients (a.i)</th>
<th>Trade names (tn)</th>
<th>Concentration of a.i</th>
<th>In vitro tested doses of a.i (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azoxystrobin</td>
<td>ORTIVA</td>
<td>25%</td>
<td>250 - 500 - 1000</td>
</tr>
<tr>
<td>Imazalil</td>
<td>IMAZAGARD</td>
<td>5%</td>
<td>40 - 45 - 50</td>
</tr>
<tr>
<td>Thiabendazole</td>
<td>TECTO</td>
<td>60%</td>
<td>5 - 7.5 - 10</td>
</tr>
<tr>
<td>Azoxystrobin + Difenoconazole</td>
<td>ORTIVA TOP</td>
<td>200 g.l(^{-1}) + 150 g.l(^{-1})</td>
<td>1000 - 2000</td>
</tr>
<tr>
<td>Fludioxonil + Difenoconazole</td>
<td>CELEST</td>
<td>25%</td>
<td>2 - 10 - 15 - 20</td>
</tr>
</tbody>
</table>

**In situ activity of tested fungicides against *F. solani* FPO-67**

Imazalil and Thiabendazole, were also assessed for their in situ efficacy against dry rot development on treated tubers in comparison to controls. One month after harvest, potato tubers of Agria were disinfected as mentioned in *in vivo* test. Then the disinfected potato tubers were sprayed with prepared suspension (2/1000) of each fungicide. Sterile distilled water was used for control. Tubers were kept in a sterile place for 12 hours; after that, an agar disc colonized by *F. solani* FPO-67 was inoculated into a slight wound (about 2 × 2mm) in potato tubers. Fungal mycelium disc was used in positive controls and PDA disc as negative control. Inoculated tubers were placed in clean plastic crates kept in net bags, and incubated in a traditional storage in Mashhad (during December through March at about 5°C). After 4 months, disease incidence of Fusarium dry rot was assessed as the percentage of infected tubers out of total number of tubers in a replicate; disease severity was assessed as mentioned in *in vivo* test. Treatments were replicated four times, each replicate included 3000g potato. The mean penetration of the fungus was evaluated by one-way analysis of
variance (ANOVA) using SPSS 16.0 for Windows.

Results

Effect of fungicides on mycelia growth of Fusarium spp.
The effect of some fungicides, incorporated in the culture media, were tested against in vitro development of two isolates of *F. solani* and *F. oxysporum*. Table 2 shows that mean colony diameter, formed after 7 days of incubation at 25 °C, varied upon tested *Fusarium* isolates and treatments revealing existence of a significant interaction (p < 0.05) between both fixed factors. All fungicides applied in vitro significantly reduced mycelia growth of all tested *Fusarium* isolates in comparison to untreated control. Imazalil and Thiabendazole showed higher efficacy and totally inhibited the mycelia growth of all tested *Fusarium* isolates. Azoxystrobin, Azoxystrobin + difenoconazole and fludioxonil + difenoconazole have inhibited by more than 45% the mycelia growth of all tested *Fusarium* isolates in comparison to untreated control, although fludioxonil + difenoconazole had a better effect especially at 10ppm. Azoxystrobin showed less efficiency as mycelia growth was reduced by 45 to 72% for all tested isolates, Fungicides effects on *F. solani* and *F. oxysporum* was different; inhibition varied from 45% to 100% depending on *Fusarium* species and different isolates within the same species.

Table 2 In vitro efficacy of some fungicides on mycelial growth of four *Fusarium* isolates.

<table>
<thead>
<tr>
<th>Treatments (concentration)</th>
<th>Mean colony diameter (mm)</th>
<th>FPO-10</th>
<th>FPO-67</th>
<th>FPO-35</th>
<th>FPO-39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imazalil (40 ppm)</td>
<td>1.75i</td>
<td>3.50g</td>
<td>0g</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Imazalil (45 ppm)</td>
<td>1.00i</td>
<td>0h</td>
<td>0g</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Imazalil (50 ppm)</td>
<td>0j</td>
<td>0h</td>
<td>0g</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (5 ppm)</td>
<td>4.00h</td>
<td>0h</td>
<td>0g</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (7.5 ppm)</td>
<td>0j</td>
<td>0h</td>
<td>0g</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (10 ppm)</td>
<td>0j</td>
<td>0h</td>
<td>0g</td>
<td>0g</td>
<td></td>
</tr>
<tr>
<td>Azoxystrobin (250 ppm)</td>
<td>23.50b</td>
<td>23.00c</td>
<td>25.00c</td>
<td>3.75c</td>
<td></td>
</tr>
<tr>
<td>Azoxystrobin (500 ppm)</td>
<td>23.75b</td>
<td>20.50d</td>
<td>22.75d</td>
<td>28.25d</td>
<td></td>
</tr>
<tr>
<td>Azoxystrobin (1000 ppm)</td>
<td>19.75d</td>
<td>19.75d</td>
<td>20.00e</td>
<td>22.75e</td>
<td></td>
</tr>
<tr>
<td>Azoxystrobin + difenoconazole (1000 ppm)</td>
<td>21.50c</td>
<td>17.25e</td>
<td>23.00cd</td>
<td>27.25d</td>
<td></td>
</tr>
<tr>
<td>Azoxystrobin + difenoconazole (2000 ppm)</td>
<td>19.50d</td>
<td>17.00ef</td>
<td>18.00e</td>
<td>22.75e</td>
<td></td>
</tr>
<tr>
<td>Fludioxonil + difenoconazole (2 ppm)</td>
<td>17.75e</td>
<td>29.75b</td>
<td>35.00b</td>
<td>36.75b</td>
<td></td>
</tr>
<tr>
<td>Fludioxonil + difenoconazole (10 ppm)</td>
<td>7.75g</td>
<td>15.75f</td>
<td>14.75f</td>
<td>19.25f</td>
<td></td>
</tr>
<tr>
<td>Fludioxonil + difenoconazole (20 ppm)</td>
<td>10.00f</td>
<td>17.50d</td>
<td>15.25f</td>
<td>21.00ef</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>49.75a</td>
<td>41.25a</td>
<td>74.25a</td>
<td>77.00a</td>
<td></td>
</tr>
</tbody>
</table>

1Values (means of four replicates) in each column followed by the same letter(s) are not significantly different according to Duncan’s multiple range test at p < 0.05.

Effect of imazalil and thiabendazole on *F. solani* FPO-67 and *F. oxysporum* FPO-39 under in vivo condition

Table 3 shows mean pathogen penetration in tubers cv. Agria. Individually inoculated by *F. solani* FPO-67 and *F. oxysporum* FPO-39, after 21 days of incubation at 25-27 °C, depending on different treatments. Obtained results revealed existence of a significant interaction (at p < 0.05) between treatments and *Fusarium* isolates. All tested doses fungicides significantly limited dry rot development on inoculated tubers by more than 50%. Maximum inhibition was reached by imazalil and thiabendazole, at three different doses, where development of dry
rot was totally stopped, in comparison to untreated control in the case of *F. oxysporum* FPO-39. In vivo development of *F. solani* FPO-67, the most aggressive *Fusarium* isolate at these incubation conditions, was inhibited by more than 53.42% by all treatments. In fact, *F. solani* FPO-67 inhibition varied from 53.42% (1/1000) to 97.44% (2/1000) in the case of imazalil. Thiabendazole at 1/1000, 1.5/1000 and 2/1000 inhibited *F. solani* FPO-67 penetration by 60.36%, 70.85% and 88.98% respectively. (Figs 1-2).

**Table 3** In vivo efficacy of imazalil and thiabendazole on *Fusarium solani* FPO-67 and *F. oxysporum* FPO-39.

<table>
<thead>
<tr>
<th>Treatments (concentration)</th>
<th>Mean of fungus penetration (mm)</th>
<th><em>F. solani</em> FPO-67</th>
<th><em>F. oxysporum</em> FPO-39</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imazalil (2/1000)</td>
<td>1.37d</td>
<td>0c</td>
<td></td>
</tr>
<tr>
<td>Imazalil (1.5/1000)</td>
<td>5.87b</td>
<td>0c</td>
<td></td>
</tr>
<tr>
<td>Imazalil (1/1000)</td>
<td>7.19b</td>
<td>5.7b</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (2/100)</td>
<td>1.70cd</td>
<td>0c</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (1.5/1000)</td>
<td>4.50bc</td>
<td>0c</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (1/1000)</td>
<td>6.12b</td>
<td>6.5b</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15.44a</td>
<td>14.7a</td>
<td></td>
</tr>
</tbody>
</table>

Values (means of four replicates) in a column followed by the same letter(s) are not significantly different according to Duncan’s multiple range test at p < 0.05.

**Figure 1** The effect of imazalil on Fusarium dry rot inhibition under in vivo condition. A: Control (*F. solani* FPO-67); E: Control (*F. oxysporum* FPO-39); B and F: Imazalil (1/1000); C and G: Imazalil (1.5/1000); D and H: Imazalil (2/1000).
Fungicides for the control of Fusarium dry rots

Effect of fungicides on dry rot development in natural conditions of a traditional storage

The effect of imazalil and thiabendazole, applied individually, was evaluated on potato tubers cv. Agria, not previously inoculated. Table 4 shows that imazalil and thiabendazole, applied prior to final storage, inhibited development of dry rot in store by more than 65%. Thiabendazole with 71.69% inhibition and 19.4% disease incidence was the most effective treatment against *F. solani* FPO-67 (Table 4, Fig. 3).

**Table 4** In situ efficacy of imazalil and thiabendazole on *Fusarium solani* FPO-67.

<table>
<thead>
<tr>
<th>Treatments (concentration)</th>
<th>Mean of fungus penetration (mm)</th>
<th>Penetration</th>
<th>Disease incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imazalil (2/1000)</td>
<td>7.30cd</td>
<td>38.43ab</td>
<td></td>
</tr>
<tr>
<td>Thiabendazole (2/1000)</td>
<td>6.14cd</td>
<td>19.40c</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>21.69a</td>
<td>25.47a</td>
<td></td>
</tr>
</tbody>
</table>

Values (means of four replicates) in a column followed by the same letter(s) are not significantly different according to Duncan’s multiple range test at \( p < 0.05 \).
Figure 3 The effect of thiabendazole on prevention of dry rot in storage condition. A: Control (Fusarium solani FPO-67); B: Imazalil (2/1000); C: Thiabendazole (2/1000).

Discussion

Dry rot is not just a minor problem like some caused by many other pathogens. It destroys tubers and leaves them completely inedible or unusable as seed in the future. Long-term storage losses have been reported to be as high as 60% while annual dry rot losses can range from 6 to 25% (Gachango et al., 2012). In this study first, the effect of imazalil, thiabendazole, azoxystrobin, azoxystrobin + difenoconazole and fludioxonil + difenoconazole were evaluated against two isolates of F. solani and F. oxysporum under in vitro condition, and quite satisfactory results were obtained. Thiabendazole significantly inhibited the fungal growth of all Fusarium isolates, even at its lower concentration (5ppm). These results were consistent with findings of Murdoch and Wood (1972). Similarly Lashin and Henriksen (1977) reported thiabendazole very effective under in vitro condition against F. solani var. coeruleum, causing dry rot of potato. Rafiq et al. (1955) reported that thiabendazole was most effective in checking the growth of F. oxysporum, F. solani, F. roseum and Rhizoctonia solani at all concentrations used. The next effective fungicide was imazalil, which stopped the mycelial growth of all Fusarium isolate at 50ppm. Similar results were obtained by Bains et al. (1999) where imazalil at 50ppm stopped the mycelia growth of F. sambucinum isolates that were resistant to thiabendazole. Imazalil also stopped the mycelia growth of F. oxysporum isolates at 40 and 45ppm. Azoxystrobin significantly limited fungal growth, in comparison to untreated control, in all used concentrations, but according to studies conducted in Tunisia, azoxystrobin at 200ppm, had no significant effect on reduction of mycelia growth of either F. solani or F. oxysporum (Daami -Remadi et al., 2006a). Considering that the fungicide had the same amount of active ingredient in both studies, it could be argued that isolates and different species in different regions may show different sensitivities to the same fungicide. Azoxystrobin also showed fungicidal activity against F. oxysporum f. sp. tuberosi isolates (Ayed et al., 2006). In the current study, fludioxonil + difenoconazole inhibited the mycelial growth of Fusarium isolates, when applied at 2ppm. At 2ppm, fludioxonil significantly (73%) inhibited mycelia development of F. oxysporum f. sp. tuberosi (Ayed et al., 2006). Fludioxonil showed higher efficacy by totally inhibiting the mycelia growth of all tested Fusarium spp. and isolates, including those of F. sambucinum resistant to benzimidazoles, when applied at 2ppm (Daami -Remadi et al., 2006a). Under in vitro condition, imazalil and thiabendazole proved statistically superior over the rest of fungicides tested at different concentrations. So, only these two fungicides were selected for in vivo tests against F. solani FPO-67 and F. oxysporum FPO-39, which were more virulent than the other two isolates. In the present work, the in vivo investigations revealed that thiabendazole and imazalil caused complete inhibition of dry rot caused by F. oxysporum FPO-39, and also had a noticeable effect on the inhibition of F. solani FPO-67. Under in situ condition, imazalil and thiabendazole had a noticeable effect on the incidence and severity of potato dry rot caused by F. solani FPO-67. The results of this study
showed that the post-harvest dry rot disease of potato tubers could be controlled by imazalil and thiabendazole fungicides which show toxic effect on *F. solani* and *F. oxysporum*. In support to the present findings, there are reports suggesting the effectiveness of fungicides against *Fusarium* species and dry rot disease of potato. Carnegie et al. (1998) found that fenpiclonil and the mixture of thiabendazole and imazalil were more effective in controlling dry rot caused by *F. solani* var. coeruleum than imazalil alone. Similar results were shown by Maughan et al. (1991) who found that the post-harvest thiabendazole or imazalil treatments have been used primarily to prevent the development of dry rot in stored potatoes. On the other hand Carnegie et al. (2001) illustrated that dipping tubers in imazalil fungicide gave significant reduction in dry rot disease. Galben® mancozeb and Galben® copper fungicides had good potential for successful inhibition against *F. solani*, where Galben mancozeb was more effective than Galben copper (Awadall et al., 2008). Hide and Cayley (2008) stated that thiabendazole, imazalil and prochloraz applied to tubers immediately after wounding almost completely prevented dry rot. Treatment 3 days after wounding was less effective and the amount of disease increased with further delay; fungicides were more effective against *F. solani* var. coeruleum and *F. sulphureum* on tubers held at 5 °C than at 10 or 15 °C before treatment and storage. Due to limited access to post-harvest products for controlling storage pathogens, devising strategies to ensure pathogen-free tubers is essential. Post-harvest application of fungicides is one of the most effective methods for controlling the spread of storage diseases. Thiabendazole is the only fungicide registered for controlling Fusarium dry rot, but it is not effective in controlling *F. sambucinum* (Ocamb et al., 2007). Although *F. sambucinum* resistant to thiabendazole has been reported in Europe and the United States (Hide et al., 1992), yet many *Fusarium* species including *F. solani* and *F. oxysporum* are susceptible to these fungicides (Gachango et al., 2012). So thiabendazole can still play an important role in the management of post-harvest diseases especially if it is used in alternation with other compounds or in combination with biological products (AL-Mughrabi et al., 2013). Since investigations were not conducted on the use of different fungicides to control potato dry rot in Khorasan province. Considering the importance of and need to control this disease, different fungicides were studied under *in vitro* and then *in vivo* and *in situ* conditions for the effective management of potato dry rot.

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Fungicides for the control of Fusarium dry rot


ارزیابی تأثیر چند قارچکش در کنترل گونه‌های مهم فوزاریوم عامل خشک‌گی خشک غده

سیب‌زمینی

معمومه وطن خواه، روح‌الله صابری ریسه، مجتبی مرادزاده اسکندری و حمید افضلی

چکیده: پنج قارچکش آزوکسی استروبین، ایمازالیل، تیابندازول، آزوکسی استروبین‌دیفنوکونازول و فلودیوکسنیل دو جدایه بیماری‌زا از Fusarium solani و F. oxysporum عامل در کنترل گونه‌های بیماری‌زا باعث کاهش رشد میسلیومی هر چه از گونه‌های فوزاریوم شد. اما تنها دو قارچکش ایمازالیل و تیابندازول حتی در کمترین غلظت مورد استفاده، بهترین نتایج را به‌دست آوردند. میانگین نفوذ دو قارچ F. solani FPO-67 و F. oxysporum FPO-39 که شدت بیماری‌زای بیشتری بود مانع کامل نفوذ F. oxysporum FPO-39 و F. solani FPO-67 بودند. در حوزه این ابزار، قبلاً بررسی که روی غده‌های سیب‌زمینی ایفا گردیده است. این قارچکش‌ها ممکن است نقش مهمی در مدیریت تلفات فوزاریوم و درمان بیماری‌های خشک غده داشته باشند.