

#### **Research Article**

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

### Masoumeh Vatankhah<sup>1</sup>, Roohallah Saberi-Riseh<sup>1\*</sup>, Mojtaba M Eskandari<sup>2</sup> and Hamid Afzali<sup>2</sup>

1. Department of Plant Protection, Vali-e-Asr University of Rafsanjan, Kerman, Iran.

2. Plant Protection Research Department, Agricultural and Natural Resources Center, Mashhad, Khorasan-Razavi, Iran.

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 5ppm 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 postharvest 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 Banihashami, 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 since1970 (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

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<sup>\*</sup> **Corresponding author**, e-mail: r.saberi@vru.ac.ir Received: 23 July 2018, Accepted: 13 April 2019 Published online: 17 June 2019

Wood, 1972). Benomyl and Thiabendazole also had little effect on spore germination but did greatly decrease mycelia growth at 5ppm (Murdoch and Wood, 1972). Thiophenate-methyl, Carbendazim, Iprodione, Metalaxyl, Procymidone and Prochloraz inhibited dry rot development in tubers inoculated with F. sambucinum and other Fusarium species (Daami-Remadi and El Mahjoub, 1997; Cherif et al., 2001). Sandipan et al. (2016) reported that Carbendazim and Benomyl significantly inhibited the fungal growth of Fusarium sp., even at its lower concentration (100ppm) and among non-systemic fungicides, MEMC significantly inhibited the fungal development even at its lower concentration (1000ppm). Carnegie et al. (1990) showed the efficacy of Thiabendazole and a mixture of Thiabendazole and 2-aminobutane (butyl amine) on F. solani var. coeruleum at harvest time. Comparison between the effects of Imazalil, Prochloraz and Fenpiclonil alone or in combination with 2-aminobutane and Thiabendazole revealed that Fenpicionil and the combination of Thiabendazole and Imazalil were more effective than Imazalil in controlling potato (Carnegie, 1998). Hymexazol, dry rot Azoxystrobin, Fludioxonil and Qoinoline significantly reduced the mycelial growth of F. oxysporum f. sp tuberosi, although the effect of Hymexazole was most pronounced (Ayed et al., 2006). Chlorothalonil, Azoxystrobin, and Hydroxyquinolin-sulfate inhibited the mycelial growth of several isolates, belonging to four Fusarium species. Fludioxonil showed higher efficacy and totally inhibited the mycelia growth of all tested Fusarium isolates. The development of F. graminearum, the most aggressive pathogen at inoculation and incubation conditions, and to a lesser degree the other Fusarium species were inhibited in vivo by more than 50% by Azoxystrobin, Hydroxyquinolin-sulfate and Fludioxonil treatments (Daami-Remadi et al., 2006a). An in vitro screening of some Tunisian isolates of Fusarium spp. for their resistance to some benzimidazoles showed that F. solani, F. oxysporum f. sp tuberosi and F. graminearum isolates were susceptible to these fungicides whereas F. sambucinum isolates were resistant (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 Fludioxonil, 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 4C 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  $\times$  5mm). Tubers were incubated in a growth chamber, at 25C 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:

[1] Penetration = (w / 2 + (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.

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

### *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  $2 \times 2mm$ ) 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 5C). 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 3000gr potato. The mean penetration of the fungus was evaluated by one-way analysis of

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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.

Treatments (concentration)	Mean colony diameter (mm) <sup>1</sup>				
	FPO-191	FPO-67	FPO-35	FPO-39	
Imazalil (40 ppm)	1.75i	3.50g	0g	0g	
Imazalil (45 ppm)	1.00i	Oh	0g	0g	
Imazalil (50 ppm)	Oj	Oh	0g	0g	
Thiabendazole (5 ppm)	4.00h	Oh	0g	0g	
Thiabendazole (7.5 ppm)	Oj	Oh	0g	0g	
Thiabendazole (10 ppm)	Oj	Oh	0g	0g	
Azoxystrobin (250 ppm)	23.50b	23.00c	25.00c	3.75c	
Azoxystrobin (500 ppm)	23.75b	20.50d	22.75d	28.25d	
Azoxystrobin (1000 ppm)	19.75d	19.75d	20.00e	22.75e	
Azoxystrobin + difenoconazole (1000 ppm)	21.50c	17.25e	23.00cd	27.25d	
Azoxystrobin + difenoconazole (2000 ppm)	19.50d	17.00ef	18.00e	22.75e	
Fludioxonil + difenoconazole (2 ppm)	17.75e	29.75b	35.00b	36.75b	
Fludioxonil + difenoconazole (10 ppm)	7.75g	15.75f	14.75f	19.25f	
Fludioxonil + difenoconazole (20 ppm)	10.00f	17.50d	15.25f	21.00ef	
Control	49.75a	41.25a	74.25a	77.00a	

<sup>1</sup>Values (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  $\dot{C}$ , 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	thiabend	azole
on Fusarium	n sola	ni FPO-6	57 and <i>F. ox</i>	yspo	rum FPC	)-39.

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

<sup>1</sup>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).



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

## 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.

Treatments (concentration)	Mean of fungus penetration (mm) <sup>1</sup>		
	Penetration	Disease incidence	
Imazalil (2/1000)	7.30cd <sup>A</sup>	38.43ab	
Thiabendazole	6.14cd	19.40c	
(2/1000) Control	21.69a	25.47a	

<sup>1</sup>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 postharvest thiabendazole or imazalil treatments have been used primarily to prevent the development of dry rot in stored potatoes. On the other hand Carnegie at 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 of 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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### ارزیابی تأثیر چند قارچکش در کنترل گونههای مهم فوزاریوم عامل پوسیدگی خشک غده سیبزمینی

معصومه وطنخواه'، روحاله صابری ریسه'\*، مجتبی مرادزاده اسکندری<sup>۲</sup> وحمید افضلی<sup>۲</sup>

۱- گروه گیاهپزشکی، دانشکده کشاورزی، دانشگاه ولیعصر (عج) رفسنجان، رفسنجان، ایران. ۲- بخش تحقیقات گیاهپزشکی، مرکز تحقیقات کشاورزی و منابع طبیعی خراسان رضوی، مشهد، ایران. پست الکترونیکی نویسنده مسئول مکاتبه:r.saberi@vru.ac.ir دریافت: ۱ مرداد ۱۳۹۷؛ پذیرش: ۲۴ فروردین ۱۳۹۸

چكيده: پنج قارچكش آزوكسى استروبين، ايمازاليل، تيابندازول، آزوكسى استروبين + ديفنوكونازول و فلوديوكسنيل + ديفنوكونازول عليه دو جدايهي بيماريزا از Fusarium solani و F. oxysporum ، عوامل ایجاد کننده پوسیدگی خشک سیبزمینی در منطقه مشهد، مورد بررسی قرار گرفتند. اندازه گیری قطر پرگنه قارچ در محیط کشت PDA بعد از ۲ روز نگهداری در دمای ۲۵ درجـه سلسـیوس نشـان داد کـه همه قارچکشها بهطور قابل توجهی باعث کاهش رشد میسلیومی هر چهار جدایه فوزاریوم شدهانـد، امـا تنها دو قارچکش ایمازالیل و تیابندازول توانستند حتی در کمترین غلظت مورد استفاده، بهترتیب ۴۰ و ۵ پی پی ام، رشد مسلیومی همه جدایه ها را به طور کامل متوقف کنند. میانگین نفوذ دو قارچ F. solani FPO-67 و F. oxysporum FPO-39 و FPO-67 و FPO-39 بیشتری نسبت به دو جدایه دیگر داشتند، بعد از ۲۱ روز نگهداری در دمای ۲۷-۲۵ درجه سلسیوس روی غدههای تیمار شده با دوزهای مختلف ایمازالیل و تیابندازول مورد بررسی قرار گرفت. نتایج بهدست آمده نشان دادند که هـر دو قـارچکش، در غلظت ۱/۵ و ۲/۱۰۰، بهطور کامل مانع نفوذ F. oxysporum FPO-39 به داخل غده شدند، اما در مورد F. solani FPO-67 تمام تیمارها باعت کاهش معنی دار پوسیدگی در مقایسه با شاهد شدند. در شرایط انبار، تیمار غدههای سیبزمینی با ایمازالیل و تیابندازول ، قبل از انبار نهایی آنها، بهترتیب باعث کاهش پوسیدگی خشک ناشی از F. solani FPO-67 از ۶۸ درصد تا۷۱/۶۹ درصد شدند. با توجه به نتایج بهدست آمده این قارچکشها میتوانند نقش مؤثری در مدیریت تلفیقی پاتوژنهای قارچی همراه غده داشته باشند.

**واژگان کلیدی**: آزوکسی استروبین، ایمازالیل، تیابندازول، دیفنوکونازول، فلودیوکسنیل، مشهد، F. solani، *F. solani*, *F. oxysporum*