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THE EFFECTS OF FUNGICIDES ON FUSARIUM OXYSPORUM F. SP. LYCOPERSICI ASSOCIATED WITH FUSARIUM WILT OF TOMATO

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Abstract: Tomato fusarium wilt is considered as one of the most important diseases of tomato both in field and greenhouse – grown tomatoes worldwide. In presented research, six fungicides; benomyl, carbendazim, prochloraz, fludioxonil, bromuconazole and azoxystrobin, were evaluated for their efficacy against the disease casual agent *Fusarium oxysporum* f. sp. *lycopersici* in vitro and in vivo. Seven different concentration (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 µg/ml) were used for assessment of their inhibitory activities against the pathogen through mycelial growth inhibition on potato media. Four concentrations of above mentioned fungicides (0.1, 1. 10 and 100 µg/ml) were tested for controlling Fusarium wilt on tomato plants in glasshouse. Fungal radial growth was measured and median effective concentration (EC_{50}) values (µg/ml) determined. The result of glasshouse tests revealed a different degree of efficacy of all tested fungicides in reducing disease infestation. Prochloraz and bromuconazole were the most effective fungicides against the pathogen both *in vitro* and *in vivo*, followed by benomyl and carbendazim. All other fungicides were less effective. Conserning the application date of fungicides it was shown that they were less effective when applied 7 days after tomato plant infection, compared with 1 day prior infection. No phytotoxic symptoms were observed after the application of prochloraz, bromuconazol and benomyl when used at recommended doses, especially on seedlings. However both fungicides fludioxonil and bromuconazole were shown to be phototoxic to tomato seedlings.

 $\textbf{Key words:} \ chemical \ control, fungicides, Fusarium \ wilt, tomato$

INTRODUCTION

Fusarium wilt of tomato (Lycopersicon esculentum Mill.), caused by Fusarium oxysporum [(Schlecht.) f. sp. lycopersici (Sacc.)] Snyder et Hansen., is one of the most prevalent, serious diseases of tomato (Reis et al. 2005; Sudhamoy et al. 2009). It is also on economically important wilting pathogen of tomato in Iran (Amini 2009). The pathogen occurs throughout most tomato-growing worldwide causing a vascular wilt that can severely affect the crop (Moretti et al. 2008), and the disease is considered as one of the main soil-borne systemic diseases (Schwarz and Grosch 2003). It causes significant losses in tomato production both in greenhouse and field - grown tomates (Nusret ozbay and Steven 2004). Several disease management strategies are available e.g. cultural technique, biological control, resistant cultivars, crop rotation and chemical control (Kamal et al. 2009). Resistant cultivars are the most effective measure of controlling Fusarium wilt (Beckman 1987; Amini 2009), but new races of the pathogen appear to overcome resistance genes in currently grown cultivars (Tello-Marquina and Lacasa 1988). Chemical control of tomato fusarium wilt in vitro and glasshouse was examined repeatedly. Fungicides including benomyl, captafol, imazalil, thiram, and prochloraz-Mn, provided inconsistent control of Fusarium crown and root rot on tomatoes, leaving problematic residues in

fruit tissues (Marois and Mitchell 1981; Jarvis 1988, 1992; Hartman and Fletcher 1991). Also application of methyl bromide and chloropicrin reduced Fusarium crown and root rot of tomato (Mc Govern and Vavrina 1998).

Mandal and Sinha (1992) found out that such compounds as copper chloride, ferric chloride, manganese sulfate, controlled Fusarium oxysporum f. sp. lycopersici by inducing resistance in susceptible tomato plants. El-Shami M.A. et al. (1993) reported that Vitavax (carboxin)-thiuram or Vitavax-captan, applied as fungicidal seed treatment, were effective in controlling Fusarium wilt disease so that, Vitavax-captan gave better disease control then Vitavaxthiuram. The effect of mixture of metamidoxime and copper oxychloride on F. oxysporum f. sp. lycopersici was tested in vitro, and the results showed that these fungicides had a strong synergistic effect and could be used as a basis for a new product to control tomato diseases (Nedelcu and Alexandri 1995). In addition, it was demonstrated that Thiram and Topsin-M were the most effective at 800 mg/g soil, reducing populations of F. oxysporum f. sp. lycopersici by 83.4% after 45 days (Dwivedai et al. 1995).

The main objective of presented study was to evaluate the possibility of controlling Fusarium wilt of tomato with the use of fungicides *in vitro* and under the glasshouse conditions.

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MATERIALS AND METHODS

Fungal pathogen growth

Tomato plants showing symptoms of Fusarium wilt were selected in order to isolate of the pathogen in 2008. The fungus was isolated from necrotic tissue of tomato stems. Sections (3-5 cm long) of tomato plant stem showing vascular discoloration were rinsed thoroughly in tap water. After surface-disinfesting in sodium hypochlorite (5%) for 2 min, the plant pieces were rinsed three times in sterile-distilled water, dried on sterile filter paper and plated onto Potato dextrose agar (PDA) medium amended with streptomycin sulphate (300 mg/l). Fungal cultures were incubated for two weeks at 24°C. The fungal isolates were cleaned up by subculturing successively and selected by single-spore isolation method on dried agar cultures. For identification of Fusarium species, isolates were cultured on Special nutrient agar (SNA) media (Nash and Snyder 1962) and identified according to Gerlach and Nirenberg (1982).

Isolates of *F. oxysporum* f. sp. *lycopersici* used in this study were grown on Potato-dextrose agar (PDA) in darkness at 22–25°C for 2 weeks. Spores from 14-day-old cultures were removed gently from the surface of each plate culture by adding sterile distilled water in order to obtain a suspension of 10⁶ spores/ml. Then, suspensions of spores were filtered through one layer of Mira cloth and spore concentration was diluted to required concentration with the aid of a haemocytometer.

Plant material

Seeds of tomato (*L. esculentum* Mill.) cv. Beliy naliv-241(universally susceptible) were surface-disinfected in 0.5% sodium hypochloride solution for 3 min, rinsed three times in sterile-distilled water prior to sowing. Then the seeds were sown in standardised medium – sand and soil (80 : 20), and grown in seedling plug trays (plug size 3.4 by 3.4 by 5 cm, 64 plugs). Trays were maintained in an air conditioned glasshouse at 23–28°C, 60–70% relative humidity, and 16 h light, 8 h darkness.

Pathogenicity tests and race determination

Pathogenicity tests and race determination were conducted using root-dip inoculation with differential tomato cultivars: Beliy naliv-241 (susceptible), Blagovest (resistant to race 1) and Benito (resistant to both races of 1 and 2). Pathogenicity of isolate was tested on tomato seedling at the three-true-leaf stage. Their roots were dipped into a conidial suspension (10⁶ spores/ml) of tested isolate for 10 min, and then the seedlings were transplanted into sterilized soils in pots (10 cm in diameter) and kept in glasshouse (Amini 2009).

In vitro inhibition of fungicides on the pathogen

Six systemic fungicides: benomyl (Fundazol WP, 500 g/l), carbendazim (Kolfugo super SC, 200 g/l), prochloraz (Sportac EC, 450 g/l), fludioxonil (Maxim SC, 25 g/l), bromuconazole (Bectra SC, 100 g/l) and azoxystrobin (Quadris SC, 250 g/l) at different concentration (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 μ g/ml) were tested individually to assess their effect on pathogen growth inhibition. The inhibitory activity of the fungicides on mycelial radial

growth of the pathogen was determined by growing the fungus isolates on Potato dextrose agar (PDA) media containing different concentrations of each fungicide in Petri dishes (9-cm diameter). The fungicides were prepared from commercial formulation and suspended in distilled water. A disc (4-mm diameters) of 7-day old pathogen mycelial culture was aseptically transferred to the center of the solidified PDA medium in plates (90-mm diameter) with different concentrations of fungicides. Control plates contained only mycelial plugs of pathogen. Then, the plates were incubated for six days at 27±2°C. Mycelial growth of the pathogen was measured on each plate and the growth in PDA medium containing fungicides was compared with the growth of the pathogen in control. Four replications of each treatment were tested and mean values calculated. Percentage inhibition of radial growth (PIRG) was determined to estimate the pathogen growth inhibition by tested fungicides. The experiment was replicated twice.

Fungal radial growth was measured and fungitoxicity was recorded in terms of percentage colony inhibition (Pandey *et al.* 1982). Percentage growth inhibition was determined as $[(D_c - D_t)/D_c] \times 100$, where D_c was the average diameter increase of fungal colony with control, and D_t was the average diameter increase of a fungal colony in treatment (Weitang Song *et al.* 2004). Then, median effective inhibitory concentration of fungicides (EC₅₀) values (µg/ml) against *F. oxysporum* f. sp. *lycopersici* were calculated by 4 software (Graphpad software, Inc. CA92037 USA).

Glasshouse assessment of fungicide efficacy

Healthy tomato seeds of Beliy naliv-241 susceptible to races 1 and 2 of FOL were surface sterilized in 0.5% sodium hypochloride solution for 3 min and rinsed with sterile distilled water. Then, seed were sown in standardised soil mixed with sand (80:20) and were grown in seedling plug trays (plug size 3.4 by 3.4 by 5 cm, 64 plugs). Trays were maintained under the glasshouse conditions at 23-28°C and relative humidity 60-70%. After 21 days, plugs containing tomato plants (three true leaves) were transplanted into 10-cm-diameter pots containing sterile filed soil infested with F. oxysporum f. sp. lycopersici at a rate of 106 CFU/g soil. Fungicides were used at the concentrations of 0.1, 1, 10 and 100 µg/ml per plant. Control plants were similarly treated with sterile distilled water and inoculated with pathogen (without fungicides). The fungicides were applied one day before FOL inoculation (preventive), seven days after inoculation (curative) and appropriate in control treatments. Experiment had six replicate pots of each treatment arranged in a completely randomized design. After 50 days, disease infestation was assassed as a total percentage of seedlings showing any symptoms of Fusarium wilt (yellowing and dropping of leaves, vascular discoloration, and height of a plant).

Disease severity assessment

Disease index was formulated to assess the disease infestation, 50 days after inoculation by using the following scale (Grattidge and O'Brien 1982): 0, (0–24%) of leaves yellowed and wilted; 1, (25–49%) of leaves yellowed and wilted; 2, (50–74%) of leaves yellowed and wilted; 3, (75–99%) of leaves yellowed and wilted; 4, (100%) dead plant.

Data analysis

All experiments were conducted in completely randomized design. Mean values were compared by the least significant difference (LSD) testing at p = 0.05. Duncan's multiple Range test at p = 0.05 was used to compare means. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, version 11). Also, EC_{50} , Confidence interval and R^2 were performed using prism 4 software (Graphpad software, Inc. CA92037 USA).

RESULTS

Fungal isolate and pathogenicity tests

An isolate of the pathogen was collected from tomato fields in Kurdistan province in year 2008. Pathogenicity test of the isolate was confirmed on tomato cv. Beliy naliv-241 (lacking a resistant gene). Diseases symptoms on infected plants appeared two weeks after inoculation. The used isolate of pathogen caused typical symptoms of Fusarium wilt, and it showed strong virulence to a DSR > 3 on cv. Beliy naliv-241. First indication of the disease was yellowing and drooping of lower leaves. This symptom often occurred on one side of infected plant or on one shoot. At last, diseased plants exhibited stunting, dark brown vascular discoloration, and death. Therefore, pathogenicity of tested isolate on tomato cultivars cv. Beliv naliv-241 was clearly distinct. The same fungus species was reisolated from the discoloured vascular tissue of diseased plants stem.

Identification of Fusarium species and race determination

Identification of *F. oxysporum* f. sp. *lycopersici* was based on morphological criteria according to Gerlach and Nirenberg (1982) references. Pathogen's mycelium was white cottony to pink, often with purple tinge or reddish coloration of the medium. Microconidia were born on simple phialides arising laterally and were abundant, oval–ellipsoid, straight to curved, 4–12x2.1–3.5 μm. Macroconidia were sparse spares to abundant, borne on branched conidiophores or on the surface of sporodochia, were thin walled, three to five septate, fusoid-subulate and pointed at both ends with a pedicellate base. Three septate spores were more common. Chlamydospores, both smooth and rough walled, were abundant and formed terminally or on an intercalary basis. Sexual stage was not observed.

The results of race determination showed that Beliy naliv-241 lacking any resistance genes wilted four weeks after inoculation, but cultivars Blagovest and Benito did not develop symptoms to any of tested isolates (Amini 2009). The results of performed experiment on race determination indicated that all isolates belonged to *F. oxysporum* f. sp. *lycopersici* race 1.

In vitro inhibition of fungicides on F. oxysporum f. sp. lycopersici

The values EC₅₀ for tested fungicides against F. oxysporum f. sp. lycopersici was calculated according to the linear relation between inhibitory probit and concentration logarithm. Confidence interval and Correlation coefficient (R) were also calculated for all data (Table 1). The EC₅₀ values for the six fungicides: prochloraz, bromuconazol, benomyl, carbendazim, fludiozonil and azoxystrobin were 0.005, 0.006, 0.008, 0.008, 0.256 and 1.56 µg/ml, respectively (Table 1). All fungicides except fludioxonil and azoxystrobin at the concentration of 10 µg/ml (a. s.), significantly reduced the mycelial growth of pathogen in culture. The results showed that prochloraz and bromuconazol proved to be the most effective in inhibiting mycelial radial growth of the pathogen, followed by benomyl and carbendazim. The fungicides fludioxonil and azoxystrobin were less effective.

Glasshouse assessment of fungicide efficacy

Symptoms of wilting and chlorosis were observed 4 weeks after inoculation on untreated plants with fungicides. Application date of fungicides was shown that they were less effective when applied 7 days after to infection compared with 1 day prior. Fungicide application post-inoculation was less effective (Table 2, 3). Application of fungicide reduced tomato fusarium wilt severity on tomato plants inoculated under the glasshouse conditions. The results of performed tests indicated that, prochloraz and bromuconazole were the most effective fungicides against the pathogen *in vivo*, followed by benomyl and carbendazim. Efficacy of both prochloraz and bromuconazole were similar. Several other products significantly reduced wilt severity but they were not as effective as prochloraz and bromuconazole (Table 2, 3).

Prochloraz and bromuconazole at the concentration of 10 μ g/ml completely reduced wilt infection on tomato plants both prior and after inoculation. At concentration 10 μ g/ml, benomyl and carbendazim one day prior inocu-

Table 1. Effect of selected fungicides (µg/ml) on 120-h radial growth of F. oxysporum f. sp. lycopersici on PDA

Fungicide	EC ₅₀ * [μg/ml]	Confidence interval	Correlation coefficient [R]
Benomyl	0.008	0.001-0.057	0.953
Carbendazim	0.008	0.001-0.064	0.951
Prochloraz	0.005	0.0004-0.077	0.963
Fludioxonil	0.256	0.021–3.1	0.973
Bromuconazole	0.006	0.0002-0.123	0.962
Azoxystrobin	1.56	0.096-24.6	0.966

^{*}EC₅₀ - median effective inhibitory concentration of fungicide

Table 2. Effect of fungicides (µg/ml) containing four different concentrations on fusarium wilt of tomato in glasshouse test, after 50 days

	Disease severity*							
Treatment	0.1 [μg/ml]		1 [μg/ml]		10 [μg/ml]		100 [μg/ml]	
	PE ^a	CE ^b	PE	CE	PE	CE	PE	CE
Control ^c	3.3 a	3.1 b	3.3 e	3.1 c	3.3 d	3.1 e	3.3 с	3.1 d
Benomyl	1.5 b	2.3 b	0.7 b	1.6 b	0.2 b	0.4 b	0 a	0 a
Carbendazim	1.6 b	2.5 b	0.7 b	1.4 b	0.3 b	0.5 b	0 a	0 a
Prochloraz	0.4 a	0.8 a	0.1 a	0.6 a	0 a	0 a	0 a	0 a
Fludioxonil	2.9 d	3.0 b	2.1 d	2.3 b	1.0 c	1.0 c	0 a	0.2 b
Bromuconazole	0.6 a	1.0 a	0.2 a	0.7 a	0 a	0 a	0 a	0 a
Azoxystrobin	2.2 c	3.0 b	1.5 c	2.8 c	1.0 c	1.5 d	0.3 b	0.8 c
LSD at 5%	0.3	0.4	0.2	0.3	0.1	0.1	0.1	0.1

a - preventive effect; fungicides were applied one days before inoculation of pathogen

Data are means of 6 replicates

Means in the column followed by different letters indicate significant differences among treatments at 0.05 according to Duncan's multiple range Test

*disease severity:

- 0, 0-24% of leaves yellowed; 1, 25-49% of leaves yellowed
- 2, 50-74% of leaves yellowed; 3, 75-99% of leaves yellowed

Table 3. Effect of fungicides (μg/ml) containing four different concentrations on reduction Fusarium wilt of tomato in greenhouse test, after 50 days

	% wilt reduction [efficacy %]							
Treatment	0.1 [µg/ml]		1 [μg/ml]		10 [μg/ml]		100 [μg/ml]	
	PE	CE	PE	CE	PE	CE	PE	CE
Benomyl	54.5	25.8	78.8	80.6	93.9	87.1	100	100
Carbendazim	51.5	19.3	78.8	87.1	90.9	83.9	100	100
Prochloraz	87.9	74.2	96.6	80.6	100	100	100	100
Fludioxonil	15.1	3.2	36.4	25.8	69.0	67.4	100	93.5
Bromuconazole	81.8	67.7	93.9	77.4	100	100	100	100
Azoxystrobin	33.3	3.2	54.5	9.7	69.0	51.6	90	74.2

PE - preventive effect; fungicides were applied one days before inoculation of pathogen

lation reduced the disease incidence and severity of Fusarium wilt by 16.5 and 11 times (93.9 and 90.9% efficacy) respectively, fludioxonil and azoxystrobin 3.3 times (69% efficacy) in comparison with infected control (Table 2, 3). Fludioxonil and azoxystrobin did not significantly reduce wilt severity at either time of application (Table 2, 3).

Reduced disease and increased yield were observed in case of some fungicides application. Disease reduction caused by fungicides applied at concentration 10 μ g/ml (with infection by pathogen) leaded to the increase of height of a plant by 2,6 to 4,0 times in comparison with infected control. At concentration 100 μ g/ml benomyl, car-

bendazim and prochloraz increased the height of a plant at 3,8 to 4,2 times in comparison with infected control, contrary to fludioxonil, bromuconazole and azoxystrobin, which decreased height of a plant (data not shown).

Morever, the result of phytotoxicity tests (without infection by pathogen) indicated that, fludioxonil at concentration 10 μ g/ml and bromuconazole and azoxystrobin at concentration 100 μ g/ml decreased the height of a plant (Table 4), whereas, benomyl, carbendazim and prochloraz did not show any phytotoxic effects, when applied at recommended dose of 10 μ g/ml.

b – curative effect; fungicides were applied one week after inoculation of pathogen

c - control; control pathogen without application of fungicides

PE - preventive effect; fungicides were applied one days before inoculation of pathogen

CE – curative effect; fungicides were applied one week after inoculation of pathogen

^{4, 100%} of leaves yellowed (dead)

CE – curative effect; fungicides were applied one week after inoculation of pathogen

Table 4. Effect of fungicides (µg/ml) on height of tomato plant after 35 days (without infection by pathogen)

Treatment	Concentrations of fungicides					
Heatment	1 [μg/ml]	10 [μg/ml]	100 [μg/ml]			
Control water	42.0	42.0	42.0			
Control pathogen	11.0	11.0	11.0			
Benomyl	20.2	31.0	38.2			
Carbendazim	23.0	33.7	36.8			
Prochloraz	31.0	33.1	30.5			
fFudioxonil	19.6	12.8	6.4			
Bromuconazole	23.4	32.4	11.0			
Azoxystrobin	15.5	21.0	12.2			
LSD at 5%	1.5	2.0	2.3			

Data are means of 6 replicates

DISCUSSION

Considering the application timing of fungicides it was shown that they were less effective when applied 7 days after tomato plants infection compared with 1 day prior. Decreasing the period between application of fungicides and infection generally resulted in their increased efficacy.

Prochloraz and bromuconazole proved to be the most effective against the pathogen of all fungicides evaluated in this study in vitro and under the glasshouse conditions. Similar result was reported on the use of prochloraz against other species of Fusarium (Song et al. 2004; Nel et al. 2007). These fungicides showed the greatest effectiveness, inhibiting F. oxysporum f. sp lycopersici growth in vitro as well as disease suppression in the glasshouse. Our experiment showed that, prochloraz and bromuconazole at the concentration of 10 µg/ml were the most effective, followed by benomyl and carbendazim that had good preventive and curative effects on tomato wilt. Allen et al. (2004) revealed that benomyl at 10 µg/ml (a.s.) completely inhibited fungal growth of F. solani, F. oxysporum and F. proliferatum. Etebarian (1992) reported that, iprodione + carbendazim, benomyl and carbendazim totally inhibited fungal growth at the concentrations of 10 and 100 ppm, after 10 days. Also results indicated that prochloraz and carbendazim were the most effective fungicides in inhibiting mycelial growth of F. oxysporum f. sp. lycopersici (Song et al. 2004; Weitang et al. 2004).

Among the systemic fungicides assessed under the glasshouse conditions for their efficacy against the disease, prochloraz was highly effective. This fungicides was previously reported as being effective under glasshouse and field conditions (Weitang *et al.* 2004), followed by bromuconazole, benomyl and earbendazim (Etebarian 1992). Systemic fungicides such as benomyl and carbendazim were also effective against anthracnose-infected lupins (Thomas *et al.* 2008). Other fungicides such as fludioxonil, and azoxystrobin were less effective.

Tomato plants treated with all tested fungicides (without infection by pathogen) at concentrations of 100 $\mu g/ml$ (active substance) showed severe symptoms of phytotoxicity, whereas the same fungicides (except fludioxonil) at

the concentration of 10 μ g/ml (active substance), were not phytotoxic to tomato plants.

Management of diseases, relies on integrated use of crop rotation, cultural techniques, biological control and disease-resistant cultivars. In addition to the chemical control of tomato disease in greenhouse (Zhonghua *et al.* 2005), other methods such as, cultural control (Vincent and Mew 1998; Paulitz and Belanger 2001), integrated control (Katayama and Kimura 1987), host – plant resistance (Dalal *et al.* 1999), transgenic resistant plant (Jia *et al.* 1999), and biological control (Jian-Hua Guo *et al.* 2004; Andreu and Caldiz 2006; Hashem 2009) are also purposeful.

Prochloraz and bromuconazole can also be used for sterilization of field equipments and vehicles in the field. The results of performed experiments revealed that strategic use of fungicides should be considered as an element of integrated management of tomato Fusarium wilt.

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POLISH SUMMARY

SKUTECZNOŚĆ FUNGICYDÓW W ZWALCZANIU GRZYBA *FUSARIUM OXYSPORUM* F. SP. LYCOPERSICI – SPRAWCY FUZARYJNEGO UWIĄDU POMIDORA

Fuzaryjny uwiąd pomidora jest jedną z najgroźniejszych chorób, występujących w uprawach polowych i szklarniowych tej rośliny. Przedstawiono ocenę skuteczności sześciu fungicydów: benomyl, karbendazym, prochloraz, fludioksonil, bromukonazol i azoksystrobin przeciwko czynnikowi sprawczemu choroby Fusarium oxysporum f. sp. lycopersici, w oparciu o wyniki testów przeprowadzonych in vitro i in vivo. Siedem różnych koncentracji fungicydów (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 μg/ml) wykorzystano do oceny stopnia zahamowania wzrostu grzybni patogena na pożywce agarowej, a cztery koncentracje (0.1, 1, 10, 100 µg/ml) testowano na siewkach pomidora w testach szklarniowych. Dokonano pomiaru wzrostu grzybni patogena i określono średnią wartość skutecznej koncentracji preparatów EC₅₀ (μg/ml). Wyniki testów szklarniowych wykazały różny

stopień skuteczności ograniczania nasilenia choroby dla wszystkich badanych fungicydów. Fungicydy prochloraz i bromukonazol okazały się najskuteczniejsze w zwalczaniu patogena zarówno w testach *in vitro*, jak też *in vivo*, a w dalszej kolejności wymieniono fungicydy benomyl i karbendazym. Pozostałe z badanych preparatów wykazały nieco słabsze działanie. Biorąc pod uwagę terminy stosowania fungicydów należy podkreślić, że były one mniej skuteczne gdy zastosowano je po upływie siedmiu

dni od momentu zainfekowania roślin, a najlepszą skuteczność uzyskano w przypadku stosowania preparatów na dzień przed infekcją roślin. Nie obserwowano symptomów fitotoksyczności po zastosowaniu fungicydów: prochloraz, bromukonazol i benomyl, gdy opryskiwano siewki pomidora zgodnie z zalecanymi dawkami preparatów. Wyjątek stanowił fungicyd fludioksonil, który okazał się fitotoksyczny dla siewek pomidora.