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Evaluation of new generation fungicidal molecules against *F. oxysporum* f.sp. *lycopersici*

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Abstract
Tomato *fusarium* wilt is seen as one of the most important tomato diseases in the field as well as in the world-wide growing greenhouse tomatoes. In presented research, six fungicides; Carbendazim (50% WP), Trifloxystrobin 25% + Tebuconazole 50% WG, Tebuconazole (25% EC), Azoxystrobin (23% EC), Propiconazole (25% EC) and Mancozeb + Carbendazim (75% WP), were evaluated for their efficacy against the disease casual agent *Fusarium oxysporum* f. sp. *lycopersici in vitro* and *in vivo*. Six different concentrations (µg / ml) were used to assess the 0.1, 1, 10, 100, 500 and 1000 ppm active ingredient (a.i) pathogen-inhibiting activities by inhibiting mycelial growth on potato media. Six concentrations of above mentioned fungicides (0.1, 1. 10 and 100 µg / ml) were tested for controlling *Fusarium* wilt on tomato plants in glasshouse. Measurement of fungal growth inhibition and determination of median appropriate dose (EC50) values (µg / ml); The glasshouse test results showed a varying degree of activity of all fungicides tested in minimizing outbreak of the disease. Out of six fungicides tested against F. *oxysporum* f. sp. *lycopersici* at different concentration *viz*., 0.1ppm, 1ppm, 10 ppm, 100 ppm, 500 ppm and 1000 ppm, Carbendazim @ 1000ppm was found to be the most effective with percent inhibition of 100% followed by Tebuconazole (98.8%), Mancozeb+Carbendazim (97.7%) Propiconazole, and Azoxystrobin (87.7%) while Trifloxystrobin+Tebuconazole was found to be the least effective (85.5%).

Keywords: chemical, tomato, *fusarium* wilt, fungicides

Introduction
Tomato *Fusarium* wilt (*Lycopersicon esculentum* Mill) formed by *Fusarium oxysporum* f. Sp. *lycopersici* (Schlecht). Snyder et Hansen., is among the most common severe tomato diseases (Reis *et al.* [30]. 2005; Sudhamoy *et al.* 2009) [33]. It is also on economically important wilting pathogen of tomato in Iran (Amini 2009) [2]. The pathogen commonly affects in most all tomato-growing causing a vascular wilt that can seriously impact the crop Moretti *et al.* (2008) [23]. The disease is taken into consideration to be one of the major systemic soil-borne diseases Schwarz and Grosch (2003) [31]. It causes major losses in both greenhouse and field tomato production- grown tomatoes (Nusret ozbay and Steven 2004) [27]. Resistant cultivars are the most powerful tool for controlling *Fusarium* wilt (Beckman 1987 [4]; Amini 2009) [2], but in typically grown varieties new pathogen. Fungicides including Carbendazim (50% WP), Trifloxystrobin 25% + Tebuconazole 50% WG, Tebuconazole (25% EC), Azoxystrobin (23% EC), Propiconazole (25% EC) and Mancozeb + Carbendazim (75% WP), Provided inconsistent regulation of *Fusarium* crown and root rot on tomatoes, leaving problem residues in the fruit tissues (Marois and Mitchell (1981) [21]; Jarvis (1988) [13], (1992) [14]; Hartman and Fletcher 1991) [11]. Usage of methyl bromide and chloropicrin have reduced *Fusarium* crown and tomato root rot Mc Govern and Vavrina (1998) [22]. Mandal and Sinha (1992) [20] found that *Fusarium oxysporum* f.sp. *lycopersici* regulated compounds such as copper chloride, ferric chloride, manganese sulphate, etc. Applied as a fungicidal seed treatment, stated that Vitavax (carboxin)-thiuram or Vitavax-captan is successful in controlling *Fusarium* wilt disease so that Vitavax-captan provided better control of disease than Vitavax-thiuram. The effect of metamidoxime-copper-oxychloride mixture on *F. oxysporum* f.sp. *lycopersici* was tested *in vitro* and the findings showed that these fungicides had a significant synergistic impact and could be used to combat tomato disease as a basis for a new drug (Nedelcu and Alexandri 1995) [25]. The effect of metamidoxime/copper oxychloride mixture on *F. oxysporim*, f.sp. *lycopersici* was studied *in vitro*, and the findings showed that these fungicides had a strong synergistic effect and could be used as a basis for a new tomato control drug (Nedelcu and Alexandri 1995) [25]. The main aim of the research presented was to assess the possibility

of controlling the use of fungicides *in vitro* and under *in vitro* conditions by *Fusarium* wilt of tomato.

Materials and methods

The fungicides Carbendazim (50% WP), Trifloxystrobin 25% + Tebuconazole 50% WG, Tebuconazole (25% EC), Azoxystrobin (23% EC), Propiconazole (25% EC) and Mancozeb + Carbendazim (75% WP) were evaluated *in vitro* against *F. oxysporum* f. sp. *lycopersici*. The fungicides were suspended in sterile distilled water and added to Potato dextrose broth (50 ml per 250 ml Erlenmeyer flask) to a final concentration of 0.1,1,10, 100, 500 and 1000 ppm active ingredient (a.i). Fungal mycelial discs (5mm diameter), prepared from the periphery of 7-day-old cultures of *F. oxysporum* f. sp. *lycopersici* were transferred to four replicated flasks and dry mass of mycelial mats were determined after 15 days of incubation at 28±2oC (Gopinath *et al.*, 2006). Then the percent inhibition of the pathogen was assessed based on the following formula,

Per cent Inhibition= (C-T/C) X 100

Where, C- Radial growth of the pathogen in the control (Medium without fungicides) T- Radial growth of the pathogen in the fungicide amended medium. Fungal pathogen growth Tomato plants showing symptoms of *Fusarium* wilt were selected in order to isolate of the pathogen in 2012. 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.

In vitro inhibition of fungicides on the pathogen six systemic fungicides

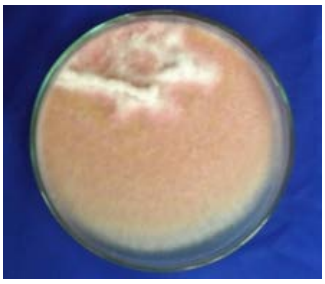
Carbendazim (50% WP), Trifloxystrobin 25% + Tebuconazole 50% WG, Tebuconazole (25% EC), Azoxystrobin (23% EC), Propiconazole (25% EC) and Mancozeb + Carbendazim (75% WP) at different concentration (0.0001, 0.001, 0.01, 0.1, 1, 10, 100 µg/ml) were tested individually to assess their effect on pathogen 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 [(Dc – Dt)/ Dc] × 100, where Dc was the average diameter increase of fungal colony with control, and Dt was the average diameter increase of a fungal colony in treatment (Weitang Song *et al.*, (2004) [37]. 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).

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, EC50, Confidence interval and R2 were performed using prism 4 software (Graphpad software, Inc. CA92037 USA).

Results

Out of six fungicides tested against *F. oxysporum* f. sp. *lycopersici* at different concentration *viz*., 0.1ppm, 1ppm, 10 ppm, 100 ppm, 500 ppm and 1000 ppm, Carbendazim @ 1000ppm was found to be the most effective with percent inhibition of 100% followed by Tebuconazole (98.8%), Mancozeb+Carbendazim (97.7%) Propiconazole, and Azoxystrobin (87.7%) while Trifloxystrobin+Tebuconazole was found to be the least effective (85.5%). Fungal isolate and pathogenicity studies Pathogen isolation was obtained from tomato fields in the region of Thondamuthur in 2012. Pathogenicity test of the isolate was confirmed on tomato cv. PKM1. Symptoms of disease in contaminated plants occurred two weeks after inoculation. The pathogen isolate used caused typical *Fusarium* wilting symptoms, and it showed high virulence. The first indication of the disease was yellowing, and lower leaves drooping. This symptom appeared often on one side of the infected plant or on one shoot. Diseased plants eventually display stunting, dark brown vascular discolouration, and death. Hence pathogenicity of isolate tested on tomato cultivars cv. The same genus of fungus was reisolated from the discolored vascular tissue of the stammering diseased plants.

The same genus of fungus had been reisolated from the mumbling diseased plants' discolored vascular tissue. Pathogen's mycelium was white cottony to pink, often with purple tinge or reddish coloration of the medium. Microconidia was raised on laterally occurring simple phialides, and was large, oval-ellipsoid, 4–12x2.1–3.5 µm straight to curved. Macroconidia were sparse to abundant spares, borne on branched conidiophores or sporodochia surfaces, thinly walled, three to five septate, fusoid-subulate, and pointed on both ends with 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 performed experiment on race determination indicated that all isolates belonged to *F. oxysporum* f. sp. *lycopersici.*



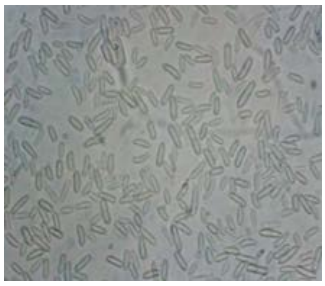



Fig 1(a): Pathogen on Petriplate **Fig 1(b, c):** Macro and micro conidia **Fig 1(d):** Chlamydospores

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 Carbendazim (50% WP), Trifloxystrobin 25% + Tebuconazole 50% WG, Tebuconazole (25% EC), Azoxystrobin (23% EC), Propiconazole (25% EC) and Mancozeb + Carbendazim (75% WP) µg/ml, respectively (Table 1). All fungicides except carbendazim 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.

Table 1: Fungicide assay: *Fusarium oxysporium* f. sp. *lycopercisi* against fungicides

S. No	Fungicides	Concentration of fungicides											
		0.1ppm		1ppm		10ppm		100ppm		500ppm		1000ppm	
		Radial Mycelia Growth (mm)	Percent Inhibition Over control	Radial Mycelia Growth (mm)	Percent Inhibition Over control	Radial Mycelia Growth (mm)	Percent Inhibition Over control	Radial Mycelia Growth (mm)	Percent Inhibition Over control	Radial Mycelia Growth (mm)	Percent Inhibition Over control	Radial Mycelia Growth (mm)	Percent Inhibition Over control
1	Carbendazim	2.7	70.0	3.1	65.5	2.5	72.2	0.5	94.4	0.0	90.0	0.0	90 .0
2	Nativo	3.8	57.7	3.2	64.4	3.0	66 .6	2.7	70.0	1.8	80.0	1.3	85.5
3	Tilt	3.3	63.3	3.0	66.6	2.4	73.3	2.0	77.7	0 .0	90.0	1.1	87.7
4	Tebuconazole	2.6	71.1	2.2	75.5	2.0	77.7	1.1	87.7	1.0	88.8	0.0	90 .0
5	Amistar	4.3	52.2	3.7	58.8	3.6	60 .0	2.2	75.5	0.2	97.7	0.0	90 .0
6	SAFF	3.3	63.3	3.0	66.6	2.5	72.2	1.6	82.2	1.0	88.8	0.0	90 .0
7	COC	3.8	57.7	2.3	74.4	2.0	77.7	2.5	72.2	1.8	80.0	0.0	90 .0
8	Control	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0	9.0	0.0

Discussion

Taking the application timing of fungicides into account, it has been shown that they were less effective when applied 7 days after infection with tomato plants compared with 1 day before. Diminishing the time between fungicide application and infection usually resulted in their increased effectiveness. Carbendazim and Natio have proved to be the most effective in *in vitro* conditions against the pathogen of all fungicides tested in this study. Similar findings have been published about the use of Tilt against other *Fusarium* species Song *et al.*, (2004) [32].

Nel *et al.*, (2007) [26]. These fungicides displayed the greatest effectiveness, inhibiting *F. oxysporum* f. sp *lycopersici* development in suppression of the disease *in vitro*. Our experiment showed that Carbendazim and Natio were the most successful at a concentration of 10 µg / ml, followed by Tilt and carbendazim which had strong preventive and curative effects on tomato wilting. Allen *et al.*, (2004) [1] revealed that fungal growth was inhibited absolutely by benomyl at 10 µg / ml(a.s.) *Alternaria solani*, *F. oxysporum* and *F. proliferatum*. Etebarian, (1992) [8] stated that, after 10 days, iprodione + carbendazim, benomyl, and carbendazim completely inhibited fungal growth at 10 and 100 ppm concentrations. Results also showed that the most effective fungicides in inhibiting mycelial growth of *fusarium* were prochloraz and carbendazyme. *F. oxysporum* f. sp. *lycopersici* Song *et al.* (2004) [32].

Weitang *et al.* (2004) [37]. Among the systemic fungicides tested for their efficacy against the disease under the invitro conditions, Carbendazim has been highly effective. These fungicides had previously been stated to be effective under conditions of invitro and field Weitang *et al.*, (2004) [37], followed by natio and carbendazim Etebarian (1992) [8]. Systemic fungicides such as carbendazim have also been successful against the lupins infected with anthracnose Thomas *et al.*, (2008) [35]. Certain fungicides were less effective, such as Mancozeb + Carbendazim. Tomato plants treated with all fungicides tested (without pathogen infection) at concentrations of 100 µg / ml (active substance) showed severe phytotoxicity symptoms; Whereas the same fungicides (except fludioxonil) were not phytotoxic to tomato plants at a concentration of 10 µg / ml (the active substance). Disease management, depends on integrated crop rotation use, cultural methods, biological control, and disease-resistant cultivars. In addition to the chemical control of tomato disease in greenhouse Zhonghua *et al.*, (2005) [38], other methods such as, cultural control (Vincent and Mew., (1998) [36]; Paulitz and Belanger., (2001) [29], integrated control (Katayama and Kimura., (1987) [19], host - plant resistance (Dalal *et al.*, (1999) [5], transgenic resistant plant

(Jia *et al.* 1999) [15], and biological control (Andreu and Caldiz., (2006) [3]; Hashem (2009) [12] are also purposeful.

carbendazim and Trifloxystrobin 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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