Mycopath (2012) 10(2)**:** 57-61 **Research Article**

In vitro chemical control of *Fusarium oxysporum* f. sp. *lycopersici*

Aroosa Khan[1], Ansa Dliferoze[1], Zia-Ullah Malik[2], *Amna Shoaib[1] and Saba Khurshid[1]

[1]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan.
[2]Evyoul group-286-H2-Johar Town, Lahore, Pakistan
**Correspondence author's email: aamnaa29@yahoo.com; amna.iags@gmail.com*

Abstract

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is notorious disease that results in adverse influence on growth and yield of tomato (*Lycopersicon esculentum* L.). In the present investigation, a wide range of commercially available fungicides were assessed for their antifungal activity against *F. oxysporum* f. sp. *lycopersici*. Laboratory experiments were conducted using malt extract broth as growth medium with recommended dose of each fungicide (active ingredient), inoculated with fungal mycelial disc and incubated at 27 °C ± 2 for 7 days on an electric shaker at 100 rpm. All the 14 fungicides significantly suppressed fungal growth resulting in 20 to 90% reduction in fungal biomass over control. Wisdom (fosetyl aluminium) and Treety (tebuconazole) were found to be the most effective in suppressing fresh and dry biomass of the fungus. Therefore, these two fungicides may be utilized to manage tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici*.

Keywords: Chemical control, fungicides, *Fusarium oxysporum* f. sp. *lycopersici*, tomato, wilt.

Introduction

Fusarium wilt of tomato (*Lycopersicon esculentum* L.), caused by *Fusarium oxysporum* (Schlecht.) f. sp. *lycopersici* (Sacc.) Snyder & Hansen, is one of the most prevalent and serious soil-borne systemic diseases in the warm vegetable growing areas of the world (Abd-Allah *et al*., 2011; Abdel-Monaim, 2012; Morid *et al*., 2012). The fungus is responsible for deteriorating the vascular system of roots, inhibiting water transport thus disturbs several of the plant's physiological processes relevant to production and quality (Malhotra and Vashistha, 1993). So far, modification in photosynthetic activity leads to inhibition in translocation of assimilates from their source of production up to the areas of growth and deposition of yield material.

With the purpose of reducing the economic losses caused by this devastating disease, producers apply a great number disease management strategies e.g. cultural technique, biological control, resistant cultivars, crop rotation and fungicides (Kamal *et al.,* 2009). Resistant cultivars are the most effective measure of controlling Fusarium wilt but new races of the pathogen appear to overcome resistance genes in currently grown cultivars (Tello-Marquina and Lacasa, 1988). Use of fungicides has always been considered an easy and attractive approach for the farmers especially in developing countries like Pakistan, where farmers are more concerned about the yield rather considering the negative impact of these fungicides. While, different classes of fungicides gaining attraction of farmer due to their relatively low cost, ease of use, and effectiveness (Dias, 2012). The objective of this study was to evaluate *in vitro* antifungal activity of broad range of commercially available fungicides against the *F. oxysporum* f. sp. *lycopersici.*

Materials and Methods

F. oxysporum f. sp. *lycopersici* was isolated from infected tomato plants collected from the fields at Experimental Area of Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Samples were taken in polythene bags and the infected roots were used for fungal isolation. Roots were washed with tap water to remove soil debris and were cut into 0.5 cm pieces. Root pieces were surface sterilized by 0.1% sodium hypochlorite solution for 1 minute and then rinsed 3-4 times with sterilized distilled water. These pieces were placed on autoclaved pantachloronitrobenzene growth medium (PCNB) in 9-cm diameter Petri plates under aseptic conditions and inoculated plates were incubated at 25±2 °C for 7 days. PCNB was prepared by adding 20 g agar, 15 g peptone, 1.0 g PCNB, 1.0 g KH_2PO_4 and 0.5 g $MgSO_4$ in 1.0 L of distilled water. The fungal colonies appeared on plates

were further purified and fungal pathogen was identified on the bases of morphological characters under compound microscope (Ignjatov *et al*., 2012).

Fungal pathogenicity was checked by growing the tomato plants in pathogen infected soil. For this, conidial suspension from 2-week old cultures of *F. oxysporum* f. sp. *lycopersici* was prepared. Mycelia/conidia were scraped from the pure fungal culture and suspended in 30 mL distilled water. Number of conidia was counted by hemocytometer (Marienfeld GmbH, Marienfeld, Germany). Conidial number in the suspension was adjusted to 4×10^6 conidia mL^{-1}. Freshly prepared conidial suspension (30 mL) was mixed with 500 g of sterilized sieved of soil filled in plastic pots followed by sowing of 5 seeds of test plant in it. The plastic pots were kept under controlled environmental conditions at relative humidity of 90-100% at 25 °C. Pots were regularly monitored for disease development. The wilt disease symptoms caused by the *F. oxysporum* f. sp. *lycopersici* on the test plant were confirmed 15 days after germination by using 0-3 rating scale (Ioannou, 2000).

For antifungal assays, fourteen fungicides representative of different chemical groups were procured from the Kanzo Ag (Evyol Group), Lahore, Pakistan (Table 1). These fungicides also included some new commercial products like Defeater, Defeater plus and Picoxystrobin that were not previously tested against *F. oxysporum* f. sp. *lycopersici*.

Malt extract (100 mL) was prepared along with recommended dose of each fungicide (active ingredient). After autoclaving, actively growing mycelial disc were taken with sterile cork borer (5 mm) from the 6 days old cultures and were transferred to the flasks aseptically. Flasks were incubated at 27 °C ± 2 for 7 days on an electric shaker at 100 rpm. Control treatment was prepared without addition of fungicide and contained just mycelial disc. The antifungal activity was evaluate

All the data were analyzed statistically by Duncan's Multiple Range Test (Steel and Torrie, 1980) by measuring fresh and dry biomass of the fungus. The fresh weight of mycelial biomass was taken after filtering it on a pre-weighted filter paper and dry weight was calculated after drying biomass in an oven at 60 ºC for 24 hours. The experiment was carried out in triplicate in a completely randomized design.

Results and Discussion

Growth characteristics of *F. oxysporum* f. sp. *lycopersici*

Fungus isolated from roots of diseased tomato plants formed white colonies (reverse yellow to brown) with branching mycelium reaching 1.5–2 cm diameter in five days at 25 ºC on PCNB medium. Macroconidia varied in size from 25–55 µm × 3–5 µm (100x). Most often they were fusiform, slightly curved, pointed at the tip and had 3-5 septa. They formed abundant unicellular, mostly non-septate, elyptical, cylindrical, straight or curved microconidia 5–12 × 2.3–3.5 µm. All these characteristics confirmed the pathogen as *F. oxysporum* f. sp. *lycopersici* (Ignjatov *et al*., 2012).

Pathogenicity test

Visible symptoms of wilt disease on infected plant appeared two weeks after inoculation. Typical wilt symptoms i.e. chlorosis, wilting, wrinkling and drying of lower leaves were observed on 75% (severe wilting) or more of total leaf number. In many cases one side of the plant was affected first. Wilt disease evaluation on the basis of disease rating scale confirmed pathogencity of *F. oxysporum* f. sp. *lycopersici* (Ignjatov *et al*., 2012).

In vitro antifungal activity of fungicides

Data regarding the effect of different fungicides on fresh and dried biomass of *F. oxysporum* f. sp. *lycopersici* is illustrated in Fig. 1 A & B. The results showed that all the fungicides significantly reduced fungal growth as compared to control. Among the various fungicides, Wisdom and Treety caused the highest inhibition of 99-100% on the fresh biomass of the fungus as compared to control and rest of the fungicides. The fresh biomass was significantly declined by 95-97% due to Hiten, Defeater 50, Pyranil and Benedict. Trifort and Picoxystrobin showed 90% reductions, whereas Bloom, Defeater 20, Falre and Definite exhibited 70-80% reduction in fresh biomass production. Vigard, Cordate and Epic were comparatively less effective against *F. oxysporum* f. sp. *lycopersici* thus exhibited 30-20% reduction in fungus growth as compared to control (Fig. 1 A).

Dry biomass of *F. oxysporum* f. sp. *lycopersici* was significantly declined by 95-100% due to Wisdom and Treety, by 90% due to Benedict, Hiten and Pyranil, and by 80-90% due to Trifort, Picoxystrobin, Defeater and Dyranil.

Bloom and Defeater significantly reduced biomass by 50-60% and, Cordate and Epic exhibited lowest antifungal activity resulted biomass was declined by 20-30% (Fig. 1 B).

Presently, diverse activity of different fungicides could be attributed to various active ingredients in them. So far, fosetyl aluminium is the organic phosphate compound and active ingredient of Wisdom. The antifungal activity of fosetyl aluminium has been previously reported against number of biotrophs and nectrotrophs (Cohen *et al*., 1987). The antifungal activity appears to be based on its phosphate compound (Fenn and Coffey, 1984) that probably act systematically by inhibiting spore germination and by blocking mycelial growth and spore production (Marks and Cassells, 1999).

Tebuconazole, Bloom, Trifort, Definite, Tebuconazole and Epic belong to triazole group. Trizole has been reported to interfere with sterol biosynthesis that results in insufficient availability of ergosterol in fungi (Hewitt, 1998). Insufficiency of ergosterol in fungal membranes likely to disturb membrane functions including activity of membrane-bound enzymes and proper synthesis of new hyphal cell walls. Consequently, severe effects of triazol on the development of hyphal and haustorial structures possibly lead to reduced growth of *F. oxysporum* f. sp. *lycopersici* (Han *et al*., 2006). Even though different triazole fungicides have a similar mechanism of action, they may show marked differences in their activity against different fungal pathogens (Scheinpflug and Kuck, 1987).

The considerable antifungal activity of Benedict is due to iprobenfos (active ingredient), that was reported to act against fungi by inhibiting the synthesis of phopholipids (Roberts and Hutson, 1999). Fentin hydroxide in Hitin is known to inhibit oxidative phophorylation and ATP synthesis in fungi (FRAC, 2006). Sheng *et al*. (2007) suggested that antifungal activity of flumorph (Defeater and Defeater plus) may be owing to the impairment of cell polar growth through directly or indirectly disrupting the organization of F-actin.

The inhibition in *F. oxysporum* f. sp. *lycopersici* growth due to Flare could be well-linked with inhibition in protein synthesis of fungi by action of its active ingredient i.e. streptomycin (FRAC, 2006). Pyranil contains pyrimethanil (aniline-pyrimidines). The biochemical mode of action of pyrimethanil is the inhibition of fungal secretion of cell wall degradation enzymes like proteinases, cellulases, pectinases and laccase. Moreover, pyrimethanil inhibits the biosynthesis of methionine via the enzyme cystathione ß-lyase. This biochemical mode of action pyrimethanil is attributed to reduced spore germination, inhibition of germ tube extension in *F. oxysporum* f. sp. *lycopersici* (Bylemans and Goodwine, 2004). Picoxystrobin is a fungicide of strobilurins group and it acts by inhibiting mitochondrial respiration by blocking the transfer of electrons in the III complex (bc1 complex) of the transporting current for mitochondrial electrons (Ammermann *et al*., 2000).

The low antifungal activity exhibited by Cordate (kasugamycin) could be due to non-specificity of fungicides against *F. oxysporum* f. sp. *lycopersici*.

Conclusion

Wisdom and Treety proved to be the most effective in inhibiting fresh and dry mycelial growth of the *F. oxysporum* sp. *lycopersici*, therefore these two fungicides could be utilized against said pathogen.

Table 1: List of fungicides used in the current study.

#	Brand	Active ingredient	Chemical formula	Chemical family	Type	Dose (100 L^{-1})
1	Defeater	Flumorph (20% WDG)	$C_{21}H_{22}FNO_4$	Morpholine	Systemic	250 g
2	Defeater Plus	Flumorph + Fosetyl-aluminium (45% + 5% WDG)	$C_{21}H_{22}FNO_4$ + $C_6H_{18}AlO_9P_3$	Morpholine + Ethyl Phosphonates	Systemic	500 g
3	Bloom	Myclobutanil (25% EC)	$C_{15}H_{17}ClN_4$	Triazoles	Systemic	40 mL
4	Wisdom	Fosetyl-aluminium (80% WDG)	$C_6H_{18}AlO_9P_3$	Ethyl phosphonates	Systemic	250 g
5	Trifort	Triadimefon (25% WP)	$C_{14}H_{16}ClN_3O_2$	Triazoles	Systemic	100 g
6	Definite	Difenoconazole (10% WDG)	$C_{19}H_{17}C_{12}N_3O_3$	Triazoles	Systemic	300 g
7	Cordate	Kasugamycin (4% WP)	$C_{14}H_{25}N_3O_9$	Cyanoimidazole	Systemic	300 g
8	Flare	Streptomycin (72% SP)	$C_{21}H_{39}N_7O_{12}$	Cyanoimidazole	Systemic	100 g
9	Benedict	Iprobenfos (50% EC)	$C_{13}H_{21}O_3PS$	Organophosphate Esters	Systemic	200 mL
10	Hiten	Fentin hydroxide (50% SC)	$C_{18}H_{16}OSn$	Tri phenyl tin compounds	Localized	250 mL
11	Pyranil	Pyrimethanil (40% SC)	$C_{12}H_{13}N_3$	Pyrimidine	Systemic	300 mL
12	Tebuconazole	Tebuconazole (6% ME)	$C_{16}H_{22}ClN_3O$	Triazoles	Systemic	750 mL
13	Epic	Epoxiconazole(12.5% SC)	$C_{17}H_{13}ClFN_3O$	Triazoles	Systemic	160 mL
14	Picoxystrobin	Strobilurin (25% SC)	$C_{16}H_{18}O_3$	Methoxyacrylates	Localized	300 mL

WP, Wettable powder; **EC,** Emulsifiable concentrate; **WDG,** Water dispersible granules; **SC,** Suspension concentrate; **SL,** Soluble concentrate; **SP ,** Soluble powder; **AS,** Aqueous solution; **ME,** Micro-emulsion

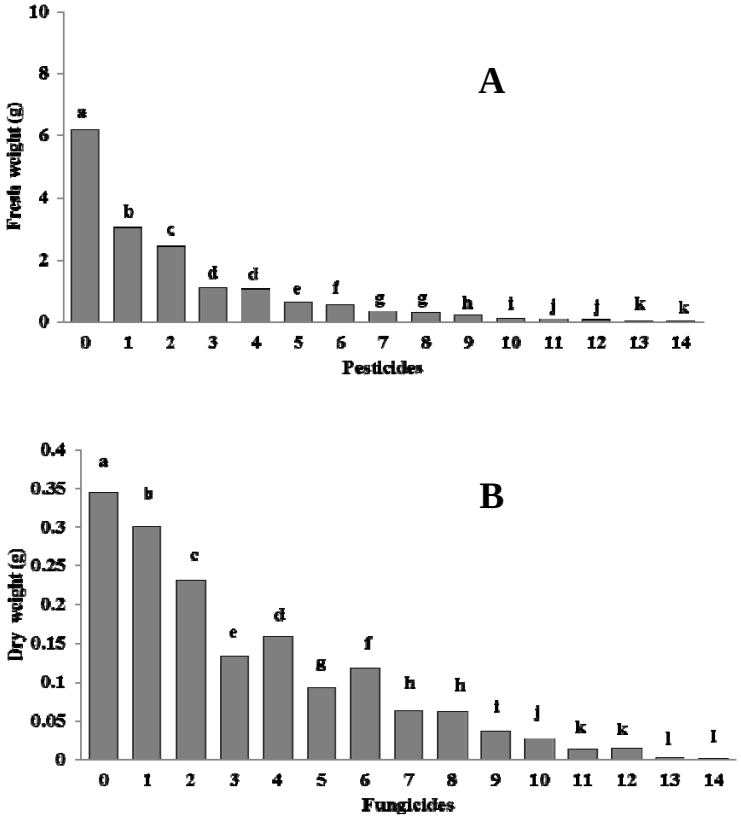

Fig. 1: Fresh (1A) and dry (1B) biomass production of *Fusarium oxysporum* f. sp. *lycopersici* on liquid media after treatment with different fungicides.
0: Control, **1:** Cordate (kasugamycin), 2: Epic (epoxiconazole), 3: Bloom (myclobutanil), 4: Defeater (flumorph), 5: Falre (streptomycin), 6: Definite (difenoconazole), 7: Trifort (triadmefon), 8: Picoxystrobin (strobilurin), 9: Defeater plus (flumorph + fosetyl-aluminium), 10: Pyranil (pyrimethanil), 11: Benedict (iprobenfos), 12: Hiten (fentin hydroxide), 13: Wisdom (fosetyl-aluminium), 14: Treety (tebuconazole)

Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

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