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In vitro inhibitory effect of fungicides and botanicals on mycelial growth and spore germination of *Fusarium oxysporum*

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ABSTRACT

Carbendazim, hexaconzol, bitertanol, myclobutanil, mancozeb, captan and zineb and extracts of *Allium sativum Allium cepa* and *Mentha arvensis* were evaluated for their effect on the inhibition of mycelial growth and spore germination of *Fusarium oxysporum.* Maximum inhibition in mycelial growth was observed in the hexaconozole at 1000 ppm followed by other fungicides at the same concentration. In case of botanicals, inhibition in spore germination was highest at concentration 'S'. It was followed by S/2, S/10, and S/100 concentrations of plant extracts as compared to control which showed least inhibition in spore germination.

Key words: *In vitro*, botanicals, fungicides, *Fusarium oxysporum*, mycelial growth, spore germination.

INTRODUCTION

Many fungi have been identified by various workers as causal organism of fungal rot diseases in all parts of the world. The principal rot diseases on tomato and other vegetables with varying intensities include early blight or *Alternaria* rot caused by *A. solani* (Ell. and Mart.) Jones and Grout, *A. tenuis* (Nees) Syn. and *A. alternata* (Fr.) Kessel, Late blight or *Phytophthora* rot caused by *Phytophthora infestans* (Mont.) Debary. *Alternaria* rot has been considered as the most common disease of tomato and other plants and causes heavy losses in quality of the fruits, thus rendering large quantity of tomato fruits unfit for consumption (Barker and Fauchs,1980.; Hassan, 1996; Singh *et al*.,1997). Chemical control measures have been tested and found effective in the control of diseases (Ogundana and Denis, 1981; Plumbley, 1985). Certain protective fungicides although hazardous to environment are still used for the control of fungal diseases (Nwankiti *et al*., 1990; Vaish and Sinha, 2003). Likewise, use of pesticides of plant origin have been suggested by some workers as alternative to synthetic chemicals in order to counter the potential hazardous effect on the environment associated with the use of synthetic chemicals (Amadioha and Obi,1999; Ejechi and IIondi,1999; Singh, *et. al*., 1997; Amadioha, 2000). Therefore, in the present investigation, inhibition of mycelial growth and spore germination of important post harvest fungus, *Fusarium oxysporum*, exposed to different concentrations of some fungicides and plant extracts of some plants were studied. The aim of the present study was to compare the effect of some selected fungicides and plant extract on *Fusarium oxysporum* mycelial growth and spore germination *in-vitro* and identify the concentration of plant extract that have fungicidal properties.

MATERIALS AND METHODS

Systemic and non-systemic fungicides *viz.*, carbendazim, myclobutanil, bitertanol, hexaconozole, mancozeb, captan and zineb were evaluated for their efficacy on mycelial growth of *Fusarium oxysporum* by food poisoning technique (Falck, 1907; Grower and Moore,1962). Appropriate quantity of each fungicide was separately dispensed in molten sterilized PDA medium to make desired concentrations for each fungicide. The mycelial discs of 5 mm diameter, taken from ten days-old culture of the fungal pathogens were aseptically placed in the center of solidified poisoned PDA. Five replications were maintained for each concentration. The Petri-plates were incubated at 24 ± 2 °C and observations on the mycelial growth of test fungus were recorded after seven days of incubation. The growth of test fungus on non-poisoned PDA served as a control. The percent inhibition in growth due to various fungicidal treatments at different concentrations was computed as follows: Mycelial growth inhibition (%) = [(dc-dt)/ dc] × 100(%)

Where dc = average diameter of fungal colony in control, and dt= average diameter of fungal colony in treatment group

In the present study different concentrations of aqueous extracts of plant leaves and bulbs of mint, *Mentha arvensis* L. onion, *Allium cepa* L. and garlic were evaluated for their effect on the spore germination of *F. oxysporum.* For the preparation of different concentrations of plant extracts, 200g each of leaves and bulbs were washed with sterilized distilled water,

grinded in Mortor and pestle using 200 ml of sterilized distilled water (Bhat and Sivaprakasan, 1994). The material was homogenized for 5 minutes and filtered through double layered muslin cloth followed by Whattman's filter paper No. 1. The filtrate was then centrifuged at 5000 rpm for 10 minutes and considered as standard solutions (S). The solution diluted 2 times (S/2), ten times (S/10), and hundred times (S/100) with sterilized distilled water, and were used to study the spore germination of *F. oxysporum.*

Spore suspension of each isolate of fungus containing at least 20-30 spores per microscopic field was prepared from ten day-old fungal culture. One drop of about 0.1ml of spore suspension was placed in a cavity glass slide containing a drop (about 0.1ml) of different concentration of plant extract. These slides were kept in moist chamber prepared by putting two folds of filter paper in both sides of Petri-plates. These Petri plates were incubated at 24±2 °C for 24 hr. Each treatment was replicated five times. The percent spore germination was recorded using formula given by Kiraly *et al*. (1974).

$$\text{Percent spore germination} = \frac{\text{No. of spores germinated}}{\text{Total no. of spores examined}} \times 100$$

The data collected during these investigations were subjected to appropriate statistical analysis using Minitab software. The data wherever needed was subjected to appropriate transformation as suggested by Gomez and Gomez (1984) before statistical analysis. The method given by Panse and Sukhatme (1978) was also used for statistical analysis of the data.

RESULTS AND DISCUSSION

Fungicides on the mycelial growth

It was revealed from the results (Table 1) that all systemic fungicides at different concentrations significantly inhibit the mucelial growth of *F. oxysporum.* However, the hexaconozole at highest concentration (1000 ppm) caused highest reduction of mycelail growth (8.80 mm) followed by carbendazim (9.40 mm), bitertanol (18.60 mm) and myclobutanil (20 mm) at the same concentration. It was also observed from the study that amongst the non-systemic fungicides, mancozeb was found most effective (14.20mm) in reducing mycelia growth of the fungi followed by captan (20.00 mm) and zineb (22.00 mm). Similar finding were reported by Daradhiyar (1980); Sommer (1982); Kalra and Sokhi (1985); Singh *et al*. (1997); Patel *et al*. (2005) and Banyal *et al.* (2008) to other fungi. However, the fungicides have been shown to completely inhibit the mycelial growth of *Fusarium oxysporum* in Richard medium (Khan *et al*., 1997; Sharma, 2006).

Plant extracts on the spore germination

It was revealed from the results (Table 2) that different concentrations of plant extracts caused significant inhibition in the spore germination. However, the maximum inhibition in the spore germination was found at highest concentration 'S' followed by S/2, S/10, and S/100 as compared to control which showed least inhibition in spore germination. The extract of *A. sativum* at highest concentration ('S') was found to be most effective in reducing the spore germination followed by highest concentration (S) of extract of *A. cepa* and *M. arvensis.* The inhibition in spore germination varies from 43.94% to 9.7% in different concentrations of *A. sativum.* In different concentrations of extract of *Allium cepa*, the inhibition in spore germination ranges from 60.51% to 17.58% whereas

Table1. Effects of different concentrations of fungicides on the mycelia growth of Fusarium oxysporum

Concentration / Treatment	Mycelial growth (mm)*				
Systemic fungicide	125ppm	250ppm	500ppm	1000ppm	Control
Carbendazim	24.00	19.40	14.80	9.40	69.40
Bitertanol	33.00	28.60	21.60	16.80	71.60
Myclobutanil	36.00	32.40	27.40	18.60	71.80
Hexaconozole	23.60	16.80	12.20	8.80	67.80
Non-systemic fungicide	500ppm	1000ppm	1500ppm	2000ppm	Control
Mancozeb	31.40	21.60	17.40	14.20	65.80
Zineb	42.00	35.20	27.00	22.00	70.20
Captan	34.60	29.40	25.00	20.00	70.20

Table 2. Effect of plant extracts on the spore germination of *Fusarium oxysporum*.

Concentration / Treatment	Spore germination (%)*				
	Control	S/100	S/10	S/2	S
Allium sativum	90.50 (72.06)	43.94 (41.52)	32.60 (34.82)	21.29 (27.48)	9.7 (18.20)
Allium cepa	91.90 (73.47)	60.51 (51.07)	51.93 (46.26)	41.26 (39.97)	17.58 (24.79)
Mentha arvensis	92.57 (74.19)	69.39 (56.41)	58.61 (49.96)	39.26 (38.80)	20.44 (26.88)

Value in parentheses indicates

inhibition of spore germination ranges from 69.39% to 20.44% in different concentration of extract of *Mentha arvensis* respectively as compared to untreated control which showed least inhibition in spore germination.

Priviously 31 plants belonging to Asteraceae family (Rai and Acharya, 1999) were tested against the cowpea wilt pathogen, *Fusarium oxysporum f.sp. ciceris*. Ozer *et al*. (2003) evaluated the pectplytic impact of *Allium cepa* "Akugun 12" against two *Fusarium* isolates FOC6 and FOC8. In addition*A. sativum* was known to ac as anti-fungal activity (Sahavaraj et al., 2006). Similar results were found by Misra and Dixit (1976) and Bowers and Locke (2000) using *Allium sativum* against eighteen different fungi including *Fusarium* spp.. Jacob and Siva Prakasan (1994) and Arya *et al*. (1995) studied the antifungal activity of the extracts of various plant species against *Fusarium pallidoroseum* and reported inhibitory effect of extracts of garlic bulbs and *Bignonia* leaves on the mycelial growth of *Fusarium pallidoroseum.* Karade and Sawant (1999), Datar (1999) and Anwar and Khan (2001) observed the same results with the plant extracts of other plants. Our findings are also in agreement with those of Bansal and Gupta. (2000), Bashir (2001) and Bhat (2002). Teqida Menesens *et. al.* (2002) reported antifungal activity of some wild plants against *Penicillium* sp.

In conclusion it was revealed from the study that both fungicides and extracts of plant origin caused inhibition in mycelial growth and spore germination of *Fusarium oxysporum.* However, plant extracts utilized above in this study for *in-vitro* inhibition of spore germination can prove better substitute under field conditions to the management of pathogenic fungi with fungicides which are hazardous to the environment.

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