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# Evaluation of tolerance in *Fusarium oxysporum* f. sp. *lycopersici* and resident biocontrol agents to fungicides for integration of biological and chemical methods of disease management

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Resident biocontrol agents viz., *Trichoderma harzianum, T. viride, Gliocladium virens* and *G. roseum* were tested against *Fusarium oxysporum* f.sp.*lycopersici* causing wilt of tomato through dual culture and production of non volatile antibiotics. *T. harzianum* showed superiority over other resident biocontrol agents in respect of inhibition of mycelial growth of the pathogen. Six fungicides, viz. bavistim (carbendazim), benlate (benomyl), thiram (tetramethyl thiram-disulfide), antracol (propineb), captaf (captan) and indofil M-45 (mancozeb) were evaluated at five different concentrations against both the pathogen and resident biocontrol agents. Antracol, indofil M-45 and captaf were highly suitable for integration with all the resident biocontrol agents as it inhibited the mycelial growth of the pathogen but not as inhibitory to the resident biocontrol agents. Bavistin and benlate connot be used for integration as it totally inhibited the mycelial growth of resident biocontrol agents even at very low concentration.

**Key Words :** Fungicided, tolerance, *Fusarium oxysporum* f. sp. *lycopersici, Trichoderma* spp. and *Gliocladium* spp.

## INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and widely grown vegetables in the world ranking second in importance to popato in many countries. Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Synder and Hansen, is the most devastating disease resulting in 10-50% crop loss (Lukyaneuko, 1991). The pathogen being soil borne persists for longer period in the soil. As propagules of such soil borne pathogen distributed randomly in soil are often not in reach of the fungicides and seed treatment may not persist in effective concentration for sufficiently long time. Soil application of fungicides is expensive, and deleterious to associated non-target soil microflora. So, search for effective biological agents for the management of plant diseases has been intensified in recent years to reduce the dependence on ecologically hazardous chemicals (Mukhopadhyay, 1996). A biocontrol agent must be effective and compatible with latest crop production technology so that its use can be integrated into the production system. *Trichoderma* and *Gliocladium* have gained considerable importance either alone or integrated with lower dose of fungicides for the management of soil borne plant pathogens (Sharma and Mishra, 1995 ; Upadhyay and Mukhopadhyay, 1986). Therefore, different fungicides were evaluated at various concentrations to know the tolerance limit of the biocontrol agent and the results are reported herein.

## MATERIALS AND METHODS

**Isolation of antagonist :** Four resident biocontrol agents, *Trichoderma harzianum, T. viride, Gliocladium virens* and *G. roseum* were isolated from rhizosphere soil of tomato by dilution plate technique (Waksman and Fred, 1922) on *Trichoderma* specific medium (Elad and Chet, 1983) modified by Saha and Pan (1997). The

isolates were maintained on potato dextrose agar (PDA) slant at 4°C for subsequent use.

**Isolation of pathogen :** Virulent culture of *Fusarium oxysporum* f. sp. *lycopersici* was isolated from wilted tomato plant by tissue segment method (Rangaswami, 1958) on potato dextrose agar medium. The culture was purified by hyphal tip culture method (Rangaswami, 1958).

**Dual culture technique :** Four bioagents were screened for their antagonistic activity in dual culture technique (Morton and Stroube, 1955) against *F. oxysporum* f. sp. *lycopersici* on PDA in petriplates. Both the antagonist and the pathogen were inoculated at the same time. Disc of 5 mm cut from the margin of young vigorously growing cultures were placed at the opposite point in the plates. The petriplates were incubated at 28±1°C. The antagonistic activity of each biocontrol agent was measured by using Bell's scale (Bell *et al.*, 1982). Hyphal interactions were studied for seven days by growing them on cellophane membrane placed over solidified PDA in petriplates (Dennis and Webster, 1971b). After both the fungi come in contact with each other, the contact zones was cut using a sharp blade and taken out along the cellophane. It was washed gently with water, mounted under lactophenol-cotton blue over a clean glass slide and observations were made under microscope.

**Effects of non-volatile compounds :** The effects of culture filtrate of the four antagonists were determined against pathogen following the method described by Dennis and Webster (1971a). The biocontrol fungi were cultured in 100 ml sterile potato dextrose broth by inoculating 6 mm mycelial disc from 3 day old culture of the antagonist and incubated at 28 ± 1°C for 8 days with constant shaking. The culture filtrate was collected in a sterilized vacuum flask and passed successively through Whatman No. 42 and millipore filter (0.4 µm pore size) using vacuum pump. The culture filtrate was then added to melted PDA (at 50°C) to obtain final concentration of 5% (v/v).

The medium containing the filtrate was poured into petriplates and inoculated with 6 mm discs of the test pathogens. PDA plate inoculated with the test pathogen not amended with culture filtrate served as check. The petriplates were then incubated at 28 ± 1°C. The radical growth was measured and inhibition percentage as a result of addition of culture filtrate was calculated by using the following formula :

$$I = \frac{C - T}{C} \times 100$$

Where, I = Inhibition percentage

C = Colony diameter in check (mm),

T = colony diameter in treatments (mm).

**Effect of fungicides on radial growth of antagonists and pathogen**

The efficacy of the six fungicides viz., bavistin (carbendazim), benlate (benomyl), thiram (tetramethyl thiuram disulfide), antracol (propineb), captaf (captan) and indofil M-45 (mancozeb) were tested at five different concentrations (5, 25, 50, 100 and 200 ppm) against both the pathogen and the antagonists *in vitro* by using poisoned food technique (Schmitz, 1930) to determine the most effective fungicide(s) for integration with bioagents which may inhibit the growth of the pathogen but not that of the antagonists. Myclial disc (6 mm) of the pathogen and antagonist from the 3 day-old culture were inoculated in fungicides amended PDA plates, separately. Medium without fungicides served as control. The inoculated plates were incubated at 28 ± 1°C. The observations on colony diameter was recorded when the check petriplates were fully covered with the growth of the pathogens and antagonist. The per cent inhibition of the growth in each treatment was calculated.

## RESULTS AND DISCUSSION

A reduction in the growth of the pathogen *F. oxysporum* f. sp. *lycopersici* was observed when it was paired with antagonists 4 cm apart on PDA (Table 1). Within the 8 days of inoculation all the bioagents overgrew the pathogen colony. *T. harzianum* showed the highest antagonistic potentiality and totally overgrwon the pathogen at 5 days. The microscopic examination at the point of contact of the pathogen and the antagonist revealed hyphal coiling, formation of loops, haustoria,

leakage of cell wall followed by cell death. *Trichoderma* and *Gliocladium* spp. represented the most common soil inhabitants exhibiting the hyper parasitic activity against wide range of soil borne plant pathogens (Pan *et. al.* 2001 ; Saha and Pan, 1996 ; Lewis *et al.,* 1996). Mukherjee and Tripathi (2000) observed the swelling and curling of hyphal tip at the point of contact between the antagonist and pathogen. None of the isolates of *Trichoderma* employed in dual culture technique showed coiling of hyphae of *F. oxysporum* f. sp. *lycopersici* but resulted in bulging of hyphae, excessive vacuolation and chlamydospore formation (Padmodaya and Reddy, 1996). Pathogen could not be reisolated from the zone of interaction indicating the total lysis of the pathogen. The results also indicated that there was sufficient selectivity of the biocontrol fungi in antagonistic potentiality towards the pathogen. Elad *et al.* (1980) reported that some species were highly antagonistic to some pathogens yet there was a distinct variability among isolate to isolate in the degree of parasitism.

**Table 1 :** Antagonistic potentiality of four biocontrol agents against pathogen through dual culture technique.

| Biocontrol fungi | Duration required for point of contact (days) | Bell's scale after (days)* | | | | | |
|---|---|---|---|---|---|---|---|
| | | 3 | 4 | 5 | 6 | 7 | 8 |
| *T. harzianum* | 2 | $R_4$-$R_3$ | $R_2$ | $R_1$ | | | |
| *T. viride* | 2 | $R_4$ | $R_3$ | $R_2$-$R_1$ | $R_1$ | | |
| *G. virens* | 2 | $R_4$ | $R_3$ | $R_3$-$R_2$ | $R_2$ | $R_1$ | |
| *G. roseum* | 2 | $R_4$ | $R_4$-$R_3$ | $R_3$ | $R_3$-$R_2$ | $R_2$-$R_1$ | $R_1$ |

* Each insertion is based on observation of five replications.

All the bioagent reduced the mycelial growth of the pathogen through the production of non-volatile antibiotics (Fig. 1). Highest inhibition of radial mycelial growth of the pathogen over the control was recorded in case of *T. harzianum* (73.33%) followed by *G. roseum* (68.89%). Dennis and Webster (1971a) and Mukhopadhyay (1996) have reported that the culture filtrate of *Trichoderma* spp. produced volatile and non-volatile antibiotics and was effective in checking the growth of many soil borne plant pathogens. Mishra (1996) observed that gliovirin and gliotoxin are the principle substances as non-volatile compounds released by *G. virens* that act on *F. oxysporum gladioli.* Non-volatile antibiotic of *T. harzianum* was less effective against *F. oxysporum lycopersici* showing 16.3% growth inhibition that volatile antibiotics (Padmodaya and Reddy, 1996).

All the fungicides were found significantly superior to the control in checking the radial growth of the pathogen (Table 2). Bavistin and benomyl inhibited the cent per cent mycelial growth of both pathogen and antagonist at 25 ppm, and above. Thiram was also toxic to both pathogen and antagonist at 100 ppm. Antracol inhibited the cent per cent mycelial growth of the pathogen and antagonist at 50 and 200 ppm, respectively. Captaf and indofil M-45 were also inhibitory to pathogen at higher dose (200 ppm) but not to antagonist followed by indofil M-45 and captaf as it totally inhibited the mycelial growth of the pathogen but not to the antagonist. Mukherjee and Tripathi (2000) reported that antracol was found most effective against the

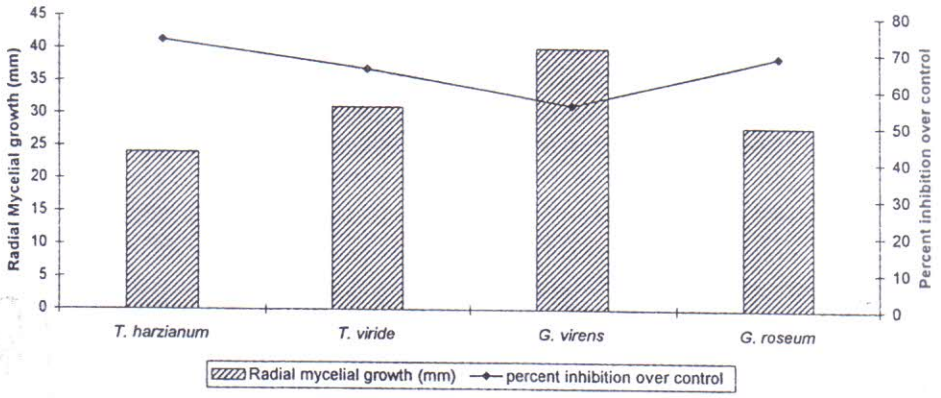


**Fig. 1 :** Effect of non volatile antibiotics of four resident biocontrol fungi on growth of *F. oxysporum.* f. sp. *lycopersici*

pathogens and non inhibitory to the antagonist, even at higher concentration of 50 µg $ml^{-1}$. Khalko *et al.* (2004) reported that dithane M-45 was highly suitable for integration with *Trichoderma* and *Gliocladium* but captaf and blitox with some limitation. Singh *et al.* (1995) reported that the growth of the biocontrol fungi *T. harzianum* were inhibited 63.0 and 49.0% at 500 ppm of dithane M-45 after 3 days of incubation. Sharma *et al.* (2001) stated that captan was also effective for integration with moderate doses as it caused 50% growth inhibition at 169 ppm. Similarly, Sharma and Mishra (1995) observed that captan showed comparatively low inhibition of *T. harzianum* than thiram. Present findings agree to the work of Kumar and Dubey (2001) and Viji *et. al.* (1997) who found

**Table 2 :** Effect of different concentration of fungicides on the growth of *Fusarium oxysporum* f. sp. *lycopersici, T. harzianum, T. viride, G. virens* and *G. roseum*

| Fungicides | Concentration | *F. lycopersici* | | *T. harzianum* | | *T. viride* | | *G. virens* | | *G. rosseum* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Growth (mm) | Inhibition (%) | Growth (mm) | Inhibition (%) | Growth (mm) | Inhibition (%) | Growth (mm) | Inhibition (%) | Growth (mm) | Inhibition (%) |
| | 5 | 22.23 | 75.19 | 16.33 | 81.85 | 19.67 | 70.33 | 24.00 | 73.33 | 18.33 | 79.63 |
| Bavistin | 25 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| (Carbendazim) | 50 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 200 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 5 | 26.00 | 71.11 | 20.00 | 77.78 | 21.00 | 76.67 | 21.00 | 76.67 | 17.67 | 80.37 |
| Benlate | 25 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| (Benomyl) | 50 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 200 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 5 | 81.00 | 10.00 | 71.67 | 20.37 | 74.67 | 17.03 | 74.00 | 17.78 | 71.67 | 20.37 |
| Thiram (Tetra | 25 | 69.67 | 22.59 | 30.00 | 66.67 | 42.67 | 52.59 | 36.33 | 59.63 | 35.00 | 61.11 |
| methyl thiram | 50 | 46.67 | 48.14 | 0 | 100 | 15.33 | 82.97 | 12.33 | 86.30 | 0 | 100 |
| disulfide) | 100 | 21.33 | 76.30 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 200 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 5 | 56.33 | 37.41 | 79.67 | 11.48 | 83.33 | 7.41 | 75.00 | 16.67 | 82.00 | 8.88 |
| Antracol | 25 | 13.67 | 84.81 | 61.33 | 31.86 | 65.00 | 27.78 | 61.00 | 32.22 | 68.67 | 23.70 |
| (Propineb) | 50 | 0 | 100 | 34.33 | 61.85 | 38.67 | 57.03 | 36.67 | 59.25 | 36.67 | 59.95 |
| | 100 | 0 | 100 | 12.33 | 86.30 | 20.00 | 77.78 | 15.33 | 82.97 | 16.33 | 81.86 |
| | 200 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 | 0 | 100 |
| | 5 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 |
| Captaf | 25 | 86.00 | 4.44 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 |
| (Captan) | 50 | 70.00 | 22.22 | 84.00 | 6.66 | 85.00 | 5.55 | 82.33 | 8.52 | 78.67 | 12.58 |
| | 100 | 57.67 | 35.92 | 76.00 | 15.55 | 72.00 | 13.33 | 75.00 | 16.67 | 73.67 | 18.14 |
| | 200 | 35.00 | 61.11 | 70.67 | 21.48 | 64.00 | 28.89 | 66.67 | 25.92 | 63.33 | 29.63 |
| | 5 | 81.67 | 9.25 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 |
| Indofil M-45 | 25 | 72.67 | 19.25 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 | 90.00 | 0 |
| (Mancozeb) | 50 | 66.33 | 26.30 | 81.67 | 9.25 | 83.33 | 7.41 | 85.67 | 4.81 | 82.00 | 8.88 |
| | 100 | 43.67 | 51.48 | 75.00 | 16.66 | 71.00 | 21.11 | 79.00 | 12.22 | 73.33 | 18.52 |
| | 200 | 19.00 | 78.89 | 73.00 | 18.99 | 63.66 | 29.27 | 75.00 | 16.67 | 68.00 | 24.44 |
| Control | — | 90.00 | — | 90.00 | — | 90.00 | — | 90.00 | — | 90.00 | — |
| CD (P = 0.05) – fungicides | | 1.73 | | 3.82 | | 1.33 | | 1.29 | | 1.18 | |
| CD (P = 0.05) – concentrations | | 1.58 | | 3.49 | | 1.04 | | 1.21 | | 1.08 | |
| CD (P = 0.05) – fungicides X concentration | | 3.95 | | 8.55 | | 2.53 | | 2.93 | | 2.65 | |

that carbendazim to be toxic to the pathogen as well as *Trichoderma* and *Gliocladium* spp. the result also showed the differential response of antagonistic fungi to antracol, indofil M-45 and other fungicides. This may be due to their inherent resistance to most fungicides and their ability to degrade chemicals.

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