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SCREENING OF *TRICHODERMA* SPECIES FOR TOLERANCE TO FUNGICIDES

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Abstract

Fungicides *viz.,* Benomyl, Topsin- M, Carbendazim and Cuprocaffro were used at different concentrations i.e., 0, 1, 10, 100, 1000 and 10,000 ppm a.i. to evaluate *Trichoderma* species viz. *T. harzianum*, *T. pseudokoningii*, *T. longibrachiatum, T. viride* for tolerance to fungicides. Topsin-M and Carbendazim were the most effective fungicides that inhibit the growth of *Trichoderma* species even at low concentration. Topsin-M completely suppressed the growth of *T. harzianum* at 10ppm.

Introduction

Some soilborne root infecting fungi are difficult to eradicate because they produce resting structure like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions (Baker & Cooke, 1974). Use of fungicides for the control of soil borne diseases is costly and also produces environment and health hazards to men and also adversely affects the beneficial microorganisms in soil (Dłużniewska, 2003). This has diverted the attention of plant pathologist towards alternate methods for the control of plant diseases.

Trichoderma species are known to suppress infection of root by soilborne pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Pythium* species on various crops (Ehtesham *et al*., 1990; Benítez *et al*., 2004; Adekunle *et al*., 2001; Lutchmeah & Cooke, 1985; Howell, 1982). Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Benítez *et al*., 2004; Dubey *et al*., 2007; Yedidia *et al*., 1999). *T. harzianum* has shown effective control of root infecting fungi and root-knot nematodes (Spiegel & Chet, 1998; Sun & Liu, 2006). *T. harzianum* isolated from rhizome rot suppressive soils reduced the disease and increased plant growth and yield (Ram *et al.,* 1999).

It has been reported that many *Trichoderma* species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide (Omar, 2006).

The combined use of biocontrol agents and chemical pesticides has attracted much attention in order to obtain synergestic or additive effects in the control of soilborne diseases (Locke *et al*., 1985). Reduced amount of fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonist (Hjeljord & Tronsmo, 1998). Srinivas & Ramakrishnan (2002) have reported that integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually.

During the present studies, *Trichoderma* species *viz*., *T. harzianum, T. longibrachiatum, T. pseudokoningii* and *T. viride* were screened for tolerance to fungicides like Topsin-M (Thiophenate methyle), Carbendazim, Cuprocaffaro (Copperoxychloride) and Benomyl.

Materials and Methods

Species of *Trichoderma viz., T. harzianum, T. Pseudokoningii, T. longibrachiatum,* and *T. viride* were obtained from Karachi University Culture Collection (KUCC). Tolerance to fungicides in *Trichoderma* species was evaluated using food poison method. Fungicides viz., Topsin-M, Carbendazim, Cuprocaffaro and Benomyl were added to Potato Sucrose Agar medium (PSA: potato-200g, sucrose-20g, Agar-20g, water 1L) to get final concentration of 1, 10, 100, 1000 and 10,000ppm a.i. PSA without fungicide served as control. A 5mm inoculum disc of *Trichoderma* species was cut from the margin of actively growing colony and placed in centre of each Petri plate. Petri plates were incubated at 25±2ºC. Radial growth of *Trichoderma* species was observed daily.

Results

***Trichoderma harzianum*:** *T. harzianum* showed growth on medium containing benomyl @ 1 and 10ppm but no growth was observed when benomyl was used @ 100, 1000 and 10,000 ppm. In 1 and 10ppm treatments, growth of biocontrol agent showed negative correlation with concentration of benomyl for upto 4 days but plates were filled on fifth day (Fig. 1). Topsin-M proved to be highly toxic and very little growth of *T. harzianum* was observed in treatments containing Topsin-M @ 1 and 10 ppm. No growth was observed at 100ppm or higher concentrations (Fig. 1). Carbendazim was found to be more effective since it completely inhibited the growth of *T. harzianum* even @ 10ppm where very minute mycelial growth was observed after 3 days of incubation where Carbendazim was used @ 1ppm (Fig. 1). *T. harzianum* grew easily in medium containing Cuprocaffaro @ 1, 10 and 100ppm and plates were filled on 4th day of incubation. Growth of *T. harzianum* was significantly reduced where Cuprocaffaro was used @ 1000 and 10,000ppm (Fig. 1).

***Trichoderma pseudokoningii*:** A negative correlation was observed between concentration of benomyl and the growth of *T. pseudokoningii* for upto 3 days of incubation. Growth in 100 and 1000ppm treatments started after 48 hours and in 10,000ppm treatment after 72 hours of incubation. Growth of the fungus in plates containing benomyl @ 10ppm or higher concentration was significantly less than the control even after 6 days of incubation (Fig. 2) Concentration of Topsin-M showed a negative correlation with growth of *T. pseudokoningii* during 6 days of incubation. Growth in 10 and 100ppm treatments started after 48 hours, in 1000ppm after 72 hours and in 10,000ppm after 96 hours of incubation. Suppression in growth was less in 1ppm treatment but use of Topsin-M @ 10ppm or more showed significant suppression in growth of *T. pseudokoningii* (Fig. 2). No growth of *T. pseudokoningii* was observed in Carbendazim @10,000ppm treatment. Growth in 10ppm started after 48 hours and in 100 and 1000ppm after 72 hours of incubation. A significant negative correlation between the growth of *T. pseudokoningii* and the concentration of Carbendazim was evident during 6 days of incubation but effect in 10, 100 and 100ppm treatments was less than the effect of Topsin-M (Fig. 2). Use of Cuprocaffaro @ 1, 10 and 100ppm showed a negative correlation with the growth of *T. pseudokoningii* for upto 5 days but plates in all the treatments were filled after 6 days of incubation. Growth in 1000 and 10,000ppm treatments started after 72 hours of incubation and was very much reduced as compared to control (Fig. 2).

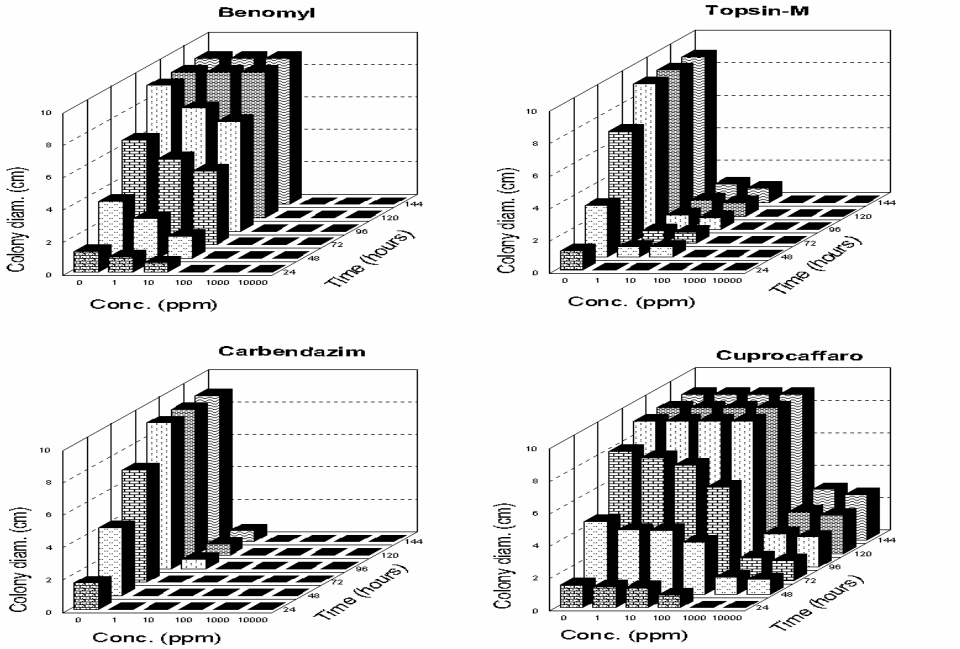

Fig. 1 Effect of fungicides on *In vitro* growth of *T. harzianum.*

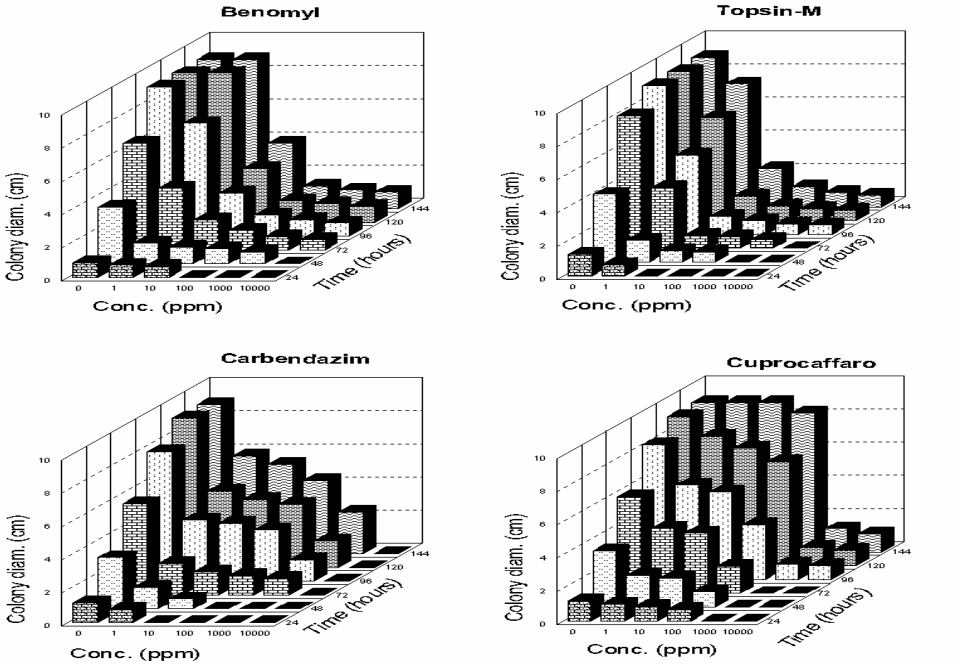

Fig. 2. Effect of fungicides on *In vitro* growth of *T. pseudokoningii.*

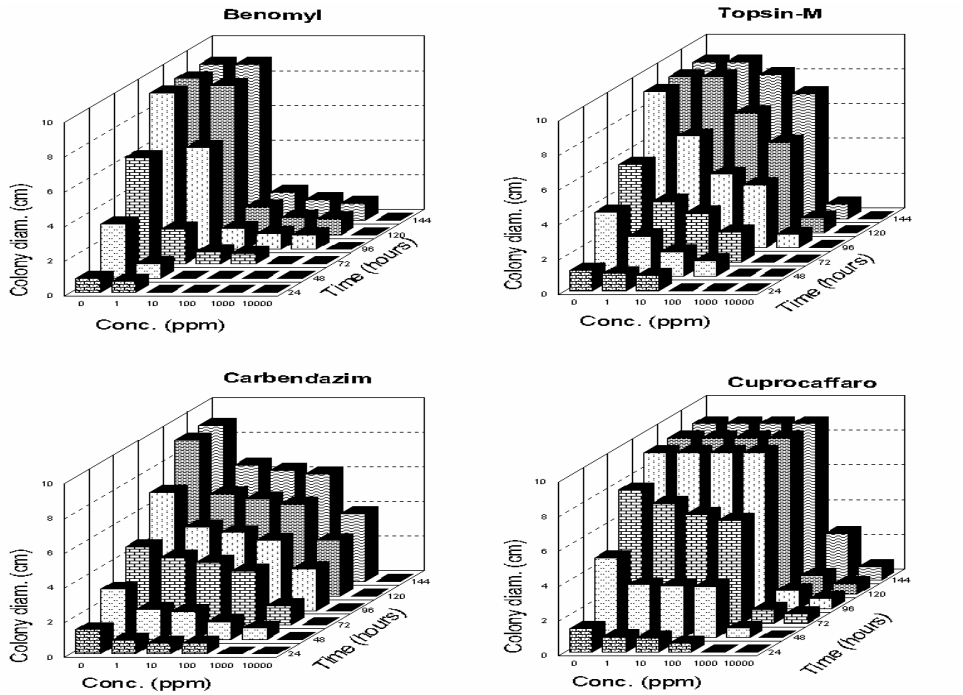

Fig. 3. Effect of fungicides on *In vitro* growth of *T. longibrachiatum.*

Trichoderma longibrachiatum: Complete inhibition of growth of *T. longibrachiatum* was observed where benomyl was used @10,000ppm. No growth was observed after first 48 hours of incubation in benomyl @10 and 100ppm treatments. In 1000ppm treatment, the growth of *T. longibrachiatum* started after 96 hours of incubation. Plates were filled in benomyl @ 1ppm treatment after 6 days of incubation, however the growth of *T. longibrachiatum* suppressed significantly where benomyl was used @ 10ppm or more (Fig. 3). Topsin-M @ 10,000ppm completely prevented the growth of *T. longibrachiatum*. Growth started after 72 hours of incubation where Topsin-M was used @ 1000ppm treatment and after 48 hours in 100ppm treatment. Plates were filled after 5 days of incubation @ 1ppm treatment. The only significant suppression in growth of *T. longibrachiatum* was observed where Topsin-M was used @ 1000 and 10,000ppm (Fig. 3). Growth of *T. longibrachiatum* was completely suppressed where Carbendazim was used @ 10,000ppm. There was a negative correlation between the growth of *T. longibrachiatum* and the concentration of Carbendazim. The plates were not filled after 144 hours even in 1ppm treatment (Fig. 3). There was a sharp decline in growth of *T. longibrachiatum* where Cuprocaffaro was used @ 1000 and 10,000ppm. Growth started after 24 hours in 1000ppm and after 48 hours in 10,000ppm treatments. Growth of *T. longibrachiatum* was not inhibited where Cuprocaffaro was used @ 1, 10 and 100ppm and plates were filled after 72 hours of incubation (Fig. 3).

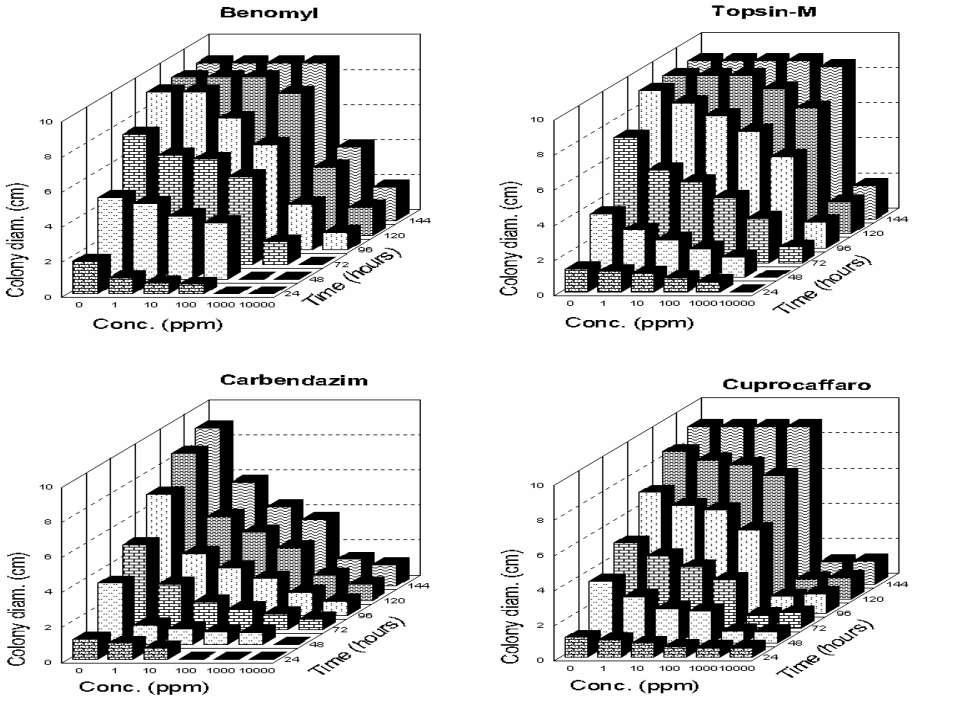

Fig. 4. Effect of fungicides on *In vitro* growth of *T. viride.*

Trichoderma viride: Growth of *T. viride* showed negative correlation with the concentration of benomyl, plates were filled after 72, 96 and 120 hours in 1, 10 and 100ppm treatments, respectively. Reductions in growth were observed in benomyl @ 1000 and 10,000ppm treatments as compared to control. Growth started after 48 hours of incubation in 1000ppm and after 72 hours in 10,000ppm treatments (Fig. 4). Topsin-M used @ 1, 10, 100 ppm was not able to suppress the growth of *T. viride.* Plates were filled @1 and 10ppm treatments after 96 hours and @100 and 1000ppm treatments after 120 hours of incubation. There was sharp decline in growth of *T. viride* when Topsin-M was used @ 10,000ppm (Fig. 4). There was a significant negative correlation between the growth of *T. viride* and the concentration of Carbendazim even after 144 hours of incubation. Growth in 100 and 1000ppm treatments started after 24 hours and in 10,000ppm treatment after 48 hours of incubation. Overall growth of *T. viride* was slow as compared to control (Fig. 4). Cuprocaffaro was also not able to inhibit the growth of *T. viride* when used @ 1, 10 and 100ppm and plates were filled after 120 hours of incubation. However, there was a sharp decline in growth of *T. viride* in 1000 and 10,000ppm treatments where growth started after 24 hours of incubation and was very much reduced as compared to control (Fig. 4).

Discussion

Most of the time, fungicides produce undesirable effects on non-targeting organisms, so the use of microorganisms that antagonize plant pathogenic fungi is risk free (Benítez,

et al., 2004). Moreover, the combination of fungicide tolerant biological control agents with reduced levels of fungicide integrated control strategies would promote the degree of disease suppression similar to that achieved with full dosage of fungicides (Monte, 2001). There are reports where the biocontrol agents, which can tolerate fungicides up to a certain level, were mixed with fungicides and resulted in eradication of diseases (De Cal *et al.,* 1994).

Among the four *Trichoderma* species *viz*., *T. harzianum*, *T. pseudokoningii*, *T. longibrachiatum* and *T. viride*, the growth of *T. harzianum* was mostly inhibited by the fungicides except Cuprocaffaro; the most effective fungicide was Carbendazim followed by Topsin-M. Similarly, *T. pseudokoningii* was suppressed by benomyl and Topsin-M. Except benomyl which inhibit the growth of *T. longibrachiatum,* no other fungicide was able to suppress the growth of this fungus. In case of *T. viride* no fungicide was able to inhibit the growth. Carbendazim suppress the growth to some extent but not completely. Latore *et al.,* (1997) suggested that antagonistic activity of biocontrol agents might be effective if it is integrated with other control practice and may result in acceptable levels of disease control with reduce level of chemicals use. The result of the present screening would help in the selection of biocontrol agents, which can be used, with reduced dose of selected fungicides for the control of plant pathogenic fungi.

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