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In-vitro evaluation of systemic fungicides against *Fusarium oxysporum* f. sp. *lycopersici* and their compatibility with bioagents

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Abstract
An *in-vitro* study was carried out for the screening of commonly used commercially available potential systemic fungicides *viz.* Topsin M and Carbendazim against *Fusarium oxysporum* F. sp. *lycopersici* at three different concentrations (50 ppm, 100 ppm and 200 ppm) which revealed that Carbendazim at 200 ppm has the strongest anti-mycotic potential followed by Carbendazim at 100 ppm showing 96.36 and 94.72 percent inhibition respectively. The *in-vitro* compatibility tests of *Trichoderma harzianum* with systemic fungicides indicated that lesser concentration of the two systemic fungicides had lesser inhibitory effect as compared to higher concentration. Carbendazim @ 50 ppm proved out to be most compatible showing minimum percent inhibition (72.59). The two potential bacterial antagonists *viz. Bacillus subtilis and Pseudomonas fluorescens* were compatible with different concentrations (50 ppm, 100 ppm, 200 ppm) of Carbendazim and Topsin M.

Keywords: Topsin M, carbendazim, *Fusarium oxysporum* f. sp. *lycopersici*, *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*

Introduction
Fusarium oxysporum F. sp. *lycopersici* is an important highly prevalent and destructive soil borne ascomycetous fungus (Sudhamoy *et al.,* 2009) [1]. The pathogen occurs worldwide throughout most of the tomato-growing countries causing vascular wilt that severely affects the crop (Moretti *et al.* 2008) [2], and the disease is considered as one of the main soil-borne systemic diseases (Schwarz and Grosch 2003) [3]. It causes significant losses in tomato production both in greenhouse and field grown tomatoes in warmer areas globally (Nusret ozbay and Steven 2004) [4]. The pathogen is soil as well as seed-borne in nature and causes vascular wilts by infecting plants through the roots and growing internally through the cortex to the stele (Bowers and Locke, 2000) [5] thereby causing xylem browning or blackening. Seedlings infected by the wilt fungus show yellowing of the lower leaves, often only on one side of the plant succeeded by reduced growth and eventually death of entire plant (Kiran kumar *et al.,* 2008) [6].

Several disease management strategies e.g. cultural technique, biological control, resistant cultivars, crop rotation and chemical control are available (Kamal *et al.* 2009) [7]. But with the purpose of reducing the economic losses caused by soil-borne diseases, generally utilization of chemical fungicides is considered as an easy and attractive approach for the farmers. Due to their relatively low cost, ease of use, and effectiveness, fungicides have become the primary means to combat fungal diseases (Vinale *et al*., 2008; Sharma, 2011; Dias, 2012) [8, 9, 10]. However, intensified utilization of fungicides has resulted in harmful effect on non-target organisms, development of resistance races of pathogens and carcinogenicity (Doley and Jite, 2012) [11]. Antagonistic microbes on the other hand are an attractive, environment friendly and inexpensive alternative over chemical fungicides (El-Bramawy and El-Sarag, 2012) [12]. The natural control of several phytopathogens is based on the presence of suppressive soils where several biocontrol microorganisms belonging to *Trichoderma*, *Pseudomonas* and *Bacillus* genera are detected (Weller *et al.,* 2002 and Huang *et al.,* 2005) [13, 14]. Numerous bacteria and fungi, including *Trichoderma* isolates, or combinations of microorganisms, collected from field tomato plants have proved to be effective in controlling Fusarium wilt in tomato (Srivastava *et al.,* 2010) [15]. Integrated use of fungicides and biological agents for the management of soil-borne diseases can prove out to be an efficient and eco-friendly approach. Supplementation of fungicides at reduced rates in combination with biocontrol agents has significantly enhanced disease control, compared to treatments with biocontrol agent alone (Frances *et al*., 2002; Buck, 2004) [16, 17].

The current study was conducted *in-vitro* to assess the antimycotic activity of commonly used commercially available potential systemic fungicides at different concentrations against *Fusarium oxysporum* f. sp. *lycopersici* and to test their compatibility with fungal and bacterial bioagents.

Materials and Methods

The *in-vitro* studies were carried out under aseptic conditions in the Bio-control Laboratory of Plant Pathology Department, Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, U.P., India.

In vitro screening of systemic fungicides against *Fusarium oxysporum* f. sp. *lycopersici*

The poisoned food technique (Shravelle, 1961) [18] was followed to evaluate the efficacy of systemic fungicides (Carbendazim and Topsin M) in inhibiting the mycelial growth of *Fusarium oxysporum* f. sp. *lycopersici.* The pathogen was grown on PDA medium for nine days prior to setting up of the experiment. The PDA medium was prepared and melted. The fungicidal suspension was added to the melted medium to obtain the required concentrations (50 ppm, 100 ppm, 200 ppm). Twenty mL of poisoned medium was poured in each sterilized Petri plate. Suitable check was maintained without addition of fungicide. Mycelial disc of 5 mm was taken from the periphery of nine days old colony and was placed at the center of the poisoned plates and incubated at 25±1°C and radial growth of the pathogen was measured in mm at intervals of 24 hours up to eight days. Three replications were maintained for each treatment. Percent inhibition of mycelial growth of the fungus was calculated by using the formula given by Vincent (1927) [19].

$$\text{Percent growth inhibition} = \frac{\text{Growth in control - Growth in treatment}}{\text{Mycelial growth in control}} \text{ X } 100$$

Compatibility of *Trichoderma harzianum* with systemic fungicides

Compatibility of *Trichoderma harzianum* with the two systemic fungicides *viz.*, Carbendazim and Topsin M was tested by using "Poison Food Technique" (Shravelle, 1961) [18]. The appropriate amount of each fungicide based on active ingredient was added to an autoclaved potato dextrose agar medium to obtain the desired concentrations (50 ppm, 100 ppm, 200 ppm) of both the fungicides. Medium without the fungicide served as control. Twenty mL medium was poured into Petri plates in 3 replicates and after solidification, each plate was inoculated with a 5 mm mycelial disc of *Trichoderma harzianum.* The inoculated Petri plates were incubated for 7 days at 25±1 °C. After incubation, radial growth was measured in mm and percent growth inhibition was calculated by applying the formula given by Vincent (1947) [20].

Compatibility of *Pseudomonas fluorescens* and *Bacillus subtilis* with systemic fungicides

The compatibility of *Pseudomonas fluorescens* with systemic fungicides (Carbendazim and Topsin M) was tested by Well Diffusion Technique (Magaldi, 2004; Valgas, 2007) [21, 22] using nutrient agar medium. 15-20 mL of NA was poured into Petri plates and allowed to solidify. The agar plate surface was then inoculated by spreading 1 mL of *Pseudomonas fluorescens* (suspension having CFU Count: $2x10^8$/ml) over the entire agar surface. Then, 4 holes with a diameter of 5 mm were punched aseptically with a sterile cork borer ensuring proper distribution of holes in the periphery and the fungicidal suspension at required concentrations (50 ppm, 100 ppm, 200 ppm) were introduced into the wells in different plates. The petri plates without fungicidal suspension served as control. The plates were then incubated at 25 ± 1 ^{0}C for 5 days and observed for the inhibition zone. Experiment was replicated thrice. The fungicidal suspension diffuses in the agar medium creating a zone of inhibition in case of incompatibility of bacterial bioagent with the fungicide. Absence of inhibition zone indicated the compatibility with respective bacterial strains (Fukui *et al*., 1994) [23]. A similar procedure was followed for testing the compatibility of *Bacillus subtilis* with systemic fungicides (Carbendazim and Topsin M).

Results and Discussions

In-vitro evaluation of different concentrations of systemic fungicides against *Fusarium oxysporum* f. sp. *lycopersici*

The results of in vitro studies revealed that Carbendazim at 200 ppm has the strongest anti-mycotic potential followed by Carbendazim at 100 ppm showing 96.36 and 94.72 percent inhibition respectively (Table 1 and Fig. 1 & 2). The findings clearly indicated that both systemic fungicides have strong anti-mycotic potential and can be effectively use to manage wilt in tomato.

Table 1: Percent inhibition of *F. oxysporum* f. sp. *lycopersici* in presence of different concentrations of Carbendazim & Topsin M

Treatments	Concentration (ppm)					
	50 ppm		100 ppm		200 ppm	
	Radial Growth*	% inhibition	Radial Growth*	% inhibition	Radial Growth*	% inhibition
Carbendazim	2.1	93.07	1.6	94.72	1.1	96.36
Topsin M	5.7	81.19	4.5	85.15	3.6	88.11
Control (untreated)	30.3					
SE	0.4					
CD@5%	2.2					

* Mean of three replications

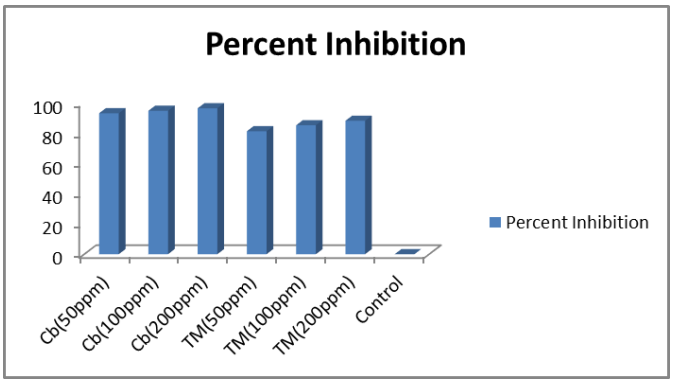


Fig 1: Percent inhibition of *Fusarium oxysporum* f. sp. *lycopersici* in presence of different concentrations of Carbendazim and Topsin M

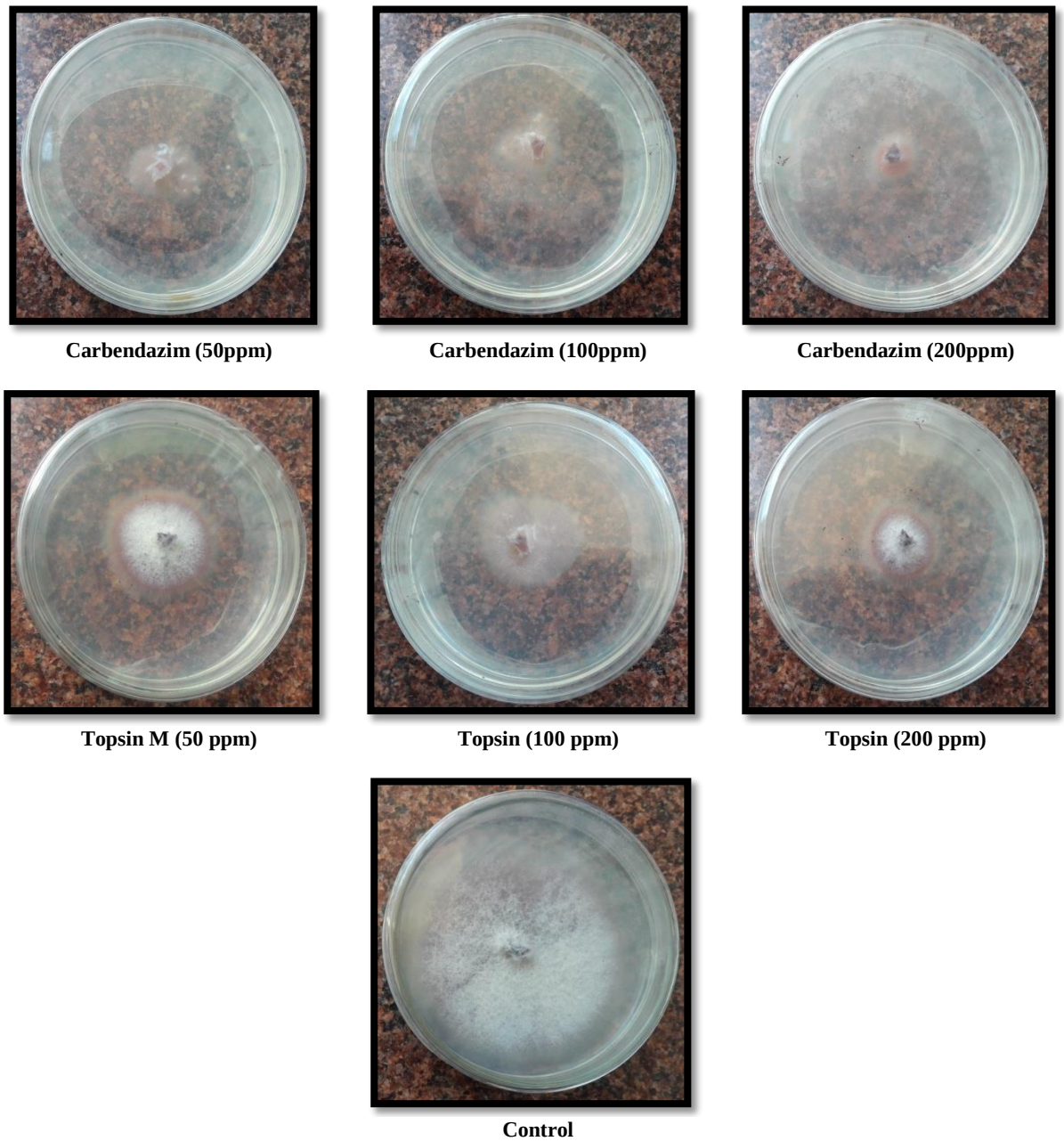


Fig 2: *In-vitro* evaluation of different concentrations of Carbendazim & Topsin M against *Fusarium oxysporum* f. sp. *Lycopersici*

Similar works that proved Carbendazim as one of the most effective fungicide has earlier been done by Song *et al.* (2004) [24] ; Harender Raj *et al.* (2005) [25]; Chhata and Jeewa Ram (2006) [26] ; Raju *et al.* (2008) [27] ; Singh (2009) [28] and Srivastava *et al.* (2011) [29].

Compatibility of *Trichoderma harzianum* with systemic fungicides

The results indicated that lesser concentration of the two systemic fungicides had lesser inhibitory effect as compared to higher concentration. Carbendazim @ 50 ppm proved out to be most compatible showing minimum percent inhibition

(72.59) whereas maximum percent inhibition (80.19) was observed with Topsin M @ 200 ppm (Table 2 and Fig. 3 & 4).

Table 2: Percent inhibition of *Trichoderma harzianum* over control in presence of different concentrations of Carbendazim and Topsin M

Treatments	Concentration (ppm)					
	50 ppm		100 ppm		200 ppm	
	Radial Growth*	% inhibition	Radial Growth*	% inhibition	Radial Growth*	% inhibition
Carbendazim	9.23	72.59	8.47	74.84	7.77	76.92
Topsin M	8.30	75.35	7.77	76.92	6.67	80.19
Control (untreated)	33.67					
SE	0.35					
CD@5%	1.07					

* Mean of three replications

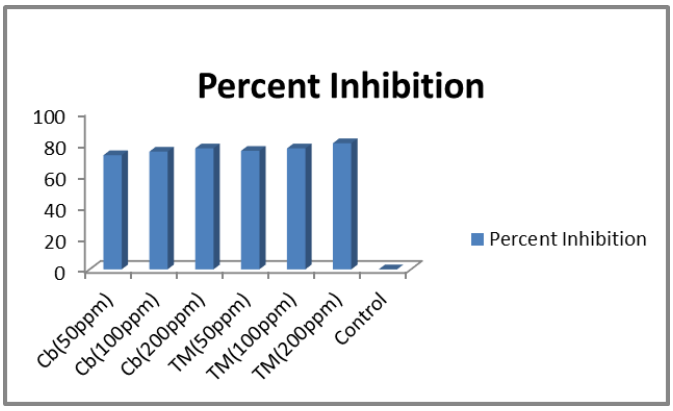
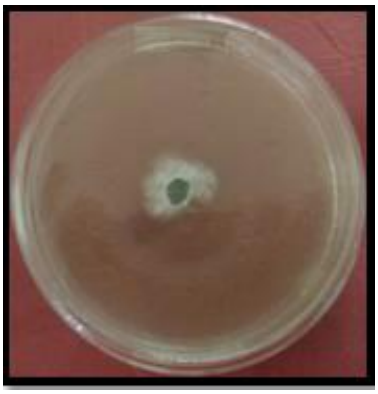
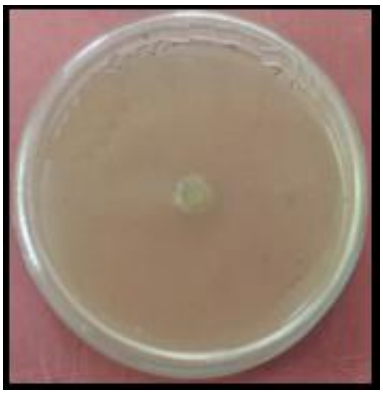


Fig 3: Percent inhibition of *Trichoderma harzianum* over control in presence of different concentrations of systemic fungicides



Topsin (50 ppm)

Topsin (100 ppm)



Topsin (200 ppm\)

Carbendazim (50 ppm)

Carbendazim (100 ppm)

Carbendazim (200 ppm)

Control

Fig 4: Compatibility of *Trichoderma harzianum* with different concentrations of Carbendazim & Topsin M

Similar result was obtained by Tiwari *et al.* (2004) [30] who evaluated the *in vitro* efficacy of different fungicides against *Trichoderma harzianum* @ 1500 ppm and found that the mycelial growth of *Trichoderma harzianum* was completely inhibited by carbendizam at high concentration (1500 ppm).

Compatibility of *Pseudomonas fluorescens* and *Bacillus subtilis* with systemic fungicides

The results revealed absence of a distinct inhibition zone around the wells indicating compatibility of the two bacterial bio-control agents (*Pseudomonas fluorescens* and *Bacillus subtilis*) with different concentrations (50 ppm, 100 ppm, 200 ppm) of systemic fungicides (Carbendazim and Topsin M) as presented in (Fig. 4 & 5).

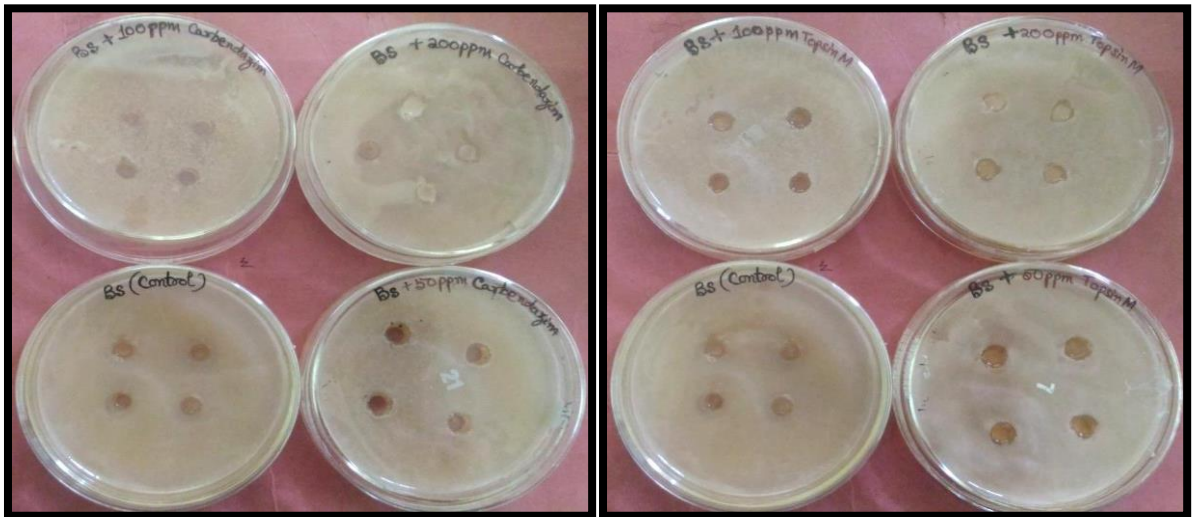


Fig 4: Compatibility of *Bacillus subtilis* with different concentrations of systemic fungicides (A) Carbendazim (B) Topsin M (Well diffusion technique)

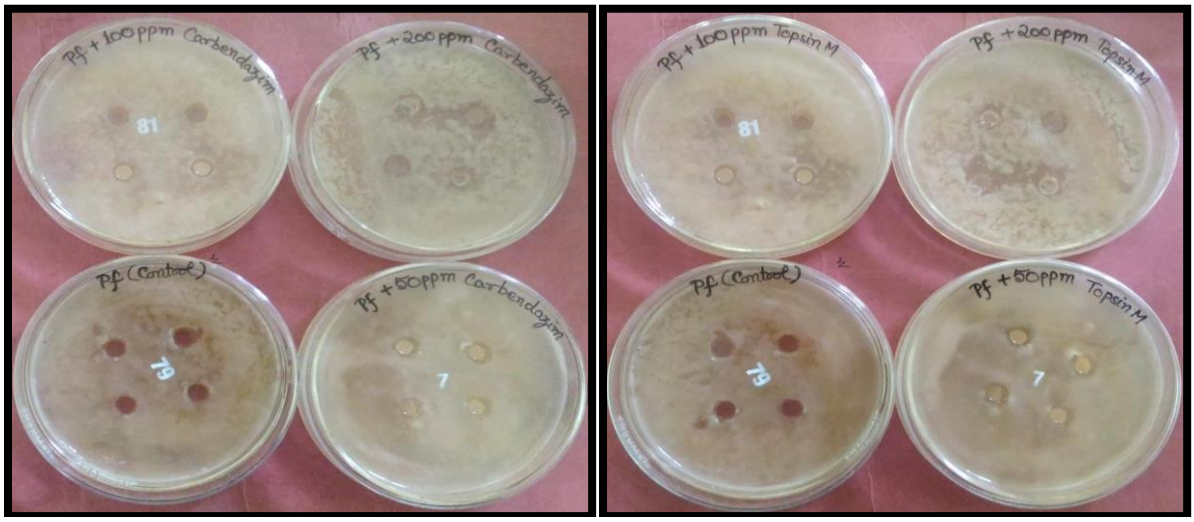


Fig 5: Compatibility of *Pseudomonas fluorescens* with different concentrations of systemic fungicides (A) Carbendazim (B) Topsin M (Well diusion technique)

Similar work has been done by Vyas (1987) [31] who reported that *Trichoderma sp.* and *B. subtilis* showed high degree of tolerance to Carbendazim and Vidhyasekharan *et al.* (1995) [32] who reported that thiram and carbendazim were not inhibitory to *P. fluorescens* under in vitro conditions. Khan and Gangopadhyay (2008) [33] also tested the compatibility of *Pseudomonas fluorescens* with the fungicides and revealed that carboxin, and carbendazim were least toxic to *P. fluorescens* strain.

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