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Management of Fusarium wilt of bell pepper through fungicides

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Abstract
Eight fungicides were evaluated *in vitro* and *in vivo* against of *Fusarium oxysporum* f. sp. *capsici*, causal agent of Fusarium wilt of bell pepper. Mycelial growth of pathogen was significantly inhibited by all tested fungicide. carbendazim at 50, 100, 150 ppm and tebuconazole + trifloxystrobin at 500 ppm were found most effective and resulted in complete inhibition in mycelial growth of the pathogen. mancozeb was found to be less effective as compared to others and resulted in 51.85 per cent mycelial growth inhibition even at high concentration of 750 ppm. Under field evaluation of fungicides against Furasium wilt disease of bell pepper, all fungicides reduced disease incidence compared to the untreated control. Soil drenching of carbendazim (0.2%), carbendazim + mancozeb (0.25%) and tebuconazole + trifloxystrobin (0.1%) were found most effective in reducing the disease completely and increasing the fruit yield. mancozeb was found least effective with 58.83 per cent reduction in disease.

Keywords: Trifloxystrobin, tebuconazole, hexaconazole, azoxystrobin

Introduction
Bell pepper (*Capsicum annuum* L. var. *grossum* Sendt.), commonly known as sweet pepper or Capsicum or Shimla Mirch, is an important high value crop with respect to nutritive value. In India, it is grown in an area of 46,000 ha with production of 327,000 MT (NHB, 2017). It is mainly cultivated in Himachal Pradesh, Uttarakhand, Uttar Pradesh, Maharashtra, Gujarat, Karnataka, Tamil Nadu and Bihar. It is grown as summer crop in Himachal Pradesh, Jammu and Kashmir, Uttarakhand, Arunachal Pradesh and Darjeeling district of West Bengal and as autumn crop in Maharashtra, Karnataka, Tamil Nadu and Bihar. In Himachal Pradesh, it is extensively grown as cash crop in Zone-I, Zone-II and Zone-III and covers an area of 2,500 hectares with production of 58,290 MT (NHB, 2017). Bell pepper is a warm season and chilling sensitive crop. It is an important off-season vegetable of western Himalayas and offers potential for boosting economy of farmers of hilly regions.

Fusarium wilt, caused by *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv, is one of the most destructive and important disease of bell pepper which reduces overall yield of crop. For the management of Fusarium wilt disease, only few conventional fungicides are available (Poddar *et al.*, 2004; Naik *et al*, 2007; Rather *et al*., 2012) [13, 10, 14], but they do not provide an adequate level of protection. Moreover, the continuous use of few selected fungicides raises the concerns regarding the development of fungicide- resistant strains of the pathogen. Hence the present investigations were carried out to evaluate new generation-fungicides, including combi-products having two different mode of action, for the management of Fusarium wilt disease of bell pepper.

Material and Methods
Infected vascular tissues from stem and root regions of bell pepper plants showing wilt symptoms were collected and isolation of associated pathogen was done by following standard isolation method under aseptic condition on potato dextrose agar (PDA) medium (Dhingra and Sinclair, 1995) [7]. Based on morphological characters viz., colony growth, color, conidia shape and size, and compared with standard description given by Booth (1971) [5] in key "The Genus *Fusarium*", the associated pathogen was identified as *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv.

In vitro evaluation of fungicides
Eight systemic and non-systemic fungicides (Table 1) were tested under *in vitro* to study the inhibitory effect of these fungicides on mycelial growth of *F. oxysporum* f. sp. *capsici* by following poisoned food technique (Falck, 1907; Dhingra and Sinclair, 1995) [8, 7].

Double strength PDA medium was prepared by doubling the quantity of all the constituents except distilled water and the medium was sterilized at 15 lb p.s.i. pressure for 20 minutes. Simultaneously, solutions of different fungicides were also prepared in sterilized distilled water at double concentration so as to get desired concentration of fungicides after mixing the fungicide solutions in the equal volume of double strength media. Solutions of different fungicides were added separately to equal quantities of double strength PDA medium aseptically before pouring in Petri plates. These plates were then inoculated with mycelial discs of 5 mm diameter taken from actively growing culture of *F. oxysporum* f. sp. *capsici* in the centre. A control treatment was also maintained in which only plain sterilized water was added to double strength medium. Experiment was conducted in Completely Randomized Design (CRD). Each treatment was replicated thrice and the inoculated plates were incubated at 25±2 °C in BOD incubator. The colony diameter of test pathogen was recorded till the control plates were full with the mycelium of test pathogen. Inhibition in mycelial growth in each treatment was calculated as described by Vincent (1947) [16].

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

where,
I = Mycelial growth inhibition (%)
C = Growth of test pathogen in absence of fungicide (cm),
T = Growth of test pathogen in presence of fungicide (cm)

In vivo evaluation of fungicides

Seven effective systemic as well as non-systemic fungicides at selected concentration from *in vitro* studies were evaluated under field conditions at the experimental farm of the Department of Plant Pathology, Dr YSP University of Horticulture and Forestry, Nauni, Solan, where disease incidence was high in previous years. Field experiment was laid out in Randomized Block Design (RBD) during 2015 crop season. Each treatment was repeated thrice using the susceptible variety 'Solan Bharpur'. Plot size was 2.0×2.0 m, having four rows 45 cm apart. Five plants were transplanted in each row and total twenty plants were transplanted per plot. Soil drenching of fungicides near stem region was done. First drenching of fungicides was done after twenty-five days of transplanting followed by second drenching at fifteen days interval. The observation on number of wilted plants was recorded at weekly interval till completion of crop season and disease incidence in each treatment was calculated by following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of wilted plants}}{\text{Total number of plants observed}} \times 100$$

The data on the yield of green fruits was also recorded on each harvesting periodically.

Results and Discussion

The associated pathogen was isolated from infected crown, stem and roots and pure culture of the fungus was obtained on potato dextrose agar (PDA) medium. The culture of pathogen was examined microscopically for morphological and cultural characteristics. On the basis of morphological characters viz. hyphae, shape and size of spores produced, and cultural characteristics viz. colour and type of colony, the fungus was identified as *Fusarium oxysporum* (Schlect.) Emend. Synd. and Hans. f. sp. *capsici* Riv. (Booth, 1971) [5].

In vitro evaluation of fungicides

Among various systemic as well as non-systemic fungicides tested under *in vitro* conditions, carbendazim resulted in complete mycelial growth inhibition at all concentrations viz. 50, 100 and 200 ppm. tebuconazole + trifloxystrobin also resulted in complete mycelial growth inhibition at 500 ppm concentration. At lower concentrations, tebuconazole + trifloxystrobin was also found effective and resulted in 95.18 and 89.25 per cent mycelial growth inhibition at 250 and 100 ppm concentration, respectively. hexaconazole + zineb (at 500 ppm), chlorothalonil (at 1000 ppm), azoxystrobin + difenconazole (at 500 ppm) and captan (at 1000 ppm) were next on order with 88.51, 84.44, 82.96 and 79.99 per cent mycelial growth inhibition, respectively. hexaconazole even at low concentration compared to others was also found effective and resulted in 76.66 per cent mycelial growth inhibition at 150 ppm concentration. mancozeb was found to be less effective as compared to others and resulted in 51.85 and 44.44 per cent mycelial growth inhibition even at high concentration of 750 and 500 ppm, respectively.

Table 1. Effect of fungicides on the mycelial growth of *F. oxysporum* f. sp. *capsici*

Fungicide	Concentration (ppm)	Radial growth (mm)	Mycelial growth inhibition (%)
carbendazim	50	0 .00	100.00 (90.00)
	100	0 .00	100.00 (90.00)
	200	0 .00	100.00 (90.00)
hexaconazole	50	24 .00	73.33 (58.89)
	100	22.66	74.81 (59.88)
	150	21.00	76.66 (61.10)
mancozeb	250	52.00	42.22 (40.50)
	500	50.00	44.44 (41.78)
	750	43.33	51.85 (46.06)
captan	250	64.00	28.88 (32.48)
	500	46.00	45.55 (42.43)
	1000	18.00	79.99 (63.45)
chlorothalonil	250	26.66	70.37 (57.02)
	500	20.00	77.77 (61.84)
	1000	14.33	84.44 (66.75)
azoxystrobin +difenconazole	100	30.66	65.92 (54.27)
	250	24.00	73.33 (58.90)
	500	15.33	82.96 (65.62)
hexaconazole +zineb	100	32.66	63.70 (52.94)
	250	23.33	74.07 (59.39)
	500	10.33	88.51 (70.20)
tebuconazole +trifloxystrobin	100	9.66	89.25 (70.85)
	250	4.33	95.18 (77.42)
	500	0 .00	100.00 (90.00)
Control	-	90.00	0.00 (0.00)
$CD_{(0.05)}$		3.87	(2.74)

Figures in the parenthesis are arc sine transformed values

Poddar *et al.* (2004) [13] reported that carbendazim resulted in maximum mycelial growth inhibition of *Fusarium* over control among various fungicides. Naik *et al.* (2007) [10] also obtained inhibitory effect of various systemic and non-systemic fungicides under *in vitro* conditions and reported that among non-systemic fungicides captan, chlorothalonil and mancozeb showed maximum inhibition of fungus at 2000 ppm concentration. Similar observations had also been recorded earlier by Rather *et al.* (2012) [14] with respect to captan, copper oxychloride, mancozeb, mancozeb +

carbendazim (SAAF), mancozeb + metalaxyl (Ridomil MZ), carbendazim, metalaxyl, carboxin, thiophanate methyl and found that carbendazim at 50, 100, and 250 ppm and captan at 500 ppm completely inhibited the mycelial growth of *Fusarium.* Similarly, efficacy of carbendazim and hexaconzole was reported by various workers and found effective against the fungus (Nisa *et al.*, 2011; Ali *et al*., 2013; Yadav *et al.*, 2014 and Barhate *et al.*, 2015) [12, 1, 17, 3].

Evaluation of fungicides under field conditions

Fungicides carbendazim, carbendazim + mancozeb and tebuconazole + trifloxystrobin were found most effective as no incidence of Fusarium wilt disease was recorded and were statistically superior from other treatments. hexaconazole + zineb and azoxystrobin + difenconazole were next best in order and resulted in 15 and 20 per cent disease incidence and were statistically at par with each other while mancozeb was found least effective and resulted in 35 per cent disease incidence. The data on fruit yield (Table 2) also revealed that maximum fruit yield (13.0 kg/ plot) was recorded in plots treated with carbendazim + mancozeb followed by carbendazim and tebuconazole + trifloxystrobin with 12.00 and 11.5 kg fruit yield per plot, respectively.

Soil drenching of carbendazim was also found effective in inhibiting the *Fusarium* fungal bits at the depths of 5cm in chilli cultivation (Naik *et al.*, 2007) [10]. These results are also in consonance with Amini and Sidovich (2010) [2] and Rather *et al.* (2012) [14] who also reported the efficacy of carbendazim drenching in the management of Fusarium wilt. carbendazim belongs to methyl benzimidazole carbamate (MBC) fungicides. MBC fungicides inhibit mitosis in fungi. MBC fungicides bind on β-tubulin in microtubules inhibiting their proliferation and suppressing their dynamic instability. MBC fungicides suppress the assembly of spindle microtubules, disturb the chromosomal alignment at the metaphase plate and microtubule-kinetochore interacion causing chromatid loss, chromosome loss or nondisjunction in target cells (Rathinasamy and Panda, 2006) [15].

The growth of *F. oxysporum* from paprika was suppressed by benomyl, tebuconazole and azoxystrobin (Cha *et* al., 2007) [6]. Inhibitory effect of tebuconazole on mycelia growth of *F. solani* had been reported by Madhavi and Bhattiprolu (2011) [9]. Tebuconazole, difenconazole and hexaconazole belong to triazole fungicides. These fungicides act as demethylase inhibitor (DMI). They interfere in the process of building the structure of fungal cell wall and finally it inhibits the reproduction and growth of the fungus. Azoxystrobin and trifloxystrobin are strobilurin fungicides which interfere with respiration in fungi. *In vitro* efficacy of tebuconazole + trifloxystrobin and azoxystrobin + difenconazole against mycelial growth of *F. oxysporum* f. sp. *capsici* had been reported by Bashir *et al.* (2018) [4]. The present study described the effectiveness of tebuconazole + trifloxystrobin, hexaconazole + zineb and azoxystrobin + difenconazole against Fusarium wilt of bell pepper under field conditions which is not reported in literature yet. In conclusion, that drenching of carbendazim, carbendazim + mancozeb and tebuconazole + trifloxystrobin to be most effective in reducing the wilt incidence and increasing the fruit yield whereas hexaconazole + zineb and azoxystrobin + difenconazole were next best in order. Hence soil drenching of these fungicides could be used effectively for the management of Fusarium wilt of bell pepper. To overcome the issue of development of fungicide-resistance in pathogen, the alternate soil drenching of these fungicide could be used.

Table 2. Field evaluation of fungicides against Fusarium wilt of bell pepper

Fungicides	Dose (%)	Disease incidence (%)	Disease reduction (%)	Yield (kg/plot)
carbendazim + mancozeb	0.25	0.00 (0.00)^{d}	100.00	13.00^{a}
carbendazim	0.2	0.00 (0.00)^{d}	100.00	12.00^{a}
azoxystrobin + difenconazole	0.1	20.00(26.07)^{c}	76.48	9.00^{c}
tebuconazole + trifloxystrobin	0.1	0.00 (0.00)^{d}	100.00	11.50^{a}
hexaconazole + zineb	0.1	15.00(22.59)^{c}	82.36	9.50^{b}
mancozeb	0.25	35.00(36.06^{b}	58.83	6.00^{d}
Control	--	85.00(67.26)^{a}	-	2.50^{e}
$CD_{(0.05)}$		(7.51)		2.044

Figures in the parenthesis are arc sine transformed values

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