

## Integrated Management of Wilt Complex Disease in Bell Pepper (*Capsicum annuum* L.)

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### Abstract

Effect of various disease management tools on seedling emergence, wilt incidence and yield of bell pepper (*Capsicum annuum* L.) Var. California Wonder was studied as part of integrated management strategies under both glass house and field conditions. Different fungicides were tested against the four wilt pathogens viz, *Fusarium oxysporum*, *Phytophthora capsici*, *Rhizoctonia solani* and *Sclerotium rolfsii* under *in-vitro* conditions. The fungicides viz, Captan, Carbendazim, Metalaxyl and Carboxin which were found effective under *in-vitro* conditions were further tested individually as well as in combinations under glass house conditions. Seed treatment followed by seedling treatment with fungicides proved ineffective in controlling wilt incidence disease, however, besides seed treatment and seedling treatment when fungicides were sprayed at crown region of the plant, wilt incidence was significantly reduced. Seed treatment+ seedling treatment + spraying of Carbendazim + Metalaxyl proved most effective and recorded 59.8% disease reduction over check under field conditions. Integration of Captan + Metalaxyl with *Trichoderma harzianum* and *T. virens* also proved superior compared to their individual treatments.

**Keywords:** integrated management, wilt complex, *Capsicum annuum*

**Abbreviations:** ST–Seed Treatment, SD–Seedling Dip, Th<sub>2</sub>-*Trichoderma harzianum*, Tv<sub>2</sub>-*T. viride*, Gv<sub>3</sub>-*T. virens*

### 1. Introduction

Bell pepper locally named as Shimla Mirch (*Capsicum annuum* L.) is infected by a number of diseases, among them wilt complex has gained major importance in the State of Jammu and Kashmir from the last 15-18 years and the losses caused by disease are up to the extent of 30-40 per cent (Anonymous, 1989). The wilt disease, incited by a number of pathogens, is the devastating soil-borne disease and hence difficult to manage. The disease has been observed to be caused by *Fusarium spp.*, *Phytophthora capsici* and *Rhizoctonia solani* (Shali, 2000; Najar, 2001). Keeping in view the etiology, exorbitant losses and lack of information regarding integrated management of wilt complex disease using bio-control agents and chemicals, the present studies were conducted to evolve the effective management strategies involving most effective chemicals and bio-control agents.

### 2. Research Methods

The pathogens viz., *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora capsici* and *Sclerotium rolfsii* were isolated from bell pepper plants affected with wilt complex disease in different areas of Jammu region. The pure cultures of pathogens were maintained on PDA/carrot agar media at 4°C. *F. oxysporum*, *R. Solani* and *S. rolfsii* were mass multiplied on pre-boiled autoclaved sorghum grains, whereas, *P. capsici* was mass multiplied on sand carrot broth medium.

#### 2.1 In-vitro Evaluation of Fungicides against Wilt Complex Pathogens

The efficacy of fungicides viz. captan, copper oxychloride, mancozeb, mancozeb + carbendazim (Saaf), mancozeb + metalaxyl (Ridomil MZ), carbendazim, metalaxyl, carboxin, thiophanate methyl were tested against each pathogen under *in-vitro* condition by poisoned food technique (Schmitz, 1930) to find out the effective fungicides. The Petri-plates containing PDA/carrot agar media (for *P. capsici*) amended with desired concentration of

fungicides were inoculated with 5 mm discs of pathogen and incubated with 25+1°C temperature. The plates without any fungicides served as check. The radial mycelial growth was recorded in each treatment and percent inhibition over check was calculated.

### 2.2 *In-vivo* Evaluation of Fungicides against Bell Pepper Wilt Complex Pathogens

The fungicides found effective *in-vitro* were tested *in-vivo* as seed + seedling treatment alone and in combinations with spraying at crown region of the plant. The studies were carried out in earthen pots containing sterilized soil and FYM (2:1). The seeds of bell pepper cv. California wonder treated with fungicides viz., carbendazim, carboxin, metalaxyl (@ 0.2%), captan, carboxin + metalaxyl (1:1), carbendazim+ metalaxyl (1:1) (@0.3%), captan + metalaxyl (1.5:1) and carbendazim + captan (1:1.5) (@ 0.35%) were sown in pathogen inoculated pots. The 45 day old seedlings were uprooted from the pots and subjected to the seedling dip in different fungicides (same as in seed treatment) for 30 minutes and were planted again in pathogen inoculated pots. The spraying of the fungicides at crown region of the plant was done at 15 days interval after the initiation of wilt disease. The fungicides viz., carboxin, carbendazim and metalaxyl were applied @ 0.1 %, whereas captan was applied @ 0.2% concentration.

### 2.3 Compatibility of Bio-agents with Fungicides

The fungicides found effective under *in-vitro* conditions viz., carbendazim, carboxin, metalaxyl were tested at concentrations i.e. 50,100,250 ppm and captan at 100, 250, 500 ppm against *Trichoderma harzianum* (Th<sub>2</sub>), *T. viride* (Tv<sub>2</sub>) and *T. virens* (Gv<sub>3</sub>) respectively, to examine their inhibitory effect on the mycelial growth of the antagonists using poisoned food technique (Schmitz, 1930<sup>3</sup>). These antagonist isolates were found most effective against wilt pathogens under both *in-vitro* and *in-vivo* conditions in other experiments. The PDA plates containing desired concentration of fungicides were inoculated at the centre with the mycelial discs (5 mm) of actively growing culture of bio-agents. The plates inoculated with bio-agents but without fungicides served as check. The plates were incubated at 26+1°C for 10 days. The observation on radial growth of the antagonists were recorded in each treatment and per cent inhibition over check was calculated.

### 2.4 Integration of Chemicals and Bio-agents against Wilt Complex Disease

#### 2.4.1 Glass House Studies

The effective/compatible fungicides (captan + metalaxyl, carbendazim + metalaxyl and carboxin + metalaxyl) and bio-agents (*Trichoderma viride* and *T. virens*) under *in-vitro* and glass house conditions were evaluated in integration to observe their effects on wilt complex disease. The seeds were first treated with reduced doses (1 g/kg seed) of fungicides followed by antagonists. The treated and untreated seeds were sown in pots containing sterilized soil. Forty-five day old seedlings were uprooted and soaked in respective fungicidal and antagonist suspensions. The seedlings were transplanted in pots inoculated with all the test pathogens. The fungicides were sprayed at crown region at 15 days intervals after the initiation of wilt disease. Observations on wilt incidence were recorded at regular intervals up to the maturity of the crop.

#### 2.4.2 Field Studies

To study the effect of seed + seedling treatment of fungicides and bio-agents alone and in combination followed by spraying at crown region of the plant against wilt complex disease, a field experiment was laid out in a randomized block design with three replications at Basht village of Chenani area of Jammu region. Seeds of bell pepper cv. California wonder were treated with fungicide and bio-agents as in pot house studies. Seeds were sown in first week of March and forty-five day old seedlings were uprooted and subjected with each of the treatment (as under glass house conditions) and transplanted during mid-April. Four sprays of fungicides at 15 days interval, starting with the initiation of wilt disease were given at crown region of plant. The antagonists (20 g each) were properly mixed with 2 kg FYM and then mixed with the top soil layer of plots (2 x 2 m). The observations on wilt incidence and yield were recorded in each treatment.

## 3. Analysis Results

### 3.1 *In-vitro* Evaluation of Fungicides against Wilt Complex Pathogens

The data presented in Table 1 indicates that carbendazim and captan completely inhibited the growth of *F. oxysporum* at 50 and 500 ppm concentrations, respectively. The growth of *P. capsici* was completely inhibited by metalaxyl at 50 ppm concentration, captan and metalaxyl+ mancozeb at 500 ppm concentration. Carboxin (50ppm) proved superior over other fungicides in inhibiting the growth of *R. solani*. The complete inhibition of mycelial growth of *R. solani* was also observed in carbendazim and thiophanate methyl at 250 ppm concentrations. None of the chemicals tested could completely inhibit the growth of *S. rolfssii*, although, captan was most effective,

followed by metalaxyl+mancozeb and mancozeb+carbendazim at 500 ppm concentration each. Similar observations have been recorded by Sharma et al. (2002) with respect to carbendazim, copper oxychloride, captan and Ridomol MZ against *F. oxysporum*. Babadoost and Islam (2003) found metalaxyl as highly inhibitory to the mycelial growth of *P. capsici* under *in-vitro* conditions. Similar results of carbendazim, captan, thiophanate methyl and carboxin against *R. solani* (Dubey & Patel, 2001) and captan against *S. rolfsii* (Chowdhary et al., 1998) have also been recorded.

### 3.2 In-vivo Evaluation of Fungicides against Wilt Complex Pathogens

Seed treatment with different fungicides and their suitable combinations revealed that all the fungicides and their combinations resulted in significantly superior emergence of bell pepper seedlings as compared to check (Table 2). The highest seedling emergence (79.5%) with 47.7 % increase over check was observed with carbendazim +metalaxyl followed by captan +carbendazim (78.5%) and captan +metalaxyl (78.0%). The increase in seedling emergence by carbendazim, captan, metalaxyl and other fungicides in different crops have been reported by different workers (Babadoost and Islam, 2002). Besides fungicidal action against pathogens the beneficial side effects of carbendazim and metalaxyl like seed germination, promotion of seedling growth etc. are well known.

The data in Table 2 indicate that seed +seedling treatment with fungicides protect the plants up to 40days after transplanting (DAT) and significantly lesser wilt incidence was observed than control. Seed +seedling treatment followed by spray of carbendazim +metalaxyl and captan +metalaxyl resulted in significantly lesser wilt incidence of 25.0 and 33.3 %, respectively, after 100 DAT, compared to 100% incidence in check at 40 DAT. The fungicidal combination of captan +carbendazim and carboxin +metalaxyl also recorded significantly lower wilt incidence (45.8 and 58.3%, respectively) as compared to check and fungicides applied only as seed +seedling dip treatment. The results were in agreement with findings of Simon et al. (1990), who reported that spraying metalaxyl at the lower stem of *Capsicum annum* plants gave effective control of wilt incidence caused by *Phytophthora capsici*.

### 3.3 Compatibility of Bio-agents with Fungicides

The results (Table 3) showed that mycelial growth of *Trichoderma viride* (Tv<sub>2</sub>) and *T. virens* (Gv<sub>3</sub>) were completely inhibited at all tested concentrations of carbendazim. The best compatibility of tested Bio-control agents (BCAs) was observed with carboxin. The mycelial growth of Tv<sub>2</sub> observed at 50 and 100ppm concentrations (carboxin) was statistically at par with untreated check, however, at 250 ppm concentration significantly lower growth (72.3mm) was recorded in Tv<sub>2</sub> compared to untreated check (90mm). The growth of antagonists were slightly inhibited by metalaxyl at its higher concentration, however, significantly lesser growth of antagonists was observed at all concentration of captan. These findings are in agreement with the finding of Mukhopadhyay et-al (1992) who also reported the insensitivity of *Trichoderma* and *Gliocladium spp.* against the carboxin. The differentiating response of antagonistic flora to various fungicides might be due to their inherent resistance to the fungicides and their ability to degrade chemicals (Papavizas, 1985).

### 3.4 Integration of Chemicals and Bio-agents against Wilt Complex Disease

#### 3.4.1 Glasshouse Studies

The Table 4 indicates that seed+seedling+spraying of carbendazim+ metalaxyl and captan+ metalaxyl were more effective than seed+seedling + soil treatment of Gv<sub>3</sub> and Tv<sub>2</sub> under glasshouse conditions. The treatment captan + metalaxyl recorded significantly higher wilt incidence (38.8%) than observed in carbendazim+ metalaxyl (31.9%), however, when *T. viride* (Tv<sub>2</sub>) and *T. virens* (Gv<sub>3</sub>) were integrated with captan+metalaxyl it resulted in lesser wilt incidence which was at par with carbendazim+ metalaxyl. The effectiveness of carboxin+ metalaxyl was also observed when used in combination with fungal antagonists. The maximum disease reduction (70.9%) over control was observed each in captan+metalaxyl+*T. viride* and captan+metalaxyl+*T. virens*. Carboxin+ metalaxyl also recorded disease reduction of 47.3% over control. Under present investigation, *T. viride* (Tv<sub>2</sub>) and *T. virens* (Gv<sub>3</sub>) showed more resistance against the fungicides rather than *T. harzianum* (Th<sub>2</sub>) which might be due to their inherent resistance to fungicides and their ability to degrade chemicals as described by Papavizas (1985). Similar to present findings the compatibility of *Trichoderma spp.* with metalaxyl has been reported earlier also (Anonymous, 2008). Khosla and Gupta (2008) observed the toxicity of carbendazim against the *Trichoderma spp.*

#### 3.4.2 Field Studies

The data presented in Table 4 also revealed the similar trend in results as under pot culture studies. Superiority of carbendazim+metalaxyl, captan+ metalaxyl+ Gv<sub>3</sub> and captan+ metalaxyl+ Tv<sub>2</sub> was confirmed as higher disease reduction of 59.8, 58.6 and 58.0 % over control and maximum yield of 138.6, 137.0 and 135.6 q/ha was observed,

respectively. The integration of BCAs with carboxin+metalaxyl resulted lesser wilt incidence compared to the individual treatment of BCAs and fungicides.

The present findings are supported by earlier works wherein integration of bio-control agents with fungicides gave significantly higher disease control in several crops than that obtained either by bio-control agent or by fungicide alone (Sawant & Mukhopadhyay 1990; Dubey, 1997). The seed and seedling treatment with captan, metalaxyl and carboxin may eradicate the wilt causing pathogens or other microflora thereby less competition for BCA to colonize the seed and root surface and proliferate (Chet et al. 1982; Ram et al. 2000). The subsequent application of compatible fungicides may support the growth of antagonists and may also prevent the plant from the attack of pathogens. The other workers also observed the additive effects of compatible fungicides and antagonists (Chattopadhyay & Sen, 1996). Gour & Sharma (2010) also integrated *T. viride* -1 or *T. harzianum* (TG-1) with metalaxyl and cymoxanil 8% + mancozeb 64% to control root rot in cotton. On the basis of present investigation it may be concluded that seed+seedlings + spraying of carbendazim+metalaxyl or captan+ metalaxyl+ Gv<sub>3</sub> or captan+ metalaxyl+ Tv<sub>2</sub> can be applied to manage the devastating wilt disease in Capsicum.

Table 1. *In-vitro* evaluation of fungicides against wilt complex pathogens

Fungicide	Conc. (ppm)	Radial growth (mm)				Inhibition over check (%)			
		<i>F.oxysporum</i>	<i>P.capsici</i>	<i>R.solani</i>	<i>S.rolfsii</i>	<i>F.oxysporum</i>	<i>P.capsici</i>	<i>R.solani</i>	<i>S.rolfsii</i>
Copper oxychloride	100	37.3	24.0	40.0	45.0	58.5	73.3	55.5	50.0
	250	28.0	18.6	33.6	36.6	68.8	79.3	62.6	59.3
	500	20.3	8.0	25.0	34.3	77.4	91.1	72.2	61.8
Captan	100	18.6	25.6	39.3	32.0	79.3	71.5	56.3	64.4
	250	10.0	15.3	31.6	22.6	88.8	83.0	64.8	74.8
	500	0.0	0.0	26.0	18.6	100.0	100.0	71.1	79.3
Mancozeb	100	30.0	25.0	26.3	45.6	66.6	72.2	70.7	49.3
	250	22.0	18.3	19.3	34.3	75.5	79.6	78.5	61.8
	500	16.0	8.0	15.0	28.3	82.2	91.1	83.3	68.5
Mancozeb + Carbendazim (Saaf)	100	18.6	31.6	24.0	40.3	79.3	64.8	73.3	55.2
Mancozeb + Metalaxyl (Ridomil MZ.)	250	10.3	21.6	13.6	32.0	88.5	76.0	84.8	64.4
	500	6.0	18.0	0.0	28.0	93.3	80.0	100.0	68.8
	100	24.3	18.0	20.6	39.0	73.0	80.0	77.1	56.6
Thiophanate methyl	250	15.3	12.6	13.0	32.6	83.3	86.0	85.5	63.7
	500	10.6	0.0	8.3	27.0	88.2	100.0	90.7	70.0
	50	23.0	76.3	19.3	67.0	74.4	15.2	78.5	25.5
Carboxin	100	16.0	72.0	9.6	62.0	82.2	20.0	89.3	31.1
	250	10.3	70.6	0.0	58.6	88.5	21.5	100.0	34.8
	50	60.3	74.6	0.0	78.6	33.0	17.1	100.0	12.6
Carbendazim	100	55.6	71.6	0.0	72.0	38.2	20.4	100.0	20.0
	250	44.0	67.6	0.0	68.6	51.1	22.4	100.0	23.7
	50	0.0	86.6	24.3	68.3	100.0	3.7	73.0	24.1
Metalaxyl	100	0.0	83.0	12.3	56.3	100.0	7.7	86.3	37.4
	250	0.0	80.3	0.0	53.0	100.0	10.7	100.0	41.1
	50	85.6	0.0	65.0	70.0	4.8	100.0	27.7	22.2
Check	100	81.3	0.0	58.6	63.3	9.6	100.0	53.5	29.6
	250	76.0	0.0	53.0	58.0	15.5	100.0	41.1	35.5
CD (P = 0.05)	-	90.0	90.0	90.0	90.0	-	-	-	-
	-	3.18	3.46	3.75	3.84	-	-	-	-

Table 2. Effect of fungicides on wilt incidence in bell pepper cv. California Wonder, under glass house conditions

Fungicides	Concentration (%)	Seedling emergence (%)	Increase over Check (%)	Wilt incidence (%) (Days after transplanting)		
				40	80	100
<b>Seed Treatment (ST) + Seedling Dip (SD)</b>						
Captan	0.3	75.6 (60.3)	40.5	58.3 (49.7)	100.0 (90.0)	100.0 (90.0)
Carbendazim	0.2	77.0 (61.3)	43.1	45.8 (42.5)	100.0 (90.0)	100.0 (90.0)
Metalaxyl	0.2	73.3 (58.8)	36.1	70.8 (57.2)	100.0 (90.0)	100.0 (90.0)
Carboxin	0.2	74.2 (59.4)	37.9	66.6 (54.6)	100.0 (90.0)	100.0 (90.0)
Captan + Metalaxyl	0.35	78.0 (62.0)	44.9	54.1 (47.3)	100.0 (90.0)	100.0 (90.0)
Captan + Carbendazim	+ 0.35	78.5 (62.3)	45.9	50.0 (45.0)	100.0 (90.0)	100.0 (90.0)
Carbendazim + Metalaxyl	+ 0.3	79.5 (63.0)	47.7	41.6 (40.1)	100.0 (90.0)	100.0 (90.0)
Carboxin + Metalaxyl	0.3	77.0 (61.3)	43.1	62.4 (52.1)	100.0 (90.0)	100.0 (90.0)
<b>Seed treatment (ST) + Seedling Dip (SD) + Spraying (15 days interval)</b>						
Captan + Metalaxyl	0.2	-	-	0.0 (0.0)	29.1 (32.6)	33.3 (35.2)
Captan + Carbendazim	+ 0.2	-	-	4.1 (11.6)	29.1 (32.6)	45.8 (42.2)
Carbendazim + Metalaxyl	+ 0.15	-	-	0.0 (0.0)	25.0 (30.0)	25.0 (30.0)
Carboxin + Metalaxyl	0.15	-	-	20.8 (27.1)	50.0 (45.0)	58.3 (49.7)
Check	-	53.8 (47.1)	-	100.0 (90.0)	100.0 (90.0)	100.0 (90.0)
<b>CD (p = 0.05)</b>	-	<b>1.78</b>	-	<b>16.9</b>	<b>9.5</b>	<b>8.0</b>

Figures in parenthesis are transformed angular values

\*The treatments excluding check, recorded 100 per cent wilt incidence, were not included in statistical analysis

Table 3. *In-vitro* evaluation of compatibility of different fungicides with the antagonists

Fungicides	Concentration (ppm)	Radial growth (mm)			Inhibition over check (%)		
		<i>T. harzianum</i> (Th <sub>2</sub> )	<i>T. viride</i> (Tv <sub>2</sub> )	<i>T. virens</i> (Gv <sub>3</sub> )	<i>T. harzianum</i> (Th <sub>2</sub> )	<i>T. viride</i> (Tv <sub>2</sub> )	<i>T. virens</i> (Gv <sub>3</sub> )
Carbendazim	50	0.0	0.0	0.0	100.0	100.0	100.0
	100	0.0	0.0	0.0	100.0	100.0	100.0
	250	0.0	0.0	0.0	100.0	100.0	100.0
Carboxin	50	90.0	90.0	90.0	0.0	0.0	0.0
	100	85.3	84.6	90.0	5.2	6.0	0.0
	250	74.6	72.3	85.0	17.1	19.6	5.5
Captan	100	75.6	75.6	80.3	16.0	16.0	10.7
	250	67.3	67.3	71.0	25.2	25.2	21.1
	500	54.0	54.0	61.6	40.0	40.0	31.5
Metalaxyl	50	85.3	84.6	88.3	5.2	6.0	1.8
	100	74.6	73.3	80.0	17.1	18.5	11.1
	250	67.3	70.0	75.3	25.2	22.2	16.3
Check	-	90.0	90.0	90.0	-	-	-
<b>CD (p = 0.05)</b>	-	<b>7.4</b>	<b>6.4</b>	<b>6.5</b>	-	-	-

Table 4. Effect of fungicides and antagonists alone and in combinations on wilt complex disease of bell-pepper cv. California Wonder

**Treatment (ST + SD + Spraying)			Glass house conditions		Field condition		
			Wilt incidence (%)	Disease reduction over control (%)	Wilt incidence (%)	Disease reduction over control (%)	Yield q/ha
T <sub>1</sub>	Captan + Metalaxyl	38.8 (38.0)	61.2	30.1 (33.2)	53.6	130.4	
T <sub>2</sub>	Carbendazim + Metalaxyl	31.9 (34.3)	68.1	26.1 (30.7)	59.8	138.6	
T <sub>3</sub>	Carboxin + Metalaxyl	59.7 (50.5)	40.3	34.0 (35.6)	47.6	122.6	
T <sub>4</sub>	<i>T. harzianum</i> (Th <sub>2</sub> )	52.7 (46.5)	47.3	51.9 (46.0)	20.1	82.1	
T <sub>5</sub>	<i>T. virens</i> (Gv <sub>3</sub> )	43.0 (40.9)	57.0	50.0 (45.0)	22.0	84.3	
T <sub>6</sub>	T <sub>1</sub> * + T <sub>4</sub>	29.1 (32.6)	70.9	27.3 (32.2)	58.0	135.6	
T <sub>7</sub>	T <sub>1</sub> * + T <sub>5</sub>	29.1 (32.6)	70.9	26.9 (31.2)	58.6	137.0	
T <sub>8</sub>	T <sub>3</sub> * + T <sub>4</sub>	45.8 (42.5)	54.2	30.8 (33.7)	52.6	129.5	
T <sub>9</sub>	T <sub>3</sub> * + T <sub>5</sub>	38.8 (38.0)	61.2	30.6 (33.5)	52.9	130.5	
T <sub>10</sub>	Control	100.0 (90.0)	–	65.0 (53.7)	–	63.1	
<b>CD (p = 0.05)</b>			<b>5.0</b>	<b>–</b>	<b>2.0</b>	<b>–</b>	<b>3.0</b>

Figures in parenthesis are transformed angular value

T<sub>1</sub> = ST + SD @ 0.35%, Spraying @ 0.25%; T<sub>2</sub>, T<sub>3</sub>, ST + SD @ 0.25%, Spraying @ 0.2%;

T<sub>4</sub>, T<sub>5</sub>, ST + SD with antagonist suspension (10<sup>7</sup> spores/ml); Soil treatment @ 20 g/plot soil.

\*Half dose of fungicides applied as ST + SD

ST – Seed Treatment; SD – Seedling Dip

\*\*The sprays were conducted at 15days interval starting from the first appearance of disease

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