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Efficacy of different fungicides for the management of chickpea wilt (*Fusarium oxysporum* f. sp. *ciceri*)

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Abstract
Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceri* is the most destructive and widespread fungal disease of chickpea. It has drastic effect on yield causing 100 per cent loss under favorable conditions. A severe outbreak of wilt of chickpea was observed causing considerable damage in the field of Ghed and Saurashtra regions of Gujarat during last few years. Experiment was conducted to find out effective fungicides for the management of chickpea wilt. Different fungicides were tested against *Fusarium oxysporum* f. sp. *ciceri viz.,* systemic, non-systemic and fungicide combination. In laboratory screening of different fungicide, Tebuconazole 25.9% EC was found to be quite effective in inhibiting the radial growth of test pathogen among systemic group of fungicides, while in non-systemic group of fungicides, Copper Oxychloride 50% WP and in case of fungicide combinations Carbendazim 12% + Mancozeb 63% WP were significantly inhibited the growth of test fungus. Fungicides found most effective under *in vitro* were further tested in greenhouse condition in pot culture. Among six fungicides tested, the combination of Carbendazim 12% + Mancozeb 63% WP showed minimum PDI followed by Carbendazim 50% WP.

Keywords: Fungicide, Chickpea, wilt, *Fusarium oxysporum* f. sp. *ciceri*

1. Introduction

Chickpea (*Cicer arietinum* L.) is one of the major pulse crops, belongs of the family Leguminosae. It is also known as Bengal gram. Chickpea is a cheap source of protein compared to animal protein. Low yield of chickpea is attributed to several diseases and insect. Despite of different diseases *Fusarium* wilt disease is most important disease of chickpea causes severe wilt. Wilt caused by *Fusarium oxysporum* Schlechtend Fr. f. sp. *ciceri* (Padwick) Matuo & K. Sato, is the most important soil borne disease of chickpea throughout the global and particularly in the Indian subcontinent, the Mediterranean and California (Nene and Reddy, 1987) [16]. The fungus is a primarily soil borne pathogen, however, few reports indicated that it can be transmitted through seeds (Haware *et al.*, 1978) [9]. The pathogen can infect at all stages of plant growth with more incidences in flowering and pod filling stage. The wilt appeared in field within three to four week after sowing, if the variety is susceptible (Haware, 1990) [6]. Early wilting causes more loss than late wilting, but seeds from late wilted plants are lighter, rough and dull than those from healthy plants (Haware and Nene, 1980) [7]. Relatively high temperature with drought may cause up to eighty percent plant mortality (Govil and Rana, 1994) [4]. The pathogen is facultative saprophytic and it can survive as mycelium and chlamydospores in seed, soil and also on infected crops residues, buried in the soil for up to five to six years (Haware *et al.*, 1986) [8]. Under favorable condition, the wilt infection can damage the crop completely and cause 100 per cent yield loss (Navas Cortes *et al.*, 2000; Halila and Strange, 1996) [14, 5]. In India annual yield loss due to *Fusarium* wilt were estimated at 10% (Singh and Dahiya 1973; Trapero-Casas and Jiménez-Díaz, 1985) [22, 24]. Present study was carried out to evaluate the fungicides against *Fusarium* wilt of chickpea both laboratory and net house condition.

2. Materials and Methods

2.1. Isolation and purification of pathogen

Chickpea plants, naturally infected and showing typical wilt symptoms were collected from different fifteen locations of Saurashtra and Ghed regions of Gujarat and brought to the laboratory. Isolation of the fungus was made by tissue isolation technique on potato dextrose agar (PDA) and incubated at 28±2 ^{0}C. The resulting fungal culture was purified by hyphal tip method. The fungus was isolated, purified and sub cultured in aseptic condition. The isolates of the pathogen were identified based on colony characters and spores morphology (Booth, 1971) [2]. The fifteen isolates were screened for their pathogenicity on chickpea cultivar JG-62 during *rabi* season 2016-17 under nethouse. Isolate which showed highest per cent disease incidence was used for evaluation of fungicides.

2.2. *In vitro* evaluation of different fungicides against test pathogen

In vitro efficacy of different fungicides against chickpea wilt pathogen was evaluated by poisoned food technique (Nene and Thapliyal, 1993) [15] as described below:

2.2.1 Poisoned food technique

Required quantity of fungicide was added in 100 ml of lukewarm PDA media and mixed thoroughly. This solution was poured into Petri plates about 30 ml in each. After solidification of media 5 mm discs of four days old culture of test pathogen were inoculated at the centre of Petri plates and then incubated at 28±2 °C. Three repetitions were maintained for each fungicide. Medium without fungicide was kept as control. Per cent inhibition of the growth of the fungus over the control was calculated as per the following formula (Vincent, 1927) [25].

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

Where,
I = Per cent inhibition of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatments.

Systemic fungicides i.e., Azoxystrobin 23% SC, Fosetyl-Al 80% WP, Tebuconazole 25.9% EC, Thiophanate methyl 70% WP, Carbendazim 50% WP and Flusilazole 40% EC were used under laboratory condition at 100, 250 and 500 ppm concentration.

Non systemic fungicides i.e., Captan 50% WP, Thiram 75%WP, Mancozeb 75% WP, Propineb 70%WP, Copper Hydroxide 77% WP and Copper Oxychloride 50% WP were used at 2000, 2500 and 3000 ppm concentration.

Ready mix fungicides i.e., Zineb 68% + Hexaconazole 4% WP, Carbendazim 12% + Mancozeb 63% WP, Azoxystrobin 11% + Tebuconazole 18.30% SC, Carboxin 37.5% + Thiram 37.5% WS, Tebuconazole 50% + Trifloxystrobin 25% WG, Cymoxanil 8% + Mancozeb 64% WP were evaluated at 250, 500 and 1000 ppm concentration under laboratory condition against chickpea wilt pathogen following poisoned food technique as described earlier. Experiment was laid out with six treatments and each treatment repeated three times. Completely Randomized block Design with Factorial Concept was used for analyzing the data.

2.3. Evaluation of fungicides in pot condition

Fungicides which gave higher per cent inhibition in growth of test pathogen during laboratory tests were evaluated in pots under nethouse condition during *rabi* season 2016-17.

2.3.1 Mass multiplication of *Fusarium oxysporum* f. sp. *ciceri*

The test pathogen *Fusarium oxysporum* f. sp. *ciceri* was mass multiplied on sterilized sorghum grains for pot culture studies. Hundred gram of sorghum grains were washed thoroughly in tap water and boiled. After that removing the excess water, grains were allowed to air dry and cooled at room temperature. Polythylene bags were filled up with about 200 g of grains. Mouth of these bags was packed with piece of PVC pipe and non-absorbent cotton. Then, it autoclaved for 20 min at 15 psi and inoculated with four days old culture of test pathogen. After seven days, the inoculum was mixed with sterilized soil in pots @ 40 g kg^{-1}.

2.3.2 Pot filling and inoculation of mass multiplied cultures

Sterilized soil was used for pot filling. About 40 g inoculum was added per kg soil. Sterilized soil and inoculum was thoroughly mixed in pot. For each fungicide, three sets of pots (20 cm width x 20 cm depth) were prepared. One set of pot constituting three pots were considered as inoculated control. Three test tubes were inserted at equidistance and about 6 cm deep in each pot for secondary inoculation. Secondary inoculation done by inoculum prepared on Potato Dextrose Broth. It was done after three weeks of sowing by removing test tubes. 30 ml liquid culture having strength 2 x 10^{7} cfu/ml was poured in hole made by removal of test tubes in each pot along with mycelial met. Hole made by removal of test tubes helps to leach inoculum directly to the root zone.

2.3.3 Drenching of fungicides and its effect on wilt disease

Two fungicides from each group *i.e.*, systemic, non-systemic and combination which was found superior in growth inhibition of test pathogen *in vitro* were used for pot culture studies. Fungicides used in pot culture studies were Tebuconazole 25.9% EC (0.01%), Carbendazim 50% WP (0.05%), Mancozeb 75% WP (0.25%), Captan 50% WP (0.25%), Carbendazim 12% WP + Mancozeb 63% WP (0.1%), Tebuconazole 50% + Trifloxystrobin 25% WG (0.1%).

Drenching of selected fungicides was made at about 30 DAS. Experiment was laid out with six treatments and each treatment repeated three times. Completely Randomized block Design was used for analyzing the data. One set of pot constituting three pots were maintained as inoculated control which was not drenched with any chemical.

$$\text{Per cent disease incidence} = \frac{\text{Total number of wilted plants per pot}}{\text{Total number of plants per pot}} \times 100$$

3. Results and Discussion

3.1 *In vitro* evaluation of systemic fungicides

Efficacy of six commonly used systemic fungicides *viz*., azoxystrobin, fosetyl-Al, tebuconazole, thiophanate methyl, carbendazim and flusilazole were evaluated against *F. oxysporum* f. sp. *ciceri* at different concentrations *viz*., 100, 250 and 500 ppm using poisoned food technique as described in section 2.2.1. The data revealed that all the fungicides at all concentrations reduced mycelial growth (Table 1) of *F. oxysporum* f. sp. *ciceri* as compared to control (Fig. - 1).

Sr. No.	Systemic Fungicides	Concentration (ppm)
1.	Azoxystrobin 23% SC	
2.	Fosetyl-AL 80% WP	**A= 100**
3.	Tebuconazole 25.9% EC	
4.	Thiophanate methyl 70% WP	**B = 250**
5.	Carbendazim 50% WP	
6.	Flusilazole 40% EC	**C = 500**
7.	Control	

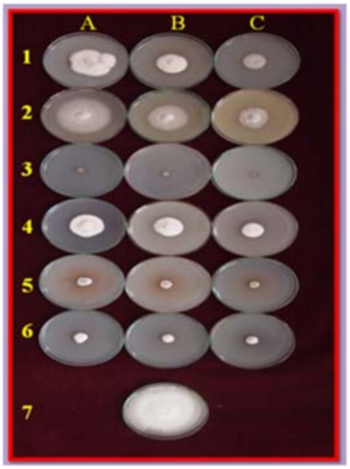

Fig 1: Growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* on PDA supplemented with systemic fungicides

It is evident from the data presented in Table 1 and Fig. 1, that maximum mean per cent inhibition of *F. oxysporum* f. sp. *ciceri* mycelial growth was recorded in tebuconazole (99.98%) followed by carbendazim (85.03%), flusilazole (84.31%), thiophanate methyl (64.52%), azoxystrobin (60.17%) and fosetyl-Al (47.58%). Fosetyl-Al was found to be least effective against *F. oxysporum* f. sp. *ciceri* (Fig. 1).

Table 1: *In vitro* evaluation of systemic fungicides

Fungicides	Growth inhibition (%) 100 ppm	250 ppm	500 ppm	Mean inhibition (%)
Azoxystrobin 23% SC	43.40 (47.21)*	52.07 (62.21)	57.49 (71.11)	50.98 (60.17)
Fosetyl-AL 80% WP	32.85 (29.43)	45.64 (51.11)	52.07 (62.21)	43.52 (47.58)
Tebuconazole 25.9% EC	89.19 (99.98)	89.19 (99.98)	89.19 (99.98)	89.19 (99.98)
Thiophanate methyl 70% WP	48.99 (56.93)	53.39 (64.43)	58.19 (72.21)	53.52 (64.52)
Carbendazim 50% WP	65.37 (82.60)	67.22 (85.00)	69.31 (87.50)	67.30 (85.03)
Flusilazole 40% EC	65.32 (82.56)	67.10 (84.83)	67.68 (85.56)	66.70 (84.31)
Mean	57.52 (66.45)	62.43 (74.59)	65.65 (79.76)	61.87 (73.60)

	Fungicide (F)	Concentration (C)	F x C
S.Em. ±	0.25	0.18	0.43
C.D at 5%	0.72	0.51	1.24
CV%	1.21		

* Data outside the parentheses are arcsine transformed whereas inside are re-transformed values

The effectiveness of tebuconazole against *F. oxysporum* f. sp. *ciceri* has been reported by Arunodhayam (2012) [1]. Whereas Singh and Jha (2003) [1], Poddar *et al.* (2004) [19], Hossain *et al.* (2013) [10], Maitlo *et al.* (2014) [13], Mahmood *et al.* (2015) [12], Patil *et al.* (2015) [18] and Suman and Mohan Kumar (2016) [23] achieved good control of *F. oxysporum* f. sp. *ciceri* in carbendazim.

There was positive correlation between concentration and inhibition of growth of pathogen. It is also observed that with increasing concentration of all fungicides, inhibition of growth of pathogen also increased, except tebuconazole (100 ppm) which give almost cent per cent inhibition at lower concentration. The carbendazim (500 ppm), flusilazole (500 ppm), thiophanate methyl (500 ppm), azoxystrobin (500 ppm) and fosetyl-Al (500 ppm) showed 99.98, 87.50, 85.56, 72.21, 71.11 and 62.21 per cent inhibition of mycelial growth, respectively which were higher than their lower concentration of 100 and 250 ppm (Fig. 1).

Looking to concentration of individual fungicides, all the fungicides differed significantly in inhibition of test pathogen, except tebuconazole which found at par at all concentration tested. *i.e.* 100, 250 and 500 ppm.

3.2 *In vitro* evaluation of non-systemic fungicides

Efficacy of six commonly used non-systemic fungicides *viz*., captan, thiram, mancozeb, propineb, copper hydroxide and copper oxychloride were evaluated against *Fusarium oxysporum* f. sp. *ciceri* at different concentrations *viz*., 2000, 2500 and 3000 ppm using poisoned food technique as described in section 2.1.1. The data revealed that all the fungicides at all concentrations reduced mycelial growth (Table 2) of *Fusarium oxysporum* f. sp. *ciceri* as compared to control (Fig. 2).

Sr. No.	Non-systemic Fungicides	Concentration (ppm)
1.	Captan 50% WP	
2.	Thiram 75%WP	A = 2000
3.	Mancozeb 75% WP	
4.	Propineb 70%WP	B = 2500
5.	Copper hydroxide 77% WP	
6.	Copper oxychloride 50% WP	C = 3000
7.	Control	

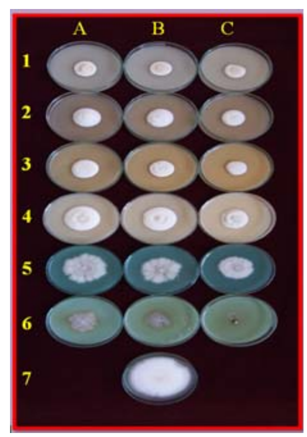

Fig 3: Growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* on PDA supplemented with non-systemic fungicide

It is inferred from the data presented in Table 2 and Fig. 2 that all the fungicides are significantly effective in inhibition of test pathogen.

The perusal of data presented in Table 2 and Fig. 2 revealed that mean per cent growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* mycelial growth was maximum in copper oxychloride (69.57%) followed by captan (65.62%), mancozeb (61.46%) and thiram (57.08%). They were significantly differed to each other. On contrary, Suman and Mohan Kumar (2016) [23] reported copper oxychloride least effective against *Fusarium oxysporum* f. sp. *ciceri*. Copper hydroxide was found least effective against *Fusarium oxysporum* f. sp. *ciceri* in present investigation.

There was positive correlation between concentration and inhibition of growth of pathogen. It is also observed that with increasing concentration of all fungicides, inhibition of growth of pathogen also increased. The fungicides copper oxychloride, captan, mancozeb, thiram, propineb and copper hydroxide showed higher 99.98, 68.13, 65.63, 60.00, 55.00 and 43.75 per cent inhibition of mycelial growth at 3000 ppm, respectively as compared to their lower concentration of 2000 and 2500 ppm (Fig. 2).

Table 2: *In vitro* evaluation of non-systemic fungicides

Fungicides	Growth inhibition (%)			Mean inhibition (%)
	2000 ppm	2500ppm	3000 ppm	
Captan 50% WP	52.98 (63.75)*	53.73 (65.00)	55.63 (68.13)	54.11 (65.62)
Thiram 75%WP	47.87 (55.00)	48.59 (56.25)	50.77 (60.00)	49.07 (57.08)
Mancozeb 75% WP	48.59 (56.25)	52.24 (62.50)	54.11 (65.63)	51.64 (61.46)
Propineb 70%WP	39.94 (41.25)	41.41 (43.75)	47.87 (55.00)	43.07 (46.66)
Copper Hydroxide 77% WP	33.20 (30.00)	35.51 (33.75)	41.40 (43.75)	36.70 (35.83)
Copper Oxychloride 50% WP	46.43 (52.50)	48.59 (56.25)	89.19 (99.98)	61.40 (69.57)
Mean	44.83 (49.79)	46.68 (52.92)	56.49 (65.41)	49.33 (56.04)
	Fungicide (F)	Concentration (C)	F x C	
S.Em. ±	0.27	0.19	0.47	
C.D at 5%	0.78	0.55	1.35	
CV%	1.65			

*Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values

The effectiveness of thiram against *Fusarium oxysporum* has been recorded by Singh and Jha (2003) [1], Shovan *et al.* (2008) [20], Dubey *et al.* (2015) [3], Patil *et al.* (2015) [18] and Hussein *et al.* (2016) [11]. Looking to concentration of individual fungicides, all the fungicides differed significantly in inhibition of test pathogen.

3.3 *In vitro* evaluation of fungicide combinations

The results of the laboratory evaluation of ready mix fungicides on the radial growth of *F. oxysporum* f. sp. *ciceri* are presented in the Table 3 and Fig. 3. The results of the experiment showed that all selected fungicide combinations at all concentrations inhibited the radial growth of tested pathogen.

Sr. No.	Ready mix Fungicides	Concentration (ppm)
1.	Zineb 68% + Hexaconazole 4% WP	
2.	Carbendazim 12% + Mancozeb 63% WP	A = 250
3.	Azoxystrobin 11% + Tebuconazole 18.30% SC	
4.	Carboxin 37.5% + Thiram 37.5% WS	B = 500
5.	Tebuconazole 50% + Trifloxystobin 25% WG	
6.	Cymoxanil 8% + Mancozeb 64% WP	C = 1000
7.	Control	

Fig 3: Growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* on PDA supplemented with fungicide combinations

It is evident from the data presented in Table 3 and Fig. 3 all the fungicides were significantly effective in inhibiting growth of pathogen. The maximum growth inhibition of 90.31 per cent was recorded in fungicide combinations, carbendazim 12% + mancozeb 63% WP followed by tebuconazole 50% + trifloxystrobin 25% WG (88.56%), azoxystrobin 11% + tebuconazole 18.30% SC (84.22%), carboxin 37.5% + thiram 37.5% WS (76.93%), cymoxanil 8% + mancozeb 64% WP (62.69%) and zineb 68% + hexaconazole 4% WP (48.74%). They were statistically differed to each other.

Maitlo *et al.* (2014) [13] reported tebuconazole 50% + trifloxystrobin 25% WG as moderately effective fungicides against *F. oxysporum* f. sp. *ciceri*.

There was also found positive correlation between concentration and inhibition of growth of pathogen. It is also observed that with increasing concentration of all fungicides, inhibition of growth of pathogen also increased. Tebuconazole 50% + trifloxystobin 25% WG, carbendazim 12% + mancozeb 63% WP, cymoxanil 8% + mancozeb 64% WP, carboxin 37.5% + thiram 37.5% WS, azoxystrobin 11% + tebuconazole 18.30% SC and zineb 68% + hexaconazole 4% WP at 1000 ppm concentration showed 99.98, 94.77, 88.95, 88.36, 86.05 and 60.76 per cent inhibition of mycelial growth, respectively which were higher as compared to their lower concentration of 250 and 500 ppm (Fig. 3).

Table 3: *In vitro* evaluation of fungicide combinations

Fungicides	Growth inhibition (%)			Mean inhibition (%)
	250 ppm	500 ppm	1000 ppm	
Zineb 68% + Hexaconazole 4% WP	40.31 (41.86)*	41.32 (43.60)	51.21 (60.76)	44.28 (48.74)
Carbendazim 12% + Mancozeb 63% WP	68.07 (86.05)	71.69 (90.11)	76.83 (94.77)	72.19 (90.31)
Azoxystrobin 11% + Tebuconazole 18.30% SC	64.02 (80.81)	67.88 (85.81)	68.07 (86.05)	66.65 (84.22)
Carboxin 37.5% + Thiram 37.5% WS	57.01 (70.35)	58.11 (72.08)	70.09 (88.36)	61.73 (76.93)
Tebuconazole 50% + Trifloxystrobin 25% WG	62.77 (79.07)	68.56 (86.63)	89.19 (99.98)	73.50 (88.56)
Cymoxanil 8% + Mancozeb 64% WP	38.95 (39.52)	50.69 (59.87)	70.62 (88.95)	53.42 (62.69)
Mean	55.19 (66.28)	59.71 (73.02)	71.00 (86.48)	61.96 (75.24)
	Fungicide (F)	**Concentration (C)**	**F x C**	
S.Em. ±	0.34	0.24	0.58	
C.D at 5%	0.96	0.68	1.67	
CV%	1.62			

*Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values

Yadav (2009) [26] evaluated different fungicides combinations against *F. oxysporum* f. sp. *ciceri* by poisoned food technique *in vitro.* He found that combination of carbendazim 12% + mancozeb 63% WP inhibit 98.50% mycelial growth and cymoxanil 18% + mancozeb 64% WP was least effective inhibit only 33.73% mycelial growth.

Suman and Mohan Kumar (2016) [23] conducted an experiment for selection of superior fungicides for the management of chickpea wilt. Among the ten fungicides evaluated, carbendazim, propiconazole, and two combination product *i.e.* carbendazim + mancozeb and tebuconazole + trifloxystrobin exhibits completely inhibited (100% inhibition) the mycelial growth of fungus at 1500 ppm concentration followed by thiophanate methyl (96.67% inhibition) and least inhibition by copper oxychloride of 76.67% inhibition.

3.4 Evaluation of fungicides under pot condition

Fungicides which gave higher per cent inhibition in growth of test pathogen were evaluated in pots under green-house condition. Two superior fungicides were selected from each group *i.e.* systemic, non-systemic and fungicide combination (Table 4).

Table 4: Evaluation of fungicides in pot culture

Sr. No.	Fungicide	Concentration (%)	Disease Incidence (%)	Per cent Increase over control
1	Control	-	89.19 (99.98)	-
2	Tebuconazole 25.9% EC	0.01	41.15 (43.33)*	53.86 (56.66)
3	Carbendazim 50% WP	0.05	28.78 (23.33)	67.73 (76.66)
4	Captan 50% WP	0.25	30.99 (26.66)	65.25 (73.33)
5	Mancozeb 75% WP	0.25	59.00 (73.33)	33.84 (26.65)
6	Carbendazim 12% + Mancozeb 63% WP	0.1	23.85 (16.66)	73.25 (83.33)
7	Tebuconazole 50% + Trifloxystrobin 25% WG	0.1	37.22 (36.66)	58.26 (63.33)
S.Em. ±			2.22	-
C.D at 5%			6.86	-
C.V%			10.48	-

* Data outside the parentheses are arcsine transformed, whereas inside are re-transformed values

Test pathogen *Fusarium oxysporum* f. sp. *ciceri* was mass multiplied on solid medium and pot filling was done as described in section 2.3.2. Total six fungicides were drenched 30 DAS. Each fungicide replicated thrice and one set of three pots was maintained inoculated control (Fig. 4).

Fig 4: Overview of pot evaluation of fungicides

Sr. No.	Fungicides	Concentration (%)
1.	Control	-
2.	Tebuconazole 25.9% EC	0.01
3.	Carbendazim 50% WP	0.05
4.	Captan 50% WP	0.25
5.	Mancozeb 75% WP	0.25
6.	Carbendazim 12% WP + Mancozeb 63% WP	0.1
7.	Tebuconazole 50% + Trifloxystobin 25% WG	0.1

Among the fungicide tested, carbendazim 12% + mancozeb 63% WP showed minimum disease incidence (16.66%) at 0.1 per cent concentration which was followed by carbendazim 50% WP with 23.33% disease incidence at 0.05 per cent concentration. They were at par with captan 50% WP at 0.25%, tebuconazole 50% + trifloxystrobin 25% WG at 0.1%, tebuconazole 25.9% EC at 0.01% and mancozeb 75% WP at 0.25 per cent concentration gave 26.66, 36.66, 43.33 and 73.33 per cent disease incidence. Maximum per cent increase over control was found in carbendazim 12% + mancozeb 63% WP (83.33) which was followed by carbendazim (76.66) (Fig. 4).

The effectiveness of carbendazim 50 WP and tebuconazole 50% + trifloxystrobin 25% WG against wilt has been reported by Maitlo *et al.* (2014) [13] and Mahmood *et al.* (2015) [12] achieved good control of chickpea wilt in pot condition by soil drenching at two different concentrations *i.e.* 200 ppm and 500 ppm and recorded 82.50 per cent and 64.72 per cent disease reduction over un-inoculated control.

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