

Original Research Article

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In vitro Efficacy of Fungicides and Bio-Control Agents against Wilt of Chickpea Caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc)

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ABSTRACT

Five non-fungicides *viz.*, captan 50 WP, copper oxychloride 50 WP, dodine 65 WP, mancozeb 75 WP, antracol 70 WP and five systemic fungicides *viz.*, bitertanol 25 WP, carbendazim 50 WP, Difenconazole 25 EC, hexaconazole 5EC, and myclobutanil 10 WP were evaluated against chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris*. The *in-vitro* evaluation of non-systemic fungicides through poisoned food technique at five different concentrations *viz.*, 50, 100, 250, 500 and 1000 $\mu\text{g ml}^{-1}$ indicated that two fungicides *viz.*, dodine and captan proved the most effective exhibiting mean mycelial growth inhibition of 66.13 per cent followed by mancozeb with 51.42 per cent, respectively. Copper oxychloride proved least effective and resulted in only 23.79 per cent mean inhibition of mycelial growth. Systemic fungicides were also evaluated at different concentrations *viz.*, 50, 100, 200, 400 and 500 $\mu\text{g ml}^{-1}$ showed that carbendazim proved the most effective exhibiting mean mycelial growth inhibition of 92.17 per cent followed by difenconazole and hexaconazole with 76.52 and 71.18 per cent, respectively. Myclobutanil 10 WP proved to be the least effective and resulted in only 64.38 per cent mean inhibition of mycelial growth. Three strains of *Trichoderma viride* designated as Tv1, Tv2 Tv3 and two strain of *Trichoderma harzianum* designated as Th1 and Th2 were also evaluated against wilt pathogen of chickpea. The *in-vitro* evaluation of bio-control agents revealed that Tv2 is most efficacious resulted in 72.00 per cent mean inhibition of mycelial growth followed by Tv1 with 68.83 per cent respectively. *Trichoderma harzianum*2 (Th2) proved least efficacious resulted in 44.40 per cent mean inhibition of mycelial growth

Keywords

Chickpea, Bio-control agent, Fungicides, *Fusarium oxysporum* f. sp. *ciceris*, *Trichoderma*.

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Introduction

Chickpea (*Cicer arietinum*) is an important pulse crop of family Leguminaceae and ranks third in the global farming. Chickpea was originated from west Asia and now it is cultivated in 55 countries of the world. Worldwide it is grown on an area of 13.5 million ha with an annual production of more

than 13 million tonnes. It is an important crop of Indian sub-continent that usually contributes more than 66 per cent in terms of global production, while Pakistan ranks 7th and produced 2.5 per cent of world production (Anonymous, 2013). Chickpea crop is affected by several pathogens but wilt

caused by *Fusarium oxysporum* f.sp. *ciceris* is the most serious disease and causes huge losses upto 100 per cent if the crop remains untreated (Dubey et al., 2007). Annual yield losses in chickpea crop were estimated to be 4.8 million tonnes worldwide due to abiotic and biotic stresses, including infectious diseases (Ryan, 1997). Under severe conditions, the wilt disease can damage the crop completely and causes losses upto 100% (Navas-cortes et al., 2000). However, yield losses of 10-15 per cent were reported as a regular feature of this severe disease (Campbell and Madden, 1990). In India, annual yield losses of 10 percent were reported (Trapero-Cases and Jimenez-diaz, 1985) due to this wilt disease. Chickpea wilt is a vascular pathogen that perpetuates both in seed as well as soil. In absence of the host, the pathogen can also survive upto 3- 6 years (Ayyub et al., 2003). This pathogen can cause infection at all the stages of plant growth with more severe at flowering and podding stage. Wilted plants showed drooping and yellowing of leaves and lie down on the ground. The fungus invades the root system, made its way through the epidermis, cortex and finally enter into xylem vessels of tap root from where it spread. As a result, the later roots might wither off as reported by Chavan et al., 2009. Eighty per cent of plant mortality has been reported by Govil and Rana, 1994, when there is relatively high temperature with drought conditions. As a result of wilt infection, the complete plant or plant part may die within few weeks of infection. Under field conditions, the typical wilting can be appeared within 3-4 weeks after sowing, if the variety is susceptible (Haware, 1990). Thus the disease can be effectively managed through different strategies such as use of resistant varieties, cultural practices, use of fungicides and through bio-control agents. Although each of these methods of disease management practices has their own importance, but none of the method give

completely success when applied alone for disease control (Chandel & Deepika, 2010). Despite many attempts to control chickpea wilt pathogen Foc, the problem is still important throughout the world. The use of fungicide is most effective and reliable method of controlling disease. Now economical and eco-friendly control measures are available to combat this threat (Bakhsh et al., 2007). Fungicides with novel chemistry are being introduced and evaluated against this wilt pathogen. Their application in the farmers field can only be recommended against the Foc pathogen after a successful laboratory evaluation. It is therefore, needs a constant watch and effort to evolve new fungicides along with some non-chemical method of controlling the wilt disease (Jamil and Kumar, 2010). Therefore, the present study is carried out to evaluate non- systemic fungicides and systemic and some bio-control agents against the disease and the results are presented herein.

Materials and Methods

The systemic and non-systemic fungicides were evaluated *in-vitro* against chickpea blight (*Fusarium oxysporum* f. sp. *ciceris*)

***In-vitro* evaluation of fungicides**

The non-systemic fungicides were evaluated at 50, 100, 250, 500 and 1000 $\mu\text{g ml}^{-1}$ and systemic fungicides at 50, 100, 200, 400 and 500 $\mu\text{g ml}^{-1}$ through poisoned food technique (Carpenter, 1942) using potato dextrose agar medium for their effects on inhibition of mycelial growth. The required concentrations of fungicides were prepared by adding appropriate amount of fungicides to sterilized molten PDA medium in conical flasks. Thirty millilitre of such amended PDA was aseptically poured in sterilized Petri plates. A 5 mm diameter mycelial disc of FOC was aseptically placed in the centre of each Petri

plate. Petri plates containing PDA media amended with equal amount of sterilized distilled water and inoculated with mycelial disc of *Fusarium oxysporum* f. sp. *ciceris* (5 mm diameter) served as check. Each treatment was replicated thrice and incubated at $28 \pm 2^\circ\text{C}$ for 48 hrs. The comparative efficacy of fungicides was calculated as per cent inhibition of mycelial growth of the test fungus in each treatment as compared to check by the following formula:

$$\text{Per cent mycelial Growth inhibition} = \frac{C - T}{C} \times 100$$

Where

C = Radial mycelial growth (mm) in check
T = Radial mycelial growth in the treatment (mm)

In-vitro evaluation of bio-control agents

The pure culture of antagonistic organisms i.e. *Trichoderma viridi* 1, *Trichoderma viridi* 2, *Trichoderma viridi* 3, *Trichoderma viridi* 4, and *Trichoderma harzianum* 1 and *Trichoderma harzianum* 2 were collected from soils of different parts of Kashmir. The pure culture was multiplied in the test tubes slants. Agar slants of these cultures were placed in refrigerator at 4 degree centigrade. For *in vitro* assay 5mm mycelial disc of both the bio-control microorganism and the pathogen were simultaneously inoculated at the opposite ends of the petriplates, containing about 20 ml of PDA medium. Three petri plates were used for each biological control agent and the same number was kept as control. Inoculated plates were incubated at $25 \pm 2^\circ\text{C}$. For 7-10 days. The data regarding the fungal hyphal growth were recorded by following percent growth Inhibition formula (already described above).

Results and Discussion

In-vitro evaluation of non-systemic fungicides on mycelial growth inhibition

Five non-systemic fungicides were evaluated *in-vitro* for inhibition of mycelium growth the pathogen (*Fusarium oxysporum* f. sp. *ciceris*). The data (Table 1; Fig. 1) revealed that all the test fungicides significantly inhibited the mycelial growth at all the test concentrations. On an overall mean basis, both dodine 65 WP and captan 50WP proved most effective exhibiting mean mycelial growth inhibition of 66.13 per cent followed by mancozeb 75 WP 51.42 per cent mean inhibition of mycelial growth while antracol 70 WP caused 41.75 per cent mean inhibition of mycelial growth, respectively. Copper oxychloride proved least effective resulting in only 23.79 per cent mean inhibition of mycelial growth. In general, the efficacy of fungicides varied significantly with change in fungicide concentration. At $50 \mu\text{g ml}^{-1}$ concentration, the mean mycelial growth inhibition was 31.16 per cent which increased to 37.96, 44.63, 53.95 and 69.12 per cent at 100, 250, 500 and $1000 \mu\text{g ml}^{-1}$ concentrations, respectively.

The highest mycelial growth inhibition at $50 \mu\text{g a.i ml}^{-1}$ concentration was exhibited by both dodine 65 WP and captan 50 WP exhibiting 45.23 per cent mean mycelial inhibition followed mancozeb 75 WP causing 36.16 per cent mean mycelial inhibition while copper oxychloride 50 WP was least efficacious and resulted in only 18.50 per cent mean mycelial inhibition. At $1000 \mu\text{g ml}^{-1}$ concentration, 100.00 per cent mycelial growth inhibition was again caused by dodine 65 WP and captan 50 WP followed by mancozeb 75 WP (76.40%) while copper oxychloride 50 WP was least effective and resulted in only 30.80 per cent inhibition at the same concentration.

Table.1 *In-vitro* evaluation of non-systemic fungicides against *Fusarium oxysporum* f. sp *ciceris* (Foc) causing wilt/root rot of chickpea

Fungicides	Per cent inhibition of radial mycelial growth at concentration ($\mu\text{g ml}^{-1}$)					
	50	100	250	500	1000	Mean
Anrtacol 70 WP	27.92 (31.89)	30.94 (37.87)	37.30 (37.87)	49.33 (44.61)	62.90 (52.49)	41.75 (40.25)
Captan 50WP	45.23 (42.26)	51.80 (46.03)	61.66 (51.75)	72.00 (58.05)	100.00 (90.00)	66.13 (54.14)
Copper oxychloride 50 WP	18.50 (25.47)	20.00 (26.56)	24.20 (29.46)	25.46 (30.30)	30.80 (33.70)	23.79 (29.19)
Dodine 65WP	45.23 (42.26)	51.80 (46.03)	61.66 (51.75)	72.00 (58.05)	100.00 (90.00)	66.13 (100.00)
Mancozeb 75 WP	36.16 (36.96)	41.40 (40.40)	46.80 (43.16)	56.36 (48.65)	76.40 (60.93)	
Mean						
CD (P=0.05)						
Fungicide	= (0.90)					
Concentration	= (0.91)					
Fungicide \times Concentration	= (2.24)					

Figures within parentheses are arc sign transformed values

Table.2 *In-vitro* evaluation of systemic fungicides against *Fusarium oxysporum* f. sp *ciceris* (Foc) causing wilt/root rot of chickpea

Fungicides	Per cent inhibition of radial mycelial growth at concentration ($\mu\text{g ml}^{-1}$)					
	50	100	200	400	500	Mean
Bitertanol 25WP	48.40 (44.08)	65.90 (54.27)	69.03 (56.18)	76.43 (60.95)	85.70 (67.78)	69.09 (56.16)
Carbendazim 50WP	78.16 (62.13)	82.70 (65.42)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	92.17 (73.75)
Difenconazol 25 EC	72.30 (58.24)	74.00 (59.34)	75.38 (60.25)	79.43 (63.02)	81.90 (64.82)	76.52 (61.01)
Hexaconazole 5EC	50.40 (45.22)	65.46 (54.00)	72.30 (58.24)	80.43 (63.74)	87.33 (69.14)	71.18 (57.53)
Myclobutanil 10 WP	36.33 (37.06)	54.70 (47.69)	70.22 (56.92)	76.23 (60.82)	84.46 (66.78)	64.38 (53.35)
Mean	57.11 (49.08)	68.55 (55.88)	77.33 (61.56)	82.50 (65.27)	87.87 (69.61)	
CD (P=0.05)						
Fungicide	= (1.27)					
Concentration	= (125)					
Fungicide \times Concentration	= (2.33)					

Figures within parentheses are arc sign transformed values

Table.3 *In-vitro* evaluation of bio-control agents against *Fusarium oxysporum* f. sp *ciceris* (Foc) causing wilt/root rot of chickpea

Bio-control agents	Per cent inhibition in radial mycelial growth of the pathogen
<i>Trichoderma viridi</i> -1	68.83 (56.90)
<i>Trichoderma viridi</i> -2	72.00 (58.05)
<i>Trichoderma viridi</i> -3	49.06 (44.66)
<i>Trichoderma viridi</i> -4	47.20 (43.28)
<i>Trichoderma harzianum</i> -1	54.20 (47.40)
<i>Trichoderma harzianum</i> -2	44.40 (41.78)
CD (P=0.05)	2.80

Figures within parentheses are arc sign transformed values

Fig.1 *In-vitro* evaluation of non- systemic fungicides against *Fusarium oxysporum* f. sp *ciceris* (Foc) causing wilt of chickpea

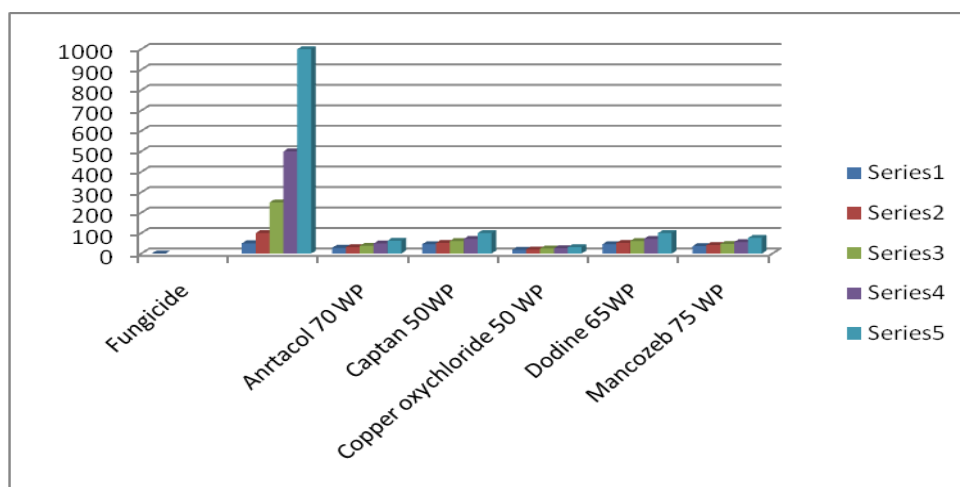
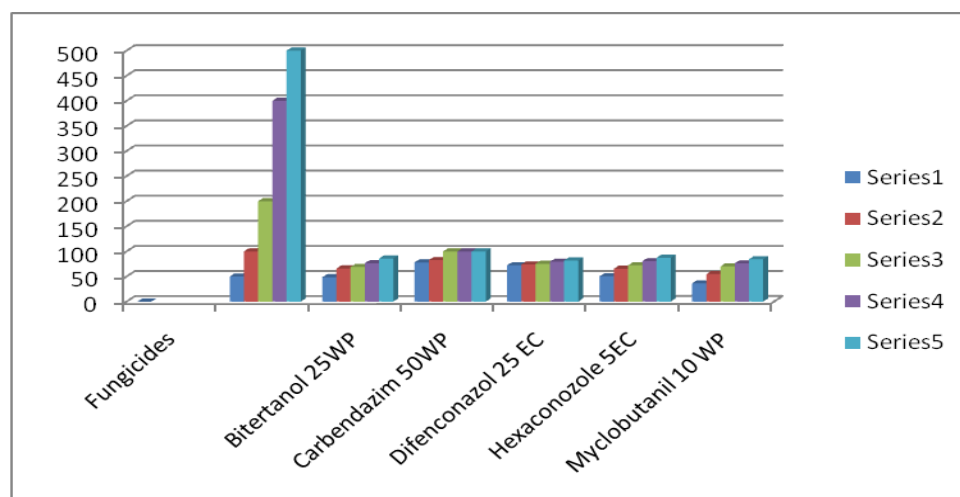


Fig.2 *In-vitro* evaluation of systemic fungicides against *Fusarium oxysporum* f. sp *ciceris* (Foc) causing wilt of chickpea



***In-vitro* evaluation of systemic fungicides on mycelial growth inhibition**

Similarly five systemic fungicides were evaluated *in-vitro* for inhibition of mycelium growth the pathogen (*Fusarium oxysporum* f. sp. *ciceris*). The data (Table 2; Fig. 2) revealed that all the test fungicides significantly inhibited the mycelial growth at all the test concentrations. On an overall mean basis, carbendazim 50 WP proved most effective exhibiting mean mycelial growth inhibition of 92.17 per cent followed by difenconazole 25 EC showing 76.52 per cent mean inhibition of mycelial growth while hexaconazole 5 EC caused 71.18 per cent mean inhibition of mycelial growth, respectively. Myclobutanil 10 WP proved least effective resulting in only 64.38 per cent mean inhibition of mycelial growth. In general, the efficacy of fungicides varied significantly with change in fungicide concentration. At 50 $\mu\text{g ml}^{-1}$ concentration, the mean mycelial growth inhibition was 57.11 per cent which increased to 68.55, 77.33, 82.50, and 87.87 per cent at 100, 200, 400 and 500 $\mu\text{g ml}^{-1}$ concentrations, respectively. The highest mycelial growth inhibition at 50 $\mu\text{g a.i ml}^{-1}$ concentration was exhibited by carbendazim 50 WP exhibiting 78.16 per cent mean mycelial inhibition followed difenconazole 25 EC causing 72.30 per cent mean mycelial inhibition while myclobutanil 10 WP was least efficacious and resulted in only 36.33 per cent mean mycelial inhibition. At 500 $\mu\text{g ml}^{-1}$ concentration, 100.00 per cent mycelial growth inhibition was caused by carbendazim 50 WP.

***In-vitro* evaluation of bio-control agents**

In dual culture test of bio-control microorganisms with Foc showed that *Trichoderma viridi* 2 (Tv2) had more mycelial growth inhibition of pathogen (Foc) with 72.00 % inhibition over control.

Trichoderma viridi 1 (Tv1) was proved to be second best followed by *Trichoderma harzianum* (Th1), *Trichoderma viridi* 3 (Tv3) and *Trichoderma harzianum* 2 (Th2) with 68.83, 54.20, 49.06 and 44.40 growth reduction over control respectively (Table 3).

Chemical control based on the use of the fungicides in spite of its all health hazards has proved to be the management strategy. Ten chemical fungicides with 50-500 ppm were screened against Foc under *in-vitro* conditions. Out of the ten fungicides carbendazim, dodine and captan were found as the most effective against the Foc. The other fungicides like difenconazole and mancozeb were moderately effective against Foc. Whereas, remaining tested fungicides were generally less effective against the targeted pathogen. Generally, a positive correlation was observed between the different concentrations of the tested fungicides and inhibition of Foc. Higher doses of fungicides were found to be more effective than their lower doses. There were several reports from elsewhere regarding *in-vitro* evaluation of chemical fungicides *F. oxysporum*. Our results are in conformity with those reported by Ilyas *et al.*, (1992), poddar *et al.*, (2004), Song *et al.*, (2004), Rajput *et al.*, (2006), Mukhtar (2007), Sultana and Ghaffar (2010) and Khan *et al.*, (2012). The fungicides which efficiently inhibit the test fungus in *in-vitro* study are supposed to be effective against the same pathogen in natural conditions. Generally, all the treatments check the activities of the inoculated fungus (Foc) and hence promote the growth of chickpea plant. Although the higher concentrations of the few fungicides completely inhibited the pathogen. Similarly biological control of the plant diseases can be defined as management of the plant diseases by reducing the inoculum of the pathogen with the help of the beneficial micro-organisms (Campbell, 1994). In the present study *Trichoderma viridi* was proved

most effective in *in-vitro* and these findings were completely in agreement with many workers who found many isolates/strains of *Trichoderma*, isolated from the rhizosphere regions of host crop plants were found effective to manage the plant pathogens (Burr *et al.*, 1998, Prasad *et al.*, 2002, Postma *et al.*, 2003 and Saikia *et al.*, 2003). Merkuz and Getachew (2012) found that isolates of *Trichoderma* were very effective against chickpea wilt disease. Singh *et al.*, in 1998 reported that seed treatment of chickpea with bio-control agent like *T. harzianum* and *G. virens* gave best results against *F.oxysporum* f.sp. *ciceri*. The present situation demands their incorporation in integrated disease management schedule for better disease management and optimum returns from crop husbandry.

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