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In vitro efficacy of fungicides against *Fusarium oxysporum* f. sp. *udum* causing wilt disease of pigeonpea

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Abstract

Pigeonpea wilt disease caused by *Fusarium oxysporum* f. sp. *udum* is one of the most devastating soil-borne disease. The aim of present investigation was to evaluate the antifungal activities of fungicides which can be used to control wilt disease of pigeonpea. The systemic fungicides viz. azoxystrobin, hexaconazole, penconazole, propiconazole, thiophanate methyl, difenconazole, carbendazim and fosetyl-AL and combi fungicides viz. carbendazim 25% + mancozeb 50 % WP, carbendazim 12 % + mancozeb 63 % WP, hexaconazole 4 % + zineb 68 % WP, hexaconazole 5 % + captan 70 % WP and metalaxyl M 4 % + mancozeb 64 % WP screened *in vitro* for their antifungal activities against *F. udum* by using poison food techniques. Among eight systemic fungicides tested, per cent average mycelial growth inhibition was ranged from 79.75 to 100 per cent. However, it was cent per cent in azoxystrobin, hexaconazole, penconazole and propiconazole (100 %), followed by thiophanate methyl (97.90 %), difenconazole (93.70 %), carbendazim (89.38 %) and fosetyl-AL (90.74 %). Among five combi fungicides tested per cent average mycelial growth inhibition was ranged from from 62 to 92.13 per cent. However, it was highest average mycelial growth inhibition in carbendazim 25% + mancozeb 50 % WP (92.13 %), followed by carbendazim 12 % + mancozeb 63 % WP (83.15 %), hexaconazole 4 % + zineb 68 % WP (78.15), hexaconazole 5 % + captan 70 % WP (76.39 %) and metalaxyl M 4 % + mancozeb 64 % WP (62.69 %).

Keywords: Pigeonpea wilt, *Fusarium oxysporum* f. sp. *udum*, *in vitro*, combi fungicides, systemic fungicides

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is most important pulse crop belong to family Fabaceae (earlier known as Leguminosae). It is also known as arhar, yellow dhal, red gram, tur etc. It is the second largest family after Poaceae (earlier known as Gramineae), in terms of food as well as vegetable protein source and of fodder. Endowed with excellent food and fodder qualities, these crops also restore soil fertility by scavenging atmospheric nitrogen, adding organic matter, enhancing phosphorus availability as well as improving physical, chemical and biological properties of the soil

The area, production and productivity of pigeonpea cultivated in India during the year 2016-17 were 5.21 m.ha, 4.23 m. tonnes, and 826 kg/ha, respectively. In Maharashtra, area, production and productivity of pigeonpea during the year 2016-17 were 15.33 L.ha, 11.70 L.tonnes and 764 kg/ha, respectively. In Marathwada region of Maharashtra, area, production and productivity of pigeonpea during year 2016-17 were 5.95 L.ha., 4.47 L. tonnes, and 759 kg/ha., respectively (Second Advance Estimate and Source: Chief Statistician; Pune, 2017) [1].

In general, there is low productivity of pulses including pigeonpea. Because, the crop is grown on marginal lands, low rainfall areas, poor management, poor crop husbandry, high rate of flower and fruit drop, non- uniform maturity, pod shattering and susceptibility to pests and diseases.

The crop is attacked by more than 100 pathogens (Nene *et al.*, 1996) [11] including fungi, bacteria, viruses, phytoplasma like organisms and nematodes. However, only a few of them cause economic losses (Kannaiyan *et al.*, 1984) [8]. The diseases of considerable economic importance are sterility mosaic, *Fusarium* wilt, *Phytophthora* blight, *Macrophomina* root rot, stem canker and *Alternaria* blight.

Fusarium wilt is the most important disease of pigeonpea in India which is responsible for yield losses up to 67 per cent at maturity and 100 per cent in case of infection at pre-pod stage (Kannaiyan and Nene, 1981) [7]. The *Fusarium* wilt in pigeonpea was first reported from Bihar by Butler (1910). Surveys conducted for the disease by Kannaiyan *et al.* (1984) [8] have indicated it to be a major problem in the states of Bihar and Maharashtra (Reddy *et al.*, 1990) [14]. *Fusarium* wilt characterized by wilting of the affected plants and characteristic internal

browning or blackening of the xylem vessels extending from root system to stems. Partial wilting of the plants (Upadhyay and Rai, 1992) [17] and patches of dead plants (Reddy *et al.*, 1993) [15] were reported to be common in the fields during advanced stages of plant growth. Present investigation was carried out with *in vitro* evaluation of fungicides for control of *Fusarium oxysporum* f. sp. *udum* causing wilt disease of Pigeonpea.

Materials and Methods

The experiment was conducted at Department of Plant Pathology, College of Agriculture Parbhani, VNMKV, Parbhani (M.S.). The pathogen was isolated from diseased leaves of Pigeonpea on PDA incubated at 27±2 °C. eight systemic fungicides *viz.* azoxystrobin, hexaconazole, penconazole, propiconazole, thiophanate methyl, difenconazole, carbendazim and fosetyl-AL and five combi fungicides *viz.* carbendazim 25% + mancozeb 50 % WP, carbendazim 12 % + mancozeb 63 % WP, hexaconazole 4 % + zineb 68 % WP, hexaconazole 5 % + captan 70 % WP and metalaxyl M 4 % + mancozeb 64 % WP were evaluated *in vitro* for their antifungal activities against wilt diseases of Pigeonpea, *Fusarium oxysporum* f. sp. *udum* using Poison food techniques (Nene and Thapliyal, 1993) [10]. The requisite quantity of each fungicides on the basis of active ingredient (a.i) was calculated thoroughly mix with autoclaved and cooled (40-45°C) PDA in conical flasks to obtain desired concentration of 500, 1000, 1500 ppm for systematic and 1500, 2000 and 2500 ppm for combi-fungicides. 20 ml of potato dextrose agar media was poured in sterilized petri plates and to be solidified. Fungal disks of 5 mm in diameter from 7 days old culture was placed in the centre of the petri dish containing medium under aseptic condition, incubated at 27±2 °C for 7 days. Three replicated plates were used for each concentration of every fungicide. Three replicated PDA plates received no fungicides served as control. Diameter of the colonies on PDA with and without fungicides was measured from the bottom side of the petri dishes. The colony diameter of the fungus pathogens on medium was recorded and per cent inhibition was calculated by using following formula (Vincent, 1927) [18].

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

Where,

C= growth of the test fungus in untreated control plates

T= growth of the test fungus in treated plates

Results and Discussion

Mycelial growth

Results revealed that at 500 ppm concentration, radial mycelial growth was ranged from 0 mm (azoxystrobin, hexaconazole, penconazole and propiconazole) to 30.67 mm (fosetyl-AL). However, the fungicide azoxystrobin, hexaconazole, penconazole and propiconazole arrested cent per cent mycelial growth. The next fungicides with significantly least mycelial growth were thiophanate methyl (5.67 mm), followed by difenconazole (14.33 mm), carbendazim (23.33 mm) and fosetyl-AL (30.67 mm).

At 1000 ppm concentration, radial mycelial growth was ranged from 0 mm (azoxystrobin, hexaconazole, penconazole, propiconazole and thiophanate methyl) to 15.67 mm (fosetyl-AL). However, the fungicides azoxystrobin, hexaconazole, penconazole, propiconazole and thiophanate methyl arrested

cent per cent mycelial growth; whereas, it was significantly least with difenconazole (2.67 mm), carbendazim (5.33 mm) and fosetyl-AL (15.67 mm).

At 1500 ppm concentration, radial mycelial growth was ranged from 0 mm (azoxystrobin, carbendazim, difenconazole, hexaconazole, penconazole, Propiconazole and thiophanate methyl) to 8.33 mm (fosetyl-AL). However, there was no mycelial growth in azoxystrobin, carbendazim, difenconazole, hexaconazole, penconazole, propiconazole and thiophanate methyl containing media.

Average radial mycelial growth of the test pathogen was ranged from 0 mm (azoxystrobin, hexaconazole, penconazole, and propiconazole) to 18.22 mm (fosetyl-AL). However, there was no mycelial growth in azoxystrobin, hexaconazole, penconazole, and propiconazole. The fungicides with next lowest average mycelial growth were thiophanate methyl (1.89 mm), difenconazole (5.67 mm) and carbendazim (9.55 mm). All the treatments were significantly superior over untreated control.

Mycelial Inhibition

Results revealed that all the systemic fungicides tested (each @ 500, 1000 and 1500 ppm) significantly inhibited mycelial growth of *F. udum*, over untreated control. Further, per cent mycelial inhibition was increased with increase in concentration of the fungicides tested (Table 2).

At 500 ppm, mycelial growth inhibition was ranged from 65.93 (fosetyl-AL) to 100 per cent (azoxystrobin, hexaconazole, penconazole and propiconazole). However, azoxystrobin, hexaconazole, penconazole and propiconazole gave cent per cent (100 %) mycelial inhibition. The next best fungicides found were thiophanate methyl (93.7 %), difenconazole (84.07 %), carbendazim (74.07 %) and fosetyl-AL (65.93 %).

At 1000 ppm, mycelial growth inhibition was ranged from 82.59 (fosetyl-AL) to 100 per cent (azoxystrobin, hexaconazole, penconazole, propiconazole and thiophanate methyl). However, it was cent per cent with the fungicides azoxystrobin, hexaconazole, penconazole, propiconazole and thiophanate methyl (each 100 %). In the order of merit the next most effective fungicides with significantly higher mycelial inhibition were difenconazole (97.04 %), carbendazim (94.07 %) and fosetyl-AL (82.59 %).

At 1500 ppm, mycelial growth inhibition was ranged from 90.74 (fosetyl-AL) to 100 per cent (azoxystrobin, carbendazim, difenconazole, hexaconazole, penconazole, propiconazole and thiophanate methyl). However, it was cent per cent in the fungicides azoxystrobin, carbendazim, difenconazole, hexaconazole, penconazole, propiconazole and thiophanate methyl (each 100 %). The next effective fungicide was fosetyl-AL (90.74 %).

Average mycelial growth inhibition recorded with the test systemic fungicides was ranged from 79.75 (fosetyl-AL) to 100 per cent (azoxystrobin, hexaconazole, penconazole and propiconazole). However, it was cent per cent in azoxystrobin, hexaconazole, penconazole and propiconazole (100 %), followed by thiophanate methyl (97.90 %), difenconazole (93.70 %), carbendazim (89.38 %) and fosetyl-AL (90.74 %).

Thus, all the systemic fungicides tested were found fungistatic against *F. udum* and significantly inhibited its mycelial growth, over untreated control. However, the systemic fungicides found most effective in the order of merit were azoxystrobin, hexaconazole, penconazole and propiconazole.

These results are in conformity with the earlier findings of

those workers who reported systemic fungicides viz., azoxystrobin, carbendazim, difenconazole, fosetyl-AL, hexaconazole, penconazole, propiconazole and thiophanate methyl at various concentrations had significantly inhibited mycelial growth of *F. udum* infecting pigeonpea (Shah *et al.*, 2006 and Chennakesavulu *et al.*, 2013) [3].

Hukmaram and Pandey (2011) tested seven fungicides *in vitro* against *Fusarium udum*, the causal agent of wilt of pigeonpea. Among the fungicides evaluated, carbendazim (500 µg ml⁻¹),

difenconazole (100 µl ml⁻¹), hexaconazole (200 µl ml⁻¹) and combi product of captan+ hexaconazole (250 µg ml⁻¹) and carbendazim + mancozeb (500 µg ml⁻¹) completely inhibited the mycelial growth of the pathogen.

Whereas, Chennakesavulu *et al.* (2013) [3] reported that all the tested fungicides at all the concentrations reduced mycelial growth of *F. udum* when compared to control. Overall per cent inhibition of mycelial growth of *F. udum* was high in carbendazim, propiconazole and tebuconazole.

Table 1: *In vitro* efficacy of systemic fungicides against *Fusarium udum*

Tr. No.	Treatments	Colony dia. *(mm) at different ppm			Av. (mm)	Per cent Inhibition* at different ppm			Av. Inhibition (%)
		500	1000	1500		500	1000	1500	
T ₁	Azoxystrobin 23 SC	0.00	0.00	0.00	00.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T ₂	Carbendazim 50 WP	23.33	5.33	0.00	09.55	74.07 (59.47)	94.07 (76.42)	100 (90.00)	89.38 (75.08)
T ₃	Difenconazole 25 EC	14.33	2.67	0.00	05.67	84.07 (66.62)	97.04 (81.93)	100 (90.00)	93.70 (78.84)
T ₄	Fosetyl-AL 80 WP	30.67	15.67	8.33	18.22	65.93 (54.34)	82.59 (65.36)	90.74 (72.30)	79.75 (63.95)
T ₅	Hexaconazole 5 EC	0.00	0.00	0.00	00.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T ₆	Penconazole 10 EC	0.00	0.00	0.00	00.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T ₇	Propiconazole 25 EC	0.00	0.00	0.00	00.00	100 (90.00)	100 (90.00)	100 (90.00)	100 (90.00)
T ₈	Thiophanate methyl 70 WP	5.67	0.00	0.00	01.89	93.7 (75.77)	100 (90.00)	100 (90.00)	97.90 (85.14)
T ₉	Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E.+	1.97	0.96	0.29	3.59	1.58	1.72	0.32	4.42
	C.D.(P=0.01)	5.89	2.88	0.88	10.75	4.79	5.16	0.96	13.24

*: Mean of three replications, Dia: Diameter, Av.: Average Figures in parentheses are angular transformed values

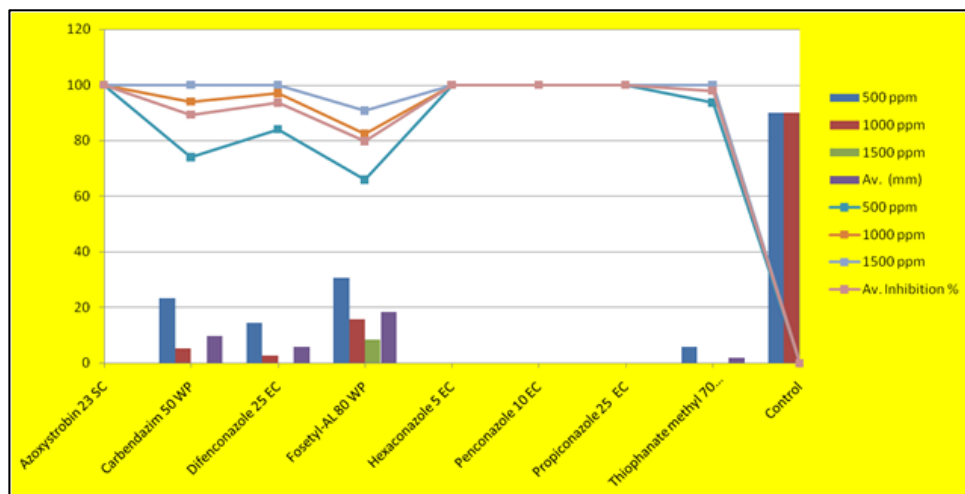


Fig 1: *In vitro* efficacy of systemic fungicides against *F. udum* *In vitro* evaluation of combi-fungicides Radial mycelial growth

Results revealed that all of the five combi-fungicides tested exhibited a wide range of radial mycelial growth of *F. udum* and was decreased drastically with increase in concentrations of the test fungicides from 1500 to 2500 ppm.

At 1500 ppm, radial mycelial growth of the test pathogen was ranged from 16.50 mm (carbendazim 25% + mancozeb 50 % WP) to 44.75 mm (metalaxyl M 4 % + mancozeb 64 % WP). However, it was significantly least in carbendazim 25% + mancozeb 50 % WP (16.50 mm). The next fungicides with significantly low mycelial growth were carbendazim 12 % + mancozeb 63 % WP (30.50 mm), followed by hexaconazole 4 % + zineb 68 % WP (36.25 mm), hexaconazole 5 % + captan 70 % WP (37.50 mm) and metalaxyl M 4 % + mancozeb 64 % WP (44.75 mm).

At 2000 ppm, radial mycelial growth of the test pathogen was ranged from 4.75 mm (carbendazim 25% + mancozeb 50 % WP) to 30.25 mm (metalaxyl M 4 % + mancozeb 64 % WP). However, it was significantly least carbendazim 25% + mancozeb 50 % WP (4.75 mm). The next fungicides with significantly low mycelial growth were carbendazim 12 % + mancozeb 63 % WP (15.00 mm), followed by hexaconazole 4

% + zineb 68 % WP (18.50 mm), hexaconazole 5 % + captan 70 % WP (24.75 mm) and metalaxyl M 4 % + mancozeb 64 % WP (30.25 mm).

At 2500 ppm, radial mycelial growth of the test pathogen was ranged from 0 mm (carbendazim 12 % + mancozeb 63 % WP, carbendazim 25% + mancozeb 50 % WP) to 25.75 mm (metalaxyl M 4 % + mancozeb 64 % WP). However, it was significantly low in hexaconazole 5 % + captan 70 % WP (1.50 mm). The next fungicides with significantly low mycelial growth were Hexaconazole 4 % + Zineb 68 % WP (4.25 mm) and metalaxyl M 4 % + mancozeb 64 % WP (25.75 mm).

Average radial mycelial growth of the test pathogen was ranged from 7.08 mm (carbendazim 25% + mancozeb 50 % WP) to 33.58 mm (metalaxyl M 4 % + mancozeb 64 % WP). However, it was least in the fungicide carbendazim 25% + mancozeb 50 % WP (7.08 mm). The fungicides with next low average mycelial growth were carbendazim 12 % + mancozeb 63 % WP (15.17 mm), hexaconazole 4 % + zineb 68 % WP (19.67 mm), hexaconazole 5 % + captan 70 % WP (21.25 mm) and metalaxyl M 4 % + mancozeb 64 % WP (33.58

mm). All treatments were significantly superior over untreated control, in respect to less mycelial growth.

Mycelial inhibition

Results revealed that all the combi- fungicides tested (each @ 1500, 2000 and 2500 ppm) have significantly inhibited mycelial growth of *F. udum* over untreated control. Further, per cent mycelial inhibition was increased with increase in concentration of the fungicides tested different ppm

At 1500 ppm, mycelial growth inhibition was ranged from 50.58 (metalaxyl M 4 % + mancozeb 64 % WP) to 81.67 per cent (carbendazim 25% + mancozeb 50 % WP). However, it was significantly highest in carbendazim 25 % + mancozeb 50 % WP (81.67 %), followed by the fungicides carbendazim 12 % + mancozeb 63 % WP (66.11 %), hexaconazole 4 % + zineb 68 % WP (59.72 %), hexaconazole 5 % + captan 70 % WP (58.33 %) and metalaxyl M 4 % + mancozeb 64 % WP (50.28 %).

At 2000 ppm, mycelial growth inhibition was ranged from 66.39 (metalaxyl M 4 % + mancozeb 64 % WP) to 94.72 per cent (carbendazim 25% + mancozeb 50 % WP). However, it was significantly highest in carbendazim 25% + mancozeb 50 % WP (94.72%), followed by the fungicides carbendazim 12 % + mancozeb 63 % WP (83.33 %), hexaconazole 4 % + zineb 68 % WP (79.44 %), hexaconazole 5 % + captan 70 % WP (72.50 %) and metalaxyl M 4 % + mancozeb 64 % WP (66.39 %).

At 2500 ppm, mycelial growth inhibition was ranged from 71.39 (metalaxyl M 4 % + mancozeb 64 % WP) to 100 per cent (carbendazim 12 % + mancozeb 63 % WP and carbendazim 25% + mancozeb 50 % WP).

However, it was significantly highest in carbendazim 12 % + mancozeb 63 % WP and carbendazim 25% + mancozeb 50 %

WP (100%), followed by the fungicides hexaconazole 5 % + captan 70 % WP (98.33 %), hexaconazole 4 % + zineb 68 % WP (95.28 %) and metalaxyl M 4 % + mancozeb 64 % WP (71.39 %).

Average mycelial growth inhibition recorded with the test combi- fungicides was ranged from 62.69 (metalaxyl M 4 % + mancozeb 64 % WP) to 92.13 per cent (carbendazim 25% + mancozeb 50 % WP). However, it was highest average mycelial growth inhibition in carbendazim 25% + mancozeb 50 % WP (92.13 %), followed by carbendazim 12 % + mancozeb 63 % WP (83.15 %), hexaconazole 4 % + zineb 68 % WP (78.15), hexaconazole 5 % + captan 70 % WP (76.39 %) and metalaxyl M 4 % + mancozeb 64 % WP (62.69 %).

Thus, five combi-fungicides tested were found fungistatic against *F. udum*. However, on the basis of order of merit combi fungicides carbendazim 25% + mancozeb 50 % WP, carbendazim 12 % + mancozeb 63 % WP, hexaconazole 4 % + zineb 68 % WP, hexaconazole 5 % + captan 70 % WP and metalaxyl M 4 % + mancozeb 64 % WP were found effective against *F. udum*, causing wilt in pigeonpea.

Combi-fungicides (systemic + contact) viz., carbendazim 12 % + mancozeb 63 % WP, carbendazim 25 % + mancozeb 50 % WP, hexaconazole 5% + captan 70 % WP, metalaxyl M 4 % + mancozeb 64 % WP and hexaconazole 4 % + zineb 68 % WP were also reported to cause significant mycelial growth inhibition of *F. udum* causing wilt disease to pigeonpea by several workers. (Haware and Kannaiyan, 1992; Poddar *et al.*, 2004; Raju *et al.*, 2008 and Gupta *et al.*, 2014) ^[10, 13, 4].

Similarly, Naresh *et al.* (2016) ^[9] reported that all the fungicides significantly reduced growth of *F. solani*. carbendazim (12 %) + mancozeb (63 %) at both the concentrations (500 ppm and 1000 ppm) completely inhibited the mycelial growth (100%) over control.

Table 2: *In vitro* efficacy of combi-fungicides against *Fusarium udum*

Tr No.	Treatments	Colony dia. *(mm) at different ppm			Av. (mm)	Per cent Inhibition* at different ppm			Av. inhibition (%)
		1500	2000	2500		1500	2000	2500	
T ₁	Carbendazim 12 % + mancozeb 63 % WP	30.50	15.00	0.00	15.17	66.11 (54.45)	83.33 (66.02)	100 (90.00)	83.15 (70.08)
T ₂	Carbendazim 25 % + mancozeb 50 % WP	16.50	4.75	0.00	07.08	81.67 (64.78)	94.72 (76.84)	100 (90.00)	92.13 (77.10)
T ₃	Hexaconazole 5 % + captan 70 % WP	37.50	24.75	1.50	21.25	58.33 (49.82)	72.50 (58.41)	98.33 (83.69)	76.39 (63.55)
T ₄	Metalaxyl M 4 % + mancozeb 64 % WP	44.75	30.25	25.75	33.58	50.28 (45.14)	66.39 (54.63)	71.39 (57.69)	62.69 (52.44)
T ₅	Hexaconazole 4 % + zineb 68 % WP	36.25	18.50	4.25	19.67	59.72 (50.62)	79.44 (63.04)	95.28 (77.74)	78.15 (63.67)
T ₆	Control	90.00	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	S.E.+	2.79	1.84	0.97	7.43	1.88	1.42	1.30	7.47
	C.D.(P=0.01)	8.34	5.50	2.92	23.15	5.62	4.26	3.89	23.26

*: Mean of four replications, Dia: Diameter, Av.: Average Figures in parentheses are angular transformed values

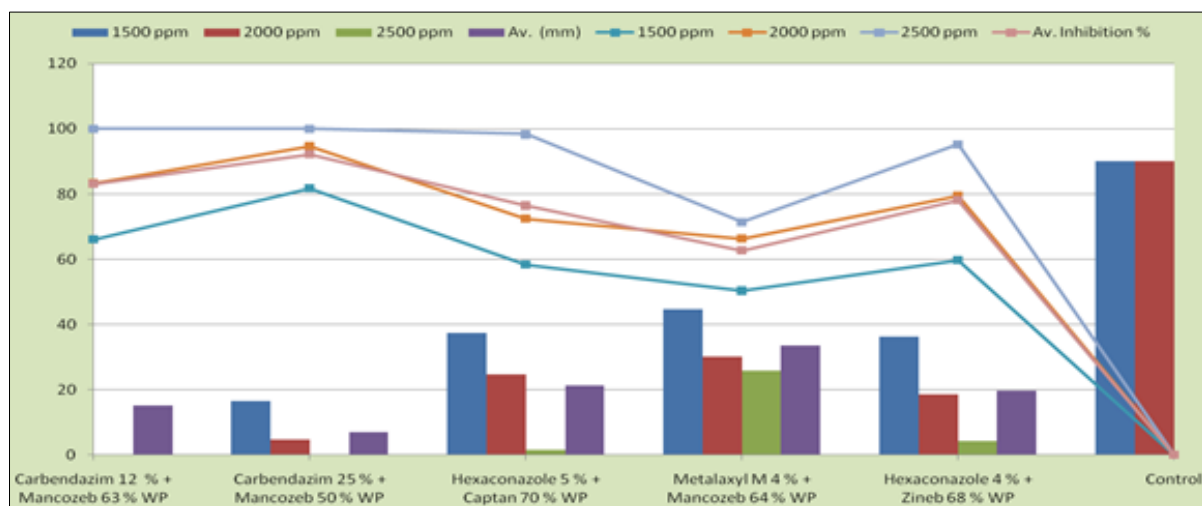


Fig 2: *In vitro* evaluation of combi-fungicides against *F. udum*

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