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In-vitro efficacy of fungicide against sudden death syndrome (wilt) disease of soybean caused by *Fusarium oxysporum* f. sp. *virguliforme*

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Abstract
Sudden death syndrome (wilt) disease of soybean caused by *Fusarium oxysporum* f. sp. *virguliforme*. *In vitro* evaluation of different ten fungicides against *Fusarium oxysporum* f. sp. *virguliforme.* All the test fungicide at the concentrations of 500, 1000, 1500, 2000, 2500 and 3000 ppm were found effective against the test pathogen. The average mycelial growth inhibition was ranged from 50 to 100 per cent. The highest average mycelial growth inhibition was recorded with the fungicides *viz*., Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin *i.e.* 100 per cent. The combi-fungicide Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with minimum mycelial inhibition *i.e.* 50 per cent amongst tested fungicides.

Keywords: *In-vitro,* soybean, *Fusarium oxysporum* f. sp. *virguliforme,* fungicide

Introduction
Soybean [*Glycine max* (L.) Merrill] is a native of northern China and is the most important legume crop in the world. Soybean is called 'Golden bean,' 'Miracle bean' and 'Crop of planet.' The crop thus, improves soil fertility and economizes crop production not only for themselves but also for the next crop grown in rotation especially, cereal crops (Nassiuma and Wasike, 2002) [6].

India, it is grown on an area of 11.25 million hectare with a production of 14.22 million metric tonnes and productivity of 1263 kg/ha in the year 2017 (Anonymous, 2017) [3]. Soybean crop can be attacked by more than 100 pathogens (Sinclair and Schurtleff, 1975) [9]. About 35 pathogens were reported to infect soybean in India (Gupta *et al*., 2001) [4]. Fungi, nematodes, viruses, bacteria, and phytoplasmas are known to cause diseases of soybean.

The soybean crop is presently suffered due to one of the important disease known as sudden death syndrome. The sudden death syndrome disease is called as wilt of soybean. The soybean wilt is caused by *Fusarium oxysporum* f. sp. *virguliforme* (Aoki 2003) [3]. Fusarium genus is a soil borne fungus that causes wilt of many crops. In many cases the fungus causing wilt in a particular crop is specific to that crop. In case of soybean, sudden death syndrome caused by the soil borne pathogen F. solani f. sp. glycines formerly called *Fusarium virguliforme* sp. in recent days which was first observed in Arkansas during 1971 (Roy *et al*. 1997) [8]. It can cause great damage, as it may reduce the average yield of soybean by up to 59 per cent (Sinclair and Backman1989) [10].

Material and Methods
The bio-efficacy of the fungicides earlier reported effective against *Fusarium oxysporum* f. sp. *virguliforme* were *in-vitro* evaluation of different systemic and combi-fungicides, 'Poisoned food Technique' developed by (Nene and Thapliyal 1993) [7] was followed. Ten different systemic and combi-fungicides fungicides were evaluated (systemic fungicides and combi-fungicide were tested @ 500, 1000, 1500, 2000 and 3000 ppm.) against *F. oxysporum* by using potato dextrose agar (PDA) as basal culture medium.

The experiment was conducted by applying CRD and all treatments were replicated thrice. Plate containing PDA without any fungicide was maintained as untreated control. All treatment plates were inoculated with 5 mm disc of one week old culture of the test

pathogen and then incubated at 28 ± 2 ^{0}c temperature. Observation on radial growth and sporulation of test fungus were recorded at 24 hrs. Interval and will be continued till growth of test pathogen in untreated control plates is fully covered. Per cent inhibition of test pathogen was calculated by applying formula given by Vincent (1927) [11].

$$\text{Percent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where

C= Growth of the test fungus in (mm) untreated control plates
T= Growth of the test pathogen in (mm) treated plates

Result and Discussion

Result revaluated that all ten fungicides were evaluated *in vitro* against *Fusarium oxysporum* f. sp. *virguliforme* which exhibited a wide range of mycelial growth inhibition of the test pathogen.

Radial mycelial growth

At concentration 500 ppm, the radial mycelium growth of the test pathogen which was ranged from 00 mm (Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin) to 50 mm (Carbendazim 12% WP + Mancozeb 63% WP). The least radial mycelium growths were recorded in Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin 00 mm which were at par with each other but significantly superior over Carbendazim, Difenconazole, Tebuconazole, Carbendazim 12% WP + Mancozeb 63% WP, Carbendazim 25% WP + Mancozeb 50% WP and control which gave 90 mm mycelial growth. These were followed by 5.33 mm (Tebuconazole), 6.66 mm (Difenconazole), 21.33 mm (Carbendazim) and 44 mm (Carbendazim 25% WP + Mancozeb 50% WP). The one another combi-fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with maximum radial mycelium growth 50 mm but still significantly superior over the control (90 mm mycelial growth).

At concentration 1000 ppm, all the tested fungicides exhibited similar trend of radial mycelium growth as that of observed at concentration 500 ppm. It was comparatively reduced the radial mycelium growth of the test pathogen which was ranged from 00 mm (Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin) to 41.66 mm Carbendazim 12% WP + Mancozeb 63% WP and control shown 90 mm mycelial growth. The least radial mycelium growths were recorded in Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin *i.e.* 00 mm which were at par to each other but significantly superior over Carbendazim, Difenconazole, Tebuconazole, Carbendazim 12% WP + Mancozeb 63% WP, Carbendazim 25% WP + Mancozeb 50% WP and control. These were followed by 2 mm (Tebuconazole), 2.66 mm (Difenconazole), 15 mm (Carbendazim), 38.33 mm (Carbendazim 25% WP + Mancozeb 50% WP). The one another combi-fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with maximum radial mycelium growth 41.66 mm but still significantly superior over the control (90 mm mycelial growth).

At concentration 1500 ppm, all the tested fungicides exhibited similar trend of radial mycelium growth as that of observed at concentrations 500 and 1000 ppm. It was comparatively reduced the radial mycelium growth of the test pathogen which was ranged from 00 mm (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) to 38.33 mm Carbendazim 12% WP + Mancozeb 63% WP and control shown 90 mm mycelial growth. The least radial mycelium growths were recorded in Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin *i.e.* 00 mm which were at par to each other but significantly superior over (Carbendazim 12% WP + Mancozeb 63% WP), (Carbendazim 25% WP + Mancozeb 50% WP) and control. These were followed by 33.66 mm (Carbendazim 25% WP + Mancozeb 50% WP) and the one combi-fungicide Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with maximum radial mycelium growth 38.33 mm but still significantly superior over the control (90 mm mycelial growth).

At concentration 2000 ppm, all the tested fungicides tested exhibited similar trend of radial mycelium growth as that of observed at concentrations 500, 1000 and 1500 ppm. It was comparatively reduced the radial mycelium growth of the test pathogen which was ranged from 00 mm (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) to 34.66 mm (Carbendazim 12% WP + Mancozeb 63% WP) and control shown 90 mm mycelial growth. The least radial mycelium growths were recorded in Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin (00 mm) which were at par to each other but significantly superior over Carbendazim 12% WP + Mancozeb 63% WP, Carbendazim 25% WP + Mancozeb 50% WP and control. These were followed by 26.00 mm (Carbendazim 25% WP + Mancozeb 50% WP). The one another combi-fungicide Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with maximum radial mycelium growth 34.66 mm but still significantly superior over the control (90 mm mycelial growth).

At concentration 2500 ppm, all the tested fungicides tested exhibited similar trend of radial mycelium growth as that of observed at concentrations 500, 1000, 1500, and 2000 ppm. It was comparatively reduced the radial mycelium growth of the test pathogen which was ranged from 00 mm (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) to 30.33 mm (Carbendazim 12% WP + Mancozeb 63% WP) and control shown mycelial growth (90 mm). The least radial mycelium growths were recorded in Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin (00 mm) which were at par to each other but significantly superior over Carbendazim 12% WP + Mancozeb 63% WP, Carbendazim 25% WP + Mancozeb 50% WP and control. These were followed by 22 mm (Carbendazim 25% WP + Mancozeb 50% WP). The one another combi-fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with maximum radial mycelium growth 30.33 mm but still significantly superior over the control (90 mm mycelial growth).

At concentration 3000 ppm, all the tested fungicides exhibited similar trend of radial mycelium growth as that of observed at concentrations 500, 1000, 1500, 2000 and 2500 ppm. It was comparatively reduced the radial mycelium growth of the test pathogen which was ranged from 00 mm (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole,

Tebuconazole, Thiophanate methyl and Azoxystrobin) to 24.33 mm (Carbendazim 12% WP + Mancozeb 63% WP) and control shown 90 mm shown mycelial growth. The least radial mycelium growths were recorded in Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin 00 mm which were at par to each other but significantly superior over Carbendazim 12% WP + Mancozeb 63% WP, Carbendazim 25% WP + Mancozeb 50% WP and control. These were followed by 19 mm (Carbendazim 25% WP + Mancozeb 50% WP). The one another combi-fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was found comparatively less effective with maximum radial mycelium growth 24.33 mm but still significantly superior over the control (90 mm mycelial growth).

Average radial mycelical growth of *Fusarium oxysporum* f. sp. *virguliforme* recorded with all eight systemic fungicides and two combi fungicides against the test pathogen ranged from 00 mm (Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin) to 36.55mm (Carbendazim 12% WP + Mancozeb 63% WP) as against 90 mm in untreated control 00 mm. The least average radial mycelium growth was recorded in Tebuconazole (1.22 mm) followed by Difenconazole (1.55 mm), Carbendazim (30.49 mm), Carbendazim 25% WP + Mancozeb 50% WP (6.05 mm). The fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was less effective with maximum average radial mycelial growth *i.e*. 36.55 mm but still significantly superior over the control (90 mm mycelial growth).

Percent mycelial growth inhibition

Result revealed that all eight systemic fungicides and two combi fungicides tested at different concentrations (500, 1000, 1500, 2000, 2500, 3000 ppm each) significantly inhibited mycelial growth of *Fusarium oxysporum* f. sp. *virguliforme* over untreated control (00 per cent). Further, the percentage mycelia growth inhibition was increased with increase in concentrations of the tested fungicides.

At concentration 500 ppm, mycelium growth inhibition of test pathogen was in the range from 44.44 Per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 Per cent (Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin) as against 00 per cent in untreated control. However, significantly the highest mycelial growth inhibitions were recorded with the fungicides *viz*., Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin (100%) which were at par to each other. These were followed by the fungicides *viz*., Tebuconazole (94.07%), Difenconazole (92.59%), Carbendazim (76.29%) and Carbendazim 25% WP + Mancozeb 50% WP (51.11%). The fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was comparatively less effective with lowest mycelium growth inhibition *i.e.* 44.44 per cent but still significantly superior over the control (90 mm mycelial growth).

At concentration 1000 ppm, all the tested fungicides exhibited similar trend of mycelia growth inhibition as that of observed at concentration 500 ppm. It was comparatively increased and ranged from 53.70 Per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 Per cent (Propiconazole, Hexaconazole, Penconazole, Thiophanate methy and Azoxystrobin) as against 00 per cent in untreated control. However, significantly the highest mycelial growth inhibitions were recorded with the fungicides *viz*., Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin (100%) which were at par to each other. These were followed by the fungicides *viz*., Tebuconazole (97.77%), Difenconazole (97.03%), per cent Carbendazim (83.32%) and Carbendazim 25% WP+ Mancozeb 50% WP (57.40%). The fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was comparatively less effective with lowest mycelium growth inhibition *i.e.* 53.70 per cent but still significantly superior over the control (90 mm mycelial growth).

At concentration 1500 ppm, all the tested fungicides exhibited similar trend of mycelia growth inhibition as that of observed at concentrations 500 and 1000 ppm. It was comparatively increased and ranged from 57.40 Per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 Per cent (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) as against 00 per cent in untreated control. However, significantly highest mycelial growth inhibitions were recorded with the fungicides *viz*., Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin (100%) which were at par to each other. These were followed by the fungicide Carbendazim 25% WP+ Mancozeb 50% WP (62.59%). The fungicide *viz*., Carbendazim 12% WP+ Mancozeb 63% WP was comparatively less effective with lowest mycelium growth inhibition *i.e.* 57.40 per cent but still significantly superior over the control (90 mm mycelial growth).

At concentration 2000 ppm, all the tested fungicides exhibited similar trend of mycelia growth inhibition as that of observed at concentrations 500, 1000 and 1500 ppm. It was comparatively increased and ranged from 61.48 Per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 Per cent (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) as against 00 per cent in untreated control. However, significantly highest mycelial growth inhibitions were recorded with the fungicides *viz*., Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin (100%) which were found at par each other. These were followed by the fungicide *viz*., Carbendazim 25% WP + Mancozeb 50% WP (61.48%). The fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was comparatively less effective with lowest mycelium growth inhibition *i.e.*71.11 per cent but still significantly superior over the control (90 mm mycelial growth).

At concentration 2500 ppm, all the tested fungicides exhibited similar trend of mycelia growth inhibition as that of observed at concentrations 500, 1000, 1500 and 2000 ppm. It was comparatively increased and ranged from 66.29 Per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 Per cent (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) as against 00 per cent in untreated control. However, significantly highest mycelial growth inhibitions were recorded with the fungicides *viz*., Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl, Azoxystrobin, Tebuconazole, and Difenconazole, Carbendazim (100%) which were found at par each other. These were followed by the fungicide Carbendazim 25% WP+ Mancozeb 50% WP (66.29%). The fungicide *viz*., Carbendazim 12% WP+ Mancozeb 63% WP was comparatively less effective with lowest mycelium growth

inhibition *i.e.* 75.55 per cent but still significantly superior over the control (90 mm mycelial growth).

At concentration 3000 ppm, all the tested fungicides exhibited similar trend of mycelia growth inhibition as that of observed at concentrations 500, 1000, 1500, 2000 and 2500 ppm. It was comparatively increased and ranged from 72.96 Per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 Per cent (Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin) as against 00 per cent in untreated control. However, significantly the highest mycelial growth inhibitions were recorded with the fungicides *viz*., Carbendazim, Propiconazole, Hexaconazole, Difenconazole, Penconazole, Tebuconazole, Thiophanate methyl and Azoxystrobin (100%) which were found at par each other. These were followed by the fungicide Carbendazim 25% WP + Mancozeb 50% WP (72.96%). The fungicide *viz*., Carbendazim 12% WP + Mancozeb 63% WP was comparatively less effective with lowest mycelium growth inhibition *i.e.* 78.88 per cent but still significantly superior over the control (90 mm mycelial growth).

Average mycelial growth inhibition recorded with all eight systemic fungicides and two combi fungicides against the test pathogen ranged from 59.37 per cent (Carbendazim 12% WP + Mancozeb 63% WP) to 100 (Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl, Azoxystrobin) as against 00 per cent in untreated control. The least average mycelia growth inhibition was recorded in Carbendazim 12% WP + Mancozeb 63% WP *i.e*. (59.37%) followed by Carbendazim 25% WP + Mancozeb 50% WP *i.e.* (66.10%), Carbendazim (93.26%), Difenconazole (98.27%) and Tebuconazole (98.64%). The five systemic fungicides *viz*., Propiconazole, Hexaconazole, Penconazole, Thiophanate methyl and Azoxystrobin were significantly effective with 100 per cent mycelial growth inhibition.

The result of present investigation have consonsnce with earlier recordes of scientist *viz*., Mayur *et al*. (2001) [5] studied the effect of systemic and non-systemic fungicides against *F. oxysporum* f. sp. ciceri and reported that among systemic, Carbendazim (100%) was most effective against *F. oxysporum* f. sp. ciceri. Anita kumari *et al*. (2014) [1] studied four fungicides Mancozeb, SAAF, Cabendazim and Cup rozin in three different concentrations, among these Carbendazim at its all concentrations were found to be most effective against the test pathogen *Fusarium oxysporum*.

Table 1: *In vitro* efficacy of different systemic and combi fungicides against mycelial growth inhibition of *Fusarium oxysporum* f. sp. *virguliforme*

Tr. No.	Treatments	Colony diameter *(mm)							Percent inhibition						
		500	1000	1500	2000	2500	3000	Mean	500	1000	1500	2000	2500	3000	Mean
T1	Carbendazim	21.33	15.00	00.00	00.00	00.00	00.00	6.05	76.29 (60.86)	83.32 (65.89)	100 (90)	100 (90)	100 (90)	100 (90)	93.26 (74.95)
T2	Propiconazole	00.00	00.00	00.00	00.00	00.00	00.00	00.00	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
T3	Hexaconazole	00.00	00.00	00.00	00.00	00.00	00.00	00.00	100 (90)	100 90	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
T4	Difenconazole	6.66	2.66	00.00	00.00	00.00	00.00	1.55	92.59 (74.20)	97.03 (80.07)	100 (90)	100 (90)	100 (90)	100 (90)	98.27 (82.44)
T5	Penconazole	00.00	00.00	00.00	00.00	00.00	00.00	00.00	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
T6	Tebuconazole	5.33	2.00	00.00	00.00	00.00	00.00	1.22	94.07 (75.90)	97.77 (81.41)	100 (90)	100 (90)	100 (90)	100 (90)	98.64 (83.30)
T7	Thiophanate methyl	00.00	00.00	00.00	00.00	00.00	00.00	00.00	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
T8	Azoxystrobin	00.00	00.00	00.00	00.00	00.00	00.00	00.00	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)
T9	Carbendazim 12% WP+ Mancozeb 63% WP	50.00	41.66	38.33	34.66	30.33	24.33	36.55	44.44 (41.80)	53.70 (47.12)	57.40 (49.25)	61.48 (51.63)	66.29 (54.50)	72.96 (58.66)	59.37 (50.40)
T10	Carbendazim 25% WP+ Mancozeb 50% WP	44.00	38.33	33.66	26.00	22.00	19.00	30.49	51.11 (45.63)	57.40 (49.25)	62.59 (52.29)	71.11 (57.48)	75.55 (60.36)	78.88 (62.64)	66.10 (54.39)
T11	Control	90.00	90.00	90.00	90.00	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	SE±	0.55	0.49	0.37	0.31	0.31	0.32	-	0.61	0.55	0.41	0.35	0.35	0.36	-
	CD at 1%	1.62	1.46	1.11	0.93	0.93	0.96	-	1.80	1.62	1.23	1.04	1.04	1.07	-

*: Average of three replications, Figures in parenthesis are arcsine transformation values

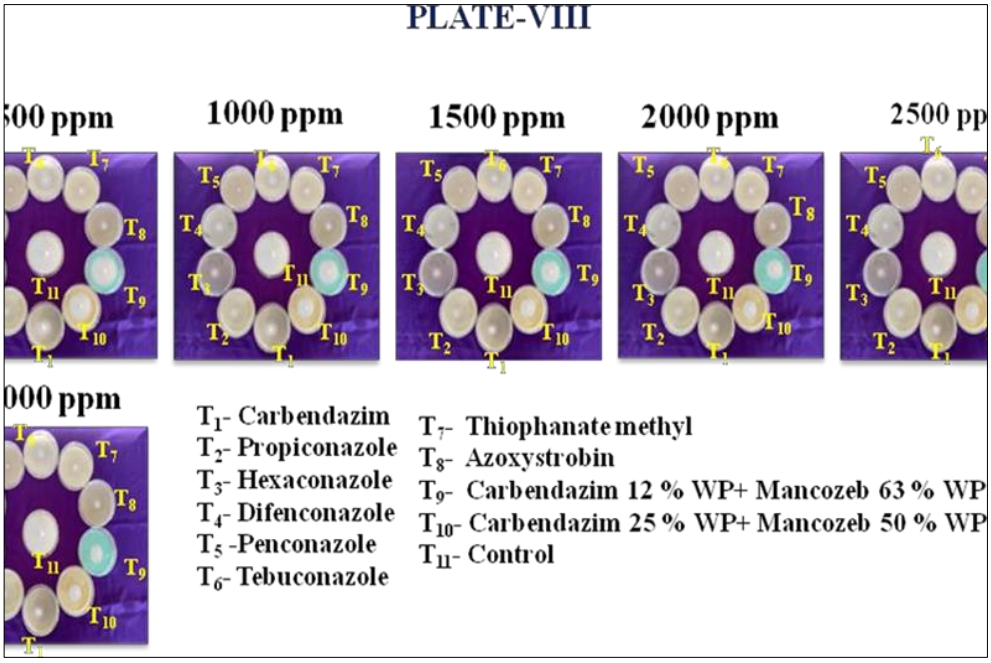

Fig 1: *In vitro* efficacy of fungicides on growth and inhibition of *Fusarium oxysporum* f. sp. *virguliforme*

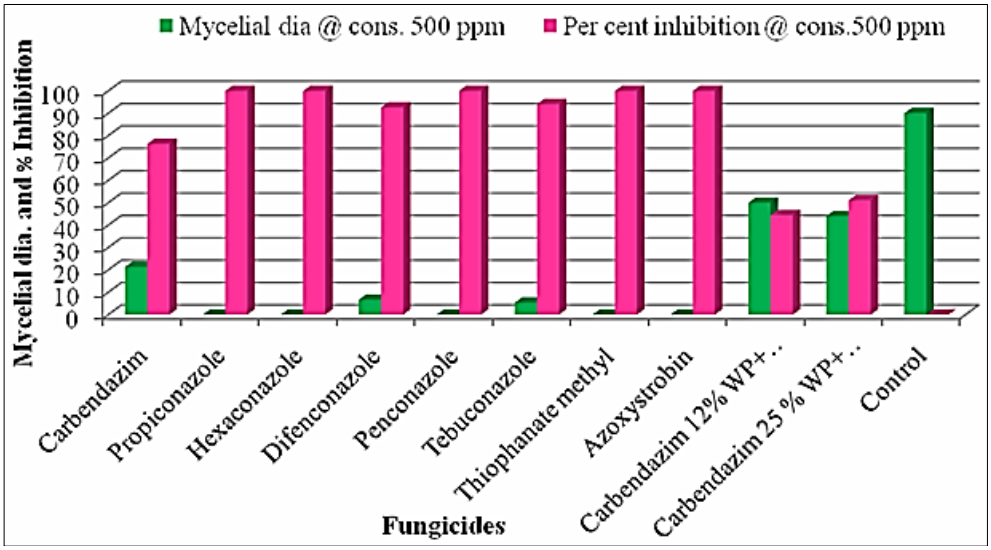

Fig 2: *In vitro* efficacy of different systemic and combi fungicides against *Fusarium oxysporum* f. sp. *virguliforme* (500 ppm)

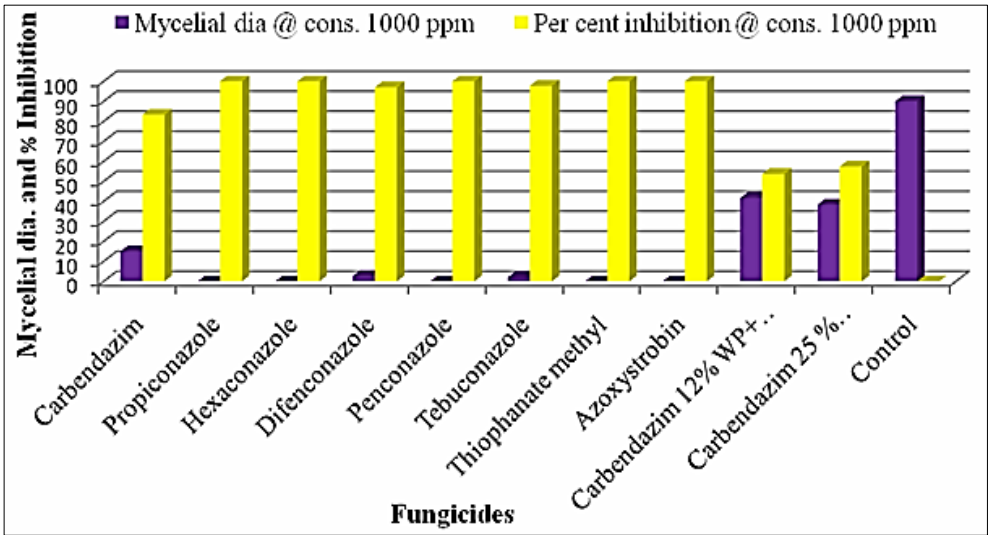

Fig 3: *In vitro* efficacy of different systemic and combi fungicides against *Fusarium oxysporum* f. sp. *virguliforme* (1000 ppm)

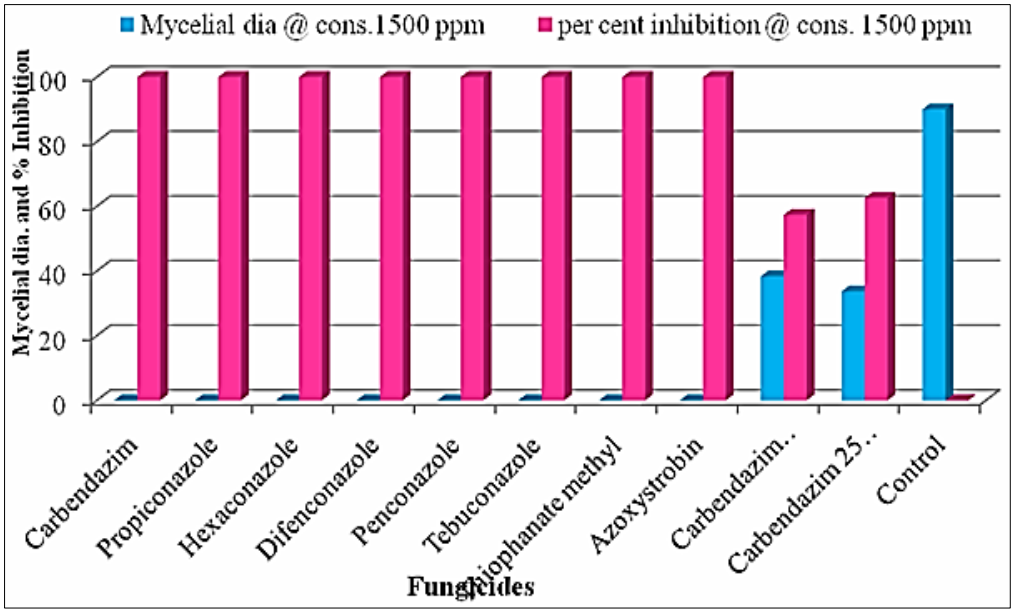

Fig 4: *In vitro* efficacy of different systemic and combi fungicides against *Fusarium oxysporum* f. sp. *virguliforme* (1500 ppm)

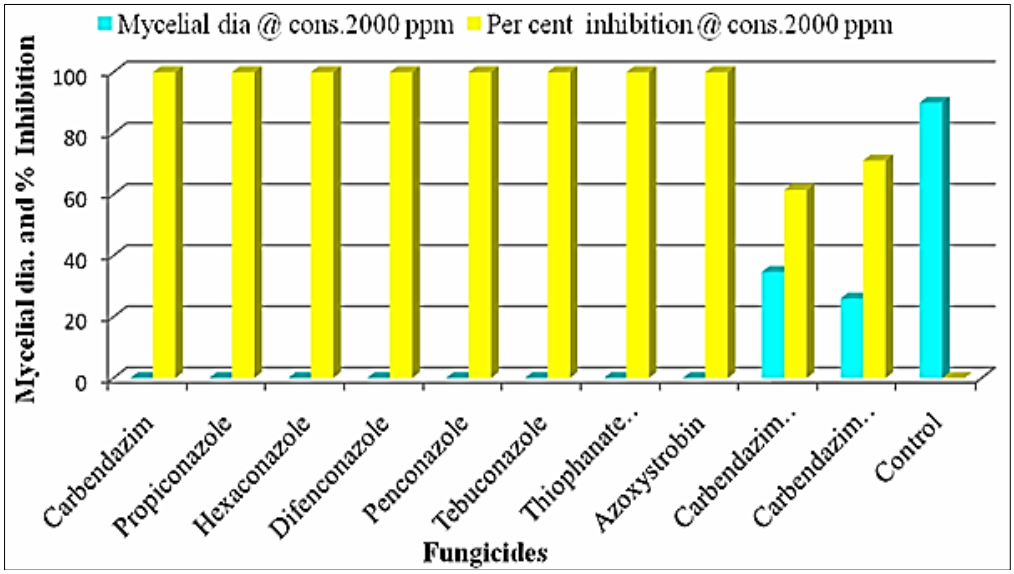

Fig 5: *In vitro* efficacy of different systemic and combi fungicides against *Fusarium oxysporum* f. sp. *virguliforme* (2000 ppm)

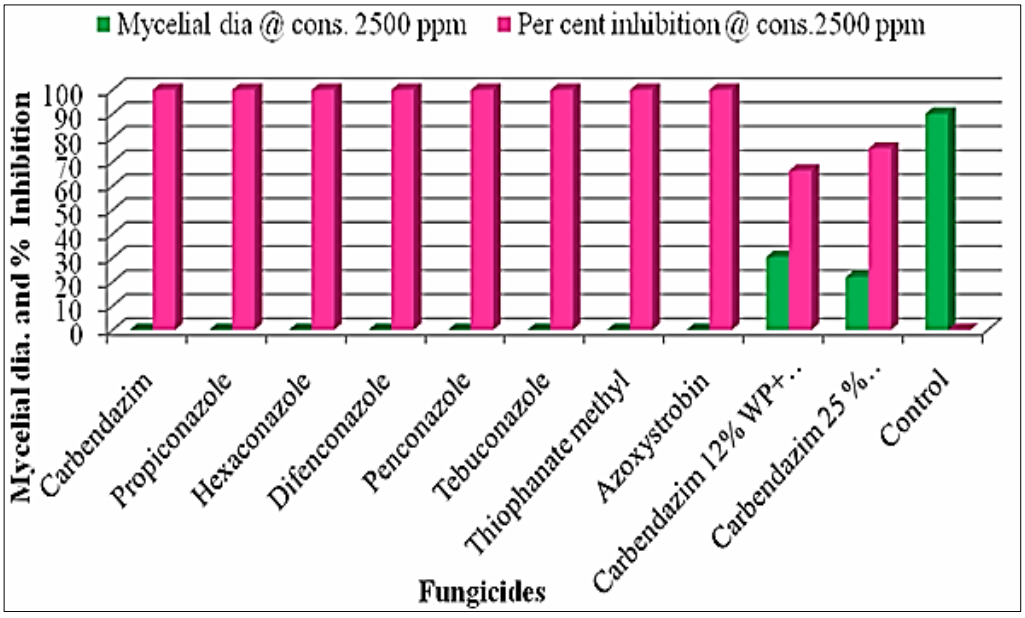

Fig 6: *In vitro* efficacy of different systemic and combi fungicides against *Fusarium oxysporum* f. sp. *virguliforme* (2500 ppm)

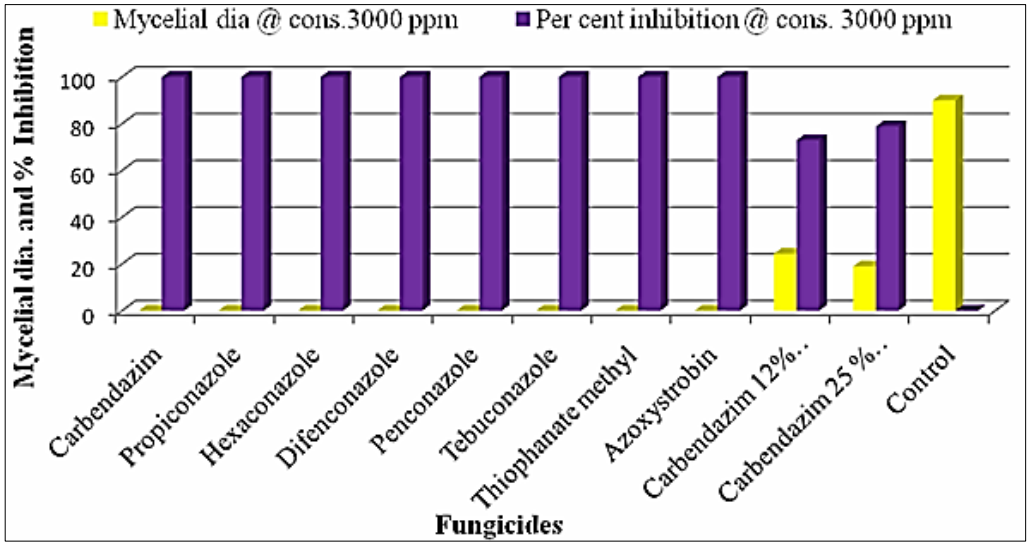

Fig 7: *In vitro* efficacy of different systemic and combi fungicides against *Fusarium oxysporum* f. sp. *virguliforme* (3000 ppm)

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