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EVALUATION OF SYSTEMIC AND COMBI SYSTEMIC FUNGICIDES FOR *IN VITRO* SUPPRESSION OF *Fusarium oxysporum* F. sp. *Melongenae*

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melongenae* is one of the important soil-borne diseases limiting brinjal production. An *in vitro* experiment was conducted to assess the efficacy of selected systemic and combi-systemic fungicides against *F. oxysporum* f. sp. *melongenae*. The pathogen was isolated from naturally infected brinjal plants collected from Chittoor district of Andhra Pradesh. Seven fungicides, *viz.*, tebuconazole, hexaconazole, propiconazole, azoxystrobin + tebuconazole, fluopyram + trifloxystrobin, isopyrazam + difenoconazole and carbendazim, were evaluated at 25, 50, 100 and 250 ppm using the poisoned food technique. Among the fungicides tested, carbendazim 50% WP exhibited the highest efficacy, completely inhibiting mycelial growth (100%) at all the concentrations tested followed by Fluopyram + trifloxystrobin, which recorded a mean inhibition of 93.38%, and tebuconazole with 92.81% mean inhibition. Azoxystrobin + tebuconazole, propiconazole, isopyrazam + difenoconazole and hexaconazole recorded mean inhibitions of 88.92, 86.06, 86.07 and 80.36%, respectively. The inhibition of mycelial growth generally increased with an increase in fungicide concentration. The results demonstrated that carbendazim was the most effective fungicide against *Fusarium oxysporum* f. sp. *melongenae* under *in vitro* conditions, followed by fluopyram + trifloxystrobin and tebuconazole. These fungicides may be further evaluated under greenhouse and field conditions for effective management of Fusarium wilt of brinjal.

Key words : Brinjal, *Fusarium oxysporum*, Systemic fungicides, *In vitro* efficacy.

Introduction

Brinjal (*Solanum melongena* L.), also known as eggplant or aubergine, is one of the most important vegetable crops widely cultivated in tropical and subtropical regions of the world. It is believed to be it is native of South East Asia, particularly India and China. India is recognized as one of the primary centers of origin and diversity of brinjal and is among the leading producers globally (Taher *et al*., 2017; Caruso *et al*., 2017). The crop can be grown under diverse environmental conditions and is suitable for both small-scale and commercial farming systems. It is the fifth most economically important solanaceous crop in the world after potato, tomato, pepper and tobacco. Globally brinjal has been cultivated in 1.96 Mha with a production of 57.54 million tonnes and a yield of 29 t/ ha. India is the second largest producer occupying ¼th cultivated area of 0.68 M Ha harvesting a total of nearly 13 million tonnes (FAOSTAT, 2024).

The crop is affected by several fungal, bacterial and viral diseases, among which Fusarium wilt caused by *Fusarium oxysporum* f. sp. *melongenae* is an important soil-borne disease. The pathogen infects the roots and colonizes the vascular tissues, resulting in yellowing, wilting, vascular discoloration and, under severe conditions, death of affected plants. The soil-borne nature and long-term survival of the pathogen make Fusarium wilt difficult to manage. Disease is more severe in soils with the low pH below 6.4 and above 7.5 pH (Jatav *et al*., 2013). The pathogen is soil borne and invades the

root system, colonizes the vascular tissues, obstructs water transport system and symptoms of Fusarium wilt of eggplant showing the characteristic symptoms, including the disease is initially manifested by yellowing of lower leaves, which gradually progress upwards and later the infected leaves turned brown, shriveled and drooping as wilting as disease progressed followed by withering of fruits and the infected plants exhibited the brownish vascular discolouration. (Manikandan *et al*., 2024). In the advanced stage of infection, a complete wilting of plant was observed.

This pathogen was initially reported in Japan (Mauto and Ishigami 1958) and next in China. (Zhuang *et al*., 2005). *Fusarium oxysporum* f. sp. *melongenae* is a soil borne fungal wilt pathogen, which induces vascular disease in eggplant causing heavy yield losses in Asian countries. The pathogen, Fusarium survive as saprophyte in soil for many years as chlamydospores, making the pathogen difficult to eradicate once established. The main objective of presented study was to evaluate the possibility of controlling Fusarium wilt of eggplant with the use of fungicides under *in vitro* conditions.

Materials and Methods

Isolation of the pathogen

During the survey conducted in Chittoor district of Andhra Pradesh, brinjal plants showing typical symptoms of Fusarium wilt, such as yellowing, wilting and vascular discoloration, were collected in fresh polythene bags from Chittor District, Andhra Pradesh (Latitude:13.23011 and Longitude: 78.581015). The infected plant samples were brought to the laboratory for isolation of the associated pathogen. Collected the infected stem and root, washed it thoroughly with running tap water and dried. Wipe the infected woody tissue with a cotton swab dipped in 70% ethanol followed by light flaming of tissue. The diseased samples was digged out small pieces of tissue (1/8 to 1/2 sizes) with flamed scalpel from freshly exposed area of advancing area of infection. Placed 3 to 4 pieces, well separated, in PDA media (supplemented with streptomycin sulphate) plates. The plates were incubated at 25^0C in an inverted position, for 5-7 days in BOD. (Aneja, 2003). The pure culture plate obtained by single spore isolation in Potato dextrose agar media. (Noman *et al*., 2017). Representative *Fusarium oxysporum* f. sp. *melongenae* isolate obtained from diseased brinjal plants collected during the survey of Chittoor district was selected for evaluating the in-vitro efficacy of seven fungicides at four concentrations (25, 50, 100 and 250 ppm) and recommended concentration using the poisoned food technique against fusarium wilt of eggplant.

Screening of fungicides for bio efficacy against *Fusarium oxysporum* f. sp. *melongenae* using solid plate assay

The fusarium isolate which was obtained from the Chittoor district was subjected to screening the bioefficacy of seven systemic + combi systemic fungicides at the doses of 25, 50, 100 and 250 ppm using the poisioned food technique. The *in vitro* efficacy of fungicides was evaluated using the poisoned food technique as described by Nene and Thapliyal (1993). Seven systemic fungicides (each @ 25,50,100,250 ppm) tested *in vitro* against test pathogen (*Fusarium oxysporum* f. sp. *melongenae*), using poisoned food technique and Potato dextrose agar (PDA). Based on the active ingredient, an appropriate quantity of the fungicides was added in previously sterilized 100 ml PDA separately in 250 ml conical flasks of test fungicides were determined and thoroughly mixed with autoclaved and cooled (45!) PDA medium to obtain desired concentrations in conical flasks separately. After solidification, the plates were inoculated by the fungal disc of 5 mm diameter cut out from seven days old culture and incubated at 27 ± 2^0C for seven days. This PDA medium was then poured aseptically in petriplates (90 mm diameter) with the sample fungicides separately (20 ml / plate) and allowed to solidify at room temperature. Three plates / treatment / replication were maintained for each of the sample fungicide and its target concentrations. Petriplates filled with plain PDA (without any fungicide) and inoculated with pure culture disc. Mycelium disc of five mm diameter from edge of actively growing seven days old culture of the fungus was drilled by sterile corkborer and placed at the centre of each plate. The unpoisoned PDA was regarded as the control. Foreach tested doses, three independent replications were kept. Plates were subjected to incubation at 25±1 C for seven days. When the fungus reached maximal growth in control plates, mycelial radial growth was recorded. Radial mycelial colony growth measurements were recorded at a 24hour interval and continued until untreated control plates were fully covered with mycelial growth of test pathogen. Per cent inhibition of the growth of the test pathogen was calculated by applying the formula given by Vincent (1927) and the data obtained were averaged and analyzed statistically.

Per cent Inhibition (I) = C -T/C × 100

Where,

C = growth (mm) of test fungus in untreated control plate.

T = growth (mm) of test fungus in treated

Experimental details: Systemic Fungicides

Design: C.R.D. (Completely Randomized Design)

Replications: Three

Treatments: Eight

Table 1 : Treatment details.

Treatment number	Treatments
1.	Tebuconazole 38.39% W/W
2.	Propiconazole 25%EC
3.	Hexaconazole 5%SC
4.	Azoxystrobin 11% +Tebuconazole 18.3%EC
5.	Fluopyram 21.37% W/W +Trifloxystrobin 21.37%W/W
6.	Isopyrazam 11.5% W/W +Difenconazole 11.5% W/W
7.	Carbendazim 50%WP
8.	Control

Results and Discussion

Efficacy of fungicides against *Fusarium oxysporum* f. sp. *melongenae* using solid plate assay

At 25 ppm, radial mycelial growth of *Fusarium oxysporum* f.sp. *melongenae* was ranged from 6.71 mm to 25mm as against 90.00 mm in untreated control. However, minimum mycelial growth was observed with the fungicide Carbendazim (0.00mm) (Fig. 7). It was followed by the fungicides viz, Fluopyrazam 11.5% W/W +Triflozystrobin (6.71 mm) (Fig. 5), Tebuconazole (7.09 mm) (Fig. 1), Azoxystrobin + Tebuconazole (14.52 mm), (Fig. 4), Propiconazole (15.95 mm), (Fig. 2) Isopyrazam 11.5 % W/W + Difenconazole 11.5% W/W (13.853mm) (Fig. 6) and Hexaconazole (22.85mm) (Fig. 3). Comparatively maximum mycelial growth was recorded with the fungicide Thiophanate methyl (22.85 mm).

At 50 ppm, radial mycelial growth of *Fusarium oxysporum* f. sp. *melongenae* was found to be decreased as compared to that of at 25 ppm and it was ranged from 0.00 mm to 17.99 mm, as against 90.00 mm in untreated control. However, with the fungicide Carbendazim no mycelial growth was observed. (Fig. 7). It was followed by the fungicides *viz.*; Fluopyrazam 11.5% W/W + Triflozystrobin (5.52 mm), (Fig. 5) Tebuconazole (6.76 mm), (Fig. 1) Azoxystrobin + Tebuconazole (14.52 mm), (Fig. 4) Propiconazole (11.14 mm), (Fig. 6) Isopyrazam 11.5% W/W + Difenconazole 11.5% W/W (11.52 mm) (Fig. 6) and Hexaconazole (17.99 mm) (Fig. 3). Comparatively maximum mycelial growth was recorded with the fungicide Hexaconazole (17.99 mm).

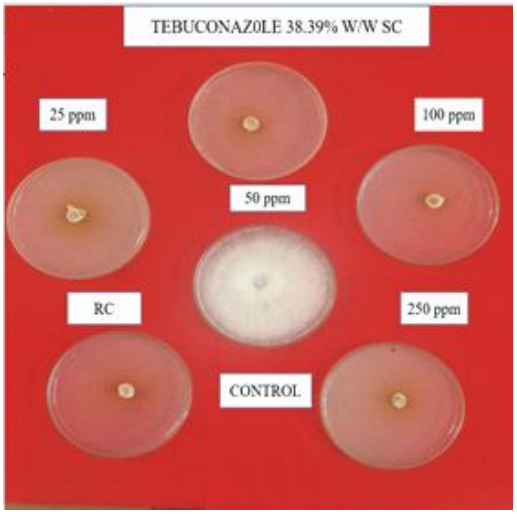


Fig. 1 : Tebuconazole 38.39% W/W SC@ RC, 25, 50, 100 and 250ppm conc.

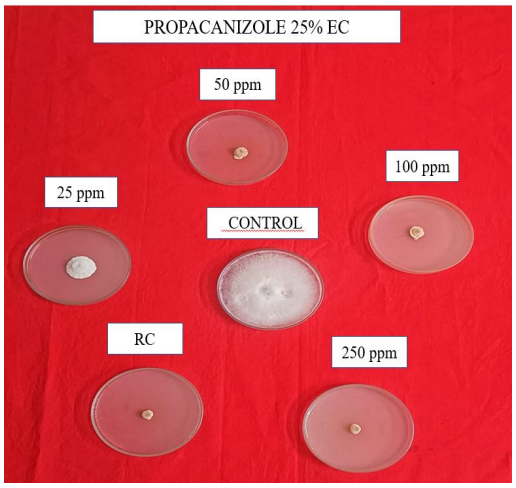


Fig. 2 : Propacanizole 25% EC @ 25, 50, 100, 250 ppm conc.

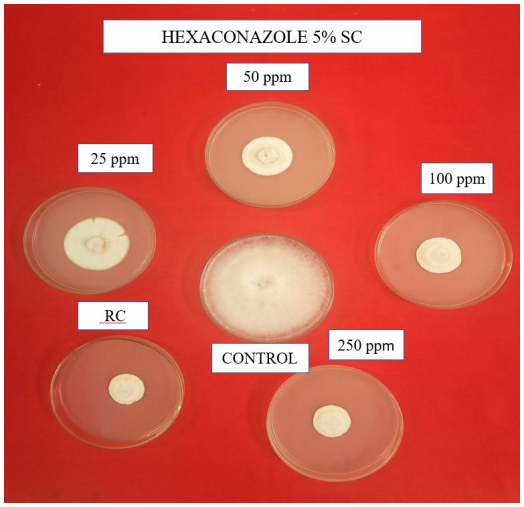


Fig. 3 : Hexaconazole 5% SC @ 25,50, 100, 250 ppm conc.

At 100 ppm, radial mycelial growth of *Fusarium oxysporum* f. sp. *melongenae* was found to be decreased as compared to 25 and 100 ppm and it was ranged from 0.00 mm to 13.52 mm, as against 90.00 mm in untreated

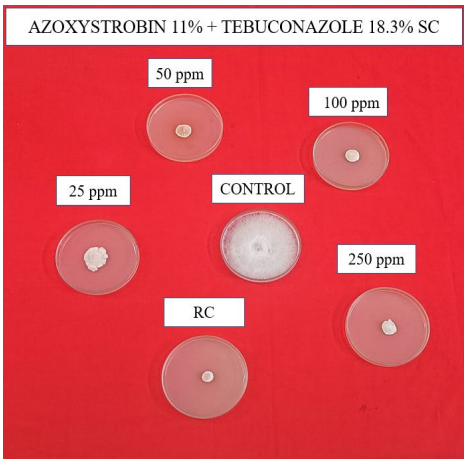


Fig. 4 : Azoxystrobin 11% + Tebuconazole 18.3%SC @ RC, 25, 50,100, 250 ppm conc.

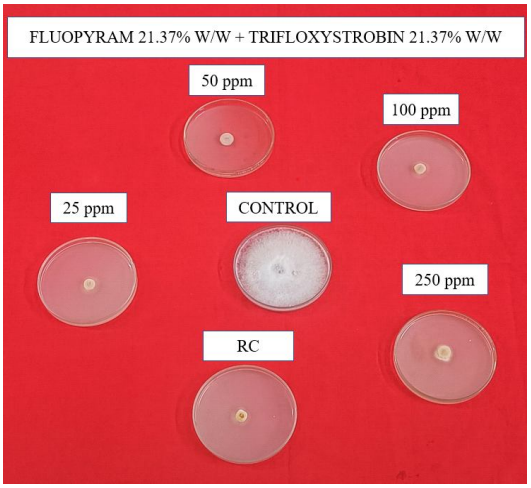


Fig. 5 : Fluopyrazam 21.37% W/W + Trifloxystrobin 21.37% W/W @ 25, 50, 100, 250 ppm conc.

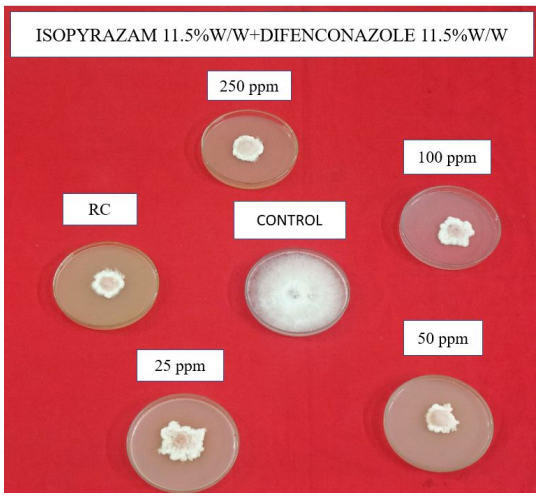


Fig. 6 : Isopyrazam 11.5% W/W + Difenconazole 11.5% W/W @25, 50, 100, 250 ppm conc.

control. However, with the fungicides *viz*., Carbendazim no mycelial growth was observed (Fig. 7). It was followed by the fungicides viz., Fluopyrazam + Trifloxystrobin (5.22 mm), (Fig 5) Tebuconazole (5.9 mm), (Fig. 1)

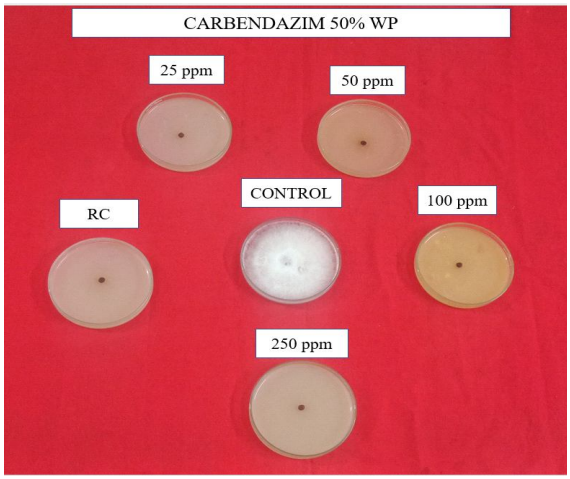


Fig. 7 : Carbendazim 50% WP @ 25, 50, 100, 250 ppm conc.

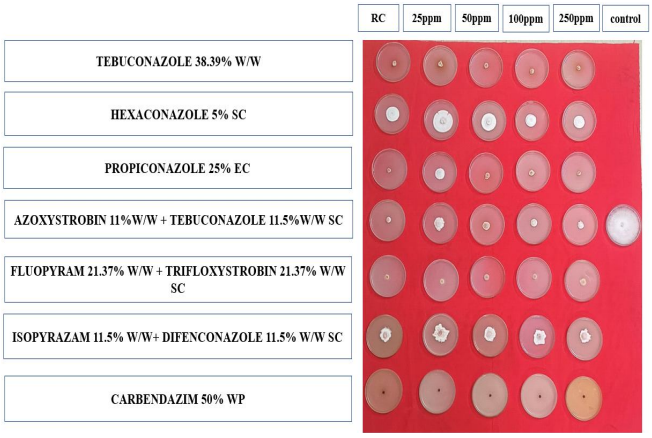


Fig. 8 : *In vitro* evaluation of systemic and combisystemic fungicides against *Fusarium oxysporum* f. sp. *melongenae* at RC, 25, 50, 100, 250 ppm.

Azoxystrobin + Tebuconazole (7.473 mm), (Fig. 4) Propiconazole (10.427 mm), (Fig. 2) Isopyrazam 11.5% W/W + Difenconazole 11.5% W/W (11.52 mm) (Fig. 6) and Hexaconazole (13.52 mm) (Fig. 3). Comparatively maximum mycelial growth was recorded with the fungicide Hexaconazole (13.52 mm).

At 250 ppm, radial mycelial growth of Fusarium was found to be decreased as compared to 25 and 100 ppm and it was ranged from 0.00 mm to 45.33 mm, as against 90.00 mm in untreated control. However, with the fungicides *viz*., Carbendazim no mycelial growth was observed (Fig. 7). It was followed by the fungicides *viz*., It was followed by the fungicides viz Tebuconazole (4.76 mm), (Fig. 1) Fluopyrazam 11.5% W/W + Triflozystrobin (4.99 mm), (Fig. 5) Azoxystrobin + Tebuconazole (6.423 mm), (Fig. 4) Propiconazole (9. 853 mm), (Fig. 2) (Isopyrazam 11.5 % W/W + Difenconazole 11.5% W/W (10.61 mm) (Fig 6) and Hexaconazole (13.5mm) (Fig. 3). Comparatively maximum mycelial growth was recorded with the fungicide Hexaconazole (13.5 mm).

Table 2 : *In vitro* evaluation of systemic and combi systemic fungicides against *Fusarium oxysporum* f. sp. *melongenae*.

Tr no.	Fungicides	Fungicides mycelial growth (mm) Dose tested in PPM						Percentage inhibition in Mycelial Growth Dose tested in PPM					
		RC	25	50	100	250	Mean	RC	25	50	100	250	Mean
1.	Tebuconazole 38.39% W/W SC	6.87	7.09	6.76	5.9	4.76	6.12	91.88 (73.42)	91.65 (73.18)	92.16 (73.72)	93.05 (74.33)	94.39 (76.27)	92.81
2.	Propiconazole 25% EC	9.80	15.95	11.16	10.42	9.85	11.84	88.46 (70.31)	81.23 (64.30)	86.89 (68.74)	87.73 (69.4)	88.40 (70.06)	86.06
3.	Hexaconazole 5% SC	13.61	22.85	17.997	13.52	13.50	16.96	84.03 (66.39)	73.11 (58.24)	78.82 (62.57)	84.09 (66.46)	85.42 (67.51)	80.36
4.	Azoxystrobin 11% + Tebuconazole 18.3% SC	6.9	14.52	9.18	7.47	7.42	9.39	91.88 (73.41)	82.91 (65.56)	89.19 (70.78)	91.2 (72.72)	92.4 (74.00)	88.92
5.	Fluopyram 21.37W/W+ Triflozystrobin 21.37 W/W	7.80	6.71	5.521	5.22	5.22	5.61	90.81 (72.32)	92.1 (73.64)	93.04 (70.60)	93.47 (75.94)	94.11 (75.16)	93.38
6.	Isopyrazam 11.5% W/W+Difenconazole 11.5%W/W	11.56	13.85	11.52	11.42	11.85	86.41	83.74 (63.34)	86.44 (66.19)	86.58 (68.36)	87.5 (68.48)	86.06 (69.40)	86.06
7.	Carbendazim 50% WP	0	0	0	0	0	100	100 (90)	100 (90)	100 (90)	100 (90)	100 (90)	100
8.	Control	90	90	90	90	90	0	0	0	0	0	0	0
	CD	0.71	0.63	0.64	0.60	0.43		0.76	0.58	0.67	0.80	1.46	
	CV	2.33	1.77	2.03	2.031	1.47		0.68	0.54	0.60	0.72	1.29	
	SE(m)	0.23	0.21	0.21	0.20	0.14		0.25	0.19	0.22	0.26	0.49	

*Mean of three replications. Figures in parentheses are Arcsine values *Mean of three replications.

Mycelial inhibition

Results revealed that all the systemic fungicides tested (each @ 25, 50, 100, 250 ppm) significantly inhibited mycelial growth of *Fusarium oxysporum* f.sp. *melongenae*, over untreated control. Further, percent mycelial inhibition was increased with increase in concentrations of the fungicides tested. Among the systemic fungicides evaluated, Carbendazim recorded maximum per cent inhibition of mycelial growth from 100 per cent at all the concentrations tested, followed by Fluopyrazam 11.5% W/W +Triflozystrobin with 92.1, 93.84, 93.47,94.11 per cent at 25, 50, 100, 250 ppm concentration, respectively with a mean of 93.88 per cent. Tebuconazole recorded 91.65, 92.16, 93.05 and 94.39 per cent inhibition at 25, 50, 100, 250 ppm concentration respectively with a mean of 92.81per cent. Fluopyrazam 11.5% W/W +Triflozystrobin recorded 82.91, 89.19, 91.2 and 92.4 per cent at 25, 50, 100 and 250 ppm concentration respectively with a mean of 88.92 per cent. Propcinazole showed 81.23, 86.29, 87.73 and 88.4 per cent at 25, 50, 100 and 250ppm concentration respectively with a mean of 88.065 per cent. Isopyrazam 11.5% W/W + Difenconazole 11.5% W/W recorded 83.74, 86.44,86.58 and 87.5 per cent 25, 50, 100 and 250 ppm concentration respectively with a mean of 86.06 per cent and Hexaconazole recorded 73.11,78.82, 84.09 and 85.42 per cent at 25, 50, 100, 250 ppm concentration respectively with a mean of 80.36 per cent. However, the least inhibition was shown by Hexaconazole with 80.36 per cent mean. It was observed that in most of the cases effect of concentration level (ppm) of fungicides on mycelial growth was inversely proportionate among the four concentrations tested (Table 1). Thus, all the systemic fungicides tested were found fungistatic against Fusarium *oxysporum* f.sp. *melongenae* and significantly inhibited its mycelial growth, over untreated control. However, the systemic fungicides found most effective in the order of merit were Carbendazim, Fluopyrazam 11.5% W/W +Triflozystrobin.

The percentage of mycelial radial growth inhibition induced by various fungicides was documented and presented in Table 1. Out of the seven including systemic and combi systemic fungicides tested against, one fungicide showed notable efficacy in inhibiting mycelial

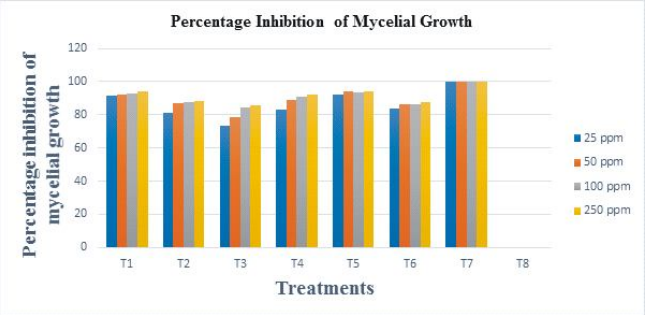


Fig. 9 : Effect of different concentrations of systemic fungicides on percentage inhibition of mycelial growth of *Fusarium oxysporum* f. sp. *melongenae.*

radial growth in plate assays at five different doses (25, 50, 100 and 250 ppm). These effective fungicides include carbendazim 50% WP of which exhibited 100 per cent inhibition of mycelial radial growth at least dose (25 ppm). Followed by Fluopyrazam 11.5% W/W +Triflozystrobin with 92.1% inhibition of mycelial radial growth. In contrast, Hexaconazole 5% SC showed the least mycelial inhibition, followed by Isopyrazam 11.5 % W/W + Difenconazole 11.5% W/W.

The study identified superior efficacy of benzmidazole group of fungicides in suppressing *Fusarium oxysporum* f. sp. *melongenae*. Benzmidazole fungicides are broad spectrum fungicides are converted at the plant surfaces to methyl benzimidazole carbamate and this compound interferes with the nuclear division of sensitive fungi. It inhibits beta – tublin synthesis and disrupts microtubule assembly, stopping nuclear division and fungal growth. (Velmurugan *et al*., 2017). The fungitoxic effect of Carbendazim 50% WP was provided by interfering with a number of cellular processes such as mitosis, meiosis, energy production and cell wall synthesis of fungi, intracellular transport of molecules and interfering in spindle formation in mitosis (Davidse *et al*., 1986).

The next best fungicide includes the Fluopyrazam 11.5% W/W + Trifloxystrobin includes the strobiliurin group of fungicides QoI (quinol oxidation) site of cytochrome bc1 complex. Results in disrupting ATP production and proton translocation resulting in inhibition of spore germination, germ tube growth and mycelial development.

The results are in accordance with the findings of several earlier workers fungicides viz Carbendazim 50% WP (Bavistin) reported by the Jatav *et al*., 2013 at the lower concentrations @ 500 ppm siginificantly inhibited the mycelial growth. Rao *et al*. (2020), who reported significant inhibition of the mycelial growth. Carbendazim showed the highest inhibitory activity, while propiconazole, tebuconazle significantly inhibited the pathogen. Similarly Ishaq *et al*. (2023) reported significant suppression with the carbendazim based Shincar showing considerable efficacy, supporting the antifungal activity of carbendazim. Bashkar *et al*. (2015) reported that carbendazim 50 WP produced 92.98% inhibition were found effective against *Fusarium solani*.

Conclusion

The present *in vitro* study demonstrated that the systemic and combi-systemic fungicides evaluated were effective in suppressing the mycelial growth of *Fusarium oxysporum* f. sp. *melongenae*, the causal agent of Fusarium wilt of brinjal. Among the fungicides tested, carbendazim 50% WP was found to be the most effective, exhibiting 100% inhibition of mycelial growth at all tested concentrations (25, 50, 100 and 250 ppm). It was followed by fluopyram + trifloxystrobin and tebuconazole, which recorded mean mycelial growth inhibition of 93.38 and 92.81%, respectively, whereas hexaconazole recorded the comparatively lowest mean inhibition of 80.36% among the fungicides evaluated.

The inhibition of mycelial growth generally increased with an increase in fungicide concentration, indicating a concentration-dependent response of *F. oxysporum* f. sp. *melongenae*. However, as the present study was restricted to laboratory conditions, further evaluation under greenhouse and field conditions is necessary to validate their efficacy and determine their suitability for integrated management of Fusarium wilt of brinjal.

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