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Yaragorla Hanumantha Rao
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Sobita Devi
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Vishnu Vardhani Vemavarapu
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

K Rishitha Chowdary
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Corresponding Author:
Yaragorla Hanumantha Rao
Department of Plant Pathology, College of Agriculture, Central Agricultural University, Imphal, Manipur, India

Effect of *Trichoderma* spp., botanicals and fungicides against *Fusarium oxysporum*

Yaragorla Hanumantha Rao, Sobita Devi, Vishnu Vardhani Vemavarapu and K Rishitha Chowdary

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Abstract
Tomato, (*Solanum lycopersicum*), flowering plant of the nightshade family (Solanaceae), cultivated extensively for its edible fruits. Labelled as a vegetable for nutritional purposes, tomatoes are a good source of vitamin C and the phytochemical lycopene. The area under tomato cultivation in Manipur accounts for about 0.15 million hectares with an average production of 2.10 million tonnes and productivity of 12.02 tonnes ha^{-1} during 2016-17. The major constraints in production of tomato are biotic and abiotic stress. Among the biotic stress Fusarium wilt incurred by *Fusarium oxysporum* f.sp. *lycopersici* inflicts tremendous losses to the crop. So the present research was carried out to study *in vitro* evaluations of native *Trichoderma* spp., botanicals and fungicides against *Fusarium oxysporum* causing Fusarium wilt of tomato which induces losses in Manipur. Food poison technique and Dual culture were aided in this investigation. The investigated results revealed that among bio control agents tested Mix (*Trichoderma asperellum* + *Trichoderma harzianum*) and *Trichoderma asperellum* effectively controlled mycelial growth of the pathogen by 80% and 72% respectively. Botanicals used in this study significantly inhibited the growth of the fungus, among which garlic (*Allium sativum)* gave the best results by showing 75% of inhibition at 10% concentration followed by garlic 5% and ginger 10% showed 60 to 62% inhibition, among fungicides Propiconazole 13.9% + Difenoconazole 13% gave the best results by showing of 100% inhibition at 0.1% concentrations.

Keywords: *Trichoderma* spp., fungicides, *Fusarium oxysporum*

Introduction
Tomato, (*Solanum lycopersicum*), flowering plant of the nightshade family (Solanaceae), cultivated extensively for its edible fruits. Labelled as a vegetable for nutritional purposes, tomatoes are a good source of vitamin C and the phytochemical lycopene. Tomato is grown for its edible fruits, which can be consumed either fresh or in the form of various processed products such as paste, powder, ketchup, sauce, soup and canned whole fruits. Tomato is also known for higher medicinal and nutritional values. The pulp and juice is digestible, promoter of gastric secretion and blood purifier. Tomato cultivation has become more popular since mid-nineteenth century because of its varied climatic adaptability and high nutritive value.

It is cultivated in an area of 4.78 million hectares all over the world with production of 177.04 million tonnes and an average yield of 19.57 tonnes ha^{-1} (FAO Stat 2016) [2].

Among the diseases of tomato, wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* which is found serious incurring heavy losses. In recent years Fusarium wilt of tomato is assumed a serious problem where the crop losses were in the tune of 10 to 80 per cent (Kapoor, 1988) [4]. Peralta *et al*., (2001) [5] reported yield losses of tomato due to wilt disease up to 40 per cent. Ramezani (2010) [6] reported mycoparasitism and inhibitory effects of five *Trichoderma* spp (*T. harzianum, T. koningi, T. longiconis, T. hamatum* and *T. viride*) on the growth of the causal agent of tomato Fusarium wilt. Observation on *in vitro* dual cultures showed that the high antagonistic effect was found in case of *T. hamatum, T. harzianum* and *T. longiconis.*

In 1982 Quadri *et al*., [7] reported that the *in vitro* efficacy of Eight fungicides against *Fusarium oxysporum* and found that Difolatan (0.2%), Thiram (0.2%), Carbendazim (0.2%), Mancozeb (0.2%) found effective against the fungi.

So the current evaluations are done to identify the effective bio agents, botanicals and fungicides against the disease causing agent of tomato.

Materials and Methodologies

Isolation of fungus

Typically Fusarium wilt infected tomato plant samples were collected from farmer's field of different locations and isolation, identification of the causal pathogen was carried out in the Department of Plant Pathology, College of Agriculture, CAU, Imphal. Diseased samples were lacerated to small pieces with the help of sterilized scalpel. The lacerated pieces were surface sterilized using 1% sodium hypochlorite solution for 1 minute followed by rinsing the pieces in three phases of sterile distilled water in order to remove the traces of sodium hyphochlorite. Later the pices were blot dried using blotting paper. The sterile pieces were aseptically transferred to sterilized petri dishes containing Potato dextrose agar (PDA). The petri dishes were incubated at 27 ± 1^0C in BOD incubator and were observed periodically for the fungal growth. Purified cultures of the fungus ware obtained by hyphal tip culture methods. Identification was done according to the key of (Leslie and Summerell, 2006) [9].

In vitro evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Fusarium oxysporum*

In-vitro antagonistic effect of three isolates of *Trichoderma* spp. *viz., (T. harzianum*, *T. asperellum* and *T.viride*) were evaluated against the test fungus. All the bio-control agents were collected from the Department of Plant Pathology, COA, CAU. Antagonistic test of bio-control agent was done, following the dual culture technique (Bell 1982). The observations were recorded based on Bell's scale
Bell's scale with slight modification:

Class I: The antagonist completely overgrew the pathogen (100%over growth)

Class II: The antagonist overgrew at least 2/3rd of the pathogen surface (75%over growth)

Class III: The antagonist colonized on half of the growth of the pathogen surface (50%over growth)

Class IV: The pathogen and the antagonist locked at the point if contact

Class V: The pathogen overgrew the mycoparasite

Class VI: The pathogen and antagonistic from inhibition A chemical fungicide, mancozeb (0.3%) will be used for the *in vitro* experiment as a check. Per cent inhibition will be calculated by using following formula suggested by Vincent (1927) [8].

$$\text{Percent Inhibition} = \frac{C - T}{C} \times 100$$

Where
C = radial growth of fungus in control
T = radial growth of fungus in treatment

Effect of Botanicals on growth against *Fusarium oxysporum*

Extracts of three locally available botanicals namely, Garlic (*Allium sativa*), Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale)* were studied *in vitro* for their effect on growth of the fungus. Each plant extract was tried at three different concentrations. Fresh plant parts were collected and washed thoroughly in running water and surface sterilized with 70% ethanol for few second then finally washed with sterile water. They were then crushed using mortar and pestle separately by mixing with sterile water at the ratio of 1:1 w/v. The extracts were filtered through muslin cloth and centrifuge at 1500 rpm for 15 minutes and the supernatants were separated. The prepared plant extracts were considered as 100% concentration. The required concentrations of plant extracts were added to hundred (100) ml Erlenmeyer conical flask containing sterilized 50 ml molten PDA medium to give the desired concentrations and shaken well and mixed thoroughly. The poisoned PDA medium were poured in petriplates @ 20 ml per plate and allowed to solidify. The plates were then inoculated aseptically by transferring 5 mm mycelial disc with the help of cork borer and sterilized needle. The plates were then kept inside BOD incubator (25+1°C) till the pathogen fully grows in the control plates. The PDA medium without plant extracts served as control. Each treatment was replicated three times. Per cent inhibition of the fungus was calculated by following the formula given by Vincent (1947) [8] mentioned above

Effect of fungicides and a fungicidal combination on growth against *Fusarium oxysporum*

Fungicides and a fungicidal combination *viz*., Propiconazole 25%, Difenoconazole 25% and Propiconazole 13.9%+ Difenoconazole 13% used in the current *in vitro* studies along with the particulars like trade name, chemical name and active ingredient of the chemical formulation. Food poison technique was used for this evaluation. The poisoned PDA medium were poured in petriplates @ 20 ml per plates and allowed to solidify. The plates were then inoculated aseptically by transferring 5 mm mycelial disc with the help of cork borer and sterilized needle. The plates were then kept inside BOD incubator (25+1°C) till the pathogen fully grows on the control plates. Each treatment was replicated three times. Per cent inhibition of the fungus was calculated by following the formula given by Vincent (1947) [8] mentioned above.

Results and Discussions

In vitro evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Fusarium oxysporum*

The effect of different *Trichoderma* spp. and a chemical fungicide on radial growth of *Alternaria solani* are presented in Table 1, Figure 1 and Plate 1. revealed that all the species of *Trichoderma* spp exhibited different antagonistic potential against the *Fusarium oxysporum*. Among three *Trichoderma* spp tested *Trichoderma harzianum* showed highest colony growth (3.7 cm) and inhibition percentage (55.87%) followed by *Trichoderma asperellum* (2.2 cm and 72.06%), *Trichoderma viride* (2.27 cm and 71.11%), Mix (*Trichoderma harzianum* + *Trichoderma asperellum*) (1.5 cm and 80.95%) and mancozeb (no colony growth and 100% respectively). These results are found to be similar with Shahida *et al*. (1994) [10] showed that the suppressive effect of *Fusarium oxysporum* against *A. solani* and *Macrophomina phaseolina* was increased in the presence of *Trichoderma asperellum Trichoderma harzianum* and other fungi on tomato and okra crop. Vinale *et al*. (2008) [11] reported that the antagonistic nature of *Trichoderma* spp. was due to release of various enzymes which can degrade cell wall and secondary metabolites of host fungus

Effect of Botanicals on growth against *Fusarium oxysporum*

Extracts of three locally available botanicals namely, Garlic (*Allium sativa*), Turmeric (*Curcuma longa*) and Ginger (*Zingiber officinale)* were studied *in vitro* for their effect on growth of the fungus Table 2. revealed the efficacy of plant extracts In *Fusarium oxysporum* best result showed by Garlic (10%) colony growth 2.06 cm and 73.75% inhibition, followed by Ginger(10%) showed (3.03 cm colony growth and 61.48% inhibition) and Garlic (5%) showed (3.1 cm colony growth and 60.63% inhibition) and Ginger (5%) showed (3.33cm colony growth and 57.67% inhibition) remaining plant extracts that's as Garlic (2.5%), Turmeric (2.5%), (5%) and (10%) showed more are less same colony growth that's is 5.1 to 5.5 cm and inhibition is 31.00 to 35.65%, and lowest results was showed by Ginger (2.5%) colony growth is 5.4cm and inhibition 30.58%. findings are in conformity with the findings of Mishra and Gupta (2006).

Effect of fungicides and a fungicidal combination on growth against *Fusarium oxysporum*

Results also revealed that in *Fusarium oxysporum* at 0.1% Propiconazole 13.9% + Difenoconazole 13% was found to be the best with 0.4 cm colony growth and 94.92% growth inhibition followed by Propiconazole 13.9%+ Difenoconazole 13% (0.05% and 0.025%) showed nearly same (0.5 cm and 93% respectively), Propiconazole 25% (0.1%, 0.05% and 0.025%) shows more are less same (1.3 to 1.7 cm colony growth 78% to 83% inhibition respectively), and Difenoconazole 25% (0.05 and 0.1%) showed 2.2-2.5 cm and 68%-72% respectively), and lowest result shown by Difenoconazole 25% (0.025%)with 3.8 cm and 51.74% respectively). Results are in conformity with the findings of Mathur *et al*. (1971) [12].

Table 1: *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Fusarium oxysporum*

Treatment No.	Treatment details	Dose (%)	*Fusarium oxysporum*	
			Colony growth(cm)*	Inhibition % over control
T1	*Trichoderma asperellum (25)*	10	2.2	72.06
T2	*Trichoderma harzianum (69)*	10	3.4	55.87
T3	*Trichoderma viride*	10	2.2	71.11
T4	Mix (T1+T2)	10	1.5	80.95
T5	Mancozeb	0.3	0	100
SE(d)				0.06
CD (0.05)				0.19

*Mean of three replications

Table 2: *In vitro* evaluation of Botanicals against *Fusarium oxysporum*

Botanicals (Parts used)	Concentration (%)	*Fusarium oxysporum*	
		Colony growth (cm)*	Inhibition % over control
Garlic (Cloves)	2.5	5.4	31.42
	5.0	3.1	60.63
	10	2.06	73.75
Ginger (Rhizome)	2.5	5.4	30.58
	5.0	3.33	57.67
	10	3.03	61.48
Turmeric (Rhizome)	2.5	5.46	31.42
	5.0	5.1	35.23
	10	5.16	34.39
SE(d)±			0.04
CD (0.05)			0.12

*Mean of three replications

Table 3: *In vitro* evaluation of Fungicides against *Fusarium oxysporum*

Fungicide	Concentration	*Fusarium oxysporum*	
		Colony growth	Inhibition over control
Propiconazole25%EC	0.1	1.3	83.49
	0.05	1.5	80.95
	0.025	1.7	78.41
Difenoconazole25% EC	0.1	2.2	72.06
	0.05	2.5	68.25
	0.025	3.8	51.74
Propiconazole13.9% + Difenoconazole13% EC	0.1	0.4	94.92
	0.05	0.53	93.65
	0.025	0.5	93.22
SE(d)±			0.05
CD (0.05)			0.15

*Mean of three replications

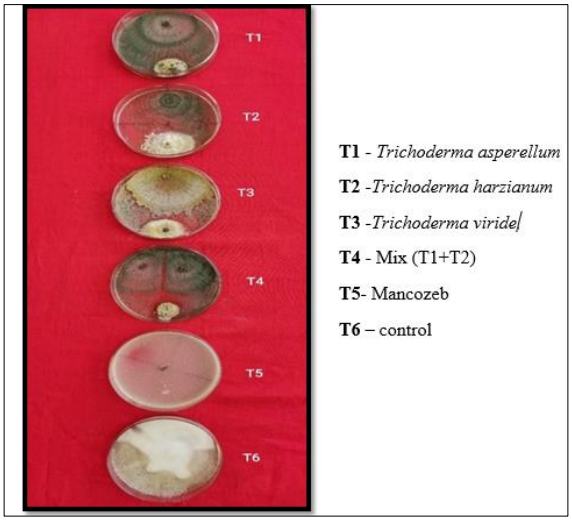


Plate 1: *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Fusarium oxysporum*

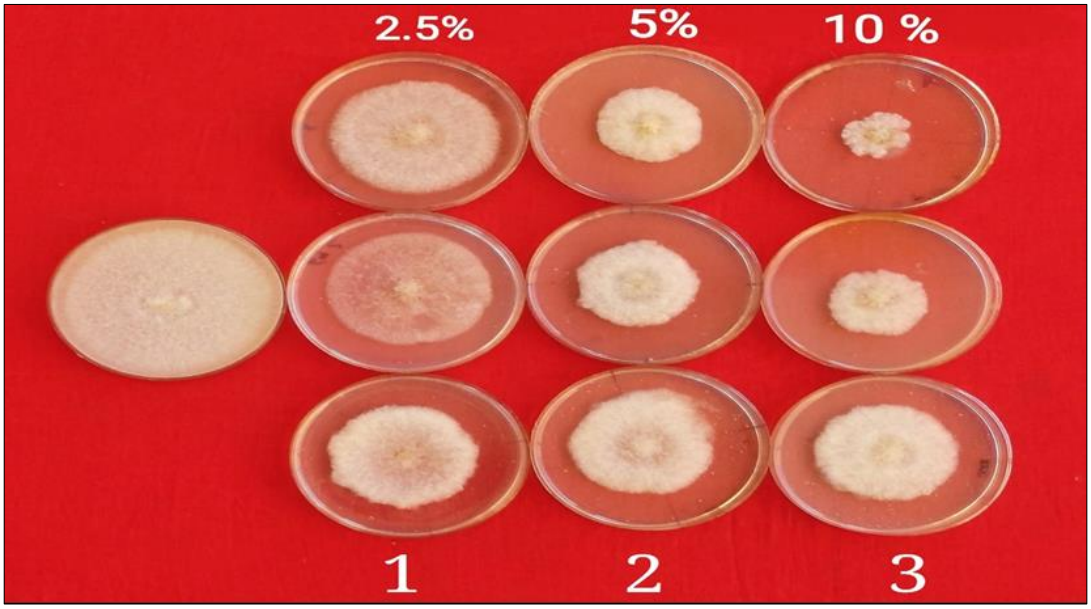


Plate 2: *In vitro* evaluation of Botanicals against *Fusarium oxysporum* 1.Garlic 2.Ginger 3.Turmeric

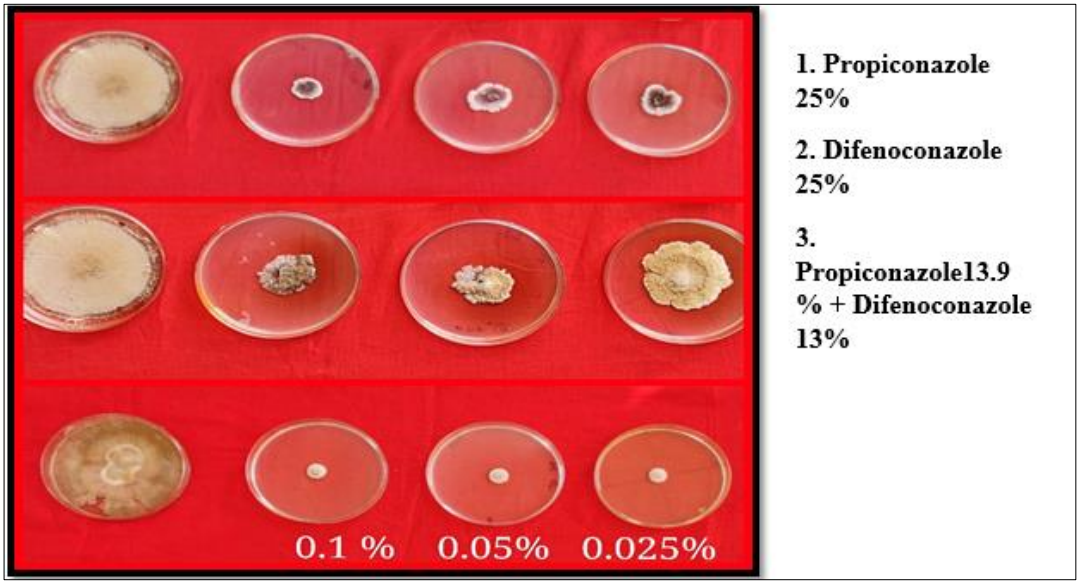
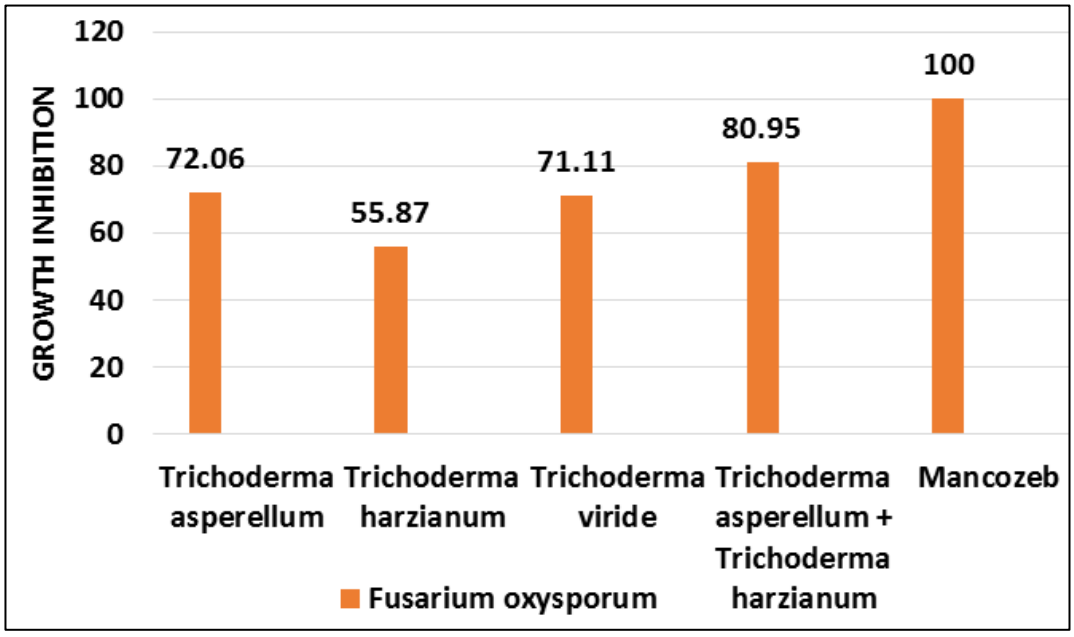
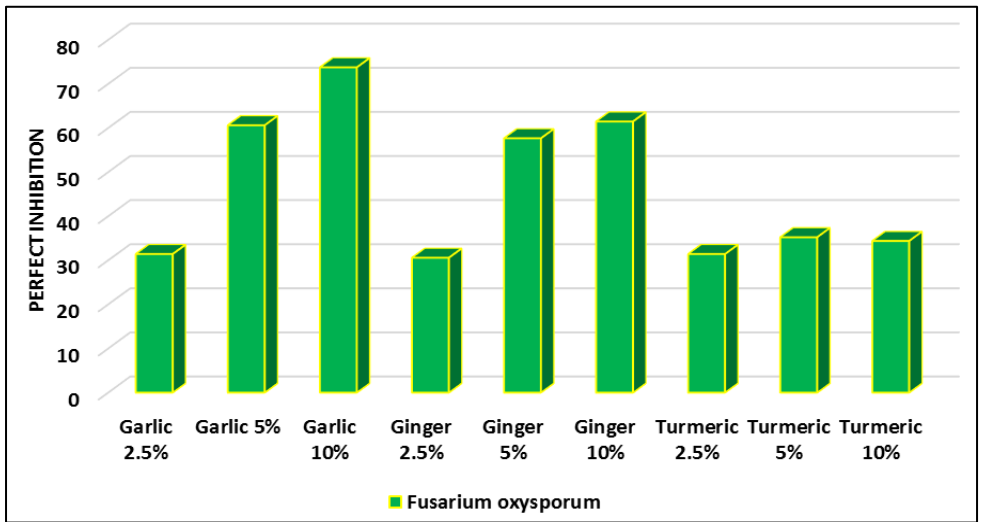


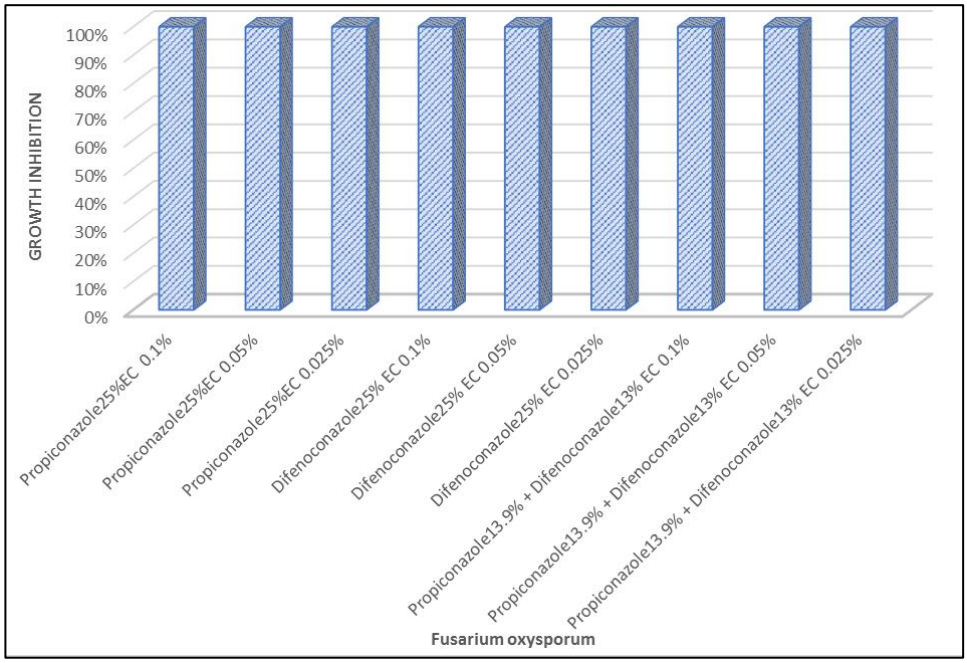
Plate 3: *In vitro* evaluation of Fungicides against *Fusarium oxysporum*



Graph 1: *In vitro* evaluation of Antagonistic effect of Native *Trichoderma* spp. against growth of *Fusarium oxysporum*



Graph 2: *In vitro* evaluation of Botanicals against *Fusarium oxysporum*



Graph 3: *In vitro* evaluation of Fungicides against *Fusarium oxysporum*

Conclusion

It is evident that all the *Trichoderma* spp. used in this investigation exhibited antagonism in suppressing the mycelial growth of *F. oxysporum*. These findings showed that for management of *F. oxysporum*, Trichoderma spp. can be used as bio control agent. All the fungicides tested effectively inhibit the growth of pathogen. Among all the plant extracts garlic and Ginger showed the best result, all the botanocals agents also significantly inhibited the growth of pathogen.

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