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BIO-EFFICIACY OF BIO-AGENTS AND BOTANICAL EXTRACTS AGAINST ROOT ROT CAUSING PATHOGEN (*FUSARIUM SOLANI)* IN MULBERRY (*MORUS ALBA* L.)

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ABSTRACT

Root rot caused by *Fusarium solani* severely affects mulberry (*Morus alba* L.) productivity and leaf yield, impacting sericulture efficiency. The present study evaluated the *in vitro* efficacy of bio-control agents and botanical extracts against *F. solani*. Ten bio-agents, including strains of *Trichoderma viride*, *T. harzianum*, *T. bravicompactum*, *Bacillus subtilis* and *Pseudomonas fluorescence*, were tested against *Fusarium solani* under *in vitro* using the dual culture technique. Among them, *T. harzianum* (Th-3) recorded the highest mycelial inhibition (74.44%), followed by *T. viride* (Tv-3) with mycelial inhibition of 68.89 per cent. Moderate inhibition was observed in *T. viride* (Tv-1), *T. harzianum* (Th-1) and *B. subtilis* (65–67%). Eleven botanical extracts evaluated through poisoned food technique showed that garlic (*Allium sativum*) extract completely inhibited mycelial growth of *F. solani* (100%) at 15 and 20% concentrations, while Agave and Subabul showed moderate activity. Neem, Onion and Pongamia exhibited limited inhibition, whereas *Lantana camara*, Jatropha and Gliricidia were least effective with respect to mycelial inhibition Overall, *T. harzianum* (Th-3) and garlic extract proved highly effective, indicating their potential as eco-friendly alternatives for managing *F. solani*-induced root rot in mulberry.

Keywords **:** *Fusarium solani*, *Trichoderma harzianum*, *Allium sativum*, mulberry, bio-agents, botanical extract.

Introduction

Mulberry (*Morus alba* L.) is a perennial crop of significant economic importance, serving as the sole food source for the silkworm (*Bombyx mori* L.) and forming the backbone of the sericulture industry (Dandin *et al.*, 2003). The quality and quantity of mulberry leaves directly influence cocoon yield and silk production. However, mulberry cultivation is constrained by several biotic stresses, including diseases caused by fungi, bacteria, viruses, nematodes and phytoplasmas, which result in considerable yield and quality losses (Sukumar and Padma, 1999). Root rot is one of the most destructive diseases in mulberry, leading to root decay, wilting, leaf yellowing, stunted growth and, in severe cases, plant death. Among the soil-borne pathogens, *Fusarium solani* is a major causal agent responsible for significant losses in leaf yield and cocoon production (Philip and Sharma, 1997; Sharma *et al.*, 2003). The disease is aggravated by high soil moisture, poor drainage, continuous cropping, and mechanical injuries to roots, making management under field conditions challenging. Chemical fungicides are widely used to control root rot, but their

repeated application can cause environmental pollution, affect soil microflora, and pose risks to silkworms. Therefore, biological control using antagonistic microorganisms (bio-agents) and plant-derived botanical extracts has gained attention as an eco-friendly and sustainable alternative. These agents can suppress pathogen growth, reduce disease incidence, and improve plant health without harmful residues.

Considering the economic importance of mulberry and the limitations of chemical control, the present study was undertaken to evaluate the bio-efficacy of selected bio-agents and botanical extracts against *Fusarium solani*, the causal agent of root rot in mulberry.

Material and Methods

The present study on Bio-efficiacy of bio-agents and botanical extracts against root rot causing pathogen (*Fusarium solani)* in mulberry (*Morus alba* L.) was carried out in the Department of Plant Pathology, College of Sericulture, Chintamani, University of Agricultural Sciences, Bengaluru, Karnataka, during 2024 - 2025. The materials used and methodology followed during the investigation are described below.

In vitro Evaluation of Bio-Agents against *Fusarium solani*

The antagonistic activity of bio-control agents, namely *Trichoderma viride*, *Trichoderma harzianum*, *Trichoderma braviconpactum, Pseudomonas fluorescens* and *Bacillus subtilis*, was evaluated using the dual culture method. For this purpose, 20 ml of sterilized potato dextrose agar (PDA) medium was poured into sterile Petri plates and allowed to solidify. A 5 mm disc from a seven-day-old culture of each fungal bio-agent was placed on one side of the plate with the aid of a sterile cork borer, while a 5 mm disc of the root rot pathogen (one-week-old pure culture) was positioned on the opposite side, maintaining a distance of 15 mm from the plate's edge. For bacterial antagonists, a sterile inoculating loop was used to streak the bacterium at one end of the PDA plate and a 5 mm disc of the pathogen was placed directly opposite. Plates inoculated only with the pathogen in the center served as controls. All inoculated plates were incubated at 28 ± 1 °C. Antagonistic efficacy was assessed by recording the radial growth of the pathogen in each treatment and comparing it with the control. The percentage inhibition of mycelial growth was calculated using the formula proposed by Vincent (1947).

In vitro Evaluation of Botanical Extracts against *Fusarium solani*

The efficiency of plant extracts or botanical extracts was tested against root rot pathogen *Fusarium solani* on Potato dextrose agar (PDA) medium by using poisoned food technique. For this, 100g of fresh plant parts (leaves/bulbs) were collected, washed with tap water subsequent washing with distilled water. The fresh sample was chopped and crushed by adding 100 ml sterile water. The crushed product was filtered through muslin cloth. The filtrate gave 100 per cent and was used as stock solution. 5, 10 and 15 ml of stock solution was mixed with 95, 90, 85 and 80 ml of PDA medium and then it was shaken for uniform mixing of plant extract. Later, the media was sterilized and allowed to cool. Twenty ml of medium was poured into sterilized Petri plates and then fungal disc of 5 mm was placed at the center of the petri Plate and incubated at 28 ± 1oC. The PDA medium without any plant extract served as control. The per cent inhibition of mycelial growth of test fungus was calculated by using following formula given by (Vincent, 1947).

$$I = (C\text{-}T/\ C) \times 100$$

Where,

I = Per cent growth inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

Results and Discussion

In vitro evaluation of bio agents against *F. solani*

The percentage inhibition of mycelial growth was computed based on the observation of radial growth of the bio agents and test fungus. The organism *T. harzianum* (Th-3) was the most effective and significant of the ten bio agents examined with a maximum mycelial inhibition of 74.44 per cent over control. *T. viride* (Tv-3) succeeded with 68.89 per cent inhibition followed by *T. viride* (Tv-1) and *T. harzianum* (Th-1) with moderate inhibition that were comparable to each other with 67.31 and 67.04 per cent mycelial inhibition. The bacterial bio agent *Bacillus subtilis* (Bs) exhibited 65.28 per cent mycelial inhibition of fungal pathogen followed by *Trichoderma bravicompactum* (Tb-1) (60.93%), *T. harzianum* (Th-2) (59.44%) and *T. viride* (Tv-2) (53.70%). Whereas, *Pseudomonas fluorescence* (Pf) and *Trichoderma bravicompactum* (Tb-2) exhibited least mycelial inhibition of 51.57 and 46.94 per cent, respectively (Table 1; Fig 1)Hyperparasitism, competition for

resources and space or antibiotics may be the causes of these fungal bioagents suppressive action (Plate 1).

The results are consistent with previous research by Narayanan *et al.* (2015) who documented the effectiveness of fungicides and possible biocontrol agents against *F. solani* inducing mulberry wilt. The evaluation of three antagonists *Trichoderma viride, Pseudomonas fluorescens* and others revealed that *Trichoderma* greatly inhibited the pathogen's mycelial proliferation.

Table 1*: In vitro* evaluation of bio agents against *F. solani*

Sl. No	Fungal bio agents	Strain	Per cent inhibition of mycelial growth
1.	*Trichoderma viridae*	Tv-1	67.31 (55.11)#
2.	*Trichoderma viridae*	Tv-2	53.70 (47.11)
3.	*Trichoderma viridae*	Tv-3	68.89 (56.08)
4.	*Trichoderma harzianum*	Th-1	67.04 (54.94)
5.	*Trichoderma harzianum*	Th-2	59.44 (50.42)
6.	*Trichoderma harzianum*	Th-3	74.44 (59.61)
7.	*Trichoderma bravicompactum*	Tb-1	60.93 (51.29)
8.	*Trichoderma bravicompactum*	Tb-2	46.94 (43.23)
9.	*Bacillus subtilis*	Bs	65.28 (53.87)
10.	*Pseudomonas fluorescence*	Pf	51.57 (45.88)
11.	Untreated control	-	0.00 (0.00)
		F test	*
		S. Em±	0.28
		CD @ 1%	0.79

#Figures in the parentheses are arcsine transformed values

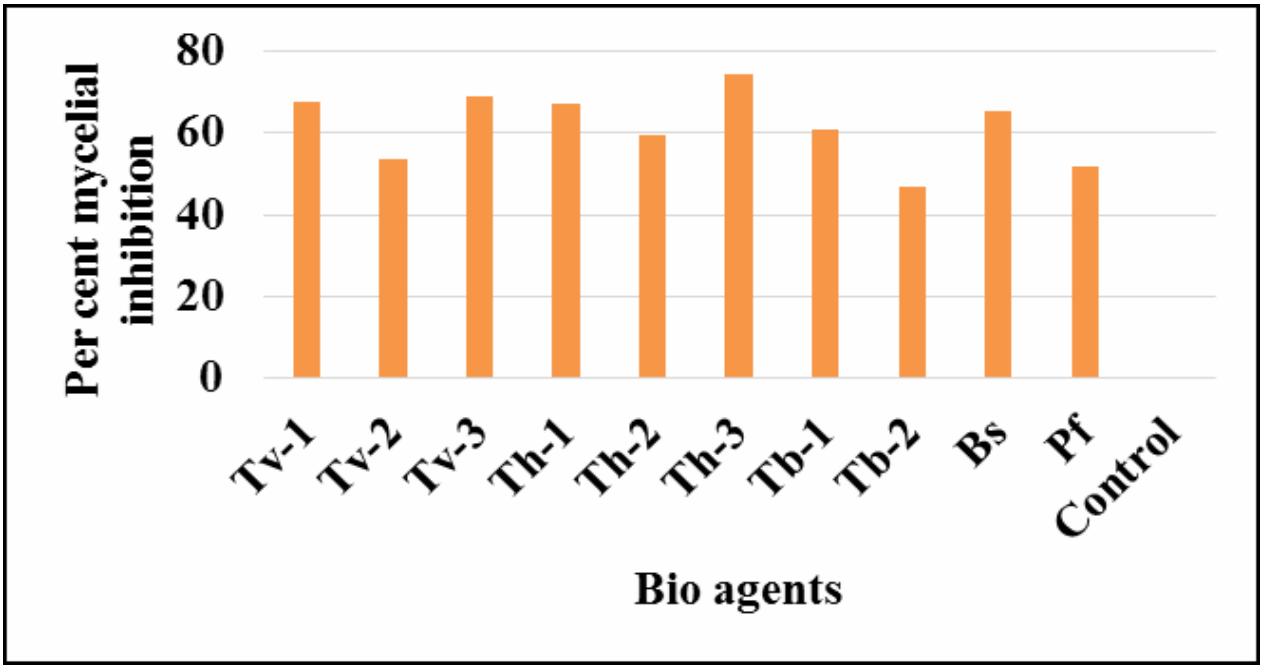

Fig. 1 : Effect of fungal bio agents against *F. solani*

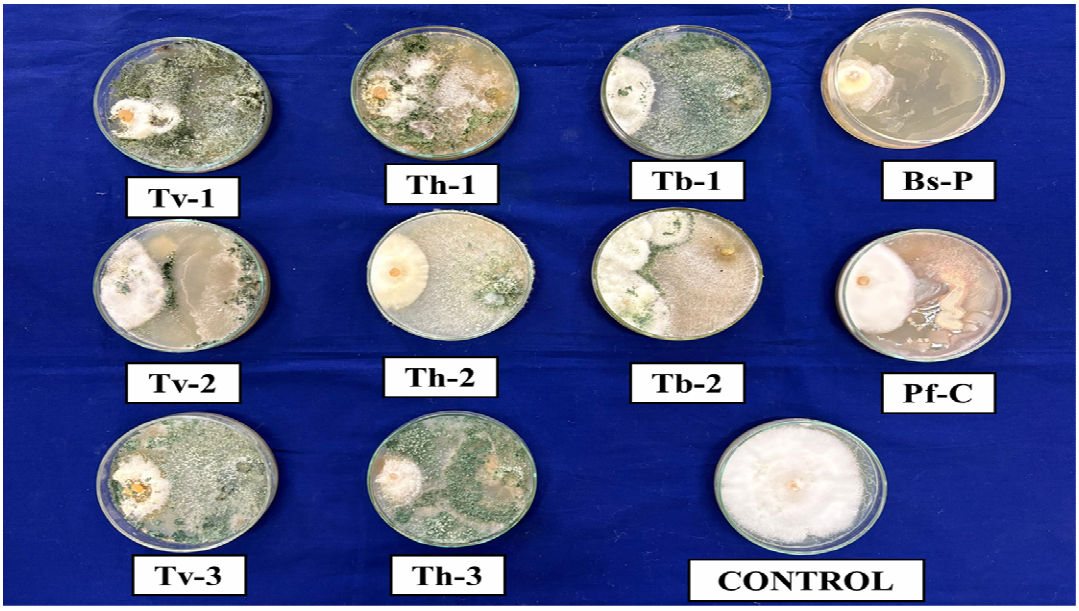

Plate 1: *In vitro* evaluation of different bio agents against *F. solani*

In vitro* evaluation of botanical extracts against *F. solani

Total of eleven botanical extracts were evaluated at four different concentrations *viz.,* 5, 10, 15 and 20 per cent employing the poison food technique under *in vitro* conditions. The effectiveness of these botanical extracts were assessed based on their ability to inhibit the mycelial growth of *Fusarium solani*. The per cent inhibition observed at each concentration for various botanical extracts is comprehensively pooled.

Among the eleven botanical extracts tested, garlic extract recorded significantly higher inhibition of mycelial growth (100%) of *Fusarium solani,* at 15 and 20 per cent concentrations. The lower concentrations of 5 and 10 per cent garlic extract still demonstrated substantial supression activity with 27.41 per cent and 51.98 per cent mycelial inhibition respectively. This trend highlights a dose-dependent antifungal activity of the extract with higher concentrations producing more pronounced suppression of fungal growth. The application of agave extract at graded concentrations of 5, 10, 15 and 20 per cent exhibited a progressive inhibitory effect on the mycelial growth of *Fusarium solani*, achieving inhibition of 15.19, 19.88, 22.35 and 29.38 per cent respectively. Subabul leaf extract showed mycelial inhibition of 19.63, 29.01, 29.26 and 33.83 per cent at the respective concentrations. The extract of onion demonstrated a moderate but consistent antifungal effect, with the inhibition values at ascending concentrations 18.89, 22.59, 22.72 and 28.77 per cent, respectively. Simarouba extract exhibited a gradual increase in mycelial inhibition from 5 per cent to 15 per cent plateauing between 15 per cent and 20 per cent. Neem extract showed moderate efficacy with inhibition values ranging from 18.15 per cent to 28.27 per cent. The touch-me-not plant extract showed only mild antifungal potential increasing from 4.69 per cent at 5 per cent concentration to 28.52 per cent at 20 per cent concentration indicating some dose responsiveness though overall inhibition remained modest. *Pongamia pinnata* showed slightly lower inhibitory effects with values between 18.40 per cent and 22.72 per cent of inhibition indicating limited but consistent activity. Conversely Jatropha, Gliricidia and *Lantana camara* extracts showed minimal to no antifungal activity with inhibition values either at 0.00 per cent or minimal activity up to 9.88 per cent observed for *Lantana camara* at the highest dose (Table 2; Fig. 2). This suggests that the bioactive compounds in these extracts were either absent or present in lesser concentrations or ineffective against *F. solani* under the test conditions.

The interaction between botanical extracts and concentration were statistically significant indicating that the effectiveness of each extract varied depending on its concentration. Garlic extract at 15 per cent and 20 per cent showed complete inhibition (100%) a response not mirrored by any other extract at any concentration emphasizing its unique potency. The complete inhibition of *Fusarium solani* by garlic extract is likely due to the presence of potent antifungal compounds such as allicin, which disrupts fungal cell membrane integrity and inhibits spore germination. Agave extract though less effective than garlic showed a gradual increase in antifungal activity indicating that its efficacy is highly concentration-dependent.

The findings were similar to that of Naveen Chandra Reddy (2023) who evaluated eleven botanical

extracts against *F. solani* and found that garlic extract has shown 100 per cent inhibition at 15 and 20 per cent concentration. Whereas, agave has shown up to 31.86 per cent inhibition at 20 per cent. Manica tomar and Sharma (2005) evaluated plant extracts to test their efficacy against *Fusarium solani* causing root rot of khair. Out of twenty different aqueous plant extracts, garlic (*Allium sativum)* aqueous extract showed a maximum inhibition (64.79%) followed by *Aloe barbedensis* (62.89%).

Table 2: *In vitro* evaluation of botanical extracts against *F. solani*

Sl. No	Botanical extracts	Per cent inhibition of mycelial growth			
		Concentrations			
	Common name	5 %	10 %	15 %	20 %
1.	Garlic	27.41 (31.55)#	51.98 (46.11)	100.00 (89.96)	100.00 (89.96)
2.	Neem	18.15 (25.20)	21.73 (27.76)	23.83 (29.20)	28.27 (32.09)
3.	Subabul	19.63 (26.29)	29.01 (32.58)	29.26 (32.73)	33.83 (35.55)
4.	Jatropha	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
5.	Pongamia	18.40 (25.37)	19.63 (26.28)	22.10 (28.02)	22.72 (28.45)
6.	Touch me not	4.69 (12.45)	12.10 (20.34)	22.10 (28.02)	28.52 (32.41)
7.	Onion	18.89 (25.74)	22.59 (28.34)	22.72 (28.43)	28.77 (32.41)
8.	Gliricidia	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
9.	Simarouba	14.57 (22.43)	19.38 (26.11)	22.96 (28.62)	23.09 (28.70)
10.	Agave	15.19 (22.92)	19.88 (26.44)	22.35 (28.19)	29.38 (32.80)
11.	Lantana	0.00 (0.00)	0.00 (0.00)	8.52 (16.91)	9.88 (18.25)
12.	Untreated control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
		Botanicals (B)	Concentration (C)	Interaction (B×C)	
	F test	*	*	*	
	S. Em±	0.28	0.16	0.56	
	CD @ 1%	1.05	0.60	2.10	

#Figures in the parentheses are arcsine transformed values

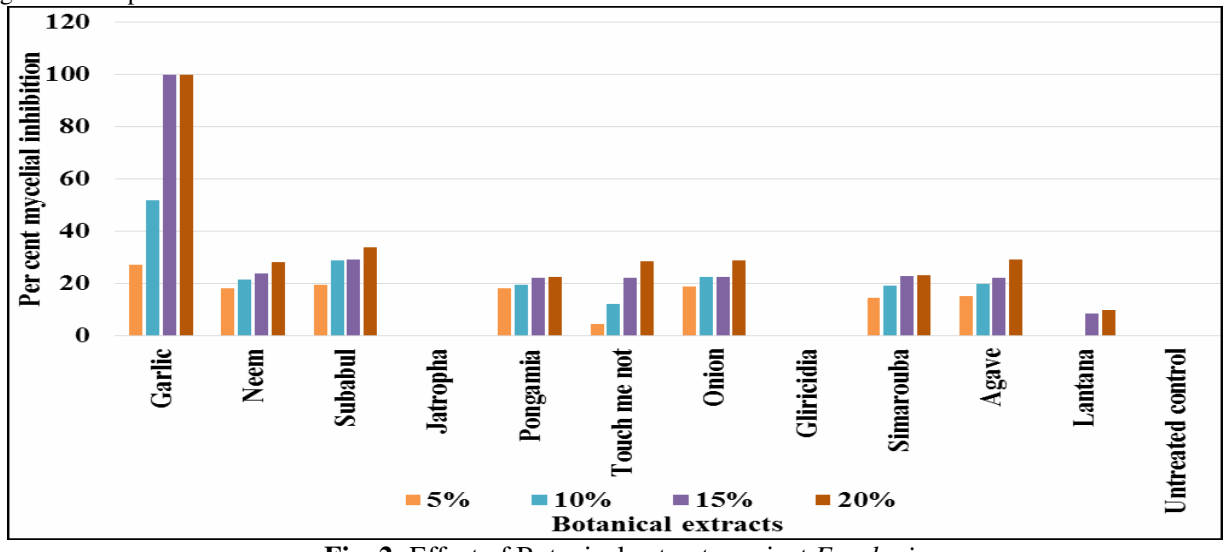

Fig. 2: Effect of Botanical extracts against *F. solani*

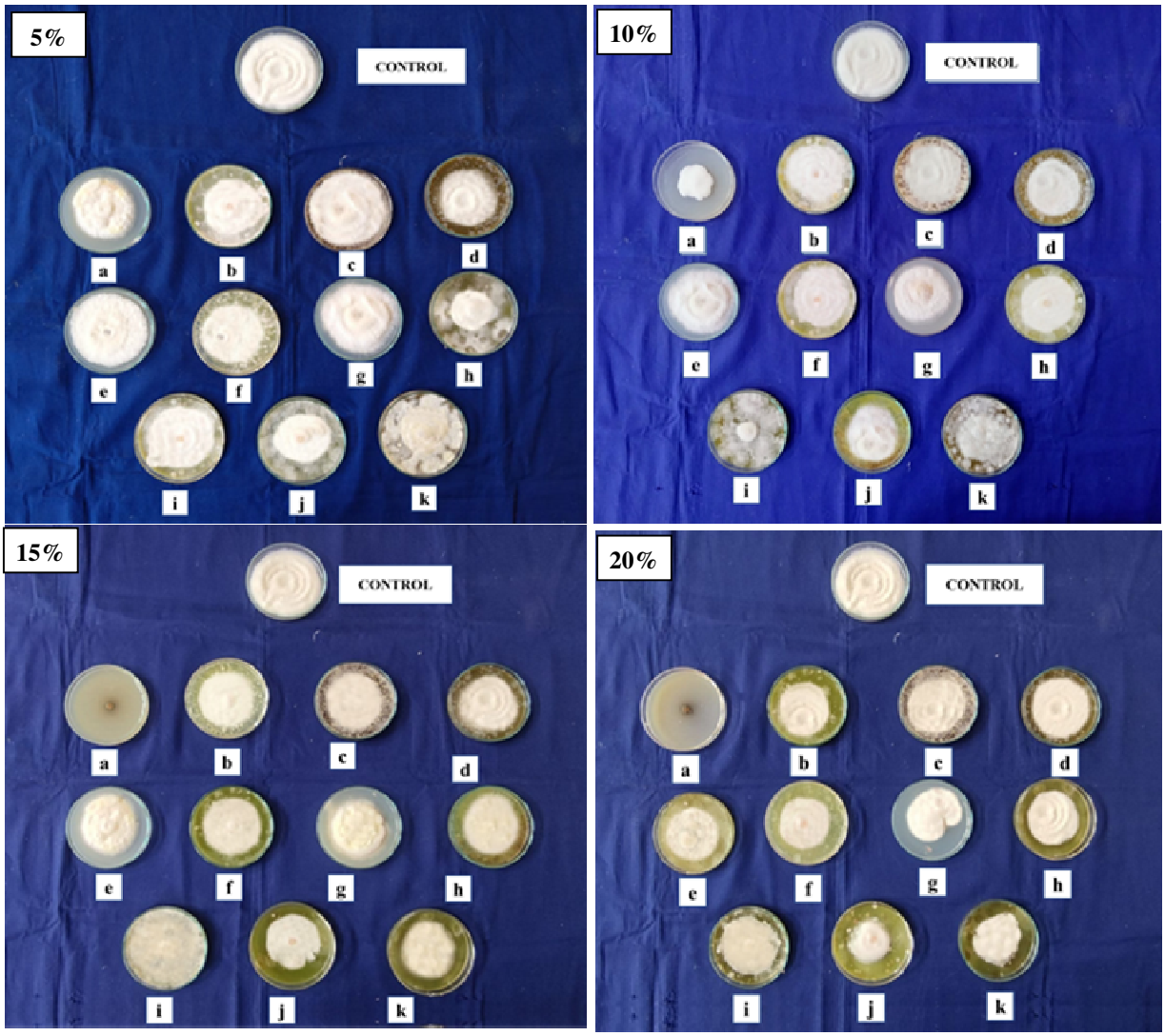

Plate 2: *In vitro* evaluation of different botanical extracts against *F. solani*: a) Garlic b) Neem c) Subabul d) Jatropha e) Pongamia f) Touch me not g) Onion h) Gliricidia i) Simarouba j) Agave k) Lantana

Conclusion

The study revealed that *Trichoderma harzianum* (Th-3) was the most potent bio-control agent in suppressing *Fusarium solani*, while garlic (*Allium sativum*) extract completely inhibited the pathogen at higher concentrations (15 and 20%). These results suggest that both *T. harzianum* and garlic extract can be effectively utilized as sustainable and eco-friendly measures for managing root rot disease in mulberry cultivation.

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