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In vitro Evaluation of Bio Control Agents and Botanicals against Mulberry Root Rot Pathogen *Lasiodiplodia theobromae*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mulberry root rot is fast spreading disease caused by *L. theobromae*. The pathogen can be managed by synthetic fungicides. However, enormous use of synthetic fungicides leads to residual toxicity which affects the growth and development of silkworm. In this context, an attempt was made to use potential bio control agents for the management of this pathogen. Seven fungal and five

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bacterial bio agents evaluated out of them *T. viride* (Tv – B2), *T. harzianum* (Th - 44), *B. subtilis* (Bs - M) and (Bs - O) proved as best for inhibition of mycelial growth of the pathogen. Out of eleven botanical extracts used, garlic proved best at 15 per cent and 20 per cent and agave at 20 per cent concentration against root rot causing pathogen *L. theobromae*.

Keywords: Mulberry; bio control agents; silkworms.

1. INTRODUCTION

Mulberry (*Morus* spp.) is fast growing perennial plant extensively grown to feed silkworms (*Bombyx mori* L.) under various type of soil and climatic conditions. Soil nutrients get depleted due to repeated harvesting of leaf and render mulberry plants susceptible to many soil borne diseases. Root rot is most important fungal disease causing considerable yield loss in mulberry. Among the soil borne diseases, root rot is majorly incited by *Fusarium solani* and *Lasiodiplodia theobromae* in Southern districts of Karnataka [1-4]. These pathogens are more alarming and they thrive well in different types of soil and cause considerable yield loss [5]. The disease spreads primarily through the diseased plant used for propagation, contaminated soil, farm implements and irrigation water [6]. These soil borne pathogens can be managed by using effective fungicides but use of chemicals in excess may cause residual effect on silkworm and environment pollution. Therefore, antagonistic fungal bio control agents like *Trichoderma* spp. and bacterial bio control agents like *Bacillus* spp., *Pseudomonas* spp. and botanicals may help in reducing the soil borne pathogen load and these were tested *in vitro* against *Lasiodiplodia theobromae*.

2. MATERIALS AND METHODS

The present study on *in vitro* evaluation of bio control agents and botanicals against mulberry root rot causing pathogen was carried out in the Department of plant pathology, College of Sericulture, Chintamani, University of Agricultural Sciences, Bengaluru, Karnataka, India during 2021 - 2022. The materials used and methodology followed during the investigation are described below

2.1 *In vitro* Evaluation of Biocontrol Agents against *L. theobromae*

The antagonistic potential of bio control agents *viz., Trichoderma viride, T. harzianum, Pseudomonas fluorescence* and *Bacillus subtilis* were tested by dual culture technique. For this 20 ml of sterilized, melted and cooled PDA medium was poured into each Petri plate and allowed to solidify. The plates were inoculated with 5 mm disc of 7 days old culture of fungal bio-control agents with the help of a sterilized cork borer and subsequently on the opposite side inoculated with pure culture of root rot pathogen by placing a 5 mm disc of one-week old pure culture keeping 15 mm distance from the periphery. The bacterial antagonists were streaked with a sterilized inoculating loop at one end of the PDA Petri plates. Just opposite to the bacterial streak, 5mm disc of the pathogen was placed with a sterilized inoculation loop. The inoculation of pathogen alone on the centre in the plates served as control. The experiment was conducted by using Completely Randomized Design (CRD). Three replications of each treatment, including the control, were maintained. These plates were incubated at 28±1°C. The efficacy of antagonistic organisms was recorded by measuring the colony diameter of the pathogen in each treatment and compared with control. Per cent mycelial inhibition over control was calculated by using the formula given by Vincent [7].

2.2 *In vitro* Evaluation of Botanical Extracts against *L. theobromae*

The efficiency of plant extracts or botanical extracts was tested against root rot pathogen *L. theobromae* 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 ± 1^{o}C. 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 [7].

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

Where,

I = Per cent growth inhibition of mycelium
C = Growth of mycelium in control
T = Growth of mycelium in treatment

3. RESULTS AND DISCUSSION

3.1 *In vitro* evaluation of Fungal Bio Agents against *L. theobromae*

The antagonistic action of selected seven fungal bio control agents against *L. theobromae* was carried out through dual culture technique based on the observation of radial growth of bio agent and fungus. The results are presented in Table 1, Fig. 1 and Plate 1. Among the fungal bio agents tested *L. theobromae, T. viride* (Tv- B2) was found to be most effective and significant over other bio control agents with maximum mycelial inhibition of 54.81 per cent over control. Next best was *T. harzianum* (Th- 55) with 54.07 per cent inhibition followed by moderate inhibition observed in *T. viride* (Tv – 2) and (Tv - 3) with 53.33 per cent each followed by *T. viride* Tv - 5 with 52.22 per cent inhibition and *T. harzianum* (Th- B2) with 44.81 per cent inhibition. The least mycelial inhibition was observed in *T. harzianum* (Th- 44) with 44.07 per cent inhibition. The inhibitory effect of these fungal bioagents may be due to hyperparasitism, competition for space and nutrients or antibiosis. The findings are in confirmation with the studies conducted by Bhadra et al. [8] who evaluated the bio-efficacy of *Trichoderma* species *viz., T. harzianum*, *T. koningii*, *T. viride, T. viride* (yellow strain) against *L. theobromae* by dual culture technique. Among four *Trichoderma* species, *T. koningii* and *T. viride* (yellow strain) were found effective with maximum inhibition of mycelium of pathogen. Suresh et al. [9] also evaluated different *Trichoderma* isolates against *L. theobromae*. Among *Trichoderma* isolates tested, *T. harzianum*, *T. koningii* and *T. viride* were found effective against pathogen.

3.2 *In vitro* Evaluation of Bacterial Bio Agents Against *L. theobromae*

The antagonistic action of selected bacterial bio control agents against *L. theobromae* was tested through dual culture technique. Based on the observations of radial growth of the bio agents and fungus, the per cent inhibition was calculated. The results are presented in Table 2, Fig. 2 and Plate 2. Among the tested bacterial bio agents against *L. theobromae*, *Bacillus subtilis* (Bs - M) was significantly superior over control with 39.62 per cent mycelial inhibition. This followed by *Bacillus subtilis* (Bs - O) with 34.44 per cent and *P. fluorescence* (Pf – O) with 25.92 per cent mycelial inhibition. Rest of the bio controls were found least effective with the mycelial inhibition of 6.29 per cent in *B. subtilis* (Bs - P) and 4.44 per cent in *P. fluorescence* (Pf - C).

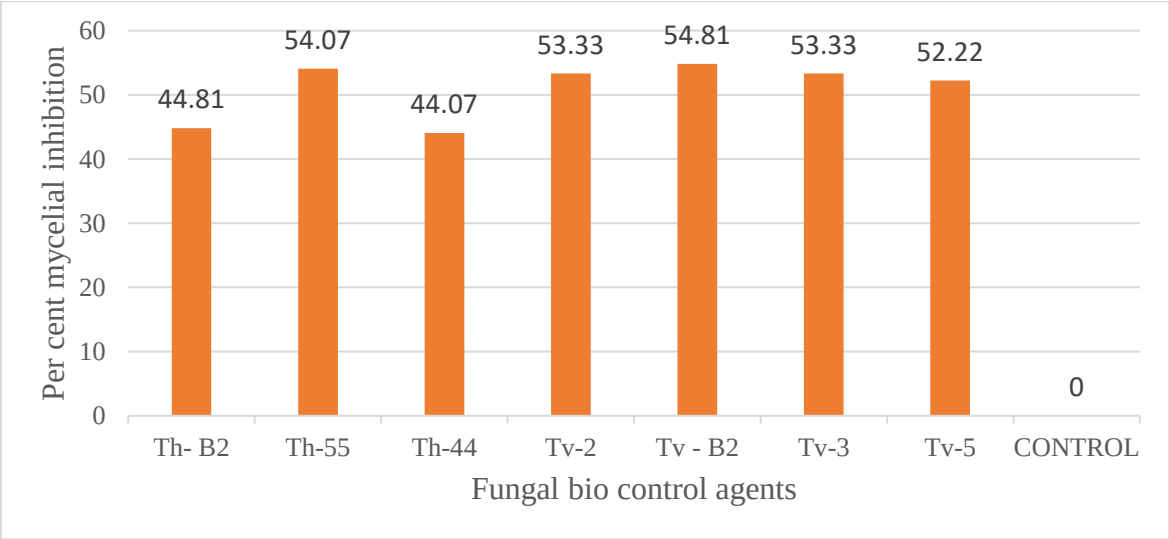

Fig. 1. Effect of fungal bio agents against *L. theobromae*

Table 1. *In vitro* evaluation of fungal bio agents against *Lasiodiplodia theobromae*

Sl. No.	Fungal Bio-agents	Isolate	Per cent inhibition of mycelial growth (%)
			L. theobromae
1	*Trichoderma harzianum*	Th- B2	44.81 (42.01) *
2	*T. harzianum*	Th-55	54.07 (47.32)
3	*T. harzianum*	Th-44	44.07 (41.58)
4	*T. viride*	Tv-2	53.33 (46.89)
5	*T. viride*	Tv-B2	54.81 (47.74)
6	*T. viride*	Tv-3	53.33 (46.89)
7	*T. viride*	Tv-5	52.22 (46.26)
8	Control	-	0.00 (0.00)
	F test		*
	S. Em±		0.95
	CD @1% (Critical difference)		2.92

** Figures in the parentheses are arcsine transformed values*

Plate 1. *In vitro* evaluation of fungal bio agents against *L. theobromae*

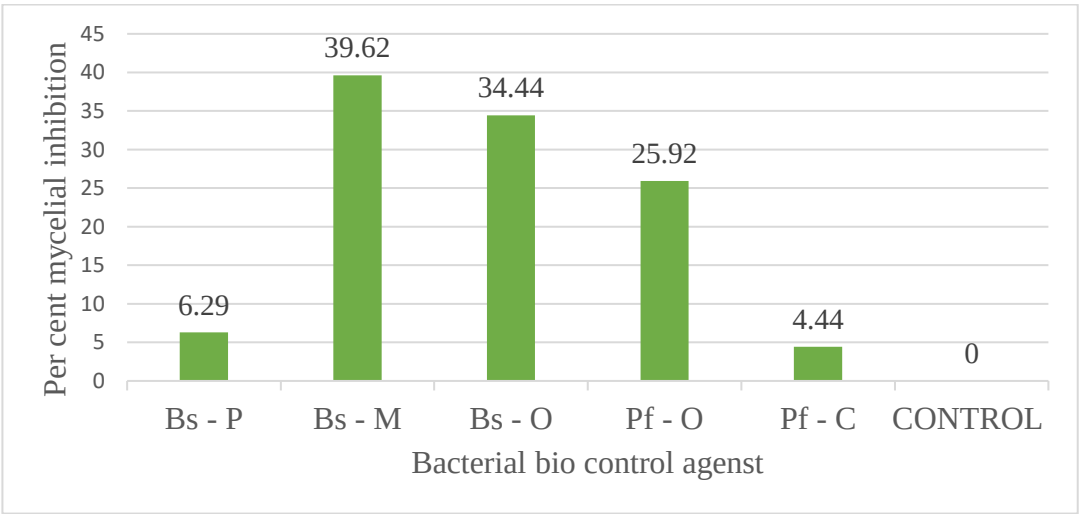

Fig. 2. Effect of bacterial bio agents against *L. theobromae*

Table 2. *In vitro* evaluation of bacterial bio agents against *L. theobromae*

Sl. No.	Bacterial bio agent	Isolate	Per cent inhibition of mycelial(%)
1	*Bacillus subtilis*	Bs – P	6.29 (13.88)
2	*Bacillus subtilis*	Bs –M	39.62 (38.99)
3	*Bacillus subtilis*	Bs- O	34.44 (39.33)
4	*Pseudomonas fluorescence*	Pf -O	25.92 (30.37)
5	*Pseudomonas fluorescence*	Pf - C	4.44 (11.95)
6	Control	-	0.00 (0.00)
	F test		*****
	S. Em±		**4.185**
	CD @ 1%		**13.358**

****** Figures in the parentheses are arcsine transformed values***

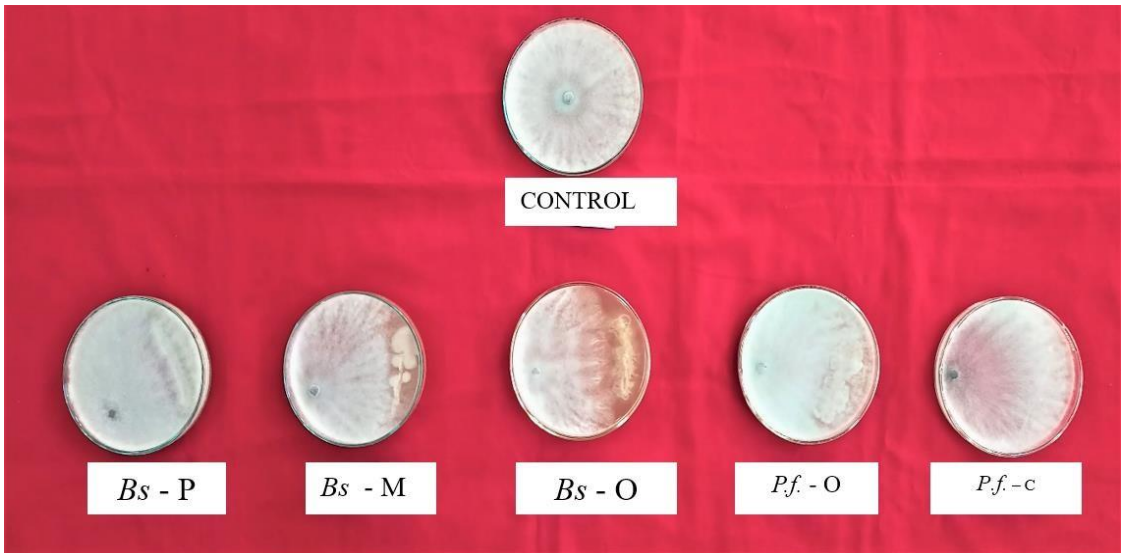

Plate 2. *In vitro* evaluation of bacterial bio agents against *L. theobromae*

3.3 *In vitro* Evaluation of Botanical Extracts against *L. theobromae*

Eleven botanical extracts were tested against *L. theobromae* at four concentrations *viz.,* 5, 10, 15 and 20 per cent by using poison food technique under *in vitro* condition. The per cent inhibition of mycelial growth of *L. theobromae* in different botanicals are presented in Table 3, Fig. 3 and Plate 3.

Out of eleven botanical extracts tested, garlic significantly inhibited the mean mycelial growth to the tune of 75.65 per cent followed by agave with 45.46 per cent inhibition. Out of four concentrations tested, cent per cent mycelial inhibition was recorded at concentration of 15 and 20 per cent followed by 28.52 and 74.07 per cent inhibition at 5 and 10 per cent concentrations respectively. Among the four concentrations of agave tested, per cent mycelial inhibition of 40.37, 42.59, 45.56 and 53.33 per cent was observed in 5, 10, 15 and 20 per cent concentrations, respectively. Onion recorded a mean per cent mycelial growth (14.17 %). 21.85 per cent of mycelial inhibition was recorded in 20 per cent concentration, and no inhibition at 5 per cent concentration, at 10 and 15 per cent 15.55 and 19.26 per cent inhibition was recorded. Whereas remaining botanicals showed minimum mean mycelial inhibition ranging from 0 to 8.24 per cent. The per cent mycelial inhibition of 5.74, 2.96, 1.02, 0.65, 0.37 per cent was observed in Touch me not plant, neem, pongemia, simaruba, lemon grass and subabul. No mycelial inhibition was recorded in ginger.

These findings were similar with that of Lakhran and Ahir [10] who evaluated different plant extracts, and oil cakes against *L. theobromae* causing dry root rot. Among the tested plant extracts, garlic extract was found most effective in reducing root rot incidence followed by neem

leaf extract. In the case of organic amendments, neem cake was found most effective in reducing the root rot incidence while wool waste and goat manure was found least effective in controlling root rot incidence. Similarly, Alice and Sundaravana [11] evaluated botanicals against *M. phaseolina* (Tassi) Goid. Under *in vitro* conditions among the oil cakes, mahua cake at 10 per cent completely inhibited the mycelial growth of the *M. phaseolina* isolates.

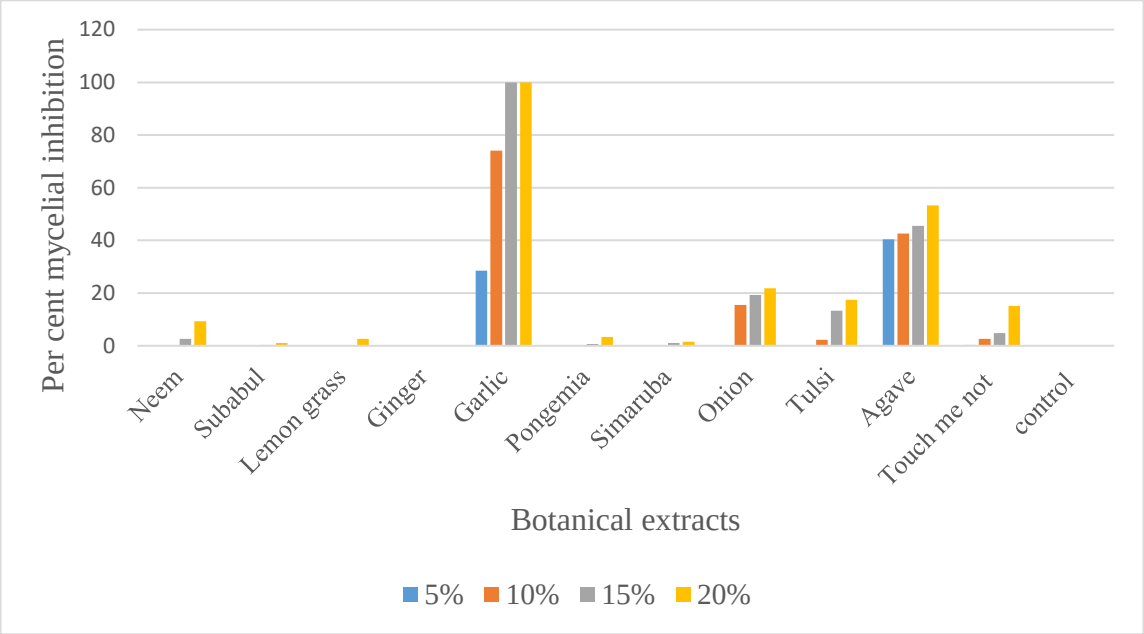

Fig. 3. Effect of Botanical extracts against *L. theobromae*

Plate 3. *In vitro* evaluation of Botanical extracts against *L. theobromae*

Table 3. *In vitro* evaluation of botanical extracts against *L. theobromae*

Sl. No.	Botanical extract	Per cent mycelial inhibition (%)				
		Concentration				Mean mycelial inhibition (%)
	Common name	5%	10%	15%	20%	
1	Neem	0.00 (0.00*	0.00 (0.00)	2.59 (8.57)	9.26 (17.66)	2.96 (6.55)
2	Subabul	0.00 (0.00)	0.00 (0.00)	0.37 (2.01)	1.11 (6.04)	0.37 (2.06)
3	Lemon grass	0.00 (0.00)	0.00 (0.00)	0.00 (0)	2.59 (8.57)	0.65 (2.14)
4	Ginger	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
5	Garlic	28.52 (32.16)	74.07 (59.70)	100.00 (90.00)	100.00 (90.00)	75.65 (67.95)
6	Pongemia	0.00 (0.00)	0.00 (0.00)	0.741 (4.032)	3.33 (9.85)	1.02 (3.47)
7	Simaruba	0.00 (0.00)	0.00 (0.00)	1.11 (6.04)	1.48 (6.88)	0.65 (3.23)
8	Onion	0.00 (0.00)	15.55 (23.21)	19.26 (26.01)	21.85 (27.85)	14.17 (19.27)
9	Tulsi	0.00 (0.00)	2.22 (8.37)	13.33 (21.39)	17.40 (24.64)	8.24 (13.36)
10	Agave	40.37 (39.43)	42.59 (40.72)	45.56 (42.43)	53.33 (46.89)	45.46 (42.37)
11	Touch me not	0.37 (2.01)	2.593 (9.21)	4.81 (12.65)	15.18 (23.15)	5.74 (11.76)

Sl. No.	Botanical extract / Common name	Per cent mycelial inhibition (%) Concentration 5%	10%	15%	20%	Mean mycelial inhibition (%)
12	Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Mean	6.2 (6.69)	12.45 (12.84)	17.07 (19.37)	20.50 (23.77)	21.90 (26.87)
		Botanicals (B)		**Concentration (C)**		**Interaction (B×C)**
	F test	*		*		*
	S. Em±	**0.648**		**0.391**		**1.296**
	CD at 1%	**1.825**		**1.1**		**3.64**

***** *Figures in the parentheses are arcsine transformed values*

4. CONCLUSION

In vitro evaluation of bio control agents and botanicals against *L. theobromae,* seven fungal bio agents and five bacterial bio agents were evaluated out of them *T. viride* (Tv – B2), *T. harzianum* (Th - 44), *B. subtilis* (Bs - M) and (Bs - O) proved as best for inhibition of mycelial growth of the pathogens. Out of eleven plant extracts tested, garlic was best for the inhibition of the root rot disease cause by *L. theobromae*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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