

# ***In vitro* evaluation of bio control agents and botanicals against mulberry root rot pathogen *L. theobromae***

## **Abstract**

Mulberry root rot is fast spreading disease caused by *L. theobromae*. These pathogens can be Managed by inorganic fungicides. However, enormous use of chemical fungicides leads to residual toxicity affects the growth and development of silkworm. In this context, an attempt was made to use of potential bio control agents for the management of these pathogens. Out of seven fungal bio agents and five bacterial bio agents evaluated viz., *T. viridae* (Tv – B2), *T. harzianum* (Th - 44), *B. subtilis* (Bs - M) and (Bs - O) were proved as best for inhibition of mycelial growth of the pathogens. Out of eleven botanical extracts garlic was proved best at 15 per cent and 20 per cent concentrations and agave at 20 per cent found effective against the root rot causing pathogens.

## **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 mulberry is susceptible to many soil borne disease. Root rot of mulberry 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. These pathogens are more alarming and they thrive well in different types of soil and causes considerable yield loss (Vineet *et al.*, 1998). The disease spreads primarily through the diseased plant samples used for propagation, contaminated soil, farm implements, irrigation water (Gupta *et al.*, 1999). 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, use of 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, 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 pathogens was carried out during 2021-22 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*, *Trichoderma harzianum*, *Pseudomonas fluorescence* and *Bacillus subtilis* were tested by dual culture technique (Webber and Hedger, 1986). 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 growth 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 serves as a 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  $28\pm 1$  °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 (1947).

### **2.2 *In vitro* evaluation of botanical extracts against *L. theobromae***

The efficiency of plant extract or botanical extracts was tested against root rot pathogen *L. theobromae* on PDA medium by using poisoned food technique. For this, fresh plant parts (leaves/bulbs) of 100 g each as mentioned below (Table 3) were collected, washed with tap water and then distilled water. The fresh sample was chopped and crushed by adding sterile water of 100 ml. The crushed product was filtered through muslin cloth. The filtrate solution gave 100 per cent and which was used as stock solution. Five, ten and fifteen 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 it to cool. Twenty ml of medium was

poured into sterilized petri plates and then fungal disc of five mm was placed at the center of the petri plate and then such plates were incubated at  $28 \pm 1^\circ\text{C}$ . The control plate was maintained on PDA medium without any plant extract. The radial growth of fungus was recorded in treatment plates when colony growth reached periphery in control plate. The per cent inhibition of mycelial growth of test fungus was calculated by using following formula given by Vincent (1947).

### 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. Per cent inhibition of mycelial growth was calculated. 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 due to hyperparasitism, competition for space and nutrients or antibiosis. The findings are in confirmation with the studies conducted by Bhadra *et al.* (2014) to know 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 in maximum inhibition of mycelium of pathogen. Suresh *et al.* (2016) evaluated different *Trichoderma* isolates against *L. theobromae*. Among *Trichoderma* isolates tested, *T. harzianum*, *T. koningii* and *T. viride* were found effective against pathogen.

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

Sl. No.	Fungal Bio-agents	Isolate	Per cent inhibition of mycelial growth (%)
---------	-------------------	---------	--

			<i>L. theobromae</i>
1	<i>Trichoderma harzianum</i>	Th- B2	44.81 (42.01) *
2	<i>T. harzianum</i>	Th-55	54.07 (47.32)
3	<i>T. harzianum</i>	Th-44	44.07 (41.58)
4	<i>T. viride</i>	Tv-2	53.33 (46.89)
5	<i>T. viride</i>	Tv-B2	54.81 (47.74)
6	<i>T. viride</i>	Tv-3	53.33 (46.89)
7	<i>T. viride</i>	Tv-5	52.22 (46.26)
8	Control	-	0.00 (0.00)
	<b>F test</b>		*
	<b>S. Em±</b>		<b>0.95</b>
	<b>CD @1%</b>		<b>2.92</b>

\* Figures in the parentheses are arcsine transformed values

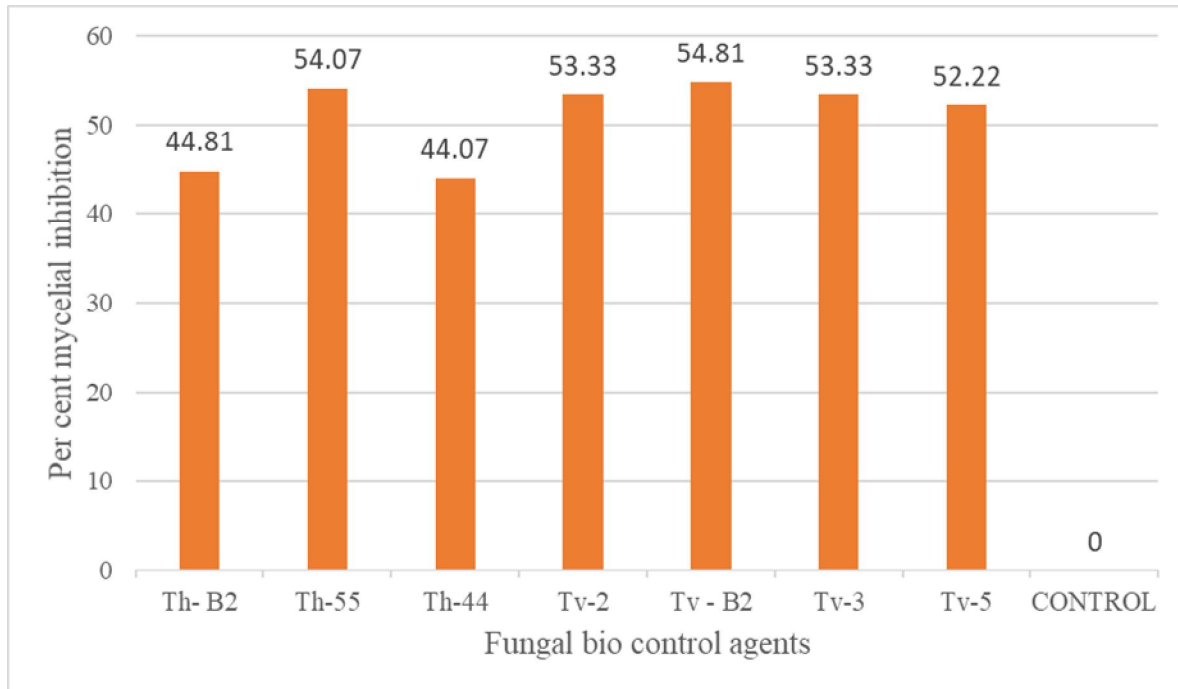


Fig. 1: Effect of fungal bio agents against *L. theobromae*.



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

### 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. Next best was *B. subtilis* (Bs - O) with 34.44 per cent followed by *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 least observed in *P. fluorescence* (Pf - C).

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

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

\* Figures in the parentheses are arcsine transformed values

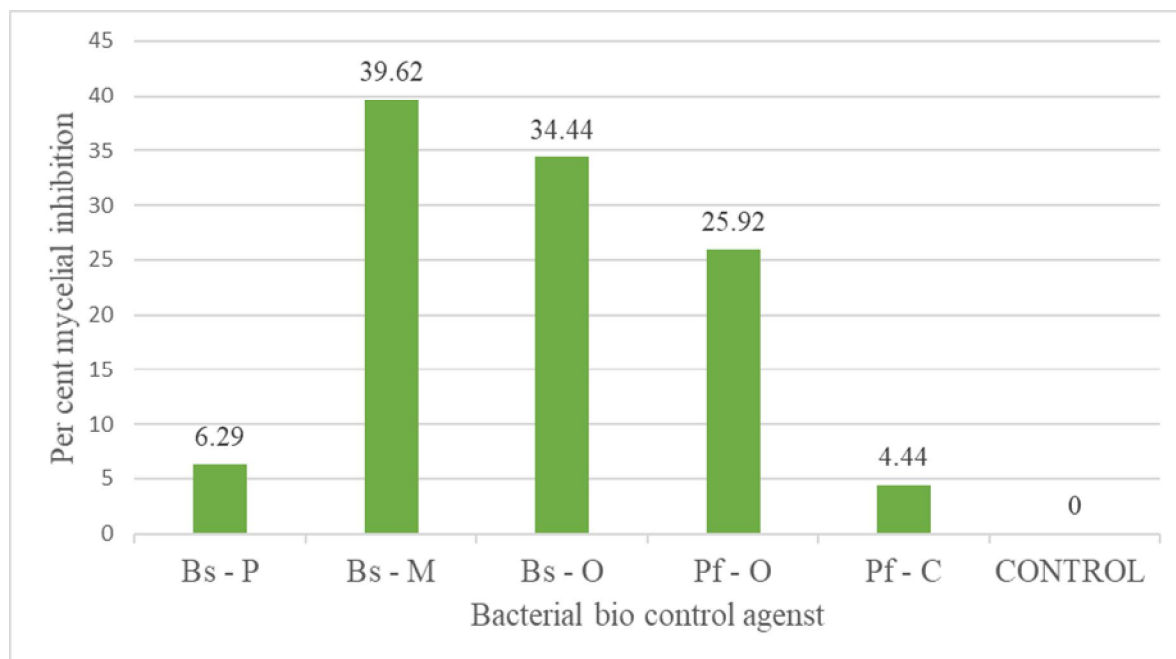


Fig. 2: Effect of bacterial bio agents against *L. theobromae*

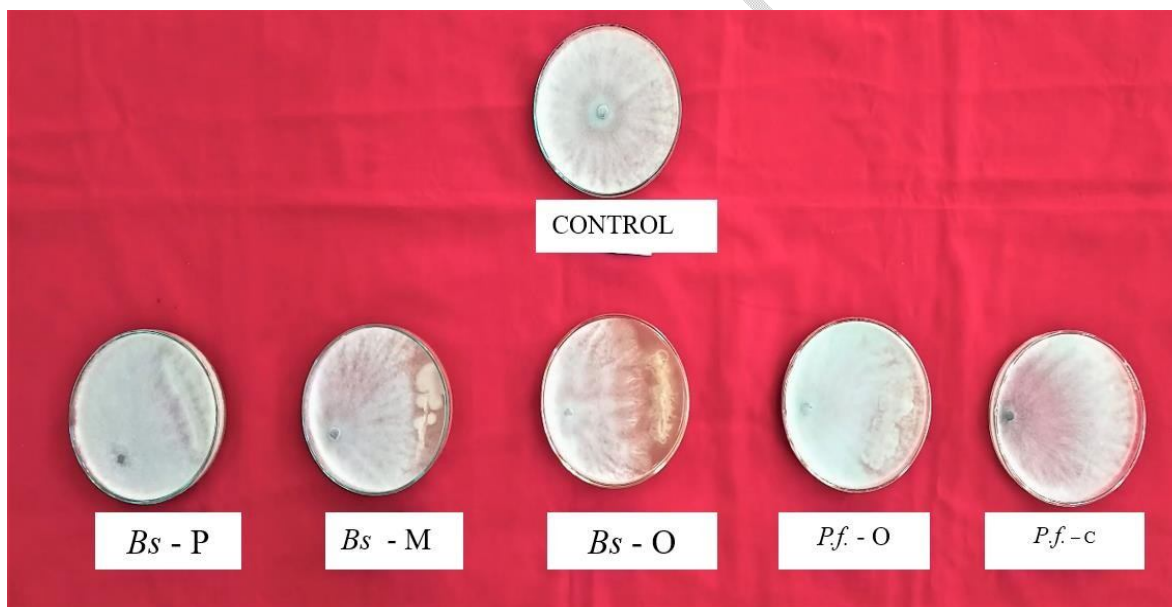


Plate 2: *In vitro* evaluation of bacterial bio agents against *L. theobromae*.

### 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 against *L. theobromae* garlic significantly inhibited the mean mycelial growth of 75.65 per cent followed by agave with 45.46 per cent inhibition. Out of four concentrations tested, cent per cent mycelial inhibition delivered at concentration of 15 and 20 per cent followed by 28.52 and 74.07 per cent inhibition was observed 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 which inhibited 14.17 per cent of mycelial growth, 21.85 per cent of inhibition was observed in 20 per cent concentration, no inhibition at 5 per cent concentration, 15.55 and 19.26 per cent inhibition was observed in 10 per cent and 15 per cent concentration. Whereas remaining botanicals showed least inhibition mean mycelial growth ranged from 0 to 8.24 per cent. Tulsi inhibited 8.24 per cent, among four concentrations in tulsi 17.40, 13.33, 2.22 and zero per cent mycelial inhibition was observed at 20, 15, 10 and 5 per cent concentrations respectively. 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. Least per cent inhibition was observed in ginger with zero per cent.

These findings were similar with that of Lakhran and Ahir (2020) 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 (2012) evaluated botanicals against *M. phaseolina* (Tassi) Goid. Under *in vitro* conditions among the oil cake, mahua cake at 10 per cent completely inhibited the mycelial growth of the *M. phaseolina* isolates.

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

Sl. No.	Botanical extract	Per cent mycelial inhibition (%)				Mean mycelial inhibition (%)
		Concentration				
	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)
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)
		<b>Botanicals (B)</b>		<b>Concentration (C)</b>		<b>Intracriion (B×C)</b>
	<b>F test</b>	*		*		*
	<b>S. Em±</b>	<b>0.648</b>		<b>0.391</b>		<b>1.296</b>
	<b>CD at 1%</b>	<b>1.825</b>		<b>1.1</b>		<b>3.64</b>

\* Figures in the parentheses are arcsine transformed values

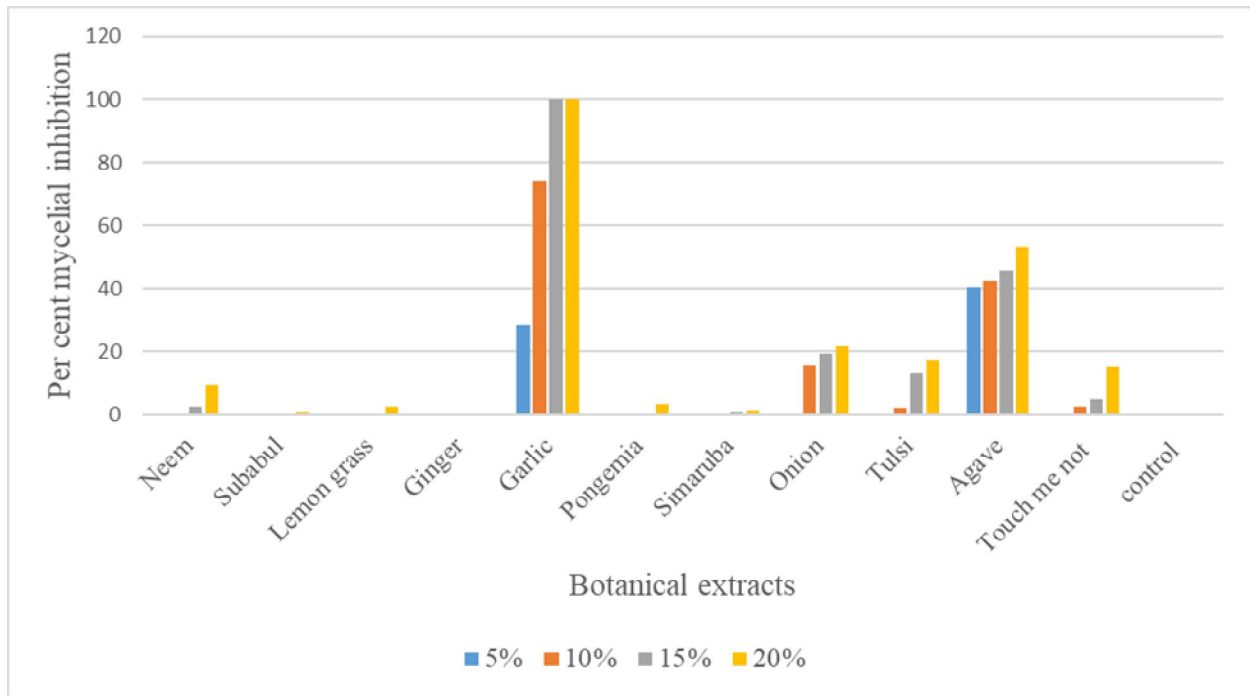


Fig. 3: Effect of Botanical extracts against *L. theobromae*.



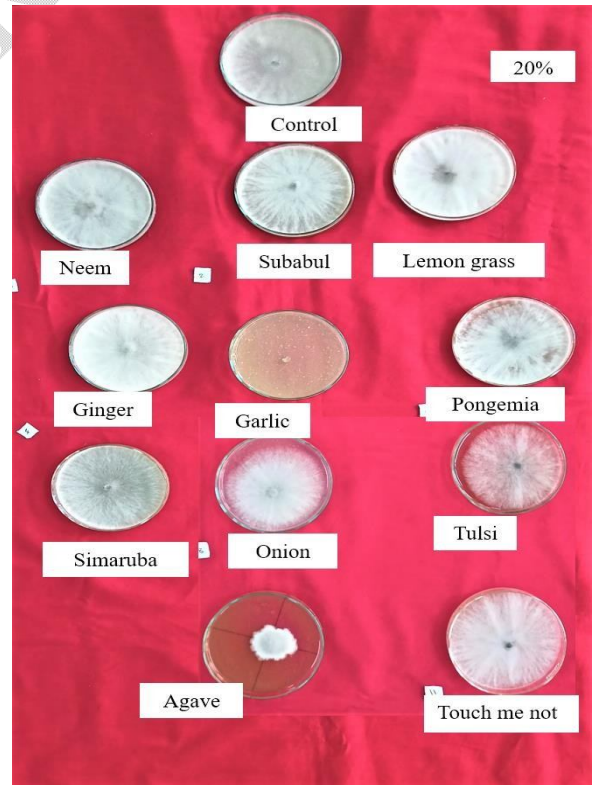


Plate 3. *In vitro* evaluation of Botanical extracts against *L. theobromae*

## 4. Conclusion

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

## References

- Alice, D. and sundravadana, S. Effects of biocontrol agents and plant products *Macrophomina phaseolina* and colchicine content in *Gloriosa superba*. *Plant Protection Science*, 2012; 48(3):110-115.
- Bhadra M., khair, A., hossain, M. A. and sikder, M. M. Efficacy of *Trichoderma spp.* and fungicides against *Lasiodiplodia theobromae*. *Bangladesh J. Sci. Ind. Res.*, 2014;49(2): 125-130.
- Gupta, V.P., sharma, D.D., Rekha, M. and chandrashekar, D.S. Integration of *Trichoderma pseudokoningii* with agro chemicals for disease management in mulberry. *Archives of Phytopathology & Plant Protection*, 1999; 32(6):521-529
- Lakhran, L. and Ahir, R.R. *In-vivo* evaluation of different fungicides, plant extracts, biocontrol agents and organics amendments for management of dry root rot of chickpea caused by *Macrophomina phaseolina*. *Legume Research-An International Journal*, 2020; 43(1):140-145.
- Suresh, V., Vidya sagar, B., Varma, P. K., Sumalatha, N. and Prasad, M. R. *In vitro* evaluation of certain fungicides, botanicals and bio control agents against *Lasiodiplodia theobromae*. *Res. J. Agric. Sci.*, 2016;7(4): 747-750.
- Vineet, K., Sharma, D. D., Babu, A. M. and Datta, R. K. SEM studies on the hyphal interactions between a biocontrol agent *Trichoderma harzianum* and a mycopathogen *Fusarium solani* causing root rot disease in mulberry. *Indian Journal of Sericulture*, 1998;37(1): 17-20.

Vincent, J. M. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*, 1947;159: 850.

UNDER PEER REVIEW