



**International Journal of  
Agricultural  
Research**

ISSN 1816-4897

## Research Article

# Integrated Management Strategies for Black Root Rot of Mulberry Caused by *Lasiodiplodia theobromae*

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## Abstract

**Background and Objective:** Black root rot of mulberry, caused by *Lasiodiplodia theobromae*, significantly reduces productivity due to rapid root degradation, vascular discoloration and plant mortality. This study aimed to isolate and characterize *L. theobromae* from infected mulberry roots and evaluate the efficacy of fungal biocontrol agents, synthetic fungicides and commercially available herbal oils against the pathogen under *in vitro* conditions. **Materials and Methods:** Infected mulberry roots were collected and used to isolate the pathogen, which was subsequently identified based on morphological and cultural characteristics. The antifungal potential of *Trichoderma* species, chemical fungicides and essential oils was assessed using standard mycelial inhibition assays. Data were analyzed using ANOVA under a completely randomized design (CRD) and treatment means were compared at a significance level of  $p \leq 0.05$ .

**Results:** Among biocontrol agents, *Trichoderma harzianum* showed the highest mycelial inhibition (72.61%), slightly surpassing *T. viride*. Hexaconazole was the most effective chemical fungicide with 84.94% mean inhibition, followed by Carbendazim and Propiconazole. Essential oils demonstrated promising eco-friendly activity: Eucalyptus oil achieved 71.33% inhibition, while Cinnamon and Neem oils exhibited substantial dose-dependent effects. **Conclusion:** The study highlights the potential of combining biological, chemical and herbal approaches for managing black root rot in mulberry. These findings support the development of an integrated disease management strategy to protect mulberry health and productivity, with future research needed to validate field-level efficacy.

**Key words:** Biocontrol agents, fungicides, herbal oils, *in vitro* antagonism, *Lasiodiplodia theobromae*, mulberry, black root rot disease, *Trichoderma*

**Citation:** Mahadeva, A., Y.M. Suma, M.N. Ramya and D. Namratta, 2026. Integrated management strategies for black root rot of mulberry caused by *Lasiodiplodia theobromae*. Int. J. Agric. Res., 21: 1-7.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Mulberry (*Morus* spp.) is a perennial crop of high economic significance, functioning as the exclusive food plant for the silkworm (*Bombyx mori* L.) and thus underpinning the sericulture industry. Sustainable cultivation and maintenance of healthy mulberry plantations are crucial for ensuring consistent cocoon production and optimal silk yield. However, mulberry is prone to several fungal pathogens, among them soil borne diseases particularly, root rot has emerged as a major constraint to its productivity and longevity<sup>1</sup>. Sharma *et al.*<sup>2</sup> listed various types of the mulberry root rot diseases. These include dry root rot caused by *Fusarium solani* and *F. oxysporum*; black root rot caused by *Botryodiplodia theobromae* Pat. (syn. *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl.); charcoal root rot caused by *Macrophomina phaseolina*; violet root rot caused by *Helicobasidium mompa*; white root rot caused by *Rosellinia necatrix*; *Armillaria* root rot caused by *Armillaria mellea*; and bacterial root rot caused by *Pseudomonas solanacearum*. Among them, the dry root rot, black root rot and charcoal root rot are reported in India. Whereas, violet root rot, white root rot, *Armillaria* root rot and bacterial root rot are reported in China, Japan, Russia, Sri Lanka and Thailand. The disease is occurred in all types of soils and varied agro climatic conditions throughout the year. The disease proliferates rapidly under conditions of high soil moisture, poor drainage and elevated temperatures, causing significant plantation losses and disrupting silkworm rearing cycles<sup>3</sup>. It appears to be in an isolated patch in few plants at beginning, later spread of the disease leading to the death of plants within short period. It also been reported that the mortality of plants with leaf yield losses up to 14% due to these root rots. Gupta *et al.*<sup>4</sup> emphasized that the mortality rate increased to between 51.40 and 61.10% when *B. theobromae* was present in combination with *F. solani* and/or *Phoma sorghina*. Chowdary and Govindaiah<sup>5</sup> observed the average mulberry leaf yield losses was up to 31.49% due to root rot disease caused by *M. phaseolina*. The root rot disease shows common symptoms like sudden withering of the whole sapling, rotting of roots and finally resulting in the death of plants<sup>4</sup>. *F. solani* and *L. theobromae* are the major pathogens in South India that cause root rot disease. Their ability to survive in different types of soil and climatic conditions leads to chronic nature of disease<sup>6</sup>. Among the root rot disease, black root rot caused by *Lasiodiplodia theobromae*, pathogen causes substantial loss in the cultivation of mulberry<sup>1</sup>. Using morphological features, molecular characterization, phylogenetic data,

and Koch's postulation study via artificial inoculation, Kanyakumari *et al.*<sup>7</sup> confirmed that *L. theobromae* causes root rot disease in mulberry. Infection by this pathogen targets the roots and basal stems of the mulberry plant, initiating severe vascular degradation. The xylem tissue of infected roots discolors, turning brown and subsequently black, followed by cortical rot<sup>8</sup>. This progressive decay impairs the plant's ability to absorb water and nutrients, resulting in foliar chlorosis, wilting and eventual mortality. The defining characteristic of *L. theobromae* infection is the visual transformation of the root surface from a healthy cream or yellow hue to a deep, charcoal-like black. As the disease advances, the root cortex degrades and loosens, allowing it to be easily peeled from the inner wood. Transverse sections reveal distinct browning or blackening of the xylem, indicating deep fungal penetration into the vascular tissue. Morphologically, this infection is distinguishable from Charcoal Root Rot (*Macrophomina*) by the absence of "ashy" microsclerotial dust on the root surface<sup>1</sup>.

The wide host range and opportunistic nature of *L. theobromae* make it a persistent threat in mulberry gardens. It not only hampers plant vigour but also necessitates frequent replanting, increasing cultivation costs, and reducing economic returns from sericulture to the stakeholders. Understanding the management of this disease is crucial for sustaining mulberry health and productivity. Hence, an effort has been made to isolate the disease-causing fungal agent, *L. theobromae*, from the infected mulberry root and the effectiveness of biocontrol agents, synthetic fungicides and herbal oil formulations was evaluated. This study aimed to isolate and characterize *Lasiodiplodia theobromae* from infected mulberry roots and to evaluate the *in vitro* effectiveness of fungal biocontrol agents, chemical fungicides and commercially available herbal oils against root rot-causing fungal pathogen.

## MATERIALS AND METHODS

The present study was conducted at Mulberry Pathology and Microbiology Lab, Karnataka State Sericulture Research and Development Institute (KSSRDI), Thalaghattapura, Bangalore-560109 during 2024-25.

**Isolation of root rot disease-causing pathogen and pathogenicity test:** Black root rot-diseased mulberry root and soil samples of infected rhizosphere were collected from the gardens in Paduvanagere, Kanakapura Taluk, Ramanagara District. The fungus *L. theobromae* was isolated from these diseased roots and soil samples and identified through both macroscopic and microscopic examination. This fungus was

then mass cultured on rice, jowar and Potato Dextrose Broth. These cultures were used to conduct pathogenicity tests to assess the fungus's disease-causing ability. Two sets of experiments were performed on mulberry in earthen pots: One set was irrigated every 10 days, while the other was irrigated once every 25 days.

**In vitro antifungal activity test:** The antifungal activity was studied by using a biocontrol fungus, fungicides and commercially available plant oils.

An antagonistic study of biocontrol agents, *T. asperellum*, *T. harzianum* and *T. viride*, was tested against the black root rot disease-causing fungus, *L. theobromae*, *in vitro*. Sterilized and cooled Potato Dextrose Agar (PDA) medium of 20 mL was poured into sterilized Petri plates. After solidification, the mycelial disc of 5 mm test fungus was inoculated at one end of the petri plate and antagonistic fungus was placed opposite to it. A control plate was also maintained where in test fungal disc was placed and the center of the medium without any biocontrol agents. Each treatment was replicated for five times and incubated at room temperature. The mycelial growth in the treated plates was recorded when fungal growth reaches periphery in control plate. The inhibition zone between test organism and antagonistic microorganism was measured and compared with the control. The percentage inhibition growth of the pathogen was calculated by using formula i.e., the rates of mycelial growth inhibition (GI%)<sup>9</sup> was:

$$GI (\%) = \frac{dc - dt}{dc} \times 100$$

where, dc is mean colony diameter of control sets and dt is the mean colony diameter of treatment sets.

The antifungal assay of fungicides and herbal oils was carried out in Petri dishes containing Potato Dextrose Agar (PDA).

**In vitro antifungal activity of fungicides against the black root rot causing fungus:** *In vitro* (Poisoned Food Technique, PFT) evaluation of 7 fungicides i.e., Carbendazim, Difenoconazole, Hexaconazole, Propiconazole, Tebuconazole, Thiophanate methyl and Tricyclazole of 6 different concentrations (0.10, 0.20, 0.40, 0.60, 0.80% and 1.00%) was conducted against root rot causing fungus, *L. theobromae*.

**In vitro antifungal activity of herbal oils against the root rot causing fungus:** *In vitro* (Poisoned Food Technique, PFT) evaluation of 6 commercially available herbal oils i.e., Neem oil, Eucalyptus oil, Clove oil, Cinnamon oil, Basil oil

and Pongamia/Karanj oil of 6 different concentrations (1.00, 1.50, 2.00, 3.00, 4.00 and 5.00%) was conducted against *L. theobromae* was conducted.

When the temperature of the growth media (PDA) reached about 40°C, specific initial concentrations of fungicides and herbal oils were added, mixed thoroughly and poured to sterilized petri plates. The test fungal disc of the 5 mm was taken from an actively grown culture and placed on center of petri plate. The control plate was maintained without any fungicides or herbal oils. Each treatment was replicated for three times. These plates were incubated until the fungal growth reached the periphery of control plate and at the same time the colony diameter of test fungus was recorded in the treatment plates. The rate of mycelial growth inhibition (GI%) was calculated using the formula mentioned above<sup>9</sup>.

**Statistical analysis:** The data were subjected to ANOVA for a completely randomized design (CRD) and statistical tools such as mean and percentage analysis were also employed to meet the study's objective. Differences among treatments were considered statistically significant at  $p \leq 0.05$ .

## RESULTS

The fungal pathogen, *L. theobromae* was isolated from root rot-infected tissues and rhizosphere soil of mulberry plants. On Potato Dextrose Agar (PDA), colonies initially appeared white to light grey, rapidly darkening to deep brown or black with abundant pycnidia formation. The mycelium was fast-growing, forming dense, fluffy or cottony mats with regular to slightly irregular margins (Fig. 1a and b). Microscopic examination revealed septate, branched hyphae that matured from hyaline, thin-walled structures to dark brown, thick-walled threads. The diagnostic conidia were thick-walled, ellipsoidal to ovoid and contained granular contents; mature spores exhibited a single median septum with characteristic longitudinal striations. Pathogenicity assays comparing two irrigation regimes (10-day vs. 25-day intervals) showed no significant difference in the progression of black root rot. Additionally, the efficacy of fungal biocontrol agents, fungicides and commercial herbal oils was evaluated against the *L. theobromae*.

**Antagonistic study of biocontrol agents:** The *in vitro* antagonistic potential of three fungal biocontrol agents (*T. asperellum*, *T. harzianum* and *T. viride*) was evaluated *L. theobromae* by assessing the percentage inhibition over a control. The percentage inhibition of mycelial growth of the fungus was calculated and the results are presented in Table 1. The highest percentage inhibition of *L. theobromae*

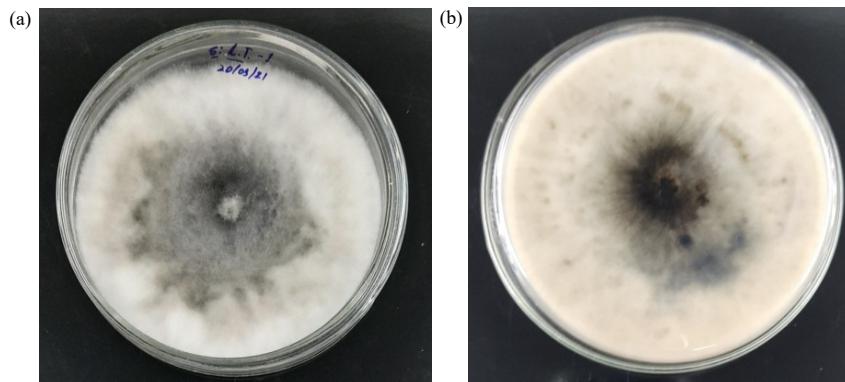


Fig. 1(a-b): Macroscopic feature of *L. theobromae* on PDA after 7 days at 25°C (a) Upper and (b) Reverse side

Table 1: *In vitro* antagonistic activity of fungal biocontrol agents against *L. theobromae*

Fungal biocontrol agent	Inhibition over control (%)
<i>T. asperellum</i>	50.64
<i>T. harzianum</i>	72.61
<i>T. viride</i>	71.54
SEM $\pm$	0.909
CD @ 5%	2.802

Table 2: *In vitro* antagonistic activity of fungicides against fungal pathogen *L. theobromae*

Fungicides	Inhibition over control (%)						
	0.10 (%)	0.20 (%)	0.40 (%)	0.60 (%)	0.80 (%)	1.00 (%)	Mean
Carbendazim	80.00	74.81	85.93	83.70	83.70	88.15	82.72
Difenconazole	55.56	52.22	51.11	51.11	56.67	53.33	53.33
Hexaconazole	80.00	84.44	81.48	88.89	87.41	87.41	84.94
Propiconazole	72.59	81.48	91.85	72.59	72.59	75.56	77.78
Tebuconazole	52.44	51.11	54.07	58.37	58.15	53.78	54.65
Thiophanate methyl	72.59	71.11	69.63	69.63	71.85	71.85	71.11
Tricyclazole	51.11	58.52	49.63	57.78	62.96	69.63	58.27
Mean	66.33	67.67	69.10	68.87	70.48	71.39	68.97
Fungicide		Concentration			Fungicide $\times$ Concentration		
SEM $\pm$	0.957	0.886			2.345		
CD @ 1%	2.698	2.498			6.609		

SEM  $\pm$ : Standard error of mean and CD: Critical difference

mycelial growth was exhibited by *T. harzianum* (72.61%), which was better than *T. viride* (71.54%) and *T. asperellum* (50.64%). Statistical analysis revealed significant differences in the mean percentage inhibition among the biocontrol agents against the pathogen.

**Antagonistic study of fungicides:** The *in vitro* efficacy of seven different fungicides i.e., Carbendazim, Difenconazole, Hexaconazole, Propiconazole, Tebuconazole, Thiophanate methyl and Tricyclazole against the fungal pathogen, *L. theobromae* of root rot disease in mulberry was evaluated by assessing the percentage inhibition over a control at 6 varying concentrations (0.10, 0.20, 0.40, 0.60, 0.80 and 1.00%). The inhibition of *L. theobromae* mycelial growth

against 7 fungicides are presented in Table 2. The fungicide Hexaconazole demonstrated the highest overall mean percentage inhibition (84.94%) across all tested concentrations, indicating its superior efficacy against the target fungus in this *in vitro* assay. Carbendazim also exhibited high efficacy with a mean inhibition of 82.72%. Propiconazole and Thiophanate methyl showed moderate efficacy with mean inhibition values of 77.78 and 71.11%, respectively. In contrast, Difenconazole and Tebuconazole displayed relatively lower mean percentage inhibition values of 53.33 and 54.65%, respectively, suggesting comparatively lower *in vitro* activity against the tested fungus. Tricyclazole showed a mean inhibition of 58.27%, placing its efficacy in the lower range among the tested fungicides. An analysis of the

Table 3: *In vitro* antagonistic activity of herbal oils against fungal pathogen *L. theobromae*

Herbal oils	Inhibition over control (%)						
	1.00 (%)	1.50 (%)	2.00 (%)	3.00 (%)	4.00 (%)	5.00 (%)	Mean
Basil oil	28.89	31.85	41.70	43.33	44.15	46.15	39.35
Cinnamon oil	56.67	42.41	70.67	72.15	72.96	75.93	65.13
Clove oil	48.89	53.33	55.56	60.22	60.89	62.59	56.91
Eucalyptus oil	60.37	64.22	73.85	74.37	76.67	78.52	71.33
Neem oil	37.70	64.59	66.67	67.19	73.48	77.93	64.59
Pongamia oil	38.07	37.70	42.59	44.82	47.41	47.04	42.94
Mean	45.10	49.02	58.51	60.35	62.59	64.69	56.71
	Herbal oils		Concentration		Herbal oils × Concentration		
SEM±	1.430		1.430		3.502		
CD @ 1%	4.039		4.039		9.894		

SEM±: Standard error of mean and CD: Critical difference

concentration-dependent inhibition revealed variations in the fungicides' performance. For instance, while Carbendazim showed high inhibition at the lowest concentration (80%), its efficacy fluctuated slightly across the higher concentrations. Hexaconazole consistently exhibited high inhibition across all concentrations. Propiconazole showed a notable increase in inhibition at 0.40% concentration (91.85%). The overall mean percentage inhibition across all fungicides and concentrations was 68.97%. The mean inhibition at each concentration level generally showed an increasing trend with higher concentrations, ranging from 66.33% at 0.10% to 71.39% at 1.00%.

The statistical inference confirms significant variation due to fungicide, pathogen, concentration and their interactions, reinforcing the importance of selecting the right combination. It implies that the efficacy of each fungicide varies not only by pathogen but also in combination with concentration and specific fungal species. Higher concentrations (0.60-1.00%) generally yielded better inhibition, but the response was fungicide-specific. Hexaconazole, Propiconazole and Carbendazim were the most effective fungicides across all three pathogens and concentrations.

**Antagonistic study of herbal oils:** The *in vitro* antagonistic effect of commercially available 6 herbal oils (Basil Oil, Cinnamon Oil, Clove Oil, Eucalyptus oil, Neem oil and Pongamia oil) at 6 different concentrations (1.00%, 1.50%, 2.00%, 3.00%, 4.00% and 5.00%) was evaluated against the mycelial growth of *L. theobromae* (Table-3).

There was a significant impact of oil type and concentration against the fungal growth. Eucalyptus oil exhibited the highest mean effectiveness at 71.33%, followed closely by Cinnamon oil (65.13%) and Neem oil (64.59%) across all concentrations. Clove oil demonstrated moderate effectiveness with a mean of 56.91%, while Basil oil and

Pongamia oil had the lowest overall performance of 39.35 and 42.94%, respectively. As the concentration increased from 1.00 to 5.00%, a general trend of increased effectiveness to curtail the growth of *L. theobromae* mycelia was observed for all oils. Notably, Eucalyptus oil and Cinnamon oil showed consistently high effectiveness across all concentrations, peaking at 78.52 and 75.93 at 5.00%, respectively. Neem oil showed a marked increase from 37.70 at 1.00% to 77.93 at 5.00%, indicating a strong dose-dependent response. Pongamia oil remained relatively low in effectiveness, showing only modest increases across concentrations. The overall mean effectiveness across all oils increased steadily, from 45.10% at 1.00% to 64.69% at 5.00% concentration, supporting the conclusion that higher concentrations generally improve performance. These results suggest that Eucalyptus oil, Cinnamon oil and Neem oil are the most potent options at higher concentrations, while Basil and Pongamia oils are less effective overall against *L. theobromae*.

## DISCUSSION

Black root rot of mulberry, caused by *L. theobromae*, is an emerging constraint to mulberry productivity and silk production due to rapid root decay, leaf yellowing and plant wilting under field conditions. The cultural and microscopic observation of the mycelia, spores of *L. theobromae* was confirmed<sup>10</sup>. In *in vitro* study the superior antagonists were observed by biocontrol microbes (*T. harzianum* and *T. viride*), fungicides (Hexaconazole, Carbendazim and Propiconazole) and commercially available herbal oil (Eucalyptus oil) against the growth of *L. theobromae*. Similar observations were made by the Indra<sup>11-13</sup>. Kazempour *et al.*<sup>14</sup> also tried bacterial isolates as a biocontrol agents to antagonize root rot pathogens in mulberry. They concluded that *P. fluorescens* and *Bacillus cereus* have an excellent potential to manage root rot causing

pathogens, *L. theobromae*, *F. oxysporum*, *F. solani* and *Rhizoctonia solani* in mulberry. Chowdary *et al.*<sup>15</sup> evaluated an *in vitro* study of *P. fluorescens* and *T. harzianum* against root rot pathogens, *F. solani*, *F. oxysporum*, *B. theobromae* and *M. phaseolina*. Further, they found combined application of bioformulation of *P. fluorescens* and *T. harzianum* with neem oil cake recorded 80.00% reduction in leaf wilting and 85.00% reduction in roots rotting in mulberry.

Vijay *et al.*<sup>16</sup> found that, *T. asperellum* was effectively inhibit the mycelial growth of *L. theobromae*. It is well known fact that the key biocontrol strategies that *Trichoderma* develops in direct conflict with fungal pathogens are mycoparasitism, competition and antibiosis. Ahluwalia *et al.*<sup>17</sup> identified 11 major secondary metabolites produced by two strains of *T. harzianum*. They confirmed their effective antifungal activity against phytopathogenic fungi, *M. phaseolina*, *R. solani*, *S. rolfsii* and *F. oxysporum*. The mycelial growth of *L. theobromae* depends on tightly coordinated pathways that regulate ergosterol biosynthesis, cytoskeletal dynamics, cell-wall formation, energy production and nucleic acid metabolism<sup>18-21</sup>. *T. harzianum* inhibits the pathogen through the production of various antifungal substances (chitinases, trichodermin and gluconase), inactivating the pathogens by dissolving the cell wall<sup>15</sup>. Herbal oils controls *L. theobromae*, by disrupting fungal growth through interactions of phytochemicals with fungal molecular targets<sup>22</sup>. Many herbal oils rich in monoterpenes, phenolics such as eugenol, cinnamaldehyde, thymol, which insert into fungal membranes, increase membrane fluidity/permeability, cause leakage of K<sup>+</sup>, proteins and nucleotides and rapidly kill or inhibit hyphal growth. The interactions involve the disruption of plasma membrane biosynthesis and morphological damage of cell wall<sup>23</sup>, ergosterol biosynthesis interference and multitarget transcriptional reprogramming<sup>24</sup>, oxidative stress and mitochondrial dysfunction<sup>25</sup>. Antifungal agents can inactivate *L. theobromae* by disrupting the structure and function of fungal cell membranes or organelles or by inhibiting nuclear material and protein synthesis, which results in the growth and multiplication of the pathogen, in turn affect the advancement of root rot disease in mulberry.

## CONCLUSION

Black root rot of mulberry caused by *L. theobromae* continues to emerge as a major limitation to mulberry cultivation and sericultural productivity due to its aggressive root decay and rapid decline of infected plants. Among the management options evaluated, *Trichoderma* spp.

(*T. harzianum* and *T. viride*), selected fungicides (Hexaconazole, Carbendazim and Propiconazole) and the commercially available herbal oil (Eucalyptus oil) demonstrated strong inhibitory activity against *L. theobromae* at *in vitro*. The integration of biocontrol agents, selective fungicides and plant-derived herbal oils offers a promising, environmentally compatible strategy for managing black root rot of mulberry. Field-level validation and development of optimized formulations will further enhance the practical application of these control measures in sustainable sericulture.

## SIGNIFICANCE STATEMENT

This study discovered the comparative efficacy of biological agents, synthetic fungicides and herbal oils in suppressing *L. theobromae*, which can be beneficial for developing sustainable and integrated management strategies for black root rot of mulberry. This study will help researchers to uncover the critical areas of synergistic disease control and eco-friendly alternatives that many researchers were not able to explore. Thus, a new theory on integrated root rot management may be arrived at.

## ACKNOWLEDGMENT

The authors wish to acknowledge the Director, Karnataka State Sericulture Research and Development Institute, Bangalore. Gratitude is extended to the Rashtriya Krishi Vikas Yojana (RKVY), Government of India, New Delhi for extending a Financial Support.

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