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# *In-vitro* assessment of fungicidal efficacy against *Lasiodiplodia theobromae*, the causal agent of root rot in mulberry (*Morus alba* L.)

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**Abstract**
Root rot of mulberry (*Morus alba* L.) caused by *Lasiodiplodia theobromae* has emerged as a major constraint in mulberry cultivation, leading to poor root development, wilting and significant yield losses in sericulture. The present investigation was conducted to evaluate the efficacy of various fungicides against *L. theobromae* under *in vitro* conditions using the poisoned food technique. Eighteen fungicides, including six contact, six systemic and six combination formulations, were tested at different concentrations. Among the contact fungicides, Mancozeb 75% WP exhibited the highest mycelial inhibition (up to 72.35%), whereas Copper oxychloride 50% WP showed the least effect. Systemic fungicides such as Propiconazole 25% EC, Tebuconazole 25% WP and Carbendazim 50% WP completely inhibited fungal growth (100%) at all tested concentrations. Similarly, combination products like Carbendazim 12% + Mancozeb 63% WP and Azoxystrobin 11% + Tebuconazole 18.3% SC also achieved complete inhibition. The findings clearly demonstrate that systemic and combination fungicides, especially triazole- and benzimidazole-based formulations, are highly effective against *L. theobromae*. These fungicides can be recommended for inclusion in integrated disease management (IDM) programs to control root rot in mulberry and sustain healthy sericulture production systems.

**Keywords:** *L. theobromae, in vitro,* fungicides, mulberry

## Introduction
Mulberry (*Morus alba* L.) plays a pivotal role in India's sericulture sector, serving as the exclusive food source for the silkworm (*Bombyx mori* L.) and thus forming the backbone of silk production. The crop is cultivated under diverse agro-climatic and edaphic conditions, many of which are conducive to the proliferation and spread of various pathogens. Mulberry is susceptible to a wide range of pathogens including fungi, bacteria, viruses and nematodes, which together contribute to significant yield and quality losses. Infections can lead to a reduction of 15-20 percent in leaf yield, and affected plants often produce nutritionally inferior leaves with diminished quality. Root rot disease alone accounts for up to 30 percent plant mortality and an additional 14 percent reduction in leaf yield, adversely impacting both quantity and quality (Sharma *et al.*, 2003) [5]. Due to the perennial nature of mulberry cultivation, soil-borne diseases are especially persistent and destructive. Among these, root rot remains one of the most devastating, owing to its epidemic potential and ability to survive and spread effectively in soil environments. The disease exhibits considerable variability in symptom expression and etiology across different regions, with manifestations such as dry root rot, charcoal root rot, violet root rot, white root rot, black root rot and bacterial root rot (Gnanesh *et al.*, 2021; Yoshida *et al.*, 2001) [3, 4]. Several fungal pathogens have been reported to cause root rot in mulberry, including *Lasiodiplodia theobromae*, *Fusarium* spp., *Macrophomina phaseolina* and *Rhizoctonia bataticola*.
In the absence of resistant mulberry varieties and considering the rapid progression of the disease, chemical control through fungicides offers an effective management option. Laboratory evaluation of fungicides provides a reliable preliminary step to identify promising molecules for subsequent field trials. Such *in vitro* screening not only helps determine the efficacy of

fungicides against specific pathogens but also facilitates further assessment regarding residue levels and safety intervals for silkworm rearing. Hence, the present investigation was undertaken to evaluate the efficacy of selected fungicides under *in vitro* conditions against *Lasiodiplodia theobromae*, the causal agent of root rot in mulberry.

## Materials and Methods

The present study on *in vitro* assessment of fungicidal efficacy against *Lasiodiplodia theobromae*, the causal agent of root rot in mulberry (*Morus alba* l.) was carried out in the Department of Plant Pathology, College of Sericulture, Chintamani, University of Agricultural Sciences, Bengaluru, Karnataka, India during 2024 - 2025. The materials used and methodology followed during the investigation are described below.

### *In vitro* evaluation of fungicides against Lasiodiplodia theobromae

Contact, Systemic and Combi product fungicides were evaluated at different concentrations under *in vitro* conditions. Six systemic fungicides at the concentration of 100, 250 and 500 ppm, six contact and combi fungicides at the concentration of 250, 500 and 1000 ppm were evaluated against the pathogen under laboratory conditions by poisoned food technique using potato dextrose agar medium.

The poisoned medium was prepared by adding required quantity of fungicides to the melted potato dextrose agar medium to obtain the desired concentration. 15 mL of poisoned medium was poured in each sterilized petri dish and suitable checks were maintained without fungicides. Five mm of ten days old fungal disc taken from the periphery of the culture were placed in the centre of poisoned medium and were incubated at 28±1°C. The experiment was conducted by using Completely Randomized Design (CRD) and each treatment were replicated thrice. The observations were recorded when the fungal growth is maximum in the untreated control. The colony diameter was measured in three directions and the average were recorded.

The per cent inhibition of mycelial growth over the control were calculated using the standard formula (Vincent, 1947) [6].

$$I = \frac{C-T}{C} \times 100$$

where,

I - Per cent inhibition of mycelial growth

C- Growth of mycelium in control.

T- Growth of mycelium in treatment.

## Results and Discussion

### *In vitro* evaluation of contact fungicides against Lasiodiplodia theobromae

Six distinct contact were examined by using poison food technique under laboratory conditions at three concentrations *viz*., 250, 500 and 1000 ppm to determine their effectiveness against *L. theobromae*. The details about the per cent mycelial growth inhibition of *L. theobromae* in various contact fungicides is shown in Table 1, illustrated in Fig. 1 and Plate 1.

Significant difference in the percentage of mycelial growth inhibition among various contact fungicides was observed (Table 1). Mancozeb 75% WP was found better than the other contact fungicides evaluated with 51.60, 69.63 and 72.35 per cent inhibition at 250, 500 and 1000 ppm, respectively. Further, Propineb 75% WP found to be next best fungicide with per cent mycelial inhibition of 30.49, 55.31 and 71.85 per cent at 250, 500 and 1000ppm, respectively. Whereas, Chlorothalonil 50% WP and Zineb 75% WP showed mycelial inhibition of 37.53, 47.28, 66.91 and 6.67, 45.06, 62.84 per cent at 250, 500 and 1000 ppm, respectively. However, Captan 50% WP (36.79%, 47.78% and 50.00%) and Copper oxychloride 50% WP (0.99%, 18.64% and 41.36%) showed the least mycelial inhibition at 250, 500 and 1000 ppm, respectively. Among the three concentrations of fungicides tested, 1000 ppm was found most efficient in preventing the organism's mycelial growth. Copper oxychloride 50% WP showed the least amount of mycelial inhibition of 41.36 per cent whereas Mancozeb 75% WP recorded highest mycelial inhibition of 72.35 per cent at 1000 ppm.

The results were supported by the findings of Suresh *et al.* (2016) [1] who evaluated different contact fungicides where in Propineb 75% WP exhibited highest inhibition against *Lasiodiplodia theobromae* in mango with inhibition percentage of 72.59 and 100.00 at 250 and 500 ppm, respectively. The next effective contact fungicide was Mancozeb 75% WP with inhibition of 66.30 and 100 per cent at 250 and 500 ppm, respectively.

**Table 1:** *In vitro* evaluation of contact fungicides against L. theobromae

| Sl. No. | Name of the fungicide | Per cent inhibition of mycelial growth | | |
|---|---|---|---|---|
| | | Concentrations (ppm) | | |
| | | 250 | 500 | 1000 |
| 1. | Captan 50% WP | 36.79 (37.33)# | 47.78 (43.71) | 50.00 (44.98) |
| 2. | Chlorothalonil 50% WP | 37.53 (37.76) | 47.28 (43.42) | 66.91 (54.86) |
| 3. | Copper oxychloride 50% WP | 0.99 (5.86) | 18.64 (25.57) | 41.36 (40.01) |
| 4. | Mancozeb 75% WP | 51.60 (45.90) | 69.63 (56.54) | 72.35 (58.25) |
| 5. | Propineb 75% WP | 30.49 (33.48) | 55.31 (48.03) | 71.85 (57.94) |
| 6. | Zineb 75% WP | 6.67 (14.89) | 45.06 (42.15) | 62.84 (52.42) |
| 7. | Untreated control | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | | Fungicide (F) | Concentration (C) | Interaction (F× C) |
| | F test | * | * | * |
| | S. Em± | 0.29 | 0.19 | 0.50 |
| | CD @ 1% | 1.11 | 0.73 | 1.92 |

#Figures in the parentheses are arc sine transformed values

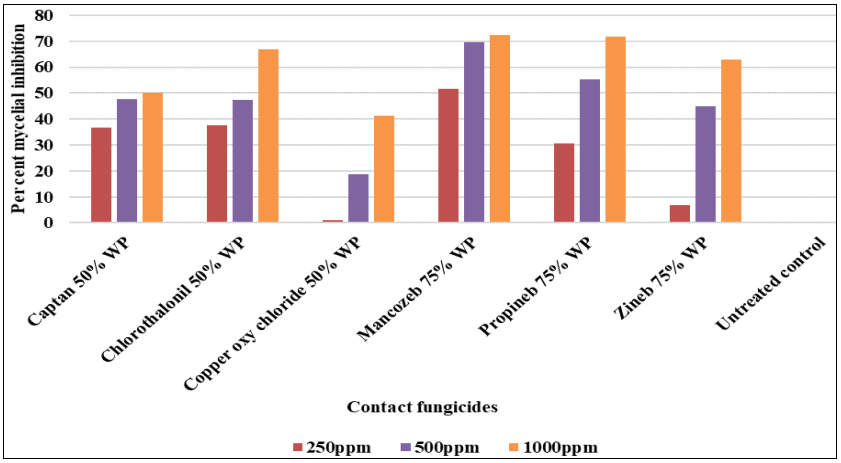


**Fig 1:** Effect of contact fungicides against *L. theobromae*

***In vitro* evaluation of systemic fungicides against Lasiodiplodia theobromae**

Six distinct systemic fungicides were examined by using poison food technique under laboratory conditions at three concentrations *viz*., 100, 250 and 500 ppm to determine their effectiveness against *L. theobromae*. The details about the per cent mycelial growth inhibition of *L. theobromae* in various systemic fungicides is shown in Table 2, illustrated in Fig. 2 and plate 2.

Among all the systemic fungicides evaluated Propiconazole 25% EC was found to be most effective fungicide, achieving complete inhibition (100%) of mycelial growth across all tested concentrations (100, 250 and 500 ppm). Tebuconazole 25% WP at 100 ppm showed 97.04 per cent mycelial suppression whereas, it exhibited 100 per cent mycelial suppression at 250 and 500 ppm, respectively. Carbendazim 50% WP was the subsequent fungicide exhibiting 96.67 at 100 ppm and 100 per cent mycelial inhibition at 250 and 500 ppm respectively. Followed by Hexaconazole 5% EC with inhibition of 85.43, 87.28 and 92.72 per cent at 100, 250 and 500 ppm, respectively. The fungicide Difenoconazole 25% EC, exhibited inhibition of 72.59, 81.73 and 87.78 per cent at 100, 250 and 500 ppm respectively. However, Azoxystrobin 23% SC exhibited the least efficacy in all three concentrations with 0.62, 1.98 and 3.95 per cent of inhibition at 100, 250 and 500 ppm, respectively.

The fungicides Difenoconazole 25% EC and Hexaconazole 5% EC showed a progressive increase in efficacy with higher concentrations. Whereas, Propiconazole 25% EC and Tebuconazole 25% WP reached maximum efficacy at 250 ppm, indicating that increasing the concentration beyond this point does not affect on mycelial growth. Azoxystrobin 23% SC even at the highest concentration showed poor efficacy and cannot not recommended.

The present findings have been reinforced by the study carried out by Naveen Chandra Reddy (2023) [2] who evaluated six different systemic fungicides against *L. theobromae* and found that Tebuconazole 25% WP and Propiconazole 25% EC were significantly superior and with 100 per cent mean mycelial inhibition at all the concentrations followed by Carbendazim 50% WP with 98.52 per cent mean inhibition. Similarly, Suresh *et al.* (2016) [1] evaluated different fungicides of which Propiconazole 25% EC exhibited 100 per cent mycelial inhibition at tested concentrations against *Lasiodiplodia theobromae*.

**Table 2:** *In vitro* evaluation of systemic fungicides against L. theobromae

| Sl. No. | Name of the fungicide | Per cent inhibition of mycelial growth | | |
|---|---|---|---|---|
| | | Concentrations (ppm) | | |
| | | 100 | 250 | 500 |
| 1. | Azoxystrobin 23% SC | 0.62 (2.61)# | 1.98 (7.96) | 3.95 (11.20) |
| 2. | Tebuconazole 25% WP | 97.04 (80.07) | 100.00 (89.96) | 100.00 (89.96) |
| 3. | Carbendazim 50%WP | 96.67 (79.46) | 100.00 (89.96) | 100.00 (89.96) |
| 4. | Difenoconazole 25% EC | 72.59 (58.41) | 81.73 (64.67) | 87.78 (69.51) |
| 5. | Hexaconazole 5% EC | 85.43 (67.54) | 87.28 (69.11) | 92.72 (74.32) |
| 6. | Propiconazole 25% EC | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) |
| 7. | Untreated control | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | | Fungicide (F) | Concentration (C) | Interaction (F× C) |
| | F test | * | * | * |
| | S. Em± | 0.44 | 0.29 | 0.76 |
| | CD @ 1% | 1.68 | 1.10 | 2.91 |

#Figures in the parentheses are arc sine transformed values

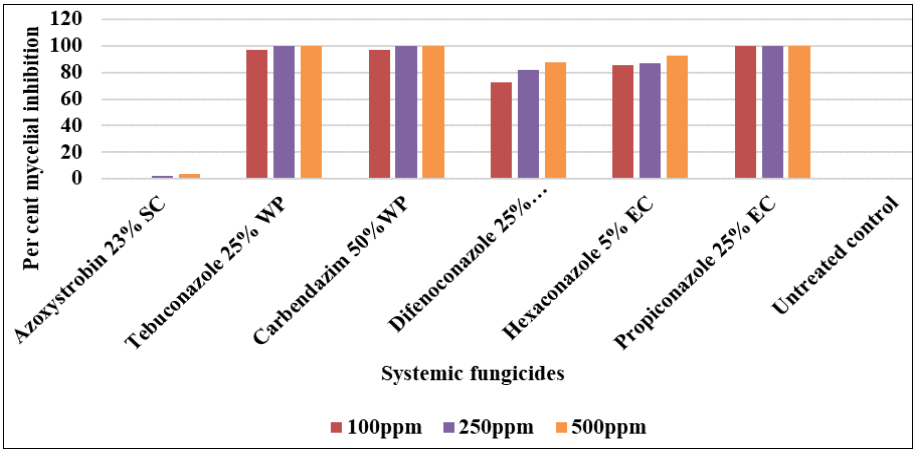


**Fig 2:** Effect of systemic fungicides against *L. theobromae*


**Plate 1:** *In vitro* evaluation of different contact fungicides against L. theobromae

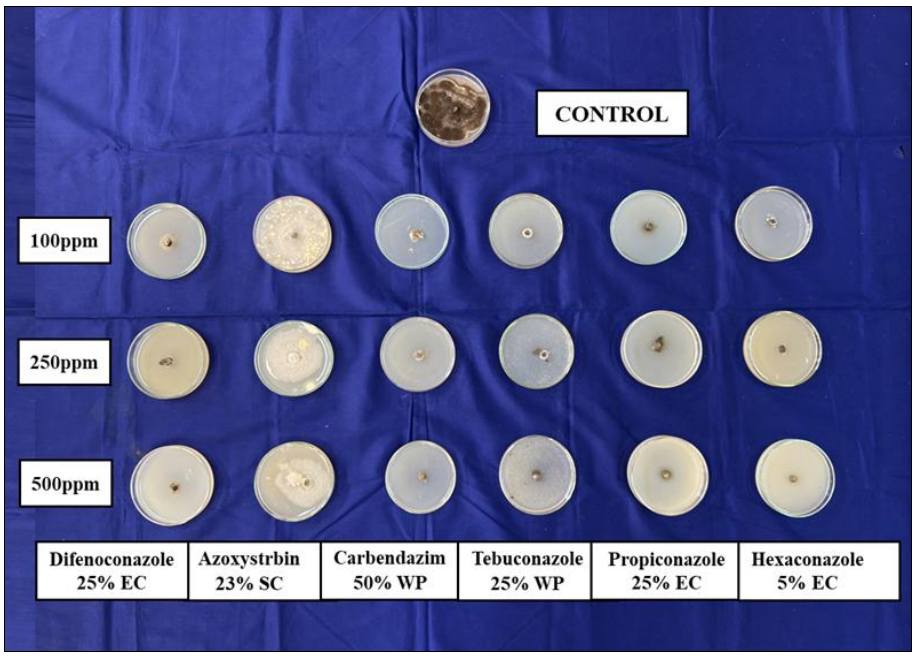


**Plate 2:** *In vitro* evaluation of different systemic fungicides against L. theobromae

### *In vitro* evaluation of combi fungicides against Lasiodiplodia theobromae

Six distinct combi fungicides were examined by using poison food technique under laboratory conditions at three concentrations *viz*., 250, 500 and 1000 ppm to determine their effectiveness against *L. theobromae.* The details about the per cent mycelial growth inhibition of *L. theobromae* in various combi fungicides is shown in Table 3, illustrated in Fig. 3 and Plate 3.

Among the six combi fungicides tested Carbendazim 12% + Mancozeb 63% WP exhibited 100 per cent mycelial inhibition at all the tested concentration, followed by Azoxystrobin 11% + Tebuconazole 18.3% SC exhibited 90.99 per cent inhibition at 250ppm and 100 per cent mycelial inhibition at 250 and 500 ppm, respectively. While Metiram 55% + Pyraclostrobin 5% WG that exhibited 74.81, 87.90, 95.68 per cent mycelial inhibition at 250, 500, 1000ppm respectively. Zineb 68% + Hexaconazole 4% WP and Cymoxanil 8%+ Mancozeb 63% WP has recorded 57.78, 73.70, 76.79 and 55.68, 67.41, 95.93 per cent mycelial inhibition at 250, 500 and 1000 ppm, respectively. However, Metalaxyl 4% + Mancozeb 64% WP showed the least t mycelial inhibition of 6.67, 12.84 and 32.47 per cent at respective concentrations.

The above results were supported by the findings of Naveen Chandra Reddy (2023) [2] who evaluated six different combi fungicides at three different concentrations against *L. theobromae.* Among tested fungicides Carbendazim 12%+Mancozeb 63%WP exhibited 100 per cent inhibition at all the three concentrations, followed by Azoxystrobin 11%+Tebuconazole 18.3% SC with mean mycelial inhibition of 98.02 per cent and it exhibited 100 per cent inhibition at 250 and 500 ppm. Similarly, Suresh *et al.* (2016) [1] assessed various combi fungicides and found the complete inhibition with Carbendazim 12%+Mancozeb 63%WP in both 250 ppm and 500 ppm concentrations, followed by Metiram 55% + Pyraclostrobin 5% WG with mycelial inhibition of 83.70 and 86.11 per cent at 250 and 500 ppm concentrations respectively.

**Table 3:** *In vitro* evaluation of combi fungicides against L. theobromae

| Sl. No. | Name of the fungicide | Per cent inhibition of mycelial growth | | |
|---|---|---|---|---|
| | | Concentrations (ppm) | | |
| | | 250 | 500 | 1000 |
| 1. | Azoxystrobin 11% + Tebuconazole 18.3% SC | 90.99 (72.51)# | 100.00 (89.96) | 100.00 (89.96) |
| 2. | Carbendazim 12% + Mancozeb 63%WP | 100.00 (89.96) | 100.00 (89.96) | 100.00 (89.96) |
| 3. | Zineb 68% + Hexaconazole 4% WP | 57.78 (49.46) | 73.70 (59.13) | 76.79 (61.18) |
| 4. | Cymoxanil 8% + Mancozeb 63% WP | 55.68 (48.24) | 67.41 (55.17) | 95.93 (78.35) |
| 5. | Metalaxyl 4% + Mancozeb 64% WP | 6.67 (14.94) | 12.84 (20.97) | 32.47 (34.72) |
| 6. | Metiram 55% + Pyraclostrobin 5% WG | 74.81 (59.86) | 87.90 (69.62) | 95.68 (78.00) |
| 7. | Untreated control | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| | | Fungicide (F) | Concentration (C) | Interaction (F× C) |
| | F test | * | * | * |
| | S. Em± | 0.19 | 0.12 | 0.32 |
| | CD @ 1% | 0.71 | 0.46 | 1.23 |

#Figures in the parentheses are arcsine transformed values

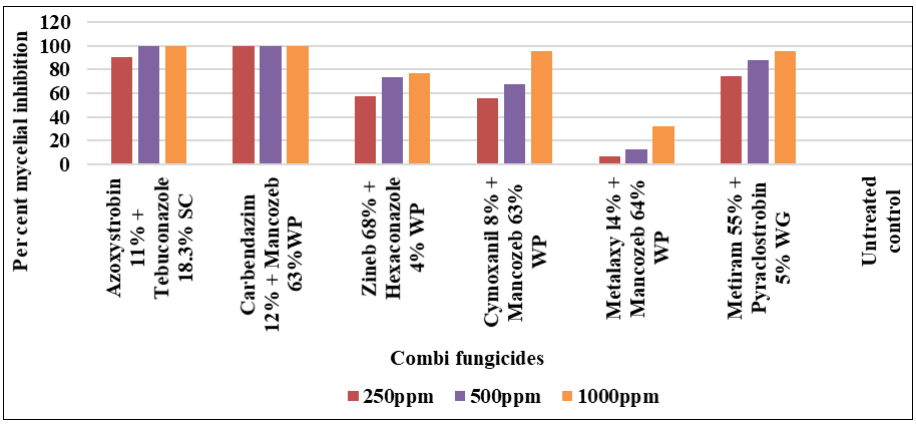


**Fig 3:** Effect of combi fungicides against *L. theobromae*

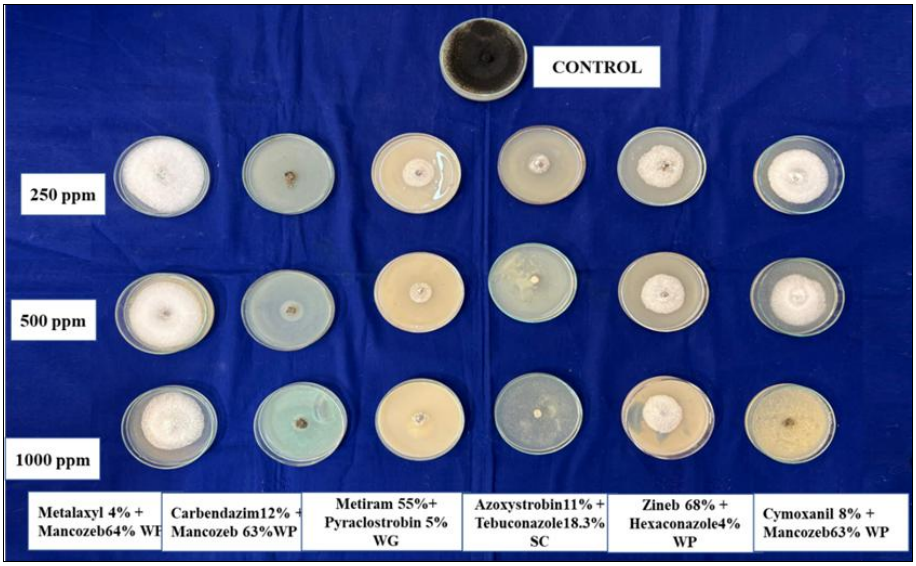


**Plate 3:** *In vitro* evaluation of different combi fungicides against L. theobromae

## Conclusion

The *in vitro* evaluation of contact, systemic and combi fungicides against *Lasiodiplodia theobromae* revealed significant variation in their efficacy. Among the contact fungicides, Mancozeb 75% WP exhibited maximum mycelial inhibition (72.35%) at 1000 ppm, followed by Propineb 75% WP, whereas Copper oxychloride 50% WP showed the least efficacy. Among systemic fungicides, Propiconazole 25% EC, Tebuconazole 25% WP and Carbendazim 50% WP achieved complete (100%) inhibition even at lower concentrations, indicating their superior systemic action. In the case of combi fungicides, Carbendazim 12% + Mancozeb 63% WP and Azoxystrobin 11% + Tebuconazole 18.3% SC recorded complete suppression of mycelial growth at all tested concentrations, followed by Metiram 55% + Pyraclostrobin 5% WG. Overall, Carbendazim 12% + Mancozeb 63% WP, Propiconazole 25% EC and Tebuconazole 25% WP proved to be the most effective fungicides and can be recommended by checking the phytoxicity effect on silkworm for managing *L. theobromae* under field conditions.

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