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**The Republic of the Union of Myanmar
Ministry of Natural Resources and Environmental Conservation
Forest Department**



**Efficacy of Different Fungicides on *Alternaria* spp. causing
Alternaria Leaf Blight of *Tectona grandis* in-vitro**



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Abstract

The incidence of foliage diseases (leaf rust, leaf spot, powdery mildew and leaf blight) was observed in teak plantation area of West Bago Yoma Region. *Alternaria* leaf blight is one of the most common diseases. *Alternaria* leaf blight is difficult to control because the fungus persists in plant debris and soil. One of the most effective methods is the use of fungicides. The present study was conducted to evaluate the efficacy of six fungicides (Mancozeb 80%WP, Chlorothalonil 72% SC, Hexaconazole 5% SC, Difenconazole 25% EC, Propiconazole 15% + Difenconazole 15% EC, Azoxystrobin 20% + Difenconazole 12.5% SC) with four concentrations (at 250 ppm, 500 ppm, 750 ppm and 1000 ppm) *in-vitro* conditions against the pathogen by using poisoned food technique. Results indicated that mean mycelial growth inhibition ranged from 46.76 to 97.78 percent among tested fungicides. Hexaconazole 5% SC (at 500 ppm), Propiconazole 15% + Difenconazole 15% EC (at 750 ppm) and Mancozeb 80% WP (at 1000 ppm) showed 100% mycelial growth inhibition. The least inhibition rate percent 34.07 is shown by chlorothalonil 72% SC (at 250 ppm). Therefore, Mancozeb 80% WP, Hexaconazole 5% SC, Difenconazole 25% EC, Propiconazole 15% + Difenconazole 15% EC and Azoxystrobin 20% + Difenconazole 12.5% SC would be effective fungicides to control *Alternaria* leaf blight of *Tectona grandis*.

Keywords: Foliage, Teak, *Alternaria* spp., Efficacy, Poisoned Food Technique, Fungicides, Mycelial Growth Inhibition

**ဓာတ်ခွဲခန်းတွင်မိုသတ်ဆေးအမျိုးမျိုးအသုံးပြုပြီး ကျွန်းရွက်ခြောက်ရောဂါဖြစ်စေသက်ရှိမှု
(*Alternaria spp.*) ကြီးထွားမှုအပေါ် ဟန့်တားနိုင်မှုစွမ်းရည်ကို လေ့လာအကဲဖြတ်ခြင်း**

ဒေါက်တာသန့်ရှင်း၊ ဒုတိယညွှန်ကြားရေးမှူး၊ သစ်တောသုတေသနဌာန
ဂျူးဂျူးသူ၊ အပင်ရောဂါပညာရှင်၊ FD-AFoCO IPDM Project
ရဲလွင်အောင်၊ ဦးစီးအရာရှိ၊ သစ်တောသုတေသနဌာန
မျိုးထက်အောင်၊ စီမံကိန်းအတွင်းရေးမှူး၊ FD-AFoCO IPDM Project
ဒေါက်တာသက်ရည်ခိုင်၊ သုတေသနမှူး၊ အပင်ရောဂါသုတေသနဌာနစု
စိုက်ပျိုးရေးသုတေသနဦးစီးဌာန

စာတမ်းအကျဉ်း

ပဲခူးတိုင်းဒေသကြီး၊ ပြည်ခရိုင်နှင့်သာယာဝတီခရိုင်အတွင်း တည်ထောင်ထားရှိသည့် ကျွန်းစိုက်ခင်းများတွင် သံချေးရောဂါ၊ ဖားဥမှိုရောဂါ၊ ရွက်ခြောက်ရောဂါနှင့် ရွက်ပြောက်ရောဂါများ ကျရောက်လျက်ရှိပါသည်။ ရွက်ခြောက်ရောဂါများအနက် ရောဂါဖြစ်စေသက်ရှိ *Alternaria* ကြောင့်ဖြစ်သော ရွက်ခြောက်ရောဂါသည် ကျရောက်မှုအများဆုံး ရောဂါတစ်ခု ဖြစ်ပါသည်။ ရောဂါဖြစ်စေသက်ရှိ *Alternaria* မှို သည် မြေဆီလွှာနှင့် သစ်ပင်အကြွင်းအကျန်များထဲတွင် အချိန်ကြာမြင့်စွာ နေထိုင်နိုင်သောကြောင့် ကာကွယ်နှိမ်နင်းရန် ခက်ခဲပါသည်။ ဓာတုမိုသတ် ဆေးများဖြင့် ကာကွယ်နှိမ်နင်းခြင်းသည် ထိရောက်သော ကာကွယ်နှိမ်နင်းနည်း တစ်ခုဖြစ်ပါသည်။ ထို့ကြောင့် ဓာတုမိုသတ်ဆေးအမျိုးမျိုး (Mancozeb 80% WP, Chlorothalonil 72% SC, Hexaconazole 5% SC, Difenoconazole 25% EC, Propiconazole 15%+Difenoconazole 15% EC, Azoxystrobin 20%+Difenoconazole 12.5% SC) ၏ ပြင်းအားများ (၂၅၀ ပီပီအမ်၊ ၅၀၀ ပီပီအမ်၊ ၇၅၀ ပီပီအမ် နှင့် ၁၀၀၀ ပီပီအမ်)ကို အသုံးပြုပြီး မိုသတ်ဆေးများ၏ ရောဂါ ဖြစ်စေ သက်ရှိကြီးထွားမှုအပေါ် ဟန့်တားနိုင်မှုစွမ်းရည် စမ်းသပ်ခြင်း ဓာတ်ခွဲခန်း သုတေသနကို Poisoned Food Technique ကို အသုံးပြု၍ ဆောင်ရွက်ခဲ့ပါသည်။ မိုသတ်ဆေးများ၏ ရောဂါဖြစ်စေ သက်ရှိကြီးထွားမှုအပေါ် ဟန့်တားနိုင်မှုသည် ၄၆.၇၆ မှ ၉၇.၇၈ ရာခိုင်နှုန်းရှိသည်ကို တွေ့ရှိရပါသည်။ Hexaconazole 5% SC (ပြင်းအား ၅၀၀ ပီပီအမ်)၊ Propiconazole 15% + Difenoconazole 15% EC (ပြင်းအား ၇၅၀ ပီပီအမ်)နှင့် Mancozeb 80% WP (ပြင်းအား ၁၀၀၀ ပီပီအမ်) သည် ရောဂါဖြစ်စေသက်ရှိ ကြီးထွားမှုအပေါ် (၁၀၀) ရာခိုင်နှုန်း ဟန့်တား နိုင်သည်ကို တွေ့ရှိရပါသည်။ ရောဂါဖြစ်စေ သက်ရှိကြီးထွားမှုအပေါ် ဟန့်တားနိုင်မှု ရာခိုင်နှုန်း အနည်းဆုံး ၃၄.၀၇ ရာခိုင်နှုန်းကို Chlorothalonil 72% SC (ပြင်းအား ၂၅၀ ပီပီအမ်) မှ

တွေ့ရှိရပါသည်။ ထို့ကြောင့် Mancozeb 80% WP၊ Hexaconazole 5% SC၊ Difenconazole 25% EC၊ Propiconazole 15%+Difenconazole 15% EC နှင့် Azoxystrobin 20 + Difenconazole 12.5% SC မှိုသတ်ဆေးများသည် ကျွန်း Alternaria ရွက်ခြောက်ရောဂါ ကာကွယ်နှိမ်နင်း ရန်အတွက် ထိရောက်မှုရှိသော မှိုသတ်ဆေးများ ဖြစ်ပါသည်။

Efficacy of Different Fungicides on *Alternaria* spp. causing *Alternaria* Leaf Blight of *Tectona grandis* in-vitro

1. Introduction

Teak (*Tectona grandis* L. f.) is a deciduous tree, which is native from India, Myanmar, Laos and Thailand. The natural populations of teak remain an important source from the economic, ecological and environmental perspectives (Venkatesh et al., 2023). Teak is suitable species for agroforestry due to its pleasant appearance, superior timber quality and timber durability (Ashajyothi et al., 2021). In Myanmar, teak plantation area was 395,492 ha covering 43.55% of total plantation area of different species (FD 2020). The teak associated fungal pathogens include *Olivia* spp., *Rhizoctonia* spp., *Alternaria* spp., *Cercospora* spp., *Phomopsis* spp., *Fusarium* spp., *Phytophthora* spp. etc. Among various diseases reported, foliage diseases mainly leaf rust, leaf blight and leaf spot were the most common ones in many areas (Ashajyothi et al., 2021). In Myanmar, the incidence of foliage diseases such as rust (*Olivea* spp.), powdery mildew (*Erysiphe* spp.), leaf spot (*Cercospora* spp.) and leaf blight (*Rhizoctonia* spp., *Alternaria* spp., *Curvularia* spp.) were observed in teak plantation area of West Bago Yoma Region. *Alternaria* leaf blight disease is one of the common diseases in teak plantation (Aye et al., 2023). *Alternaria* leaf blight is difficult to control because the fungus persists in plant debris and soil. One of the most effective methods for disease control is the use of fungicides (Bangar et al., 2019).

2. Objectives

To investigate the efficacy of six fungicides at four concentrations (250, 500, 750 and 1000 ppm) on the mycelial growth of *Alternaria* spp. in-vitro.

3. Materials and Methods

3.1 Collection of diseased samples

Infected leaves showing *Alternaria* blight symptom were collected during survey (August 2022 - July 2023) from FD-AFoCO project site of West Bago Yoma Region, Myanmar. Infected leaves were kept into polyethylene bags. Samples were brought to Pathology Laboratory, Forest Protection Section, Forest Research Institute (FRI), Yezin. *Alternaria* disease symptom on leaves initially appeared as round dark brown necrotic lesions which were surrounded by yellow haloes. When disease infection increased, these lesions coalesced and developed into larger circular or irregular spots (Plate 1). In severe infections, infected leaves dried and eventually dropped.



Plate 1. Leaf blight symptom of *Tectona grandis*

3.2 Isolation and identification of pathogen

The diseased samples were cut into small pieces along with growing margins of disease. These pieces were surface sterilized with 70% sodium hypo chloride for 3 minutes and cleaned with sterilized distilled water for 1 minute for 2-3 times to remove the traces of surface sterilant. Then sterilized pieces were blotted dry on sterile filter paper and aseptically transferred to sterilized potato dextrose agar (PDA) plates. These plates were incubated at room temperature for 3-5 days for mycelial growth formation. Observed growths were sub-cultured by single spore technique to obtain pure culture. Identification of *Alternaria* spp. was carried out by its morphological features (Woudenberg et al., 2013).

3.3 Evaluation of different fungicides against *Alternaria* spp. *in-vitro*

Two systemic (Hexaconazole, Difenoconazole), two non-systemic (Mancozeb, Chlorothalonil) and combi-product (Propiconazole + Difenoconazole, Azoxystrobin + Difenoconazole) fungicides were used in evaluating the efficacy on mycelial growth of *Alternaria* spp. Poisoned food technique was used for assessing these fungicides on *Alternaria* spp. by using PDA as the medium. The principle involved in poisoned food technique is to poison the nutrient medium with a fungi-toxicant and then allowing the test fungus to grow on it and finally recording the extent of growth (Nene and Thapliyal 1997). Four concentrations i.e 250ppm, 500ppm, 750ppm and 1000ppm were used for each fungicide (Table 1). Required quantities of individual fungicides based on active ingredients were added separately into potato dextrose agar so as to get desired concentration of fungicides. To avoid bacterial contamination, Streptomycin (15 mg L⁻¹) was added to each flask before pouring. Twenty ml of poisoned medium was poured into sterile petri dishes.

Table 1. List of fungicides and their concentrations

Sr. no.	Fungicides	Trade Name	Concentration (ppm)
1.	Mancozeb 80% WP	Dithane M-45	250, 500, 750 & 1000
2.	Chlorothalonil 72% SC	Cleaner	250, 500, 750 & 1000
3.	Hexaconazole 5% SC	Hezagon	250, 500, 750 & 1000
4.	Difenoconazole 25% EC	Zolar	250, 500, 750 & 1000

5.	Propiconazole 15% + Difenoconazole 15% EC	Tiger	250, 500, 750 & 1000
6.	Azoxystrobin 20% + Difenoconazole 12.5% SC	Dize	250, 500, 750 & 1000

Mycelial discs of 5 mm diameter were cut from actively growing culture of fungus with the help of a sterilized cork-borer. A single disc was aseptically placed at the center of each PDA plate that already poured with poisoned medium. Control was maintained without adding any fungicides to the medium. Each treatment was replicated thrice. Plates were incubated at room temperature. Observation on colony diameter (mm) of pathogen was recorded when control treatment was fully covered with mycelial growth. The efficacy of a fungicide was expressed as percent inhibition of mycelia growth over control which was calculated by using the following formula (Vincent, 1927).

$$\text{Percent Inhibition (I)} = \frac{(C - T)}{C} \times 100$$

Where,

C = Growth (mm) of the fungus in untreated control plates

T = Growth (mm) of the fungus in treated plates

4. Results and Discussion

4.1 Identification of Pathogen

The colonies were initially light grey in color and turned olive green to dark brown with whitish margin. When the medium was completely covered by fungi, the whole surface of the colony turned dark green or black with numerous sporulation (Plate 2). The fungus produces long chains conidia. Mycelia were light brown with brown conidiophores. Conidiophores have septa, straight to geniculate and prominent conidial scars. Conidia were brown in color and obclavate. Conidia have short beak with 3-5 transverse septa and 0-3 longitudinal septa (Plate 3). Conidial morphology of the fungus was similar with characteristics of *Alternaria* on teak (Ai et al., 2015) and description of *Alternaria* (Woudenberg et al., 2013).

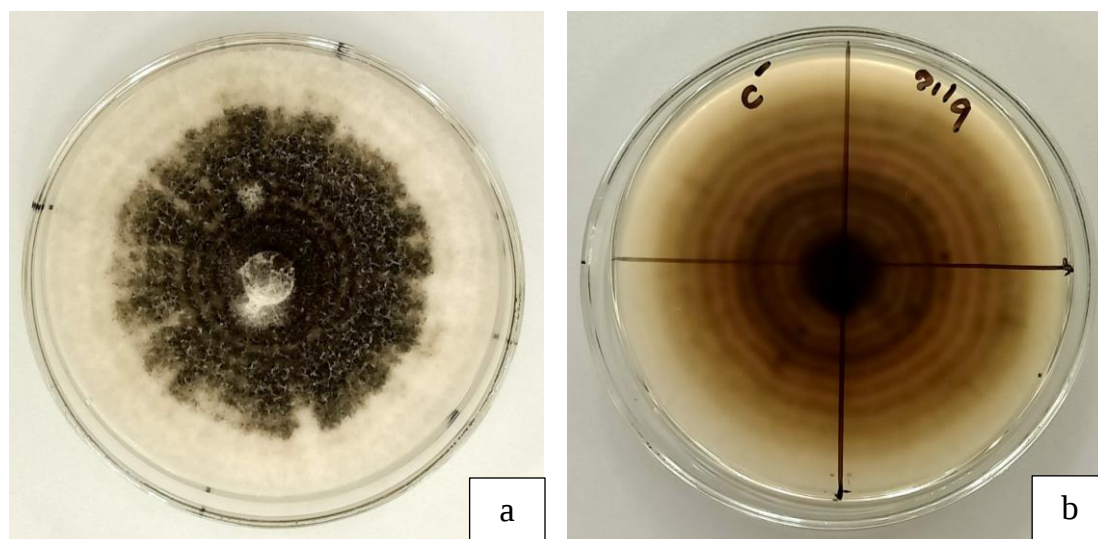


Plate 2. Front view (a) and back view (b) of colony morphology of *Alternaria* spp.

on PDA at 10 DAI

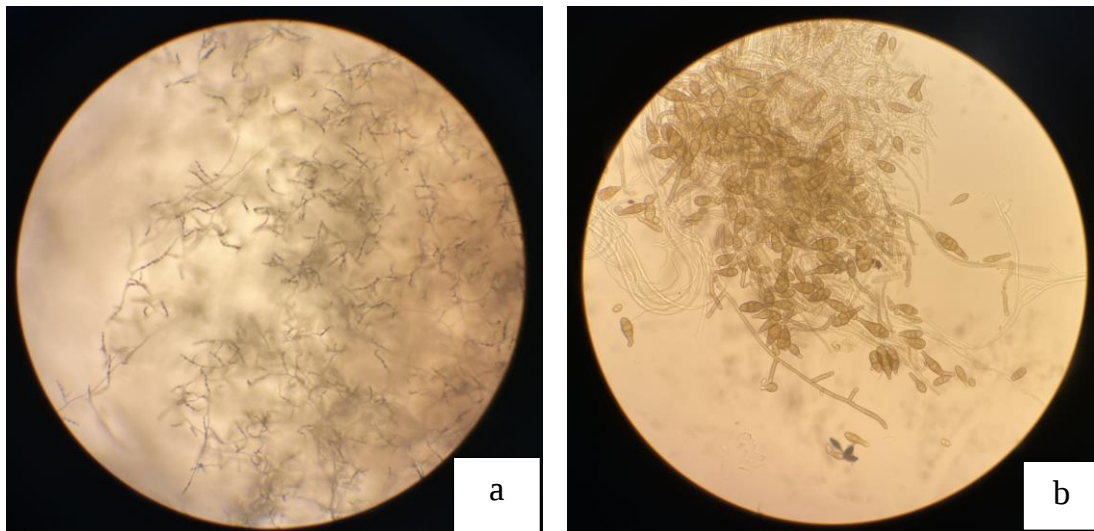


Plate 3. Conidia and conidiophores of *Alternaria* spp. 10x (a) and 40x (b)

4.2 Evaluation of different fungicides on mycelial growth of *Alternaria* spp. *in-vitro*

Table 2. Effect of different fungicides on mycelial growth of *Alternaria* spp. *in-vitro*

Fungicides	Colony Diameter (mm)			
	C1: 250ppm	C2: 500ppm	C3: 750ppm	C4: 1000ppm
T1: Mancozeb 80% WP	19.33	12.67	4.00	0.00
T2: Chlorothalonil 72% SC	59.33	58.50	37.67	36.17
T3: Hexaconazole 5% SC	8.00	0.00	0.00	0.00
T4: Difenconazole 25% EC	16.00	11.67	10.33	10.00
T5: Propiconazole 15% + Difenconazole 15% EC	9.67	9.00	0.00	0.00
T6: Azoxystrobin 20% + Difenconazole 12.5% SC	16.17	13.50	12.33	12.33
T7: Control	90.00	90.00	90.00	90.00
P value	0.0000			
CV %	17.78			
LSD _{0.05}	4.34			

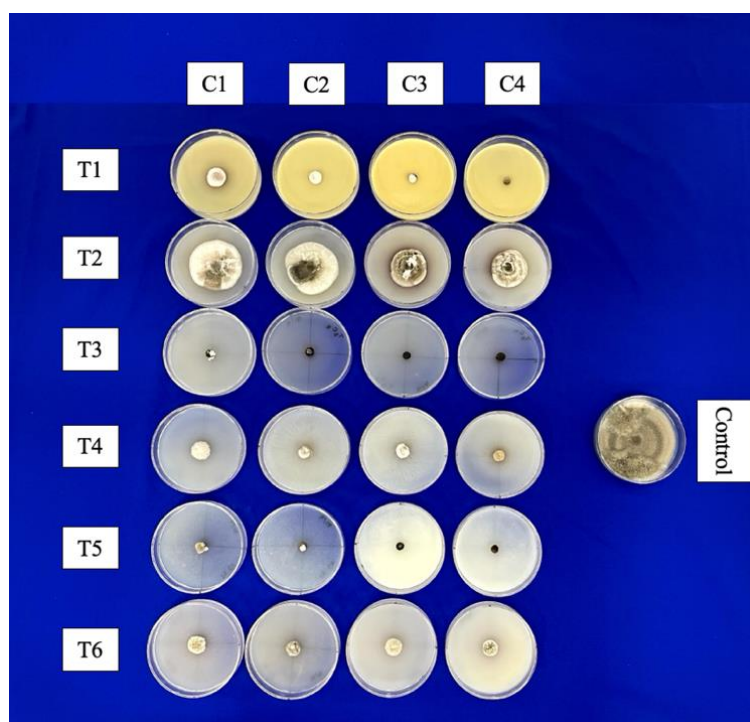


Plate 4. Efficacy of six fungicides on the mycelial growth of *Alternaria* spp. at four concentrations

Six fungicides viz., Mancozeb, Chlorothalonil, Hexaconazole, Difenconazole, Propiconazole + Difenconazole, Azoxystrobin + Difenconazole were evaluated against the mycelial growth of *Alternaria* spp. at four concentrations viz., 250, 500, 750 and 1000 parts per million (ppm) by poisoned food technique at 10 days after inoculation (DAI). Result (Table 2, Plate 4) revealed that test fungicides exhibited a wide range of mycelial growth of *Alternaria* spp., which was found to be decreased drastically with increase in concentrations of different fungicides. At 250 ppm, mycelial growth of *Alternaria* spp. ranged from 8.00 to 59.33 mm. However, it was least with Hexaconazole 5% SC (8.00 mm), followed by Propiconazole 15% + Difenconazole 15% EC (9.67 mm), Difenconazole 25% EC (16.00 mm), Azoxystrobin 20% + Difenconazole 12.5% SC (16.17 mm), Mancozeb 80% WP (19.33 mm) and Chlorothalonil 72% SC (59.33 mm). At 500 ppm, mycelial growth ranged from 0.00 to 58.50 mm. However, Hexaconazole 5% SC completely inhibited the growth of the pathogen. It was followed by Propiconazole 15% + Difenconazole 15% EC (9.00 mm), Difenconazole 25% EC (11.67 mm), Mancozeb 80% WP (12.67 mm), Azoxystrobin 20% + Difenconazole 12.5% SC (13.50 mm) and Chlorothalonil 72% SC (58.50 mm). At 750 ppm, mycelial growth was between 0.00 to 37.67 mm. However, Hexaconazole 5% SC and Propiconazole 15% + Difenconazole 15% EC entirely inhibited the pathogen growth. The highest mycelial growth (37.67 mm) observed from Chlorothalonil 72% SC. At 1000 ppm, mycelial growth ranged from 0.00 to 36.17 mm. However, Hexaconazole 5% SC, Propiconazole 15% + Difenconazole 15% EC and Mancozeb 80% WP inhibited the growth of the fungus.

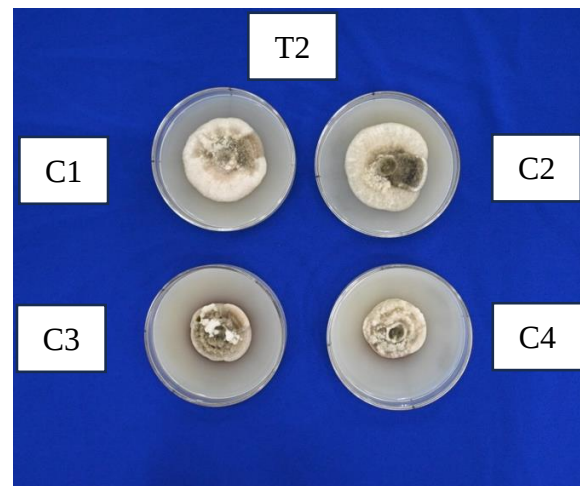
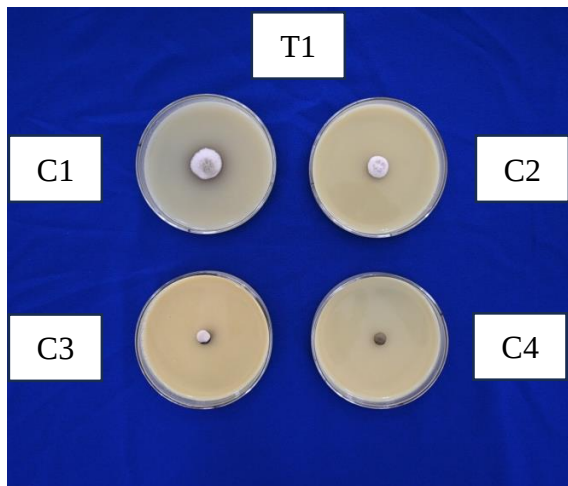


Plate 5. Inhibition effect of Hexaconazole (T3) and Difenconazole (T4) on mycelial growth of *Alternaria* spp. at four different concentrations

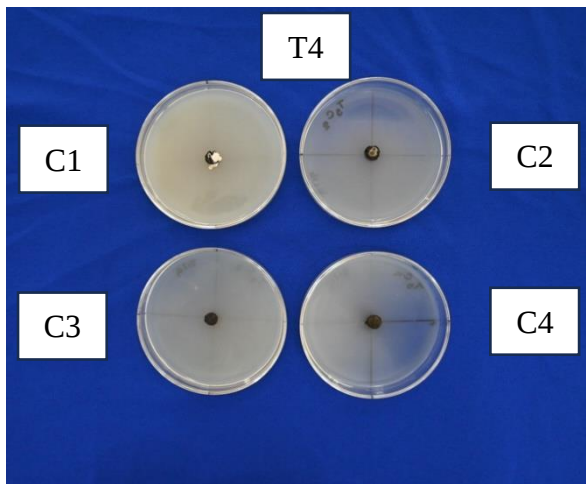
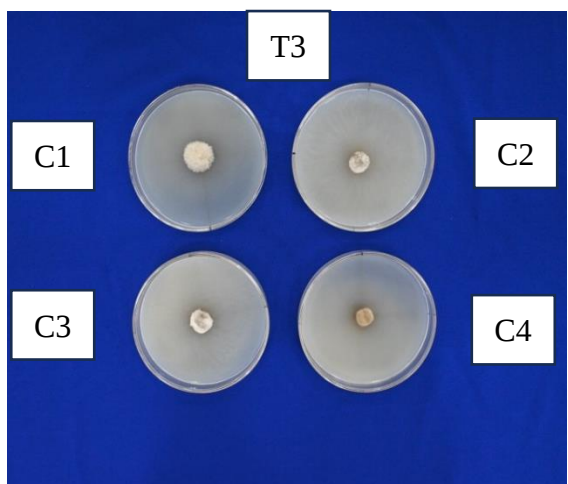


Plate 6. Inhibition effect of Hexaconazole (T3) and Difenconazole (T4) on mycelial growth of *Alternaria* spp. at four different concentrations

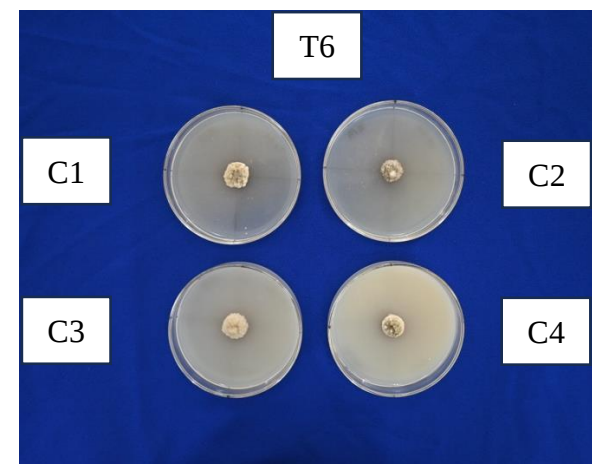
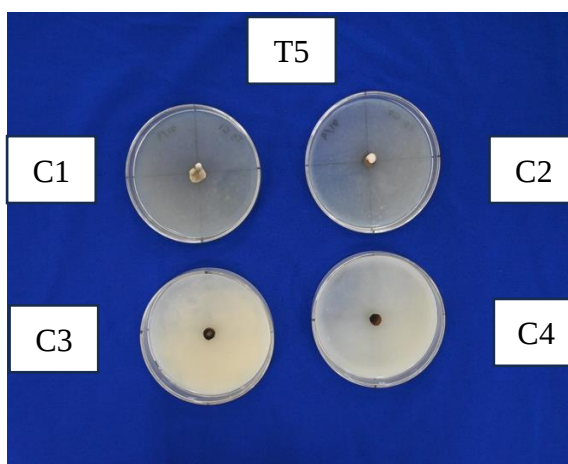


Plate 7. Inhibition effect of T5: Propiconazole + Difenconazole (T5) and Azoxystrobin + Difenconazole (T6) on mycelial growth of *Alternaria* spp. at four different concentrations

Table 3. Inhibition percent of six fungicides on mycelial growth of *Alternaria* spp.

Fungicides	Mycelial growth inhibition (%)
T1: Mancozeb 80% WP	90.00 C
T2: Chlorothalonil 72% SC	46.76 E
T3: Hexaconazole 5% SC	97.78 A
T4: Difenconazole 25% EC	86.67 D
T5: Propiconazole 15% + Difenconazole 15% EC	94.81 B
T6: Azoxystrobin 20% + Difenconazole 12.5% SC	84.91 D
P value	0.0000
CV %	3.52
LSD _{0.05}	2.41

All tested fungicides are capable of inhibiting the growth of *Alternaria* spp. at all the concentrations. A progressive increase in inhibition on mycelial growth of pathogen was observed with increase in concentration of fungicides (Plate 5, Plate 6, Plate 7). The percent inhibition of mycelial growth ranged from the minimum of 46.76 to the maximum of 97.78. Among these fungicides, Hexaconazole 5% SC was found superior in inhibiting the growth of *Alternaria* spp. with a mean percent inhibition of 97.78 % and was significantly different as compared to the other fungicides. Propiconazole 15% + Difenconazole 15% EC (94.81%) was second best followed by Mancozeb 80% WP (90.00%). Difenconazole 25% EC (86.67%) and Azoxystrobin 20% + Difenconazole 12.5% SC (84.91%) were the next best chemicals in that order. However, Chlorothalonil 72% SC recorded least percent inhibition of mycelial growth at all concentration with a mean 46.76 percent growth inhibition (Table 3).

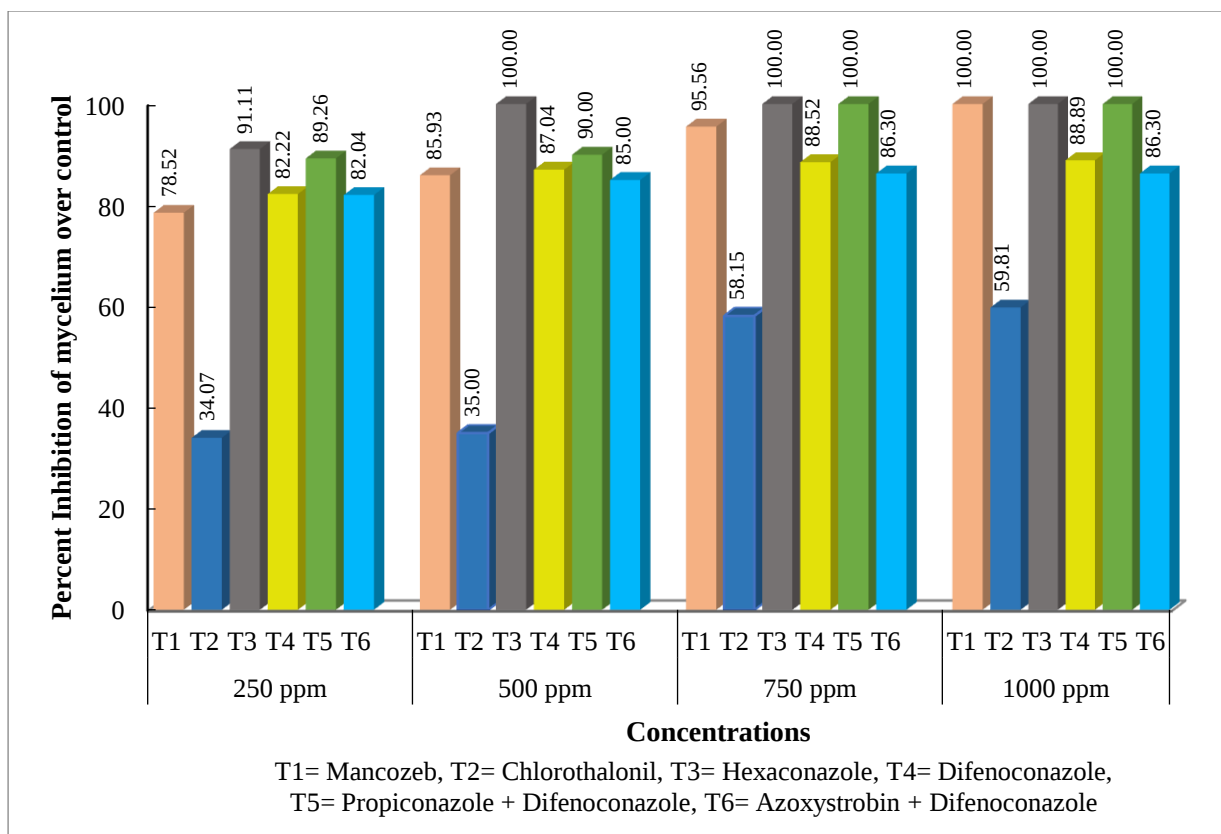


Figure 1. In-vitro efficacy of six fungicides against *Alternaria* spp. at four concentrations (LSD_{0.05} = 4.83, CV = 3.52)

At 250 ppm, Hexaconazole 5% SC showed maximum percent inhibition (91.11%) followed by Propiconazole 15% + Difenconazole 15% EC (89.26%) and least percent inhibition was recorded by Chlorothalonil 72% SC (34.07%). At 500 ppm, Hexaconazole 5% SC resulted with highest inhibition (100%), followed by Propiconazole 15% + Difenconazole 15% EC (90.00%). Least inhibition was observed in Chlorothalonil 72% SC (35.00%). At 750 ppm, 100% inhibition of pathogen was achieved from the treatments of Hexaconazole 5% SC and Propiconazole 15% + Difenconazole 15% EC. Chlorothalonil 72% SC was appeared as least effective fungicide which showed only 58.15% inhibition of pathogen. At 1000 ppm, Mancozeb 80% WP, Hexaconazole 5% SC and Propiconazole 15% + Difenconazole 15% EC showed 100% inhibition of mycelial growth followed by Difenconazole 25% EC with 88.89% inhibition. Least percent inhibition was recorded by Chlorothalonil 72% SC (59.81%) (Figure 1).

These results were in conformity with the findings of several earlier researchers. Bangar et al., (2019) reported that Mancozeb 75% WP (0.25%) was completely inhibited the growth of *Alternaria brassicae* causing Alternaria leaf spot of cauliflower. Babhare et al., (2023) showed that significantly highest and percent (100%) inhibition against *Alternaria cucumerina*, causing leaf blight of bottle gourd was recorded from Propiconazole 25% EC and Tebuconazole 25.9% EC, followed by Hexaconazole 5% SC (96.36%). Jewaliya et al., (2021) reported that Hexaconazole was the most effective against *Alternaria alternata* which showed 97.96% inhibition of mycelial growth.

5. Conclusion

Based on the results of current experiment, it can be concluded that Mancozeb 80% WP, Hexaconazole 5% SC, Difenoconazole 25% EC, Propiconazole 15% + Difenoconazole 15% EC and Azoxystrobin 20% + Difenoconazole 12.5% SC would be effective fungicides to control *Alternaria* leaf blight of *Tectona grandis* in Laboratory. However, these findings need to be studied under field conditions.

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