



# Field evaluation of fungicides for management of *Lasiodiplodia* leaf blight of coconut

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## Abstract

Various fungal and phytoplasma diseases are adversely impacting coconut yield. Among them, leaf blight, primarily attributed to *Lasiodiplodia theobromae*, has emerged as a significant contributor to yield losses ranging between 10 to 25 percent. This study aimed to assess the efficacy of novel fungicide formulations in mitigating leaf blight in coconut palms. An experimental plot, organized using a Randomized Block Design, was established, consisting of five distinct treatments, each replicated four times. The findings reveal that systemic fungicides, specifically propiconazole and tebuconazole, exhibited notable efficacy in curtailing leaf blight when applied at concentrations of 250 ppm and 1000 ppm, respectively. The effective fungicide concentrations are considerably lower than other fungicides tested in the field trials. The results revealed that sequential root feeding of propiconazole 25.9% EC @ 5% (100ml/palm) and tebuconazole 25.9% EC @ 5% (100ml/palm) at three months interval reduced the leaf blight incidence (5.66 % reduction) over control compared to root feeding of same fungicides continuously at quarterly interval (3.02 %) with in six months on currently it boosted the nut yield to 135 nuts per palm per annum, compared to the 99 nuts per palm per annum recorded in the control palms.

**Keywords:** Coconut, Systemic fungicides, Leaf blight, *Lasiodiplodia theobromae*, Management

The coconut (*Cocos nucifera* Linn.) is a perennial oilseed crop of significance in Indian agriculture, revered for its multifaceted utility. Every part of the coconut tree serves a distinct purpose in human life. However, the coconut output faces a formidable challenge from various diseases, with leaf blight caused by *Lasiodiplodia theobromae* emerging as a critical threat. Particularly in key coconut-growing regions of Tamil Nadu, there has been a distressing surge in the prevalence of this disease, causing yield losses ranging from 10 to 25 percent.

Leaf blight wreaks havoc on coconut plants, inflicting damage on mature nuts, leaves, and seedlings. The lower leaves of affected coconut palms exhibit a distinctive pattern of leaflet drying, commencing at the tips and progressing downward. Moreover, the nuts exhibit wavy dark grey to brown lesions, indicating fungal infiltration from mesocarp into the kernel, leading to eventual

decomposition of the endosperm. The affected nuts exhibit premature breakage, appearing dry, shrunken, and disheveled. Pycnidia, the asexual fruiting structures, and the development of greyish-black mycelium are hallmark features of the pathogen (Wang-Ching Ho and Wen-Hsiung Ko, 1997; Johnson *et al.*, 2014).

The confluence of leaf blight and eriophyid mite infestation exerted a substantial impact on coconut palms in Tamil Nadu in 1998 (Lakshmanan and Jagadeesan, 2004). Additionally, the rapid spread of leaf blight in the Thanjavur area of Tamil Nadu has been documented (Surulirajan *et al.*, 2014). Warwick *et al.* (1990) reported a reduced incidence and severity of the leaf blight disease in the Brazilian accession known as "Green Dwarf Jiqui". Despite its global distribution, *L. theobromae* predominantly thrives in tropical and subtropical regions, demonstrating a broad host range by infecting both gymnosperms and angiosperms,

manifesting as a parasite, saprophyte, or endophyte. In tropical and subtropical climates, *Lasiodiplodia*, a member of the Ascomycota in the Botryosphaeriaceae family, is widespread among woody plants (Punithalingam, 1980). Species within this genus have been implicated in various plant diseases, affecting a diverse array of woody plants and presenting diverse disease symptoms (Burgess *et al.*, 2006).

Beyond its visible manifestations, this fungus can reside endophytically within asymptomatic plant material, evading detection during quarantine processes and posing a substantial threat to crops. Consequently, chemical control of leaf blight emerges as a viable solution. Certain studies have indicated the relative success of using systemic fungicide sprays to manage leaf blight disease (Ushamalini *et al.*, 2019; Warwick and Abakerli, 2001). However, previous research suggests that controlling the disease may require two or three sequential applications of fungicide, although the level of control achieved is relatively modest (Santana *et al.*, 2013; Ascari *et al.*, 2021). To address the significant research gap in utilizing novel fungicide compounds for coconut leaf blight, an experimental investigation was conducted. The literature underscores the importance of providing farmers with precise fungicide recommendations, which can result in substantial cost savings. This research aims to evaluate the effectiveness of fungicides against *L. theobromae*. Encouraging results from this screening prompted the field evaluation of two potent systemic fungicides, tebuconazole and propiconazole, administered

sequentially at different intervals throughout the year.

## Materials and Methods

### Isolation and establishment of pure culture of the pathogen

Diseased coconut leaf parts displaying characteristic symptoms of leaf blight were carefully collected for analysis. The hyphal tip isolation method described by Dhingra and Sinclair (1985) was utilized on Potato Dextrose Agar (PDA) medium in Petri plates to establish a pure culture of

*L.theobromae*. Subsequently, the hyphal tips were transferred to fresh PDA plates and incubated at a constant temperature of  $27 \pm 2^\circ\text{C}$ . Regular transfers onto PDA medium under sterile conditions were conducted to maintain the culture's viability. After removal of advanced hyphae, the remaining material was cultured on PDA-containing test tube slants for seven days at room temperature. Following incubation, slants were carefully inspected for contaminants and then stored at  $4^\circ\text{C}$  in a refrigerator for future use. Totally 27 isolates were collected from different parts of Tamil Nadu.

*L.theobromae* CRSLT03, isolated found more virulent and prove the symptom development was taken up for further fungicidal screening.

### In vitro Screening of Fungicides

The effectiveness of various fungicides (Table 1), both alone and in combination, against

**Table 1. List of fungicides and their test concentrations**

S. No.	Treatments (Systemic Fungicides)	Formulation	Concentrations (ppm)
1	Tebuconazole	25.9% EC	50, 100, 250, 500, 750, 1000, 1250,
2	Propiconazole	25.9% EC	1500
3	Penconazole	10% EC	
4	Carbendazim	50% WP	
5	Kresoxym methyl	44.3% SC	
6	Azoxystrobin	23% SC	
7	Thiophanate methyl	70% WG	
8	Debcarb+Carbendazim	1.7%+0.3% WP	
9	Mycobutanol	10% WP	
10	Trifloxystrobin + Propineb	75% WP	

*L.theobromae* was evaluated using the poisoned food technique as described by Nene and Thapliyal (1993). This experiment was conducted twice in a sterile environment. A completely randomized design (CRD) with three replications was employed for each of the three treatments. The percentage suppression of mycelial growth in each treatment was calculated using Vincent's formula (1947).

$$\text{Percentage Inhibition} = \frac{C - T}{C} \times 100$$

Where:

*C* – fungal growth in the control plate.

*T* – fungal growth in the treated plate.

### Poisoned Food Technique

Each fungicide was individually mixed into sterilized Potato Dextrose Agar (PDA) to achieve the desired concentration. The poisoned medium was then poured into sanitized Petri plates. Mycelial discs, measuring five mm in diameter, were cut from seven-day-old cultures using a sterile cork borer and placed in the center of each agar plate. Control plates devoid of fungicide were also prepared. Three replications were maintained for each concentration. Once the fungus in the control plates reached maximum growth, the radial growth was measured at room temperature. The percentage suppression of mycelial growth compared to the control was calculated using Vincent's formula (1947) to determine the efficacy of the fungicides.

### Evaluation of Sequential Use of Fungicides in Field Conditions

The effective fungicides selected from the in vitro screening, namely Tebuconazole 25.9% EC

$$PDI = \frac{\text{Sum of all individual ratings}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

### Calculation of C: B ratio

Cost benefit ratio was calculated using formula and following the work done by Mahapatra and Das (2017).

and Propiconazole 25.9% EC, were subjected to field evaluation at different time intervals. The experiment was conducted in Puliyanakandi village, Anaimalai block, Coimbatore district, and included five treatments with four replications arranged in a Randomized Block Design (RBD). The treatments included:

$T_1$  : Root feeding of Tebuconazole 25.9% EC @ 5% (100 ml per tree) four times a year at three-month intervals.

$T_2$  : Root feeding of Propiconazole 25.9% EC @ 5% (100 ml per tree) four times a year at three-month intervals.

$T_3$  : Root feeding of Tebuconazole 25.9% EC @ 5% (100 ml per tree) in the first and third quarters, and Propiconazole 25.9% EC @ 5% (100 ml per tree) in the second and fourth quarters of the year.

$T_4$  : Root feeding of Propiconazole 25.9% EC @ 5% (100 ml per tree) in the first and third quarters, and Tebuconazole 25.9% EC @ 5% (100 ml per tree) in the second and fourth quarters of the year.

$T_5$ : Control with no fungicide application.

Leaf blight incidence on a 0–5 scale was measured before and after treatments on randomly chosen palms. Pre-and post-treatment observations involved assessing the incidence of leaf blight on approximately 25 leaflets from the lower 10 leaves of the test palm. Disease severity was scored based on a 0-5 scale [0 Scale (No disease incidence); 1 Scale (< 10% of disease incidence); 2 scale (11–25% of disease incidence); 3 Scale (26–50% of disease incidence); 4 Scale (51–75% of disease incidence); 5 scale (>75% of disease incidence)]. The percentage disease index (PDI) was calculated for each treatment using the following formula:

$$C : B \text{ ratio} = \frac{\text{Additional income form protection}}{\text{Cost of protection}}$$

### Statistical Analysis

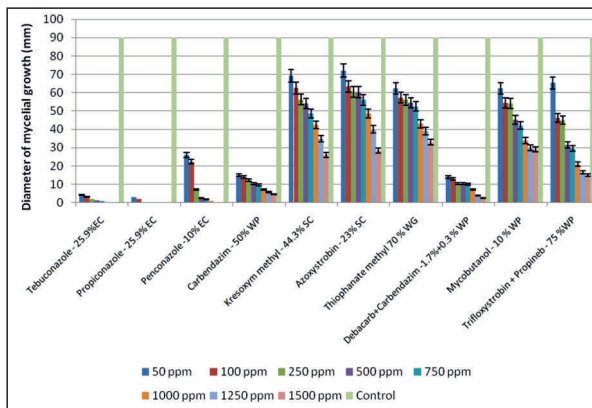
The *in vitro* experiments were replicated three times, while the field experiments were carried out in Puliyankandi village over a three-year period (2020-2022). Ten trees exhibiting leaf blight symptoms were randomly chosen for each treatment and assessed. Prior to ANOVA analysis, the percentage values of disease indices were subjected to arcsine transformation. The means  $\pm$  standard errors were calculated, and the data were analyzed using ANOVA at a significance level of  $P < 0.05$ . Post-ANOVA, mean comparisons were conducted using Duncan's Multiple Range Test (DMRT).

### Results and Discussion

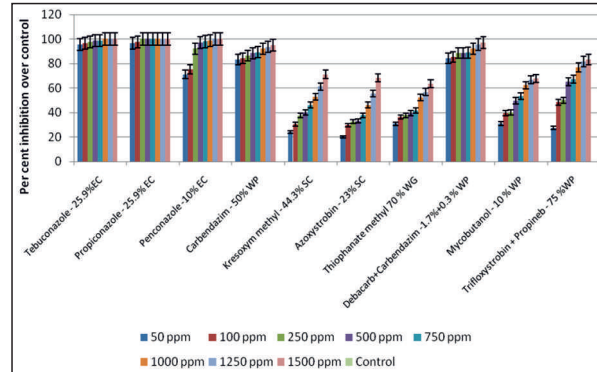
#### *In vitro* Evaluation of Fungicides

In this study, the effectiveness of ten fungicides against *L. theobromae* was evaluated using the poisoned food technique with different concentrations. The outcomes of the *in vitro* screening revealed that propiconazole demonstrated inhibitory effects on fungal growth at concentrations as low as 250 ppm, maintaining efficacy up to 1500 ppm. Conversely, tebuconazole exhibited efficacy starting from 1000 ppm, while penconazole initiated control of the fungus at concentrations of 1250 ppm (Fig 1). Due to their effectiveness at concentrations as low as 250 ppm, propiconazole, tebuconazole, and penconazole were selected for further field studies (Fig 2).

**Fig1. Assessment of fungicides against *Lasiodiplodia theobromae*, the pathogen causing Coconut Leaf Blight, under *in-vitro* conditions**



**Fig2. Assessment of fungicides efficacy against *Lasiodiplodia theobromae* under *in-vitro* conditions**



The inhibitory effect of carbendazim on the mycelial growth of *L. theobromae* is consistent with previous studies (Khazada *et al.*, 2005). Similarly, carbendazim has been shown to be effective against *Botryodiplodia theobromae* in prior research (Yadav and Majumdar, 2004). However, recent findings by Baloch *et al.* (2017) demonstrated that the combination of tebuconazole and trifloxystrobin inhibited mycelial growth at 100 ppm. Notably, the mycelium of *L.theobromae* displayed structural abnormalities in response to fungicide treatment, including shrinkage and shriveling, possibly due to the leakage of protoplasmic contents from the hyphae. This observation aligns with Mahmood *et al.* (2002), who reported complete inhibition of *L theobromae* colony growth at 50 ppm of thiophanate-methyl and 100 ppm of benomyl.

In the field study, sequential application of propiconazole @ 5% (100 ml per palm) during first and third quarter of the year, followed by tebuconazole @ 5% ml (100 ml per palm) during second and fourth quarter of the year, significantly reduced leaf blight in coconut. Six months post-treatment, leaf blight incidence decreased to 5.58%, accompanied by an increased nut yield of 135 nuts per year per palm, compared to 99 nuts per palm per year in the control group (Table 2). These results are consistent with those of Johnson *et al.* (2017), who found that root feeding combined with carbendazim at a rate of 2 g/100 ml of water effectively reduced disease incidence. Fungicides have also been successful in preventing *L.theobromae*-induced stem end rot in mango fruits (Muller and Burt, 2000). Mahmood *et al.* (2002) reported that foliar spraying Thiophanate-methyl

**Table 2.** Evaluation of sequential use fungicides against leaf blight disease

Treatments	Leaf blight intensity (PDI)			Leaf blight severity (%)			Nuts yield (palm/year)	C:B ratio
	Before imposition of treatment	6 Months later	Reduction after imposition of treatment	Before imposition of treatment	6 Months later	Reduction after imposition of treatment		
Tebuconazole 25.9% EC @ 5% (100 ml/ Tree) Root feeding four time in a year at three months interval	24.49	21.46	3.02 (9.9)	100	82.9	17.1	109.5	1:1.05
Propiconazole 25.9% EC @ 5% (100 ml/ Tree) Root feeding four time in a year at three months interval	27.78	24.39	3.38	100 (10.5)	82.9	17.1	113.5	1:1.25
Root feeding of Tebuconazole 25.9% EC @ 5% (100 ml/ Tree) First and Third Quarter + Propiconazole 25.9% EC @ 5% (100 ml/ Tree) Second and Fourth Quarter	32.49	28.38	4.11 (12.8)	100	82.9	17.1	122.0	1:1.38
Root feeding of Propiconazole 25.9% EC @ 5% (100 ml/ Tree) First and Third Quarter + Tebuconazole 25.9% EC @ 5% (100 ml/ Tree) Second and Fourth Quarter	31.95	26.28	5.66 (13.9)	100	74.7	25.2	132.5	1:1.40
Control	33.45	33.68	-0.22 (2.5)	-			98.5	-
Sed			0.76				3.11	
CD(P=0.05)			1.64				6.55	

at a rate of 1g/l decreased *L. theobromae* infestation to 10%, with a second application completely halting the fungus. However, the effectiveness of carbendazim in the field is diminishing, with many fungi developing resistance to it (Tzec-Sima *et al.*, 2022). Tebuconazole and propiconazole are

extensively used in agriculture as foliar sprays (Loughman and Thomas, 1992; Cabras *et al.*, 1997). These triazoles inhibit the formation of ergosterol, an essential component of fungal cell membranes, by binding to the heme of the cytochrome P-450 enzyme 14 $\alpha$ -demethylase with

the N-1 substituent of the azole ring (Joseph-Horne *et al.*, 1995). This disruption of fungal membranes typically results in the death of the organism (Eaton and Hale, 1993). While triazoles can inhibit the growth of many fungi, they can also be modified or degraded by various fungal species (Woo *et al.*, 2010).

Assessing the effectiveness of various fungicides on *B. theobromae* revealed a reduction in colony growth with increasing fungicide concentration. Tebuconazole 50% + Trifloxystrobin 25% WG emerged as the most effective, displaying colony diameters of 2.03, 3.50 and 4.80 mm at 100, 50 and 30 ppm, respectively. Propineb 70% WP exhibited moderate effectiveness with colony diameters at the same concentrations. Tebuconazole 50% + trifloxystrobin 25% WG and propineb 70% WP outperformed Fosetyl Al 80% WP and penconazole 100% EC (Adnan *et al.*, 2017). Safdar *et al.* (2015) observed significant inhibition of *B. theobromae* colony growth with various fungicides, with carbendazim proving the most successful. Sahi *et al.* (2012) reported that fungicides against *B. theobromae* exhibited increased efficacy at higher dosage rates, with chlorothalonil and thiophanate-methyl showing the greatest efficacy in preventing mycelial growth.

There is a significant lack of information regarding the control of *L.theobromae* in sapote mamey grafts, particularly concerning the physical or chemical treatment of vegetative material used in grafting. In Mexico, the incidence of dieback in sapote mamey grafts caused by *L.theobromae* exceeds 70% (Tovar *et al.*, 2012). Despite the demonstrated sensitivity of *L. theobromae* to fungicides in multiple in vitro tests, there is a notable absence of specific studies addressing experiments for controlling *L. theobromae* isolates from sapote mamey (Bester *et al.*, 2007; Da Silva *et al.*, 2012). The discussion here highlights the gap in knowledge regarding the management of *L.theobromae* in sapote mamey grafts, emphasizing the need for further research to develop effective control strategies in this specific context. It acknowledges the existing literature on the sensitivity of *L.theobromae* to fungicides but underscores the lack of targeted studies addressing control measures for this particular application.

In a study targeting *Alternaria triticina*, propiconazole, hexaconazole, carboxin 37.5% + thiram 37.5% DS, and neem leaf extract were tested for their impact on the pathogen's growth. The results indicated varying degrees of growth inhibition, with propiconazole demonstrating the highest inhibition followed by hexaconazole. The use of chemical fungicides such as thiophanate methyl, carbendazim, hexaconazole, ridomil, and propicanazole has been suggested for suppressing *Alternaria* (Kakraliya *et al.*, 2017). These synthetic fungicides effectively inhibit pathogens by interfering with metabolic processes, rupturing cell membranes, or permeability.

Examining the yield effects and cost-benefit analysis revealed that Propiconazole had the highest yield at 37.00 q/ha, subsequently by *Pseudomonas fluorescens* (30.44 q/ha). Chemical inducers impacted plant growth significantly, with some treatments reducing growth but contributing to the highest yield. Among the treatments, vitavax at 0.25% had the best and most economical yield, with a cost-benefit ratio of 1:1.52. This was followed by *Trichoderma viride* (1:1.43) and propiconazole @ 0.1% (1:1.42), also performed well, while the control (1:1.03) had the lowest cost-benefit ratio.

These findings collectively underscore the potential of combining synthetic fungicides and beneficial microorganisms, such as *P. fluorescens* and *T. viride*, or the use of specific fungicides, like propiconazole, hexaconazole, and vitavax, to significantly enhance yields and offer cost-effective disease management solutions.

## Conclusion

In our comprehensive study aimed at evaluating the effectiveness of various modern fungicidal agents for the management of coconut leaf blight, it has become evident that systemic fungicides, specifically propiconazole and tebuconazole, displayed remarkable inhibitory effects on the mycelial growth of *Lasiodiplodia theobromae*. Propiconazole was found to be highly effective at a concentration of 250 ppm, while Tebuconazole exhibited strong inhibitory action at 1000 ppm. Further analysis of our experimental data revealed that a treatment regimen involving the sequential application of propiconazole @ 5% (100 ml per

palm) during first and third quarter of the year, followed by tebuconazole @ 5% ml (100 ml per palm) during second and fourth quarter of the year was exceptionally successful in curtailing the incidence of leaf blight disease in coconut plantations. After a six-month observation period, the incidence was substantially reduced to a mere 5.58%. Moreover, this treatment regimen had a substantial positive impact on nut yield, with an increase to 135 nuts per palm per year, as compared to the 99 nuts per palm per year recorded in the control group. This would provide valuable guidance to farmers to adopt sequential root feeding of fungicide for effective coconut leaf blight management, ultimately benefiting the coconut industry in the region.

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