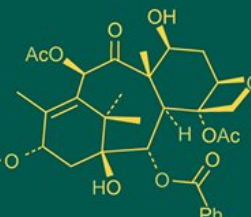
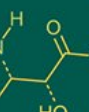
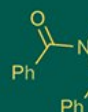
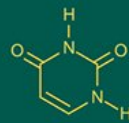


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693

ISSN Online: 2617-4707

NAAS Rating (2026): 5.29

IJABR 2026; 10(9): 138-142

www.biochemjournal.com

Received: 04-07-2026

Accepted: 07-08-2026

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In vitro evaluation of fungicides against different diseases of kokum caused by *Pestalotiopsis Maculans* and *Colletotrichum Gloeosporioides*

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DOI: <https://www.doi.org/10.33545/26174693.2026.v10.i9b.9883>

Abstract

Kokum (*Garcinia indica* Choisy) is an important minor fruit crop of the Western Ghats and Konkan region of India. Fungal diseases associated with kokum can adversely affect plant health and productivity, necessitating the identification of effective management measures. The present investigation was undertaken to evaluate the *in vitro* efficacy of selected fungicides against *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides* associated with kokum. Seven fungicides, namely Carbendazim 50% WP, Propiconazole 25% EC, Copper oxychloride 50% WP, Myclobutanil 10% WP, Captan 50% WP, Chlorothalonil 75% WP and Cymoxanil 8% + Mancozeb 64% WP, were evaluated using the poisoned food technique in a Completely Randomized Design with three replications. Propiconazole 25% EC at 0.1% and Cymoxanil 8% + Mancozeb 64% WP at 0.2% completely inhibited the mycelial growth of *P. maculans*, recording 100% inhibition. Captan and Carbendazim recorded 88.15 and 87.78 per cent inhibition, respectively. Against *C. gloeosporioides*, Propiconazole 25% EC at 0.1% was the most effective treatment, recording complete inhibition, followed by Carbendazim 50% WP (82.04%) and Cymoxanil 8% + Mancozeb 64% WP (80.93%).

Keywords: Kokum, pestalotiopsis maculans, colletotrichum gloeosporioides, fungicides

Introduction

Kokum (*Garcinia indica* Choisy), a tropical fruit-bearing tree belongs to the family Clusiaceae and native to the Western Ghats of India, holds significant economic and medicinal value. It is one of the most significant fruits cultivated in the Konkan region, typically grown in arable wastelands characterized by high rainfall and a hot, humid climate. The environmental conditions in the Konkan region of Maharashtra are particularly well-suited for kokum cultivation, making it a prominent cash crop in this area and its surrounding regions.

The proximate composition of Kokum rind is moisture (80.0 g/100 g), protein (1.0 per cent), fat (1.4 per cent), tannin (1.7 per cent), pectin (0.9 per cent), total sugars (4.1 per cent), organic acids such as hydroxy citric acid, lactone and citric acid; anthocyanins, cyanidin-3-glucoside and cyanidin-3-sambubioside; poly isoprenylated phenolics, garcinol and isogarcinol (traces) (Jagtap *et al.*, 2015) [9]. When fruits are unripe, they are green in colour and when completely ripe their colour changes from scarlet to dark purple. The kokum fruit is oval, varies in size and weighs between 50 to 180 grams. The fruits are 2.5 to 3.75 cm in diameter, globule or spherical, dark purple when ripe and contain 5-8 seeds (Nayak *et al.*, 2010) [14].

In India, kokum is widely used as a spice and serves as a natural acidulant in the preparation of juices, pickles and curries. The fruit is rich in antioxidants, which help neutralize free radicals and protect body cells from oxidative damage, while also promoting cell regeneration and repair. Kokum juice is especially favoured during the hot summer months due to its natural cooling properties. A popular beverage known as 'Amrut Kokum', made with sugar syrup, is traditionally used to prevent sunstroke. In Ayurvedic medicine, various parts of the kokum plant including the rind, leaves and other tissues are utilized to treat inflammatory conditions, rheumatic pain and digestive disorders. Kokum oil also serves as an emollient and antiseptic. It is used to reduce fever and alleviate allergic reactions. kokum

is one of the important fruits of Western Ghats of India and has tremendous medicinal and nutraceutical values. It is rich in anti-oxidants. A number of recent studies have examined the potential of g Garcinol, a non-nutritive dietary component, against different cancer types such as breast cancer, kidney cancer, lung cancer, pancreatic cancer, prostate cancer and tongue cancer (Saadat and Gupta, 2012) [19].

Despite its economic, medicinal and nutraceutical importance, kokum cultivation is affected by various fungal diseases that can adversely influence plant health and productivity. Among the pathogens associated with kokum, *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides* are important fungal pathogens. Effective management of fungal diseases largely depends on the use of suitable fungicides. However, information on the efficacy of fungicides against these pathogens associated with kokum is limited. Therefore, the present investigation was undertaken to evaluate the *in vitro* efficacy of selected fungicides against *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides* under laboratory conditions.

Materials and Methods

Isolation and proving pathogenicity of causal organisms

Naturally infected kokum leaves showing typical disease symptoms were collected from the Horticulture Nursery, College of Horticulture, Dapoli and brought to the laboratory for isolation. The diseased portions were cut into approximately 5 mm pieces containing both healthy and diseased tissues. The pieces were surface disinfected with 0.1% sodium hypochlorite, followed by three successive washes with sterile distilled water. The tissue pieces were aseptically placed on potato dextrose agar (PDA) medium and incubated at 27 ± 2°C for seven days. The emerging fungal growth was sub-cultured to obtain pure cultures. Pathogenicity was tested on one-month-old healthy potted kokum plants using the pin-prick method. Spore suspension prepared from ten-day old cultures was sprayed on leaves until runoff. Inoculated plants were covered with polythene bags to maintain humidity, while plants sprayed with sterile water served as control.

In vitro evaluation of fungicides

The present study was conducted at the Department of Plant Pathology, College of Agriculture, Dapoli, Dist. Ratnagiri. *In vitro* study of fungicides against *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides* was carried out.

Seven fungicides representing different fungicidal groups were evaluated against the two pathogens. The fungicides and concentrations used were Carbendazim 50% WP at 0.1%, Propiconazole 25% EC at 0.1%, Copper oxychloride 50% WP at 0.25%, Myclobutanil 10% WP at 0.1%, Captan 50% WP at 0.2%, Chlorothalonil 75% WP at 0.2% and Cymoxanil 8% + Mancozeb 64% WP at 0.2%. An untreated medium served as the control.

The fungicides were evaluated using the Poisoned Food Technique described by Nene and Thapliyal (1993) [15]. The experiment was conducted in Completely Randomized Design (CRD) with three replications. Mycelial growth was recorded as mean colony diameter, and the percentage inhibition of mycelial growth over the untreated control was calculated. Observations were recorded seven days after incubation.

The percentage inhibition of mycelial growth was calculated by using the formula given by Vincent (1947) [23].

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

Where,

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

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

Results and Discussion

Isolation and proving pathogenicity of causal organisms

Diseased kokum leaves yielded two distinct fungal isolates on PDA medium after seven days of incubation at 27 ± 2 °C. Based on cultural and morphological characteristics, the isolates were identified as *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides*. Pathogenicity testing confirmed that both isolates were capable of producing characteristic symptoms on artificially inoculated kokum leaves within 10-12 days, whereas the uninoculated control plants remained healthy. The respective pathogens were successfully re-isolated from the artificially infected leaves, and their cultural and microscopic characteristics corresponded with those of the original isolates, thereby confirming Koch's postulates. The pathogenicity of *C. gloeosporioides* was comparable with the findings of Abera *et al.* (2016) [1]. and Golakiya *et al.* (2020) [6]. Similarly, the pathogenicity of *Pestalotiopsis* spp. has been established by Ismail *et al.* (2013) [8]. and Bhagariya (2019) [2].

In vitro evaluation of fungicides against *Pestalotiopsis maculans*

The results presented in Table No.1 and illustrated in Plate No. I and Fig. No. 1, indicated that all the fungicides tested significantly suppressed the growth of *Pestalotiopsis maculans*. Among the fungicides evaluated, Propiconazole 25% EC at 0.1% and Cymoxanil 8% + Mancozeb 64% WP at 0.2% were the most effective treatments, completely inhibiting mycelial growth and recording 100% inhibition. Captan 50% WP at 0.2% produced 88.15% inhibition, closely followed by Carbendazim 50% WP at 0.1%, which recorded 87.78% inhibition. Chlorothalonil 75% WP at 0.2% resulted in 73.33% inhibition, whereas Myclobutanil 10% WP at 0.1% recorded 67.96% inhibition. Copper oxychloride 50% WP was the least effective treatment, recording 38.70% inhibition of mycelial growth.

The strong activity of Propiconazole observed in the present study agrees with earlier reports. Hoque *et al.* (2016) [7]. reported complete inhibition of *Pestalotiopsis palmarum* with Propiconazole 25% EC, while Devi *et al.* (2017) [5]. recorded complete inhibition of *Pestalotiopsis mangiferae* and Kyada (2006) [11], recorded 99.77% inhibition of *Pestalotiopsis guepinii* with propiconazole.

The present findings are further supported by Nakum (2017) [13]. and Das *et al.* (2020) [3], who reported cent per cent inhibition of *Pestalotiopsis anacardii* and *P. mangiferae*, respectively, by Cymoxanil 8% + Mancozeb 64% WP under *in vitro* conditions. Likewise, Bhagariya (2019) [2]. reported that propiconazole was among the most effective systemic fungicides, while Cymoxanil 8% + Mancozeb 64% effectively suppressed the mycelial growth of *Pestalotiopsis clavispora*.



Plate 1: In vitro evaluation of fungicides against *Pestalotiopsis maculans*

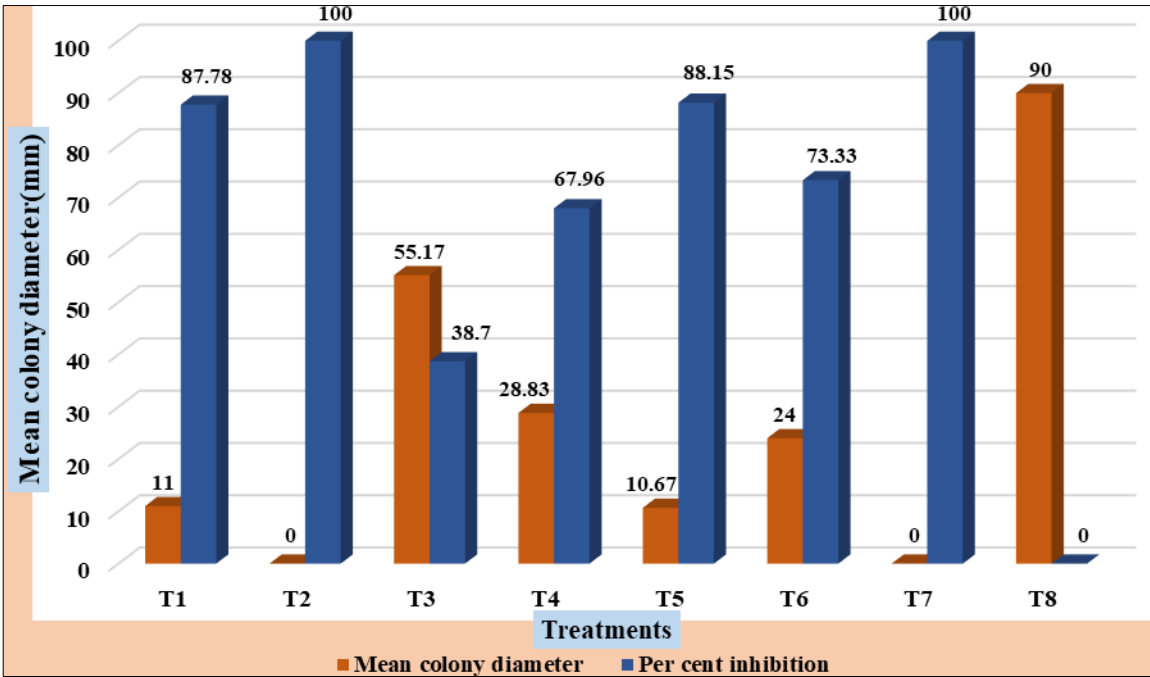


Fig 1: In vitro evaluation of fungicides against *Pestalotiopsis maculans*

Table 1: In vitro evaluation of fungicides against *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides*.

Tr. No.	Fungicides	Conc. (%)	Mean colony dia.* (mm)		Per cent inhibition	
			Pm	Cg	Pm	Cg
T ₁	Carbendazim 50% WP	0.1	11.00	16.17	87.78	82.04
T ₂	Propiconazole 25% EC	0.1	00.00	00.00	100	100
T ₃	Copper oxychloride 50% WP	0.25	55.17	50.00	38.70	44.44
T ₄	Myclobutanil 10% WP	0.1	28.83	26.00	67.96	71.11
T ₅	Captan 50% WP	0.2	10.67	33.17	88.15	63.15
T ₆	Chlorothalonil 75% WP	0.2	24.00	71.83	73.33	20.19
T ₇	Cymoxanil 8% + Mancozeb 64% WP	0.2	00.00	17.17	100	80.93
T ₈	Control	-	90.00	90.00	-	-
SEm±			0.33	0.29		
C. D. @ 1%			1.38	1.20		

*Average of three replications, Pm-*Pestalotiopsis maculans*, Cg-*Colletotrichum gloeosporioides*

In vitro evaluation of fungicides against *colletotrichum gloeosporioides*

The fungicides also differed significantly in their ability to inhibit the growth of *C. gloeosporioides*. Propiconazole

25% EC at 0.1% was the most effective treatment and completely inhibited mycelial growth, recording 100% inhibition. Carbendazim 50% WP at 0.1% ranked second with 82.04% inhibition, followed by Cymoxanil 8% +

Mancozeb 64% WP at 0.2%, which recorded 80.93% inhibition of mycelial growth. The results pertaining to the efficacy of the tested fungicides against *Colletotrichum gloeosporioides* are presented in Table No. 1 and illustrated in Plate No. II and Fig. No.2

The complete inhibition of *Colletotrichum gloeosporioides* by Propiconazole 25% EC and the high efficacy of Carbendazim 50% WP recorded in the present investigation are in agreement with the findings of Singh (2012) [21], Ranjitha *et al.* (2019) [18], Sharma *et al.* (2019) [20], and Vandana *et al.* (2021) [22], who reported cent per cent (100%) inhibition of *C. gloeosporioides* by propiconazole under *in vitro* conditions. Patil *et al.* (2010) [17], also reported complete inhibition by propiconazole, while

carbendazim recorded 91.67% inhibition. Similar observations were made by Dev *et al.* (2016) [4], who reported cent per cent inhibition of *C. gloeosporioides* with both propiconazole and carbendazim under *in vitro* conditions. Comparable results were also reported by Kothikar and Koche (2017) [10], in *Colletotrichum dematium* and by Padghan *et al.* (2023) [16], in *Colletotrichum capsici*, where propiconazole also exhibited cent per cent inhibition. Mishra and Pan (2008) [12], reported cent per cent (100%) growth inhibition of both *Pestalotia psidii* and *Colletotrichum gloeosporioides* by Propiconazole 25% EC under *in vitro* conditions, supporting its high efficacy against both pathogens.



Plate 2: *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides*

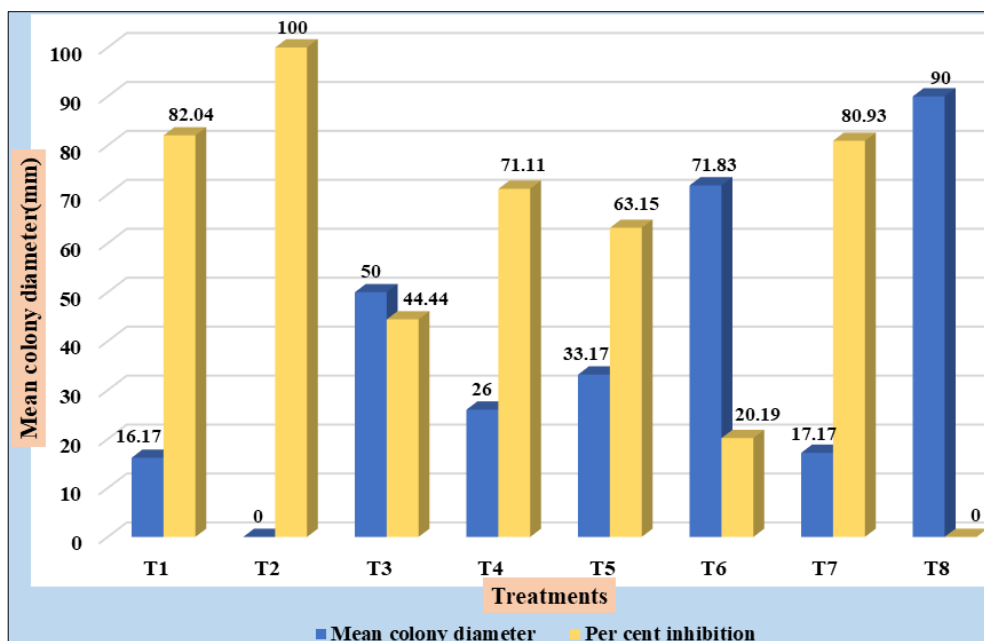


Fig 2: *In vitro* evaluation of fungicides against *Colletotrichum gloeosporioides*

Conclusion

The present investigation demonstrated significant variation in the *in vitro* efficacy of the tested fungicides against *Pestalotiopsis maculans* and *Colletotrichum gloeosporioides*

associated with kokum. Propiconazole 25% EC at 0.1% concentration was the most effective fungicide against both pathogens and completely inhibited their mycelial growth. Cymoxanil 8% + Mancozeb 64% WP at 0.2% also produced

complete inhibition of *P. maculans*. Carbendazim 50% WP showed comparatively high activity against both pathogens.

Acknowledgments

The authors gratefully acknowledge the Department of Plant Pathology, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, for providing the laboratory facilities and necessary support for conducting the present investigation.

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