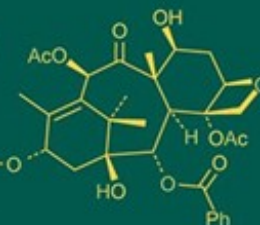
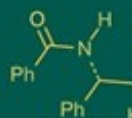
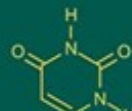
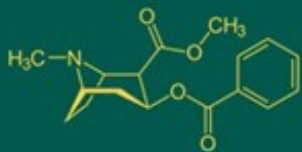


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In vitro efficacy of fungicides against fungal pathogens associated with boll rot of cotton

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ABSTRACT

Boll rot is a destructive fungal disease complex of cotton causing significant yield and fibre quality losses. Four pathogens *Aspergillus niger*, *Fusarium* sp., *Rhizopus* sp. and *Alternaria* sp. were isolated and tested against seven fungicides in vitro using the poisoned food technique. Propiconazole 25% EC was the most effective, showing the lowest overall colony diameter (2.37 mm) and 100% inhibition of *A. niger*, *Rhizopus* sp. and *Alternaria* sp., while providing 89.43% inhibition of *Fusarium* sp. Azoxystrobin + Tebuconazole and Tebuconazole were the next most effective, whereas Propineb was least effective. Propiconazole showed strong potential for managing the cotton boll rot complex.

Keywords: Fungicides, Poisoned food technique, Propiconazole, *Aspergillus niger*, *Rhizopus*, Boll rot

1. Introduction

Cotton (*Gossypium* sp.) is cultivated in tropical and subtropical regions and plays a crucial role in global agricultural economies by providing fibre, edible oil, livestock feed and industrial raw materials. In recent years, global cotton production has remained relatively stable, with approximately 26 million tonnes of lint produced worldwide during the 2025-26 season, indicating continued dominance of cotton as a major natural fibre crop in the international textile sector (ICAC, 2026) [1]. India continues to occupy a prominent position as the second-largest cotton producer globally, with an estimated production of about 23.2 million 480-lb bales in 2024-25 and 23.5 million bales projected for 2025-26, cultivated across approximately 11.2 million hectares (USDA-FAS, 2025-26) [2]. Indian cotton production is spread across a wide array of agro-ecological zones, ranging from irrigated, high-input farming systems to low-input, rainfall-dependent regions. Nearly sixty per cent of the country's cotton growing area is rainfed, which means that fluctuations in monsoon patterns, prolonged dry spells and uneven rainfall distribution directly influence crop growth and boll retention (Venugopalan *et al.*, 2019) [3]. The boll development phase in particular is highly sensitive to environmental stress and any disruption during this stage affects fibre formation and ultimately determines economic yield.

Cotton is exposed to several diseases during its lifecycle, including *Fusarium* wilt, *Verticillium* wilt, *Alternaria* leaf spot, bacterial blight, grey mildew and cotton leaf curl disease (Hillocks, 1992; Ali *et al.*, 2019) [5, 4]. While foliar diseases generally reduce photosynthetic efficiency and overall plant vigour, diseases affecting the reproductive structures, especially bolls, pose a much greater economic threat. Among these, boll rot is one of the most destructive problems faced by cotton growers. Unlike many diseases caused by a single pathogen, boll rot constitutes a complex involving multiple fungal and bacterial organisms that invade the boll tissue under favourable environmental conditions (Mani *et al.*, 2017) [6].

The boll rot complex includes pathogens such as *Fusarium*, *Aspergillus*, *Rhizopus*, *Penicillium* and *Xanthomonas* species (Kumar *et al.*, 2016) [7]. These organisms typically enter the boll through natural openings or injuries caused by insects, rain induced cracks or mechanical abrasions. In many cases, the role of insect pests especially the pink bollworm is critical, as larval feeding creates ideal entry points for infection (Gopalakrishnan *et al.*, 2020) [8].

Under such circumstances, boll rot can cause severe yield losses, with certain reports documenting reductions ranging from 15% to more than 50% (Patel *et al.*, 2015) [9]. Although research on specific pathogens related to boll rot is available, comprehensive work on the entire disease complex its epidemiology, environmental triggers, varietal responses and integrated management is still limited (Meena *et al.*, 2018; Thombre *et al.*, 2021) [10, 11]. This gap was particularly evident in major cotton-growing regions such as Maharashtra, where climatic variability, prolonged humidity and pest interactions often intensify the disease. Farmers frequently resort to indiscriminate chemical applications without clear information on causal organisms or effective control strategies, leading to inconsistent results and higher production costs.

With this background, the present study was undertaken to evaluate the comparative *in vitro* efficacy of seven commonly used fungicides, representing different chemical groups and modes of action, against the four fungal pathogens *Aspergillus niger*, *Fusarium* sp., *Rhizopus* sp. and *Alternaria* sp. associated with boll

rot of cotton in this region, with the objective of identifying the most promising candidate(s) for field level validation.

2. Materials and Methods

2.1 Experimental site

The present investigation was carried out during *Kharif*, 2025-26 in the laboratory of the Department of Plant Pathology, Government College of Agriculture, Dhule, Maharashtra.

2.2 Collection of disease samples

The rotted cotton bolls were collected from different cotton growing areas of Dhule, Nandurbar and Jalgaon district for isolation of pathogens.

2.3

Isolation of pathogens

The diseased portion of the rotted cotton boll was washed thoroughly in tap water to remove dirt and then blot dried. For isolation of the pathogens, the diseased portion of the cotton boll was cut into small pieces of 2 to 3 mm size with sterilized blade in such a way that half portion of healthy tissue must be present with it. These small pieces were surface sterilized in 0.1% mercuric chloride solution (HgCl_2) for 30 seconds followed by washing in three successive changes of distilled water to remove the traces of mercuric chloride and then dried on clean blotting paper. Three to four such pieces were aseptically transferred to Petri plates containing potato dextrose agar medium. The plates were incubated at $27 \pm 2^\circ\text{C}$ for seven days and examined at regular intervals to see the growth of fungus surrounding the pieces. Soon after the fungal growth was noticed, well isolated fungal colony free from contamination was sub-cultured and transferred to agar slants by hyphal tip method. The pure culture obtained was maintained for further studies.

2.4 Purification of the isolated organisms

Purification of the fungal species was done by transferring small bit of hypha to PDA slants in aseptic condition.

2.5 In vitro evaluation of fungicides (poisoned food technique)

The efficacy of different fungicides for controlling the pathogen responsible for boll rot of cotton was determined by poison food technique on solid media. The medium was distributed as 100 ml in each 250 ml Erlenmeyer flasks and sterilized in autoclave at 15 lbs pressure for 15 minutes. The measured quantity of various fungicides was added in the flask after sufficient cooling so as to get the required final concentration with suitable control. A series of concentration were prepared. The fungicides were thoroughly mixed by stirring the flasks. The medium was then poured into Petri plates with three replications for each treatment.

Seven days old culture grown on potato dextrose agar medium was used as inoculum. The plates were inoculated with 5 mm of each fungal disc removing with a sterilized cork borer and was transferred aseptically at the centre of the petri plates containing PDA medium with measured quantities of fungicides. Plates with PDA medium without fungicide served as control. These petriplates were then incubated at $27 \pm 1^\circ\text{C}$ for 7 days. Observations on colony diameter and sporulation of the test pathogens were recorded. The per cent inhibition of mycelial growth of inoculated pathogens were worked out by using a following formula.

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

Where,

I = Percent inhibition on fungal growth.

C = Growth in mm on the 7th day after inoculation in control.

T = Growth in mm on the 7th day after inoculation in treatment.

2.6 Statistical analysis

Data on colony diameter and per cent inhibition were statistically analysed following *Panase et al. (1978)* [12]; standard error (S.E.m $\pm$) and critical difference (C.D. at 1%) were computed for each pathogen.

3. Results and Discussion

The effectiveness of seven fungicides, namely Copper oxychloride 50% WP (0.25%), Tebuconazole 25.9% EC (0.05%), Carbendazim 50% WP (0.1%), Propiconazole 25% EC (0.1%), Propineb 70% WP (0.1%), Pyraclostrobin 20% WG (0.25%) and Azoxystrobin 11% + Tebuconazole 18.3% SC (0.01%), was assessed under *in vitro* conditions against four fungal pathogens, *Aspergillus niger*, *Fusarium* sp., *Rhizopus* sp. and *Alternaria* sp., using the poisoned food technique to determine their ability to suppress the growth of boll rotting fungi. The findings are summarized in the *Table No. 2* and presented in Plate No. 1, 2, 3, 4 and *Fig 1*.

Among the tested fungicides, Propiconazole 25% EC (T_4) was found to be the most effective treatment against all four pathogens, recording the lowest overall mean colony diameter of 2.37 mm. It caused complete inhibition (100%) of *Aspergillus niger*, *Rhizopus* sp. and *Alternaria* sp., with zero colony growth. Against *Fusarium* sp., it recorded a colony diameter of 9.51 mm with 89.43% inhibition. These results demonstrated the superior broad spectrum antifungal activity of Propiconazole.

The next most effective treatment was Azoxystrobin 11% + Tebuconazole 18.3% SC (T_7), which recorded an overall mean colony diameter of 3.37 mm for all pathogens. It completely inhibited (100%) *Aspergillus niger*, *Fusarium* sp. and *Alternaria* sp., while *Rhizopus* sp. recorded a colony diameter of 13.49 mm with 85.01% inhibition. Similarly, Tebuconazole 25.9% EC (T_2) was also highly effective, recording an overall mean colony diameter of 4.43 mm. It resulted in complete inhibition (100%) of *Aspergillus niger*, *Fusarium* sp. and *Alternaria* sp. Against *Rhizopus* sp., it restricted colony growth to 17.73 mm, corresponding to 80.29% inhibition.

Copper oxychloride 50% WP (T_1) showed moderate effectiveness, with an overall mean colony diameter of 16.79 mm against the pathogens. It completely inhibited *Rhizopus* sp. (100% inhibition) and showed strong suppression of *Fusarium* sp. (91.82% inhibition) and *Alternaria* sp. (84.18% inhibition), with colony diameters of 7.36 mm and 14.23 mm, respectively. However, it was comparatively less effective against *Aspergillus niger*, which recorded a colony diameter of 45.57 mm and 49.37% inhibition.

Pyraclostrobin 20% WG (T_6) recorded an overall mean colony diameter of 19.79 mm. It was highly effective against *Alternaria* sp., with a colony diameter of 5.43 mm and 93.97% inhibition. It also showed considerable inhibition of *Fusarium* sp. (83.51%) and *Aspergillus niger* (77.02%), with colony diameters of 14.83 mm and 20.68 mm, respectively. Its effectiveness was comparatively lower against *Rhizopus* sp., which recorded a colony diameter of 38.23 mm and 57.51% inhibition.

Carbendazim 50% WP (T_3) recorded an overall mean colony diameter of 27.13 mm. It completely inhibited (100%) both *Aspergillus niger* and *Fusarium* sp. However, it was less effective against *Rhizopus* sp., which recorded a colony diameter of 60.26 mm with only 33.04% inhibition. Against *Alternaria* sp., a colony diameter of 48.27 mm and 46.36% inhibition were recorded.

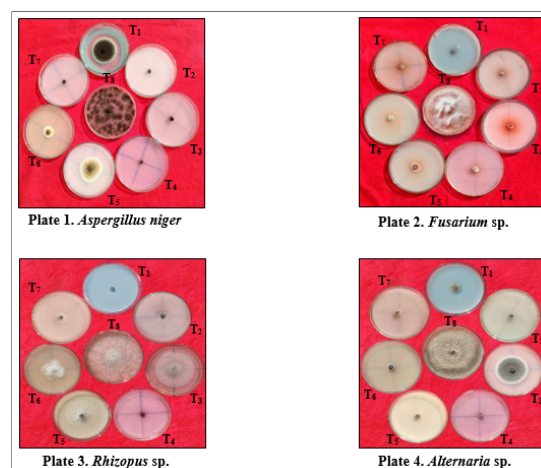
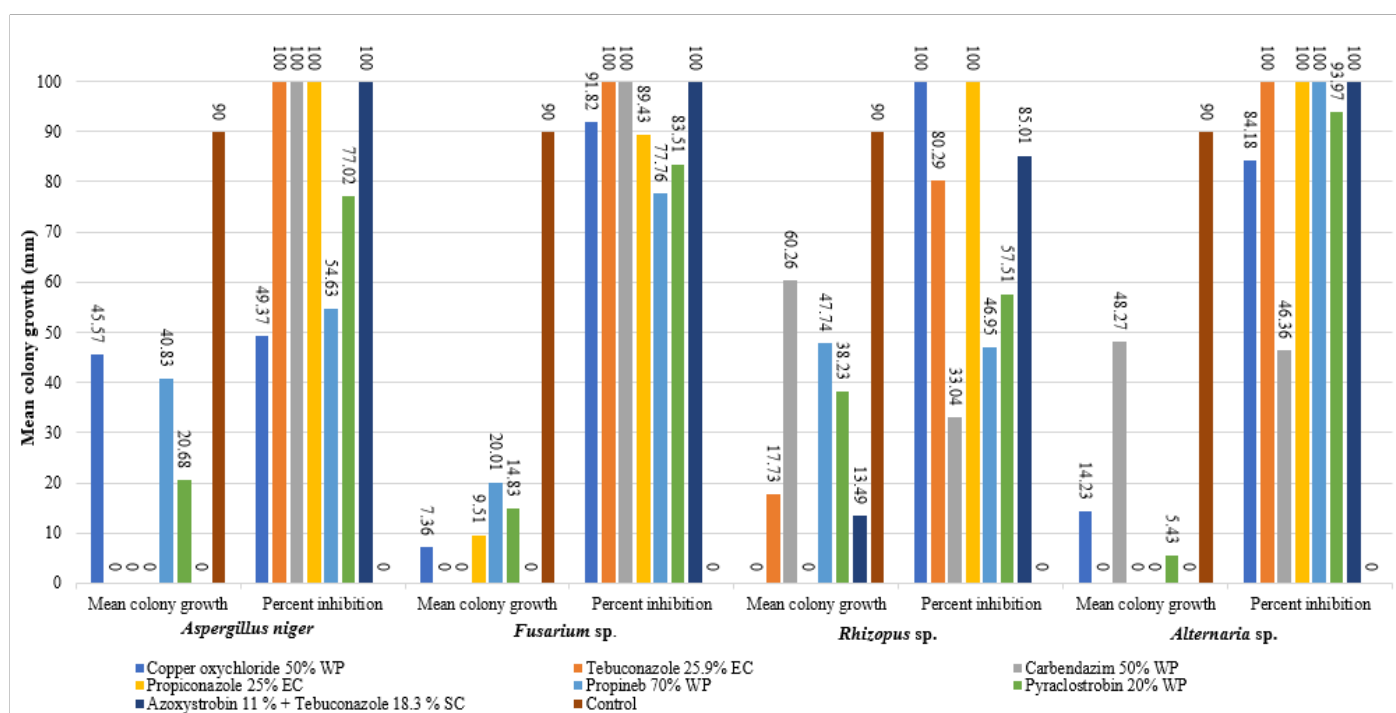
Table 1. List of fungicides evaluated under *in vitro* condition

Sr. No.	Fungicide	Concentration
1	Copper oxychloride 50% WP	0.25%
2	Tebuconazole 25.9% EC	0.05%
3	Carbendazim 50% WP	0.1%
4	Propiconazole 25% EC	0.1%
5	Propineb 70% WP	0.25%
6	Pyraclostrobin 20% WG	0.1%
7	Azoxystrobin 11% + Tebuconazole 18.3% SC	0.1%
8	Control (Untreated)	-

Propineb 70% WP (T₅) was the least effective fungicide based on overall mean colony diameter, recording the highest mean colony diameter of 27.14 mm among the fungicidal treatments. It completely inhibited *Alternaria* sp. (100% inhibition), while *Fusarium* sp. showed a colony diameter of 20.01 mm with 77.76% inhibition. Comparatively lower inhibition was observed against *Aspergillus niger* (54.63%) and *Rhizopus* sp. (46.95%), which recorded colony diameters of 40.83 mm and 47.74 mm, respectively.

Overall, Propiconazole 25% EC proved to be the most effective fungicide under *in vitro* condition, followed by Azoxystrobin 11% + Tebuconazole 18.3% SC and Tebuconazole 25.9 % EC. These systemic fungicides, particularly triazoles either alone or in combination with strobilurins, demonstrated excellent inhibitory activity against the boll rotting fungi. Carbendazim, Pyraclostrobin and Copper oxychloride exhibited moderate performance while Propineb showed comparatively lower broad spectrum effectiveness.

Similar line of result was obtained by Northover *et al.* (2002) [13] they reported that fludioxonil and tebuconazole significantly reduced *Rhizopus* rot incidence. They concluded that postharvest fungicide application is effective when combined with proper handling. Arun Kumar (2008) [14] reported that among nine fungicides evaluated under *in vitro* conditions, propiconazole and hexaconazole at all tested concentrations (0.1%, 0.2% and 0.3%) completely inhibited the mycelial growth of *A. alternata* infecting cotton. Perane *et al.* (2015) [15] observed 88.75% inhibition of mycelial growth of *A. macrospora* with propiconazole 25% EC.

**In vitro efficacy of fungicides against boll rotting fungi of cotton****Figure 1.** *In vitro* efficacy of fungicides against boll rotting fungi of cotton

4. Conclusion

The present study demonstrates that among seven fungicides evaluated by the poisoned food technique against the four pathogens of boll rot complex of cotton (*Aspergillus niger*, *Fusarium* sp., *Rhizopus* sp. and *Alternaria* sp.), Propiconazole 25% EC (0.1%) provided the most

complete and consistent broad-spectrum inhibition, followed by Azoxystrobin 11% + Tebuconazole 18.3% SC and Tebuconazole 25.9% EC. However, Carbendazim 50% WP and Propineb 70% WP, while effective against specific pathogens, failed to control the full complex adequately. Propiconazole 25% EC is therefore recommended as the most suitable fungicide for chemical management of boll rot in the North cotton belt of Maharashtra.

Table 2. *In vitro* evaluation of fungicides against boll rotting fungi

Sr. No.	Treatment	<i>Aspergillus niger</i>		<i>Fusarium sp.</i>		<i>Rhizopus sp.</i>		<i>Alternaria sp.</i>		Mean colony diameter of four pathogens (mm)
		Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	Mean colony diameter (mm)	Per cent inhibition	
T ₁	Copper oxy-chloride 50% WP	45.57	49.37 (44.64)*	7.36	91.82 (73.38)*	00	100 (90)*	14.23	84.18 (66.57)*	16.79
T ₂	Tebuconazole 25.9% EC	00	100 (90)*	00	100 (90)*	17.73	80.29 (63.65)*	00	100 (90)*	4.43
T ₃	Carbendazim 50% WP	00	100 (90)*	00	100 (90)*	60.26	33.04 (35.09)*	48.27	46.36 (42.91)*	27.13
T ₄	Propiconazole 25% EC	00	100 (90)*	9.51	89.43 (71.03)*	00	100 (90)*	00	100 (90)*	2.37
T ₅	Propineb 70% WP	40.83	54.63 (47.66)*	20.01	77.76 (61.86)*	47.74	46.95 (43.25)*	00	100 (90)*	27.14
T ₆	Pyraclostrobin 20% WG	20.68	77.02 (61.35)*	14.83	83.51 (66.04)*	38.23	57.51 (49.32)*	5.43	93.97 (75.78)*	19.79
T ₇	Azoxystrobin 11 % + Tebuconazole 18.3 % SC	00	100 (90)*	00	100 (90)*	13.49	85.01 (67.22)*	00	100 (90)*	3.37
T ₈	Control	90	-	90	-	90	-	90	-	-
	S. Em. (±)	0.61	0.68	0.48	0.50	0.57	0.63	0.93	1.03	-
	CD (P = 0.01)	2.43	2.70	1.91	1.98	2.25	2.50	3.69	4.10	-

*. Figures in parentheses are arc sine transformed values

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