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Evaluation of new molecules of fungicides against *Colletotrichum truncatum* causing pod blight of soybean

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Abstract
Pod blight of soybean caused by *Colletotrichum truncatum*, is a destructive disease resulting in severe yield and quality losses under warm and humid conditions. The present investigation was conducted to evaluate systemic, non-systemic and combi fungicides against *C. truncatum* under *in vitro* conditions using the poisoned food technique. Significant variation was observed among fungicide groups. Among non-systemic fungicides, Mancozeb 75% WP recorded the highest mycelial growth inhibition, followed by Chlorothalonil and Propineb. Systemic fungicides of the triazole group, particularly Tebuconazole 25.9% EC, Propiconazole 25% EC and Difenconazole 25% EC completely inhibited the mycelial growth of the pathogen at higher concentrations. Combi-products proved most effective overall with Tebuconazole 50%+ Trifloxystrobin 25% WG achieving near-complete inhibition, followed by Azoxystrobin+ Difenconazole and Pyraclostrobin+ Epoxiconazole. The results suggest that triazole-based systemic fungicides and strobilurin-triazole combinations are highly effective and can be integrated in pod blight management strategies.

Keywords: *Colletotrichum truncatum*, pod blight, soybean, systemic fungicides, non-systemic fungicides, combi fungicides, poisoned food technique

Introduction

Soybean (*Glycine max* L. Merrill) is an economically important legume crop valued for its high protein (40-42%) and oil (20-22%) content. It contributes significantly to global edible oil and livestock feed production. In India, soybean is cultivated on about 12.9 million ha with an annual production of 15.1 million tonnes, mainly in Madhya Pradesh, Maharashtra, Rajasthan and Karnataka (Anon, 2024). Despite its importance, soybean productivity is constrained by several diseases, among which pod blight caused by *Colletotrichum truncatum*, is one of the most destructive.

The disease is prevalent in warm and humid regions and leads to pod infection, shriveled seeds, reduced seed viability and yield losses up to 60% under favorable conditions (Hartman *et al*., 1999) [3]. Symptoms include irregular lesions on pods, premature drying, necrosis and seed discoloration. The pathogen is primarily seed-borne and survives in infected debris, ensuring its persistence across seasons (Sinclair, 1992) [8].

Chemical management forms an important component of integrated disease management (IDM). However, increasing resistance development due to over-reliance on single-site fungicides such as benzimidazoles necessitates evaluation of newer systemic fungicides and their combinations with protectants or Strobilurins. Hence, the present study was undertaken to evaluate systemic, non-systemic and combi fungicides against *C. truncatum* under *in vitro* conditions.

Material and methods

Isolation of the pathogen

Soybean pods and stems showing typical blight and anthracnose symptoms were collected from major soybean-growing areas of northern Karnataka. Tissue isolation was carried out on potato dextrose agar (PDA) medium after surface sterilization with sodium hypochlorite (0.1%).

Pure cultures were obtained by hyphal tip isolation and maintained on PDA slants at 4°C. The pathogen was identified based on cultural and morphological characteristics as *C. truncatum*, and pathogenicity was confirmed.

Poisoned food technique: The poisoned food technique (Nene and Thapliyal, 1993) [6] was employed for fungicide evaluation. PDA medium was amended with different concentrations of test fungicides prior to pouring into sterile Petri plates. A 5 mm disc of 7-day-old culture of *C. truncatum* was placed at the center of each plate and incubated at 27 ± 2 °C for 7 days. Untreated PDA plates served as control.

Three groups of fungicides were evaluated

1. Non-systemic fungicides- Mancozeb 75% WP, Chlorothalonil 75% WP, Propineb 75% WP, Copper oxychloride 50% WP.
2. Systemic fungicides - Carbendazim 50% WP, Thiophanate-methyl 70% WP, Azoxystrobin 23% SC, Propiconazole 25% EC, Hexaconazole 5% EC, Difenconazole 25% EC, Tebuconazole 25.9% EC.
3. Combi fungicides-Tebuconazole 50%+ Trifloxystrobin 25% WG, Azoxystrobin 18.2%+ Difenconazole 11.4% SC, Pyraclostrobin 13.3%+ Epoxiconazole 5% SE, Carbendazim 12%+ Mancozeb 63% WP, Tebuconazole 10%+ Sulphur 65% WG, Tricyclazole 75%+ Mancozeb 25% WP.

Colony diameter was measured when the control plate was fully covered. Per cent inhibition of mycelial growth was calculated using Vincent's formula (1947) [10]. Data were statistically analyzed using Completely Randomized Design (CRD) with three replications, and treatment means were compared at 1% level of significance.

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

Where I=Per cent inhibition,

- C = Growth of mycelium in control
- T = Growth of mycelium in treatment.

Results and discussion

Non-Systemic fungicides

All non-systemic fungicides significantly inhibited mycelial growth compared to control. Mancozeb recorded the highest inhibition values of 78.58 per cent, 81.83 per cent and 85.61 per cent at 0.15 per cent, 0.25 per cent and 0.30 per cent concentrations respectively, followed by Chlorothalonil, Propineb and Copper oxychloride. Similar findings were reported by Jagtap *et al.* (2012) [4], who demonstrated Mancozeb superiority against *C. truncatum* under both *in vitro* and field conditions. Multi-site fungicides like Mancozeb are effective protectants and also delay resistance development (Subedi *et al*., 2015) [9] (Table 1, Fig 1).

Table 1: *In-vitro* evaluation of non-systemic fungicides against *C. truncatum* causing pod blight of soybean

Sl. No.	Non-Systemic fungicides	Mycelial inhibition over control (%)			
		Concentration (%)			Mean
		0.15	0.25	0.3	
1	Mancozeb 75% WP	78.58 (62.43)*	81.83 (64.77)*	85.61 (67.71)*	82.06 (64.94)*
2	Propineb 75% WP	51.38 (45.79)	54.75 (47.72)	60.09 (50.82)	55.40 (48.10)
3	Chlorothalonil 75% WP	44.34 (41.75)	61.46 (51.62)	68.42 (55.81)	58.07 (49.64)
4	Copper oxychloride 50% WP	33.65 (33.45)	51.58 (45.91)	60.44 (51.03)	48.55 (44.17)
	Mean	51.99 (46.14)	62.41 (52.18)	68.64 (55.95)	—
	Source	Fungicide (F)	Concentration (C)	F x C	
	S. Em. ±	0.09	0.07	0.14	
	C.D at 1%	0.34	0.30	0.59	

*Arc sine transformed values

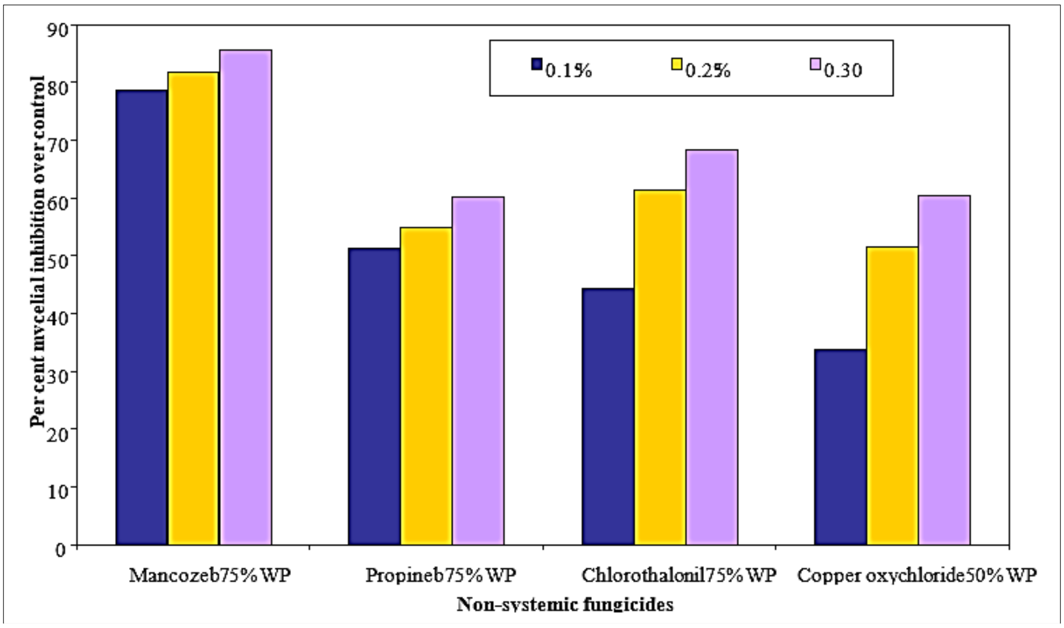

Fig 1: *In vitro* evaluation of non-systemic fungicides against C. *truncatum*, the causal agent of soybean pod blight.

Systemic fungicides

Among the treatments, Tebuconazole 25.9% EC emerged as the most effective fungicide, exhibiting exceptional inhibition of mycelial growth at all concentrations. It recorded inhibition values of 96.56 per cent, 97.77 per cent and 99.30 per cent at 0.025 per cent, 0.05 per cent and 0.1 per cent, respectively, with an overall mean inhibition of 97.87 per cent. Hexaconazole was moderately effective, while Azoxystrobin and benzimidazoles like Carbendazim showed comparatively poor inhibition, indicating possible resistance development (Raghuvanshi *et al*., 2023) [7]. Previous studies also emphasized the superior efficacy of triazoles in managing anthracnose and pod blight (Gurjar *et al*., 2021) [2] (Table 2, Fig 2).

Table 2: *In-vitro* evaluation of Systemic fungicides against *C. truncatum* causing pod blight of soybean

Sl. No.	Systemic fungicides	Mycelial inhibition over control (%)			
		Concentration (%)			Mean
		0.025	0.05	0.1	
1	Carbendazim 50% WP	33.63 (35.44)*	40.83 (39.71)*	42.96 (40.95)*	39.14 (38.73)*
2	Thiophenyl methyl 70% WP	20.56 (26.96)	38.76 (38.50)	41.27 (39.97)	33.53 (35.38)
3	Azoxystrobin 23% SC	40.68 (39.63)	46.57 (43.03)	49.62 (44.78)	45.62 (42.49)
4	Propiconazole 25% EC	95.66 (77.98)	96.52 (79.24)	98.70 (83.44)	96.96 (79.86)
5	Hexaconazole 5% EC	60.73 (51.20)	65.70 (54.15)	69.70 (56.60)	65.37 (53.95)
6	Difenconazole 25% EC	94.89 (76.94)	95.65 (77.96)	98.41 (82.76)	96.31 (78.93)
7	Tebuconazole 25.9% EC	96.56 (79.32)	97.77 (81.41)	99.30 (85.20)	97.87 (81.61)
	Mean	63.24 (52.68)	68.83 (56.06)	71.42 (57.69)	—
	Source	Fungicide (F)	Concentration (C)	F x C	Source
	S. Em. ±	0.16	0.11	0.28	S. Em. ±
	C.D at 1%	0.63	0.42	1.09	C.D at 1%

*Arc sine transformed values

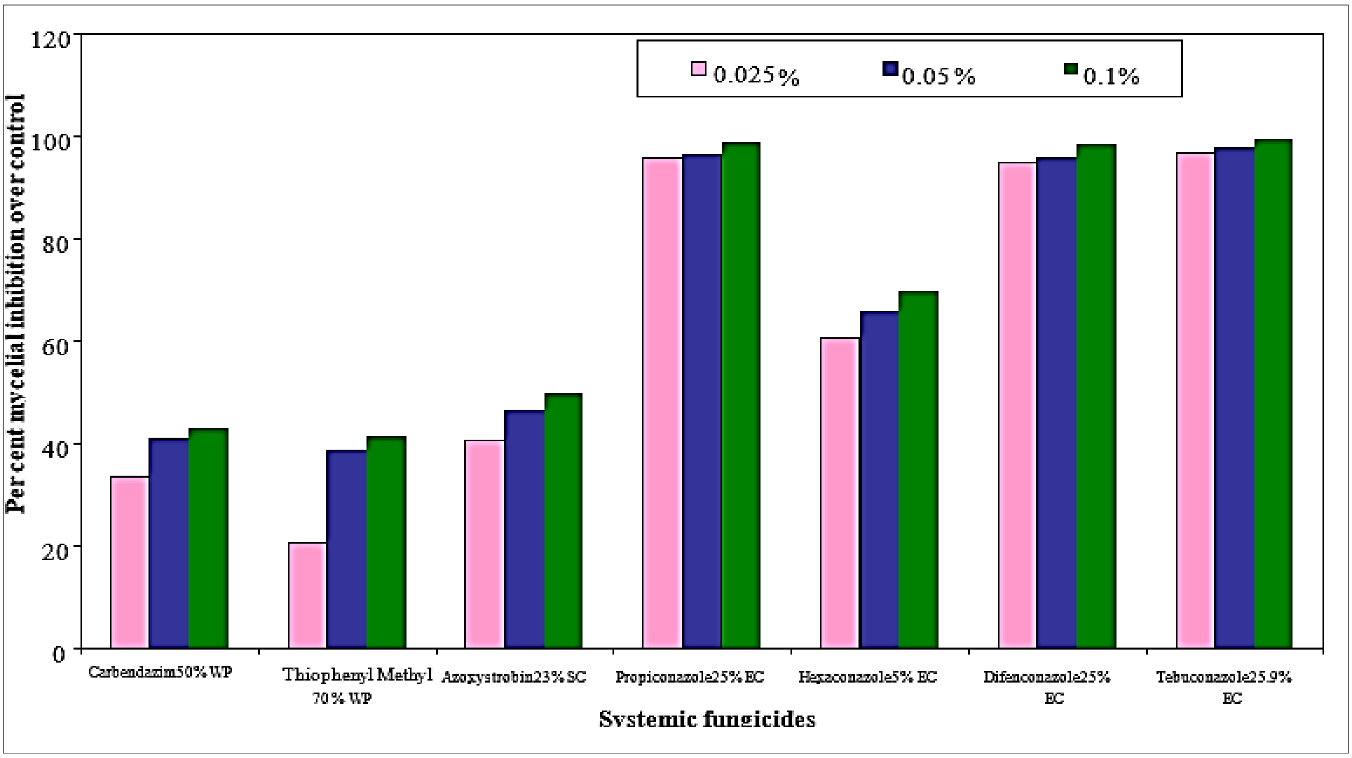

Fig 2: *In vitro* evaluation of systemic fungicides against *C. truncatum*, the causal agent of soybean pod blight.

Combi-fungicide

Combi-products proved significantly superior over single fungicides. Tebuconazole 50%+ Trifloxystrobin 25% (75% WG) proved to be the most effective in inhibiting the mycelial growth of C. *truncatum*. It achieved inhibition values of 98.01 per cent, 98.43 per cent and 99.38 per cent at 0.1 per cent, 0.15 per cent and 0.2 per cent, respectively, with a grand mean of 98.60 per cent, followed by Azoxystrobin+ Difenconazole and Pyraclostrobin+ Epoxiconazole. Carbendazim+ Mancozeb and Tebuconazole+ Sulphur were less effective but still provided higher inhibition than single benzimidazoles. The dual modes of action in combi fungicides confer both protective and curative properties, reduce disease pressure, and help delay fungicide resistance (Mandloi *et al*., 2023) [5] (Table 3, Fig 3).

Table 3: *In-vitro* evaluation of Combi- fungicides against *C. truncatum* causing pod blight of soybean

Sl. No.	Combi product fungicides	Mycelial inhibition over control (%)			
		Concentration (%)			Mean
		0.1	0.15	0.2	
1	Carbendazim 12%+ Mancozeb 63% (75% WP)	71.71 (57.87)	75.26 (60.17)	78.60 (62.45)	75.19 (60.13)
2	Pyraclostrobin 13.3%+ Epoxiconazole 5% (18.3% SE)	93.61 (75.35)	95.97 (78.42)	96.69 (79.42)	95.42 (77.64)
3	Azoxystrobin 25%+ Difenconazole 12.5% (37.5 SC)	94.58 (76.54)	95.37 (77.57)	97.57 (81.04)	95.84 (78.23)
4	Tebuconazole 50%+ Trifloxystrobin 25% (75% WG)	98.01 (81.88)	98.43 (82.80)	99.38 (85.48)	98.60 (83.20)
5	Tricyclazole 18%+ Mancozeb 62% (80% WP)	93.62 (75.37)	94.86 (76.90)	95.81 (78.18)	94.76 (76.77)

6	Tebuconazole 10%+ Sulphur 65% (75% WG)	69.77 (56.65)	73.72 (59.16)	79.58 (63.13)	74.35 (59.57)
	Mean	86.88 (68.77)	88.94 (70.57)	91.27 (72.82)	—
	Source	Fungicide (F)	Concentration (C)	F x C	Source
	S. Em ±	0.20	0.14	0.34	S. Em ±
	C.D at 1%	0.77	0.54	1.34	C.D at 1%

*Arc sine transformed values

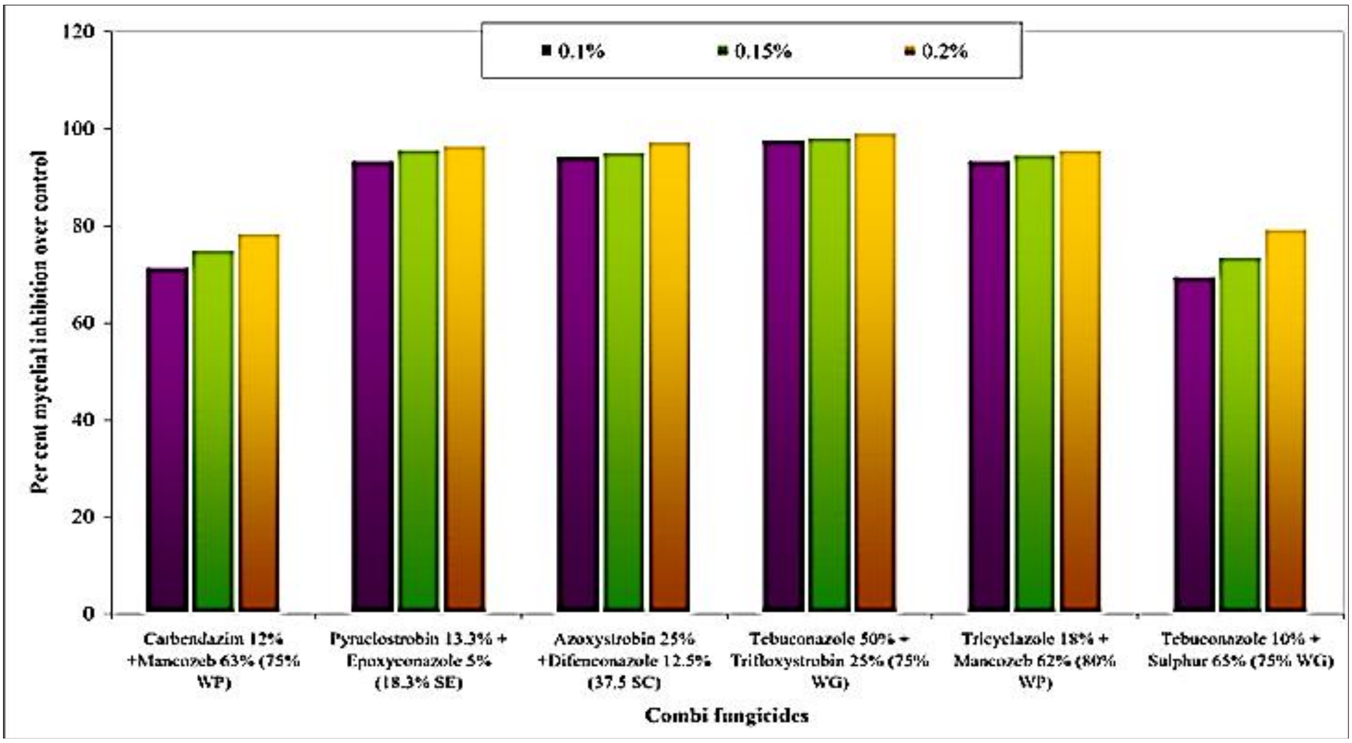

Fig 3: *In vitro* evaluation of combi fungicides against C. *truncatum* causing pod blight of soybean.

Conclusion

In vitro evaluation of non-systemic fungicides revealed Mancozeb 75% WP as the most effective, with a mean mycelial inhibition of 82.06%, followed by Chlorothalonil (58.07%) and Propineb (55.40%). Among systemic fungicides, triazoles such as Tebuconazole (97.87%), Propiconazole (96.96%) and Difenconazole (96.31%) recorded the highest inhibition, while Carbendazim showed the lowest due to likely resistance in pathogen populations. Combi-fungicides showed the best overall performance, with Tebuconazole 50%+ Trifloxystrobin 25% achieving 98.60 per cent inhibition, followed by Azoxystrobin+ Difenconazole (95.84%) and Pyraclostrobin+ Epoxiconazole (95.42%). Thus triazole fungicides and their combinations with Strobilurin emerge as promising options for effective pod blight management. These findings can be utilized in integrated disease management (IDM) strategies along with resistant cultivars, cultural practices and bio agents for sustainable management.

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