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# *In vitro* evaluation of different fungicides against *Colletotrichum truncatum* causing anthracnose of green gram

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**Abstract**

A study has been conducted at Department of Plant Pathology, University of Agricultural Sciences Raichur on evaluation of new fungicide molecules against *Colletotrichum truncatum*, causing anthracnose of green gram. Seven systemic, seven contact and seven combi fungicides were evaluated against *C. truncatum* under *in vitro* by poison food technique. Among the contact fungicides, propineb 70% WP was recorded highest mean mycelial inhibition (82.71%) followed by copper oxy chloride 50% WP (78.51%). Among the systemic fungicides, thiophanate methyl and triazole group of fungicides like propiconazole 25% EC, difenoconazole 25% EC and tebuconazole 25.9% EC recorded highest mycelial inhibition of *C. truncatum* at all the three concentrations (0.05, 0.1 and 0.15%). Among the combi fungicides, cent per cent inhibition was recorded in tricyclazole 18% + mancozeb 62% WP and carbendazim 12% + mancozeb 63% WP at 0.3 per cent concentration.

**Keywords:** *Colletotrichum truncatum*, *in vitro* evaluation, fungicides, and mycelial inhibition

**Introduction**

Green gram [*Vigna radiata* (L.) Wilczek] commonly known as mungbean is an important pulse crop of India cultivated mainly in *Kharif* season. It is also considered as “Golden Bean” because of its nutritional values and suitability for increasing the soil fertility by way of addition of nitrogen (30 kg/ha/annum) (Murakami *et al.*, 1991) [9]. Green gram is a rich source of protein (23-24%), carbohydrate (54-56%), minerals (4%) and vitamins (3%) (Afzal *et al.*, 2008) [1]. In India the area of green gram is 40.7 lakh ha with a production of 19.01 lakh tonnes and 467 kg/ha of productivity. Karnataka stands in 3<sup>rd</sup> position in area and Rajasthan (17.19 lakh ha) stands first position followed by Maharashtra (4.53 lakh ha). In Karnataka, the area under green gram cultivation is 3.97 lakh hectares with a production of 0.96 lakh tonnes and an average productivity of 242 kg per hectare (Anonymous, 2018) [2]. In India, the green gram anthracnose was first reported from Jorhat of Assam state in 1951 (Majid, 1953) [8]. The disease has been reported from all major mungbean growing regions of India in mild to severe form. Losses in yield due to anthracnose have been estimated to be in the range of 24 to 67 per cent (Deeksha and Tripathi, 2002) [3] and 18.2 to 86.57 per cent disease index of anthracnose have been reported in Northern Karnataka (Laxman, 2006) [7]. The average seed yield loss of 40.18 per cent and stalk yield loss of 46.90 per cent was noticed due to anthracnose of mungbean (Kulkarni, 2009) [6]. Considering, the emerging and devastating nature of the disease and economic loss of the crop, an attempt was made in the present investigation to evaluate the newer fungicides for the better management of the disease.

**Materials and methods**

The study has been conducted on evaluation of new molecules of contact, systemic and combi fungicides against *C. truncatum* in the Department of Plant Pathology, University of Agricultural Sciences Raichur, during 2018-19. Efficacy of seven systemic fungicides were evaluated at 0.05, 0.1 and 0.15 per cent concentrations. Whereas, contact and combi fungicides (seven each) were evaluated at 0.1, 0.2 and 0.3 per cent concentrations by poison food technique (Nene and Thapliyal, 1993) [10] and using PDA as a basal medium.

The required quantities of individual fungicides were added separately into molten and cooled potato dextrose agar to get the desired concentration of the fungicides.

Later 20 ml of such poisoned medium was poured into sterile Petri plates. Mycelial discs of 5 mm size from actively growing culture of the test fungus was cut out by a sterile cork borer and one such disc was placed at the centre of each agar plate. Control was maintained without adding any fungicides to the medium and each treatment was replicated thrice. Then such plates were incubated at room temperature for 12 days and radial colony growth (mm) was measured. The efficacy of a fungicide was expressed as per cent inhibition of mycelial growth over control that was calculated by the following formula suggested by Vincent (1947) [14].

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

Where,

I = Per cent inhibition

C = Radial growth of fungus in control

T = Radial growth of fungus in treatment

### Statistical analysis

Statistical analysis was carried out as per the procedures given by Panse and Sukhathme (1985) [11]. Actual data in percentage were converted to arc sine values, before analysis according to the table given by Snedecor and Cochran (1967).

### Results and discussion

#### *In vitro* evaluation of fungicides against *C. truncatum*

The results revealed that, among the seven different non systemic fungicides, propineb was found most effective at all the three concentrations with highest mycelial inhibition at 0.3 per cent concentration (89.62%) with mean mycelial inhibition of 82.71 per cent found significantly superior when compared to other non-systemic fungicides. The next best fungicides were copper oxy chloride which recorded 89.62 per cent at the concentration of 0.3 per cent with mean mycelial inhibition of 78.51 per cent found significantly

superior followed by mancozeb (73.08%) and captan (73.07%) which were found on par with each other. At the concentration of 0.2 per cent, copper oxy chloride (76.29%), mancozeb (74.81%), copper hydroxide (67.40%) and captan (71.84%) were statistically on par with each other. The least mycelial inhibition was recorded in zineb at 0.1 per cent concentration (27.40%) followed by chlorothalonil (59.00%) with the mean mycelial inhibition of 51.59 and 59.00 per cent respectively (Table 1) (Fig. 1).

Of the seven non systemic fungicides tested, three fungicides viz., propineb, copper oxy chloride and mancozeb were most effective with maximum mycelial inhibition. Similar results were obtained in the investigations made by Kinjal and Gohel (2016) [5] who found that propineb completely inhibited the mycelial growth of *C. gloeosporioides* and proved to be most effective over rest of the treatments. Laxman (2006) [7] and Kavyashree (2014) [4] reported that, copper oxy chloride 50 WP (0.25%) and mancozeb as the most effective fungicides against *C. gloeosporioides*.

The table 2 showed that, all the seven systemic fungicides were found significantly superior over the control in inhibiting the mycelial growth of *C. truncatum*. The two systemic fungicides, thiophanate methyl (97.77%) at the concentration of 0.10 per cent and propiconazole (97.03%) at all the three different concentrations (0.05, 0.1 and 0.15%) were found highly effective in inhibiting the radial mycelial growth of *C. truncatum* which were statistically on par with each other but significantly superior over remaining treatments. The next best fungicides were carbendazim (96.29%) and difenoconazole (96.29%) at the concentration of 0.15 per cent and with the mean mycelial inhibition of 94.07 and 92.83 per cent respectively, followed by tebuconazole (94.07%) at the concentration of 0.15 per cent with mean mycelial inhibition of 90.11 per cent. The fungicides with lowest mean mycelial inhibition were azoxystrobin (68.14%) and hexaconazole (75.55%) at the concentration of

**Table 1:** *In vitro* evaluation of contact fungicides on mycelial growth of *C. truncatum*

Tr. No.	Fungicides	Per cent inhibition			Mean
		Concentrations (%)			
		0.1	0.2	0.3	
T1	Captan (70% WP)	67.40 (55.19)	71.84 (57.97)	79.99 (63.45)	73.07 (58.73)
T2	Chlorothalonil (75% WP)	47.40 (43.50)	54.07 (47.33)	75.55 (60.37)	59.00 (50.17)
T3	Copper oxy chloride (50% WP)	69.62 (56.57)	76.29 (60.89)	89.62 (71.22)	78.51 (62.37)
T4	Copper hydroxide (77% WP)	66.66 (54.73)	67.40 (55.18)	76.29 (60.89)	70.11 (56.85)
T5	Mancozeb (75% WP)	64.44 (53.41)	74.81 (59.90)	79.99 (63.45)	73.08 (58.73)
T6	Propineb (70% WP)	74.07 (59.40)	84.44 (66.79)	89.62 (71.22)	82.71 (65.42)
T7	Zineb (75% WP)	27.40 (31.49)	54.06 (47.33)	73.32 (58.93)	51.59 (45.90)
	Mean	59.57 (50.51)	68.98 (56.14)	80.62 (63.87)	
		S. Em. ±			CD at 1%
	1. Fungicides (F)	0.64			1.84
	2. Concentrations (C)	0.42			1.20
	3. F × C	1.11			3.19

\*Original value \*\*Arcsine transformed value

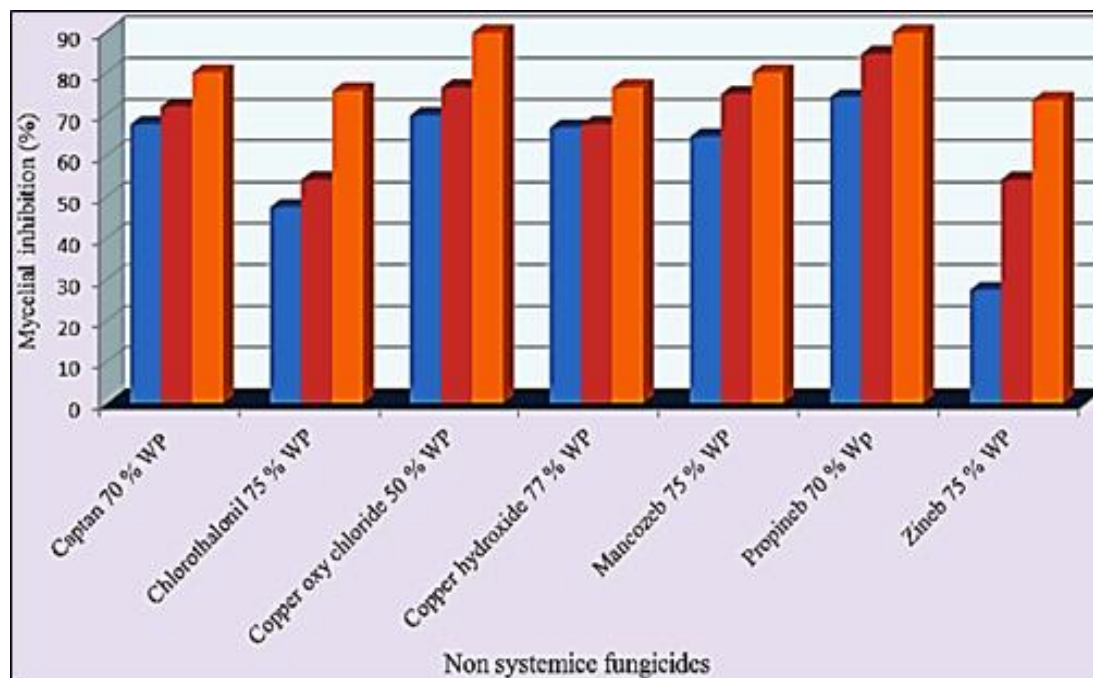


Fig 1: Efficacy of non-systemic fungicides on mycelial inhibition of *C. truncatum*

Table 2: *In vitro* evaluation of systemic fungicides on mycelial growth of *C. truncatum*

Tr. No.	Fungicides	Per cent inhibition Concentrations (%)			Mean
		0.05	0.10	0.15	
		T1	Azoxystrobin (23% SC)	68.14 (55.65)	
T2	Carbendazim (50% WP)	91.85 (73.44)	94.07 (75.95)	96.29 (79.28)	94.07 (75.95)
T3	Difenoconazole (25% EC)	89.62 (71.22)	92.59 (74.23)	96.29 (79.28)	92.83 (74.45)
T4	Hexaconazole (5% SC)	75.55 (60.37)	83.70 (66.25)	98.51 (84.26)	85.92 (67.95)
T5	Propiconazole (25% EC)	97.03 (80.20)	97.03 (80.20)	97.03 (80.20)	97.03 (80.20)
T6	Tebuconazole (25% EC)	85.18 (67.97)	91.10 (72.73)	94.07 (75.95)	90.11 (71.66)
T7	Thiophanate methyl (70% WP)	97.03 (80.20)	97.77 (81.40)	97.03 (80.20)	97.27 (80.47)
	Mean	86.34 (68.30)	90.16 (71.70)	94.59 (76.54)	
			S. Em. ±		CD at 1%
	1. Fungicides (F)		0.77		2.21
	2. Concentrations (C)		0.50		1.45
	3. F × C		1.34		3.83

\*Original value \*\*Arc sine transformed value

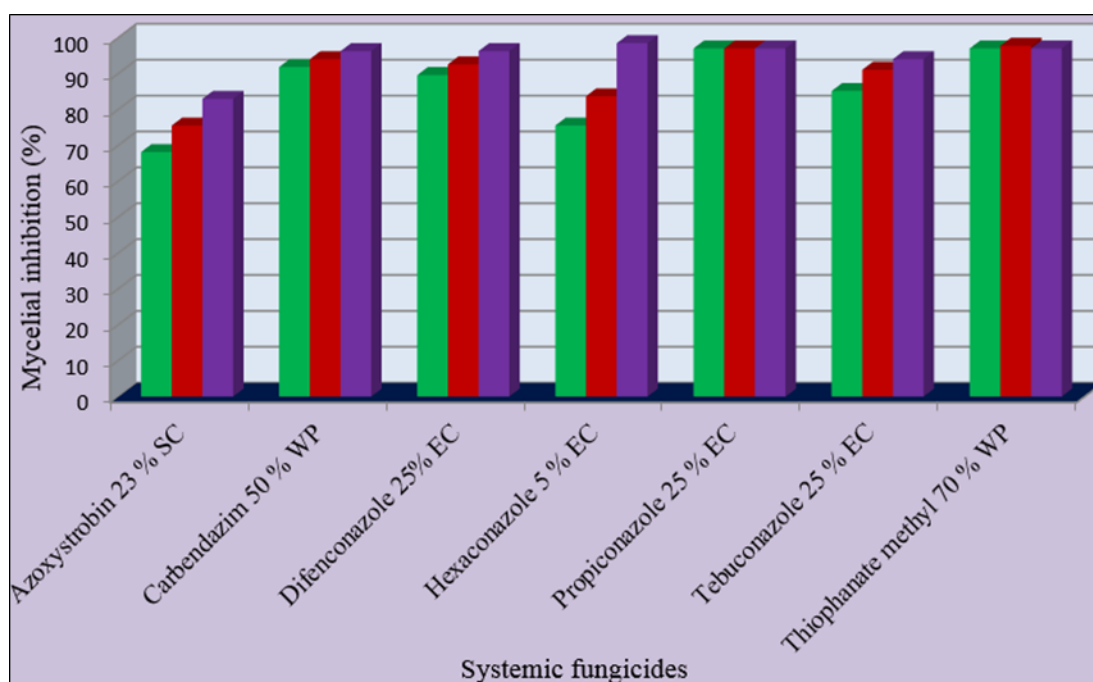


Fig 2: Efficacy of systemic fungicides on mycelial growth of *C. truncatum*

0.05% with the mean mycelial inhibition of 75.55 and 85.92 per cent respectively (Fig. 2). The results obtained in the present investigation are in accordance with Laxman (2006) [7] who tested eight different systemic fungicides in *in vitro* against *C. truncatum*, most of them showed complete inhibition of mycelial growth at the concentration, of 0.05 and 0.1 per cent except tricyclazole and hexaconazole. Kulkarni (2009) [6] also conducted *in vitro* evaluation of fungicides which revealed that, propiconazole, carbendazim, thiophanate methyl, benomyl, hexaconazole and carbendazim + mancozeb were found effective. Carbendazim showed good effect (96.29%) against *C. truncatum* as it is effective against hyaline spore producing fungus.

Triazole fungicides were proven to show good inhibition on growth of *C. truncatum*. The triazole group of fungicides are the potent inhibitors of 'ergosterol biosynthesis', the major membrane sterol of fungi and they also block the cytochrome P450-dependent enzyme C-14 alpha-demethylase, which is needed to convert lanosterol to ergosterol.

Kavyashree (2014) [4] reported that, propiconazole 25% EC, at all the three concentrations *viz.*, 0.05, 0.1 and 0.15 per cent found most effective systemic fungicide against *C. gloeosporioides* when compared with other fungicides. Kinjal

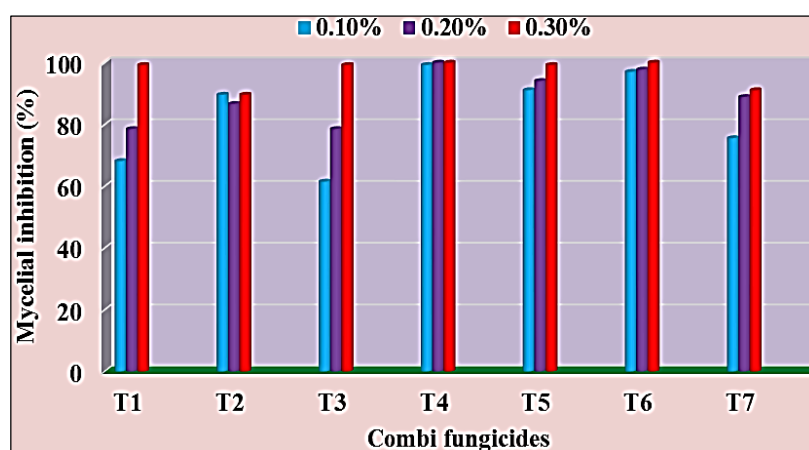
and Gohel (2016) [5] conducted the *in vitro* studies for efficacy of nine fungicides at three concentrations against *C. gloeosporioides* revealed that, propiconazole, carbendazim (12%) + mancozeb (63%) and propineb completely inhibited the mycelial growth and proved to be most effective over rest of the treatments. The results were found slightly contradicted with studies made by Roopadevi and Jamadar (2015) who found thiophanate methyl as the least effective fungicide when compared to other fungicides however they have reported propiconazole 25% EC, at all the three concentrations *viz.*, 0.05%, 0.1% and 0.15% as the most effective systemic fungicide against *C. gloeosporioides*. The systemic fungicides belonging to strobilurin group *i.e.*, azoxystrobin 23.5% SC, has not shown much efficacy in control of growth of *C. truncatum*. The interaction between fungicides and concentrations on inhibition of mycelial growth was found significant.

Development of resistance in many pathogens to fungicides with single point action has lead way to the development of new fungicides where chemicals with two different mode of action, which showed synergistic effect for the control of pathogens. So an effort made to evaluate different combi fungicides against *C. truncatum*.

**Table 3:** Efficacy of combi fungicides on inhibition of mycelial growth of *C. truncatum* under *in vitro* conditions

Tr. No.	Fungicide	Per cent inhibition			Mean
		Concentrations (%)			
		0.1	0.2	0.3	
T1	Captan 70% + hexaconazole 5% WP	68.14 (55.65)	78.51 (62.38)	99.25 (87.13)	81.96 (64.85)
T2	Pyraclostrobin 5% + metiram 55.5% WP	89.62 (71.37)	86.66 (68.61)	89.62 (71.37)	88.63 (70.28)
T3	Hexaconazole 5% + zineb 75%	61.47 (51.64)	78.51 (62.38)	99.25 (87.13)	79.74 (63.24)
T4	Tricyclazole 18% + mancozeb 62% WP	99.25 (87.13)	100.00 (90.00)	100.00 (90.00)	99.75 (87.12)
T5	Azoxystrobin 11% + tebuconazole 18.3%	91.10 (72.73)	94.07 (75.95)	99.25 (87.13)	94.80 (76.80)
T6	Carbendazim 12% + mancozeb 63% WP	97.03 (80.20)	97.77 (81.40)	100 (90.00)	98.26 (82.40)
T7	Trifloxystrobin 25% + tebuconazole 50% EC	75.55 (60.37)	88.88 (70.57)	91.10 (72.73)	85.17 (67.34)
	Mean	83.16 (65.76)	89.14 (70.74)	96.92 (79.88)	
				<b>S. Em. ±</b>	<b>CD at 1%</b>
	1. Fungicides (F)			0.88	2.52
	2. Concentrations (C)			0.57	1.65
	3. F × C			1.53	4.36

\*Original value \*\*Arc sine transformed value



**Fig 3:** Efficacy of combi fungicides on mycelial inhibition of *C. truncatum*

- T1- Captan 70% + hexaconazole 5% WP
- T2- Pyraclostrobin 5% + Metiram 55.5% WP
- T3- Hexaconazole 5% + zineb 75% WP
- T4- Tricyclazole 18% + mancozeb 62% WP
- T5- Azoxystrobin 11% + tebuconazole 18.3%
- T6- Carbendazim 12% + mancozeb 63% WP
- T7- Trifloxystrobin 25% + tebuconazole 50% EC

All the combi fungicides were found effective at 0.3 per cent concentration. Of the seven combi fungicides tested against *C. truncatum*, complete inhibition of mycelial growth was recorded in the combi fungicide tricyclazole 18% + mancozeb 62% WP (merger) (100%) at 0.2 and 0.3 per cent concentration which was followed by carbendazim 12% + mancozeb 63% WP (SAAF) (100%) at 0.3 per cent

concentration with the mean mycelial inhibition of 99.75 and 98.26 per cent respectively which were statistically on par with each other and significantly superior over pyraclostrobin 5% + metiram 55.50% WP and trifloxystrobin 25% + tebuconazole 50% EC. The next best fungicide was azoxystrobin 11% + tebuconazole 18.3% with the mean mycelial inhibition of 94.80 per cent followed by pyraclostrobin 5% + metiram 55.50% WP (88.63%). Trifloxystrobin 25% + tebuconazole 50% EC showed the mean mycelial inhibition of 85.17 per cent followed by captan 7% + hexaconazole 5% WP (81.96%). At the concentration of 0.2 per cent combi fungicides like pyraclostrobin 5% + metiram 55.50% WP, captan 70% + hexaconazole 5% WP and hexaconazole 5% + zineb 75% were statistically on par with each other. The lowest mycelial inhibition was recorded in hexaconazole 5% + zineb 75% (61.47%) at 0.1 per cent concentration and with the mean mycelial inhibition of 79.74 per cent (Table 3) (Fig. 3).

The results obtained in the present investigation are in confirmation with the findings of Laxman (2006)<sup>[7]</sup> and Kulkarni (2009)<sup>[6]</sup> who reported bioefficacy of carbendazim + mancozeb against *C. truncatum* at all the three concentrations (0.05, 0.1 and 0.15%) found most effective combi fungicide when compared with other treatments.

Kinjal and Gohel (2016)<sup>[5]</sup> conducted the *in vitro* studies for efficacy of nine fungicides at three concentrations against *C. gloeosporioides* and found that, carbendazim (12%) + mancozeb (63%), azoxystrobin + difenoconazole and trifloxystrobin + tebuconazole as the effective combi fungicides. The results were in contradiction with research findings of Roopadevi and Jamadar (2015) who found hexaconazole 5% + zineb 75% (Avtar) as the most effective combi fungicide but in the present investigation the same fungicide was found least effective.

## Conclusion

The results revealed that, out of the seven contact fungicides tested, propineb 70% WP was the best fungicide recorded with highest mean mycelial inhibition followed by copper oxy chloride. Among seven systemic fungicides evaluated against *C. truncatum*, thiophanate methyl and triazole group of fungicides like propiconazole 25% EC, difenoconazole 25% EC and tebuconazole 25.9% EC recorded highest mycelial inhibition of *C. truncatum* at all the three concentrations (0.05, 0.1 and 0.15%). Of the seven combi fungicides evaluated, cent per cent inhibition was recorded in tricyclazole 18% + mancozeb 62% WP and carbendazim 12% + mancozeb 63% WP at 0.3 per cent concentration. The next best fungicide was azoxystrobin 11% + tebuconazole 18.3%.

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