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Effectiveness of Fungicides against *Colletotrichum lindemuthianum* Causing Anthracnose of Green Gram [*Vigna radiata* (L.) Wilczek]

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ABSTRACT

Background: Anthracnose is currently a severe threat to green gram cultivation in India. *In vitro* bioassay and glasshouse studies were performed to evaluate the effectiveness of different fungicides against *Collectotrichum lindemuthianum*.

Methods: The current investigation was conducted at the Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat in 2020-21. The poison food technique assessed the efficacy of systemic, non-systemic and combination fungicides against *C. lindemuthianum*. The management study was also carried out from the best-performing bioassay treatments and evaluated in the glasshouse.

Result: At 500 ppm, carbendazim 50 WP and propiconazole 25 EC inhibited 100% of mycelial growth out of six systemic fungicides tested while in non-systemic fungicides, mancozeb 75 WP and chlorothalonil 75 WP inhibited the test pathogen the most at 2500 ppm. Among the combi-fungicides tested at 500 ppm, carbendazim (12%) + mancozeb (63%) 75 WP and captan 70% + hexaconazole 5% 75 WP inhibited pathogen mycelial growth the most significantly more than the untreated control. In glasshouse studies, the lowest disease index was found in two foliar sprays of carbendazim 50 WP @ 1.0 g/l, propiconazole 25 EC @ 2.0 ml/l and captan 70% + hexaconazole 5% @ 0.6 g/l spaced 15 days apart. On the other hand, seed treatment of carbendazim 12% + mancozeb 63% @ 3.0 g/kg seed and mancozeb 75 WP @ 3.0 g/kg seed had the lowest disease index compared to control.

Key words: Anthracnose, Disease index, Fungicides, Poison food technique, Seed treatment.

INTRODUCTION

Green gram [*Vigna radiata* (L.) Wilczek, Syn.: *Phaseolus aureus* Roxb., *Phaseolus radiata* L.] is India's third most important pulse crop after Bengalgram and redgram. Green gram is grown not only for seed, but also for green manure and fodder. On a dry weight basis, green gram contains about 25 to 28 per cent protein, 1 to 1.5 per cent oil, 3.5 to 4.5 per cent fibre, 4.5 to 5.5 per cent ash and 62 to 65 per cent carbohydrate (Meena *et al.,* 2021). The crop also suffers from many diseases caused by fungi, bacteria, viruses, nematodes and abiotic stresses (Chaudhari and Gohel, 2016). Anthracnose (*Colletotrichum lindemuthianum* Sacc. and Magnus), the most economically significant of all diseases, reduced green gram yield notably (Khaire and Hake, 2018) which causes severe leaf spotting known as "shot holes" progressing to defoliation and significantly reduces yield. Infected pods harm the seeds and affect germination. Due to anthracnose, the average seed yield losses and average stalk yield losses are 40.18% and 46.90% respectively (Sunil *et al.,* 2009). Anthracnose in green gram is more common during the *kharif* season in south Gujarat due to predominant favourable weather conditions throughout the crop season. Contemplate to the emerging and devastating nature of the disease and economic loss of the crop in Gujarat, the current study was planned to assess fungicides for the management of anthracnose in south Gujarat.

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MATERIALS AND METHODS

Fungal pathogen isolation and purification

The diseased sample was collected from Pulse Research Station, NAU, Navsari and a microscopic examination was carried out in the lab. The diseased sample was divided into small explants, along with some healthy tissues and surface sterilised with a solution of 0.1 per cent mercuric chloride for one minute followed by three washes with sterile distilled water. Then the explants were placed on potato dextrose agar (PDA) plates in an aseptic condition and incubated at 28±2°C. The fungal growth was transferred

aseptically to PDA slants and purified using the hyphal tip method after seven days of incubation.

In Vitro* evaluation of various fungicides against *C. lindemuthianum

The Poison Food Technique was employed to evaluate various fungicides *in vitro* against *C. lindemuthianum. In vitro* evaluation of different systemic fungicides, contact fungicides and combi-fungicides against *C. lindemuthianum* was carried out at the laboratory, Department of Plant Pathology, N.M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat. Poisoned media was prepared by blending different concentrations of respective fungicides with 100 ml of PDA. Seven days old test pathogen culture disc (5 mm) was placed in the centre of the poisoned media plate whereas the plate without fungicides was kept as control. The inoculated plates were then incubated at 28±2°C. After 7 days of incubation, mycelial growth (mm) and growth inhibition (%) were observed and recorded. The per cent growth inhibition (PGI) of the pathogen in each treatment was calculated by the formula given by Vincent (1947):

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

Where,

PGI = Per cent growth inhibition.

C = Colony diameter (mm) in control plate.

T = Colony diameter (mm) in treated plate.

Testing effective chemicals in pot condition against anthracnose of green gram

A glasshouse experiment was conducted to test the effectiveness of fungicides during their respective tests. The fungicides found effective in *in vitro* tests were used in pots individually for the management of anthracnose of green gram under greenhouse conditions. The healthy seeds of green gram were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) solution and washed thoroughly with sterile distilled water to remove traces of the mercuric chloride. Surface sterilized seeds were dipped in the spore suspension (1.0×10^6 spores/ml) for 30 minutes and were sown in pots filled with autoclaved soil. The design used was a Completely Randomized Design (CRD). The number of replications was three and the number of treatments was seven (Two systemic, two non-systemic and two combi-fungicides were tested along with control). Mancozeb @ 3.0 g/kg, carbendazim 12% + mancozeb 63% @ 3.0 g/kg, were used as seed treatment before sowing of green gram seeds. Carbendazim @ 1.0 g/l, propiconazole @ 2.0 ml/l, captan 70% + hexaconazole 5% @ 0.6 g/l and chlorothalonil @ 3 g/l were used as foliar sprays twice at 30 days and 45 days after germination whereas control with only distilled water. Observations were recorded using the following formula given by Wheeler (1969):

$$\text{Per cent disease index} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaf observed} \times \text{Maximum rating}} \times 100$$

RESULTS AND DISCUSSION

Effect of different systemic fungicides on growth inhibition of *C. lindemuthianum*

To assess the systemic fungicide effectiveness against *C. lindemuthianum in vitro*, various concentrations (100, 250, 500 ppm) of carbendazim (50 WP), carboxin (75 WP), propiconazole (25 EC), azoxystrobin (23 EC), hexaconazole (5 EC) and thiophanate methyl (70 WP) were tested. After fifteen days of incubation, observations were taken on mycelial growth and the percentage of growth inhibition [Table 1, Fig 1, 4(a)]. The majority of the above mentioned fungicides were found to be effective in preventing mycelial growth of *C. lindemuthianum* over control. But within each concentration, a significant difference was observed. Carbendazim showed the highest growth inhibition percentage (96.67%; 97.30%; 100%) over control at all different concentrations used (100, 250 and 500 ppm) respectively. Surprisingly, propiconazole showed absolute inhibition at 500 ppm. The poor inhibition was depicted by hexaconazole and azoxystrobin at all different concentrations (Fig 1). It was discovered that the growth inhibition percentage and increase in the chemical concentration were positively correlated. At 500 ppm, carbendazim and propiconazole did not allow mycelial growth of the pathogen and significantly reduced the growth at 100 and 250 ppm. As a result, both fungicides were more efficient against *C. lindemuthianum*. The remaining fungicides, however, were found to be comparatively moderate and less effective. In comparison to other fungicides, hexaconazole was found to be the least effective at all concentrations.

The above results are following Hedge (1998), who found that carbendazim effectively prevented chilli fruit rot brought on by *C. capsici*. *In vitro* testing of fungicides against *C. dematium* was done by Varaprasad (2000) and systemic fungicides carbendazim (0.1%) and kitazin (0.3%) were found to be effective at preventing fungal growth. Ten fungicides were tested *in vitro* by Madhusudhan (2002) against *C. truncatum*. Both benomyl and carbendazim, at concentrations of 0.025, 0.05 and 0.1%, inhibited fungal mycelium. Propiconazole, hexaconazole and carbendazim were also reported to be effective fungicides against green gram anthracnose by Joshi and Tripathi (2002) and Laxman (2006).

Effect of different non-systemic fungicides on growth inhibition of *C. lindemuthianum*

To assess the non-systemic fungicide effectiveness against *C. lindemuthianum in vitro*, various concentrations (1000, 2000, 2500 ppm) of copper oxychloride (50 WP), mancozeb (75 WP), chlorothalonil (75 WP), zineb (75 WP) and sulphur (80 WP) were tested. After fifteen days of incubation, observations were made on the average colony diameter and the per cent growth inhibition (Table 1, Fig 2, 4(b)). Mancozeb showed significantly higher per cent of growth inhibition (54.25%; 64.44%; 84.44%) at all concentrations (1000; 2000; 2500 ppm) respectively. Significantly higher

Table 1: I*n vitro* evaluation of systemic, non-systemic and combi- fungicides against *C. lindemuthianum.*

Systemic fungicides				Non-systemic fungicides				Combi-fungicides			
Technical names of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition (%)	Technical names of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition (%)	Technical names of fungicides	Concentration (ppm)	Average colony diameter (mm) @	Per cent growth inhibition (%)
Carbendazim 50 WP	100	3.00	96.67	Copper	1000	47.33	47.41	Carbendazim	100	12.92	85.64
	250	2.43	97.30	oxychloride	2000	43.50	51.66	(12%) +	250	10.08	88.8
	500	0.00	100.00	50 WP	2500	39.50	56.11	Mancozeb (63%)	500	7.33	91.85
Carboxin 75 WP	100	39.25	56.38	Mancozeb	1000	41.17	54.25	Carboxin	100	40.17	55.36
	250	21.00	76.67	75 WP	2000	32.00	64.44	(37.5%) +	250	21.17	76.47
	500	12.25	86.38		2500	14.00	84.44	Thiram (37.5%)	500	12.17	86.47
Propiconazole 25 EC	100	24.58	72.69	Chlorothalonil	1000	42.00	53.33	Captan (70%)	100	40.00	55.56
	250	17.25	80.83	75 WP	2000	37.50	58.33	+ Hexaconazole	250	22.25	75.27
	500	0.00	100.00		2500	23.33	74.07	(5%)	500	5.83	93.52
Azoxystrobin 23 EC	100	44.28	50.8	Zineb	1000	40.75	54.72	Hexaconazole	100	35.00	61.11
	250	42.33	52.97	75 WP	2000	35.00	61.11	(4%) + Zineb	250	23.08	74.35
	500	25.25	71.94		2500	30.00	66.67	(68%)	500	15.00	83.33
Hexaconazole 5 EC	100	44.50	50.56	Sulphur	1000	65.50	27.22	Pyraclostrobin	100	24.33	72.96
	250	38.25	57.5	80 WP	2000	55.00	38.89	(5%) + Metiram	250	19.40	78.44
	500	35.75	60.27		2500	45.00	50.00	(55%)	500	15.08	83.24
Thiophanate methyl	100	22.33	75.19	Absolute	-	90.00	-	Absolute	-	90.00	-
70 WP	250	16.00	82.22	control				control			
	500	15.67	82.58								
Absolute control	-	90.00	-								
SEm ±		0.67		SEm ±		0.54		SEm ±		0.44	
CD at 5%		1.91		CD at 5%		1.57		CD at 5%		1.28	

percentage of growth inhibition were observed with zineb (54.72%) and chlorothalonil (53.33%) at 1000 ppm concentration. Comparatively sulphur (27.22%; 38.89%; 50.00%) showed less effectiveness against *C. lindemuthianum* at all concentrations (1000; 2000; 2500 ppm) respectively. The percentage of growth inhibition and the increase in chemical concentration were noticed to be positively correlated.

Comparable discoveries were made by Hedge (1998), who found that the non-systemic fungicide mancozeb at a concentration of 3000 ppm effectively controlled the *C. capsici* that causes chilli fruit rot. *In vitro* testing of fungicides against chickpea anthracnose (*C. dematium)* was done by Varaprasad (2000) and mancozeb (@ 0.2 and 0.3%) in the contact group of fungicides was found to be effective in inhibiting fungal growth. Mancozeb was also found to be the most effective contact fungicide against *C. trancatum* (Laxman, 2006).

Effect of different combi-fungicides on growth inhibition of *C. lindemuthianum*

Six combi-fungicides, which include carbendazim (12%) + mancozeb (63%), carboxin (37.5%) + thiram (37.5%), captan (70%) + hexaconazole (5%), hexaconazole (4%) + zineb (68%) and pyraclostrobin (5%) + metiram (55%), were *in vitro* evaluated for their effectiveness against *C. lindemuthianum*. The observation of average colony diameter and the percentage of growth inhibition was noted [Table 1, Fig 3, 4(c)]. Carbendazim 12% + mancozeb 63% showed higher growth inhibition (85.64%; 88.80%) over control at 100 and 250 ppm

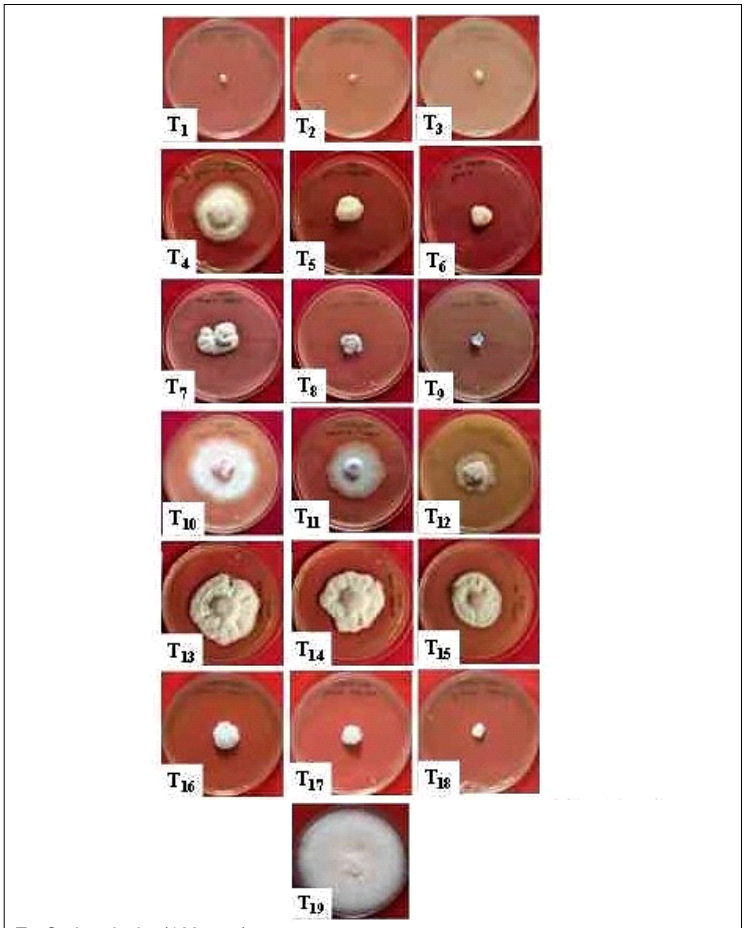

Fig 1: Growth inhibition of *C. lindemuthianum* at different concentrations of systemic fungicides.

respectively. The fungicides captan (70%) + hexaconazole (5%) (@ 55.56%) and carboxin (37.5%) + thiram (37.5%) (@ 55.36%) were found to be the least effective in preventing the growth of *C. lindemuthianum* at 100 ppm and captan (70%) + hexaconazole (5%) (75.27%) and hexaconazole 4% + zineb 68% (74.35%) at 250 ppm. Surprisingly, Captan 70% + hexaconazole 5EC (93.52%) emerged as effective in controlling pathogens than other fungicides at 500 ppm concentration. At 100 and 250 ppm, pyraclostrobin 5% + metiram 55% significantly outperformed the control of the pathogen, but both pyraclostrobin 5% + metiram 55% (83.24%) and hexaconazole 4% + zineb 68% (83.33%) were significantly less effective against *C. lindemuthianum* at 500 ppm. Growth inhibition percentage and chemical concentration increase was revealed to be positively correlated.

Above findings are in accordance with Madhusudhan (2002), who found that SAAF (carbendazim 12% + mancozeb 63%) significantly reduced the growth of *C. trancatum* at 0.25% and 0.5%, respectively, by 99.22 and 85.92%. Chaudhari and Gohel (2016) reported carbendazim (12%) + mancozeb (63%) (1000, 2000, 2500 ppm) completely inhibited the mycelial growth and thus appeared significantly superior against *C. gloeosporioides* causal fungus of green gram anthracnose.

Testing of effective fungicides in glasshouse

The potency of the effective fungicides discovered *in vitro* assay was further assessed in glasshouse experiment. Two systemic fungicides, carbendazim and propiconazole, two non-systemic fungicides, mancozeb and chlorothalonil, two combi-fungicides,

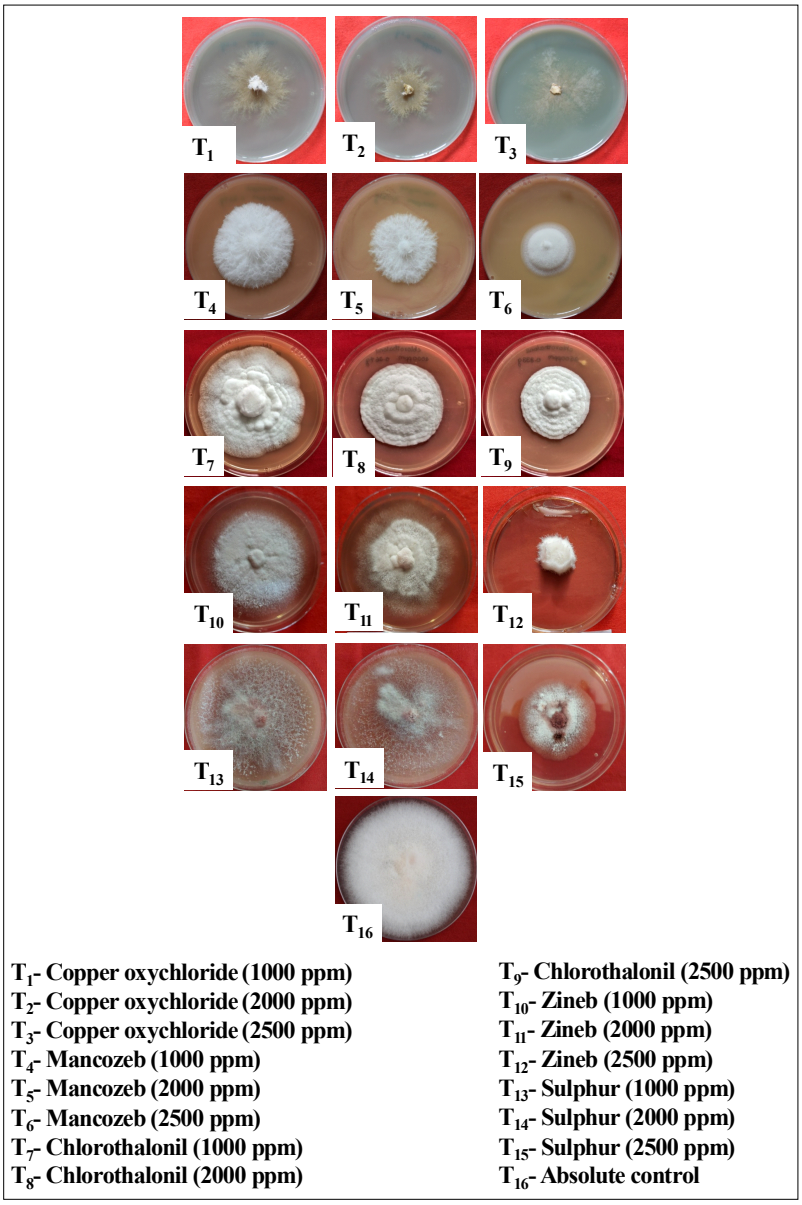

Fig 2: Growth inhibition of *C. lindemuthianum* at different concentrations of contact fungicides.

captan 70% + hexaconazole 5% and carbendazim 12% + mancozeb 63%, were tested with a control in a pot experiment under glasshouse conditions. For the management of green gram anthracnose, seed treatment and foliar spraying were used and it showed that all treatments significantly reduced the severity of the anthracnose of green gram disease. The observation after 7 days of treatment on per cent disease index was noted (Table 2, Fig 4d). The lowest percentage of disease index was found in two foliar sprays of carbendazim 50 WP @ 1.0 g/l (10.70%), propiconazole 25 EC @ 2.0 ml/l (13.34%) and captan 70% + hexaconazole 5% @ 0.6 g/l (26.22%) spaced 15 days apart. On the other hand, seeds treated with carbendazim 12% + mancozeb 63% @ 3.0 g/kg seed (24.03%) and mancozeb 75 WP @ 3.0 g/kg seed (35.55%) had the lowest disease index compared to the control (69.86%).

Many researchers conducted similar experiments in order to manage green gram anthracnose. Bharadwaj and Thakur (1991) used carbendazim (0.1%), captafol (0.25%) and mancozeb (0.25%) for managing leaf spot and pod blight of urd bean caused by *C. dematium* under field condition. Varaprasad (2000) employed an integrated approach for the control of chickpea blight disease. Seed treatment with carbendazim @ 2 g/kg + two foliar sprays of SAAF (@ 0.05%) at 15 days intervals displayed the maximum reduction in disease incidence. Joshi and Tripathi (2002) reported that seed treatment followed by two prophylactic sprays of bavistin or tilt @ 0.1% each at 15 days intervals showed minimum disease severity and maximum grain yield followed by contaf (0.1%) and indofil M-45 (0.2%) to manage black gram anthracnose. Chauhan *et al.* (2014) conducted an

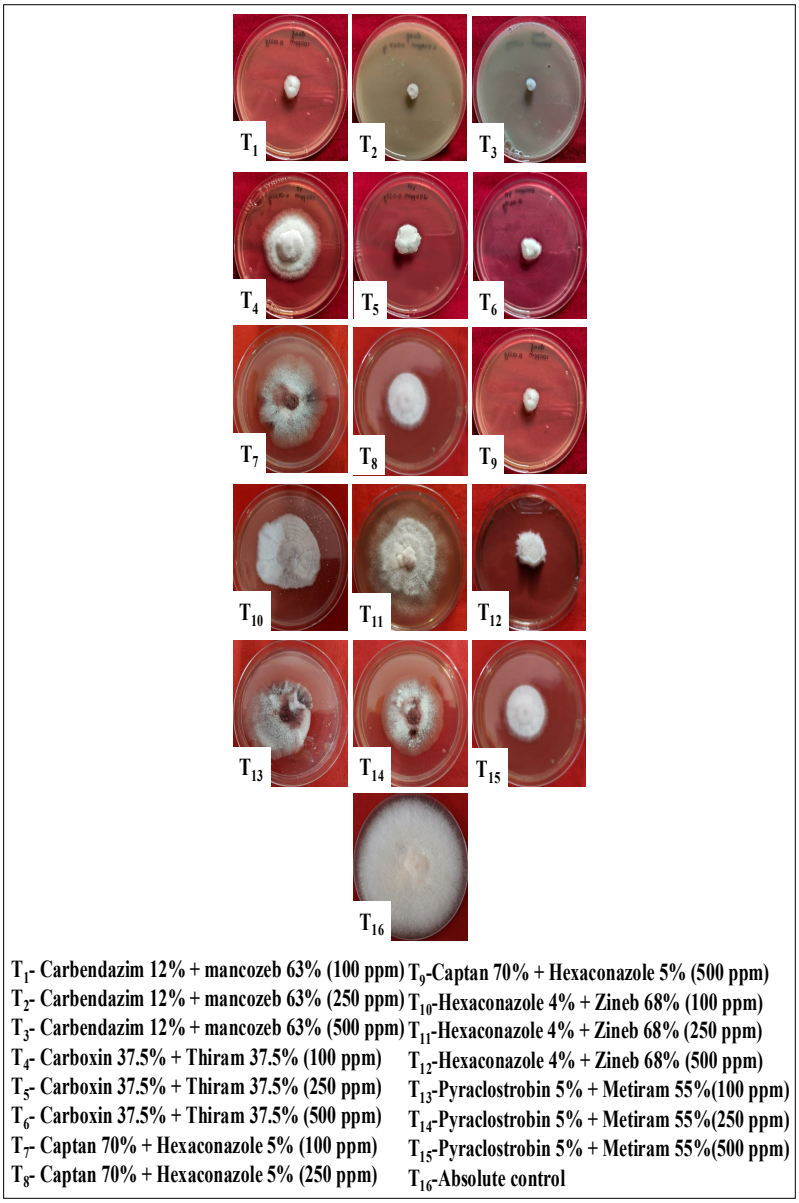

Fig 3: Growth inhibition of *C. lindemuthianum* at different concentrations of combi-fungicide.

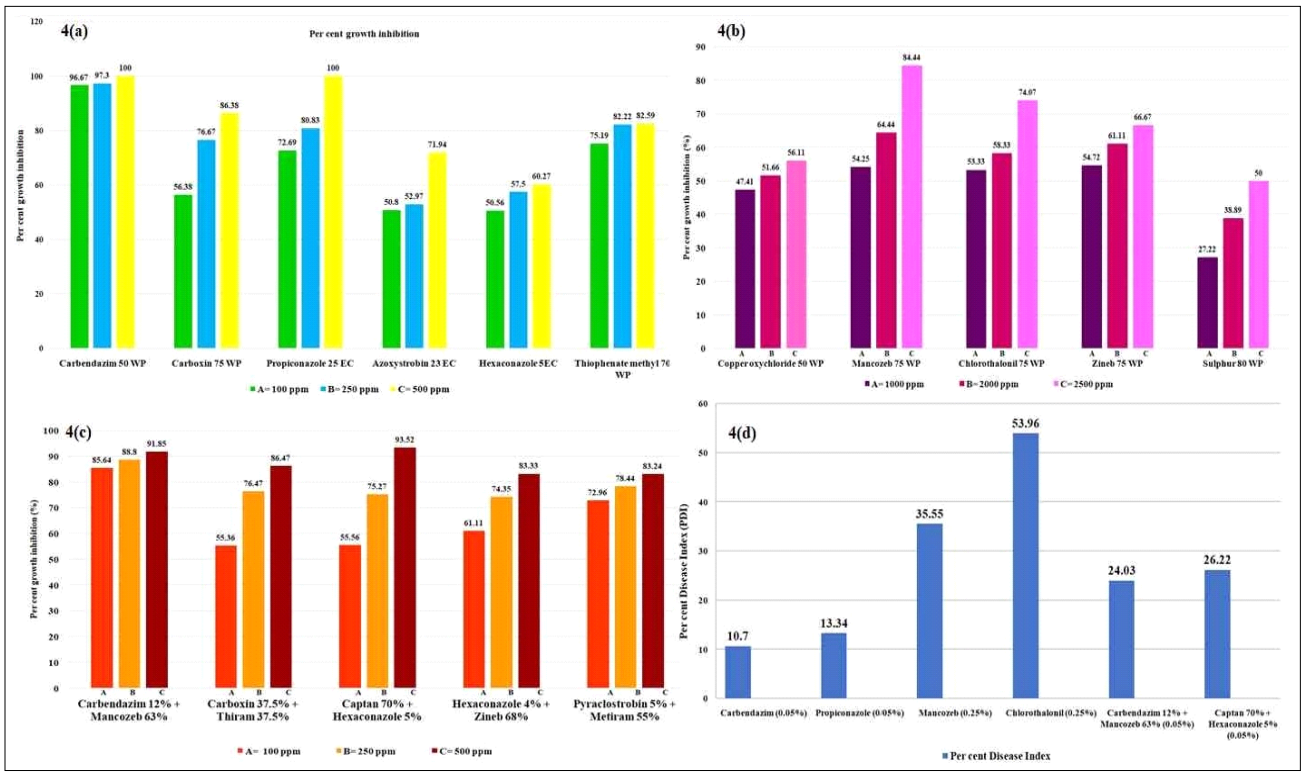

Fig 4: Per cent growth inhibition of *C. lindemuthianum* for various systemic 4(a), contact 4(b) and combi-fungicides 4(c). 4(d) Per cent disease index of *C. lindemuthianum* of efficiently performing fungicides.

Table 2: Management of anthracnose of green gram in glasshouse.

Treatments	Concentration	Method of application	Per cent disease index (%)
Carbendazim (0.05%)	1.0 g/l	Spraying	10.70
Propiconazole (0.05%)	2.0 ml/l	Spraying	13.34
Mancozeb	3.0 g/kg	Seed treatment	35.55
Chlorothalonil (0.25%)	3.3 g/l	Spraying	53.96
Carbendazim 12% + Mancozeb 63%	3.0 g/kg	Seed treatment	24.03
Captan 70% + Hexaconazole 5% (0.05%)	0.6 g/l	Spraying	26.22
Control	-	-	69.86
SEm±			0.01
CD at 5%			0.03
CV %			0.05

efficacy test of fungicides against chilli anthracnose caused by *C. capsici*. The minimum disease intensity of 19.67% was reported in carbendazim at 0.05% with two foliar sprays, sprayed at 15 days interval which was statistically at par with spraying of 0.2% mancozeb (20.71%), 0.20% carbendazim + mancozeb (22.5%) and 0.20% copper oxychloride (23.16%).

CONCLUSION

Fungicide testing provides preliminary information on the efficacy of fungicides against pathogens in a short period, serving as a guide for field testing. *In vitro* studies found that carbendazim, propiconazole, mancozeb, chlorothalonil, captan 70% + hexaconazole 5% and carbendazim 12% + mancozeb 63% were effective fungicides. Two systemic fungicides stood first in controlling pathogens at 500 ppm, followed by combi-fungicides and non-systemic fungicides. Foliar sprays of carbendazim 50 WP @ 1.0 g/l, propiconazole 25 EC @ 2.0 ml/l and captan 70% + hexaconazole 5% @ 0.6 g/l spaced 15 days apart in a glasshouse had the lowest disease index. Foliar sprays can be categorized as “systemic” or “non-systemic”. Systemic fungicides, like Carbendazim, interfere with DNA biosynthesis during fungal cell division, but their persistence and resistance development can lead to resistance. Combi-fungicides are a good option for managing anthracnose of green gram in south Gujarat.

Conflict of interest

All authors declared that there is no conflict of interest.

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