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Management of Cotton Leaf Spot Through Fungicides: An In Vitro Evaluation Against *Curvularia lunata*

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Abstract

Cotton (*Gossypium* spp.) is one of the most economically important fibre crops cultivated worldwide; however, its productivity is severely challenged by a range of foliar diseases. Among these, Curvularia leaf spot incited by *Curvularia lunata* has emerged as a significant threat to cotton cultivation in Gujarat, causing substantial deterioration in plant health and yield potential. Considering the increasing importance of this disease, the present investigation was undertaken to evaluate the fungitoxic efficacy of selected non-systemic, systemic, and combination fungicides against *C. lunata* under laboratory conditions. The study was conducted using the poisoned food technique in a Completely Randomized Design with three replications, wherein fifteen fungicides were assessed at different concentrations for their ability to suppress mycelial growth of the pathogen. The results revealed pronounced differences in antifungal activity among the fungicides tested. Among the non-systemic fungicides, mancozeb 75 WP exhibited the highest inhibitory effect on fungal growth. Systemic fungicides demonstrated remarkable efficacy, with propiconazole 25 EC, difenconazole 25 EC, and hexaconazole 5 EC achieving complete inhibition of mycelial growth at 500 ppm. Notably, among the combination fungicides, metalaxyl + mancozeb 72 WP proved exceptionally potent, recording cent per cent inhibition of *C. lunata* at all concentrations evaluated. A clear concentration-dependent increase in growth suppression was observed, highlighting the influence of fungicide dosage on pathogen inhibition. Overall, systemic and combination fungicides outperformed non-systemic fungicides in restricting the growth of *C. lunata*, underscoring their superior fungitoxic potential. The findings of the present study provide valuable insights into the selection of effective fungicidal molecules and offer a scientific basis for developing robust and sustainable management strategies against Curvularia leaf spot, thereby contributing to improved cotton health, productivity and profitability.

Key words: *Cotton, Curvularia leaf spot, Poisoned food technique, Mycelial growth inhibition, Disease management.*

Introduction

Cotton is the world's most widely grown fibre crop, which belongs to the genus Gossypium in the family *Malvaceae* (Anonymous, 2017). It is oldest among the commercial crops of the world and also known as "THE KING OF FIBERS" and "WHITE GOLD". It has delicate, white, soft and fluffy fibre that is made of about 87 to 90 per cent of cellulose. Cotton has been cultivated as over thousands of years for both the food and fibre. It is versatile crop, its fibre is used as raw material in textile, pulp and paper industries and oil extracted from the cotton seed is used in food, cosmetics, chemicals and pharmaceuticals (Proto *et al*., 2000).

The symptoms of Curvularia leaf spot appear initially as small circular brown to brownish black spot surrounding with yellow hallow, later it become dark yellow to brown hallow surrounding to brownish black spots (Joshi *et al.,* 2023).

Curvularia is a wide spread air borne facultative weak pathogen, which mostly survives as a saprophyte in tropical and sub-tropical areas. It is a dematiaceous, filamentous fungus. *Curvularia* spp. are darkly pigmented fungi with spores (conidia) efficiently adapted for most aerial dissemination. Some species have caused devastating disease epidemics of important food crops such as rice, wheat and maize (Sivanesan, 1987). The Curvularia leaf spot injures or kills the leaf tissues and thereby reduces the area of chlorophyll which involved in photosynthesis. If considerable leaf area is killed, then vigour and yields are reduced drastically.

Unfortunately, in our efforts for maximization of crop production by evolving various operations in crop production system very often tends to increase the potential disease hazards of some diseases which are either new or of major importance to the crop, Curvularia leaf spot disease is one example of a minor disease gaining such importance. Anamorph of *Curvularia lunata* is *Cochliobolus lunatus* (Nelson and Haasis, 1964) has been known to cause a leaf spot of cotton in India (Sharma and Chauhan, 1985). This fungus is found throughout the tropics and has a wide host range in tropical countries of South East Asia such as Thailand, Cambodia, Vietnam, Indonesia and Nepal. Looking into the occurrence of the Curvularia leaf spot disease in cotton crop ,it has the potential to spread drastically over a large area.

Curvularia lunata is an important fungal pathogen causing leaf spot disease in cotton, leading to reduction in photosynthetic area and ultimately affecting crop yield and quality. The disease has been observed with increasing incidence under warm and humid environmental conditions prevailing in many cotton-growing regions of Gujarat. Continuous cultivation of susceptible varieties and favorable climatic conditions may further enhance the spread and severity of the disease. Although several fungicides are available for disease management, information regarding their effectiveness against *Curvularia lunata* under laboratory conditions is limited. Therefore, evaluation of different fungicides against the pathogen is essential for identifying effective chemicals for the management of Curvularia leaf spot of cotton.

Materials and methods

The experiment was conducted in the Department of Plant Pathology, Post Graduate Laboratory, N. M. College of Agriculture, Navsari Agricultural University (NAU), Navsari, Gujarat. The study was laid out in a Completely Randomized Design (CRD) comprising sixteen fungicidal treatments (Systamic, Non- systamic and Combi-product) with three replications for each treatment. The efficacy of different fungicides against the test pathogen was evaluated under in vitro conditions using the poisoned food technique. The observations recorded from the experiment were subjected to statistical analysis as per the Completely Randomized Design to determine the effectiveness of the fungicidal treatments.

Different fungicides (Table 1 to 3) were tested for their effect on the growth of pathogen using the poisoned food technique (Sinclair and Dhingra, 1985). The technique involves cultivation of test organisms on a medium containing the test chemicals.

In all the experiments, PDA was used as a basal medium. The required quantity of each chemical was incorporated aseptically in 100ml of PDA in 250ml flasks at the time of pouring the media in Petri plates.

The medium is shaken well to give uniform dispersal of the chemicals and then in each Petri plate 5ml of medium was poured aseptically and allowed to solidify. The Petri plates were inoculated with a 5mm diameter mycelial disc cut from the periphery of 10days old fungal cultures. The mycelial disc was placed in the centre of the plates in an inverted portion to make a direct contact with the poisoned medium and incubated at 27±2ºC for 10days after inoculation simultaneously, a suitable control was also maintained by growing the fungus on chemical free PDA medium. Observations on the linear growth were recorded when full growth of the fungus is observed in the control Petri plate (Patel, 2019 and Patel, 2022).

The Per cent Growth Inhibition (PGI) of growth of the fungus in each treatment was calculated by using the formula given by Skidmore and Dickenson (1976); Bhaliya and Jadeja (2014).

$$PGI = (C - T)/C \ X \ 100$$

Where,

P G I = Per cent Growth Inhibition
C = Growth in control (mm)
T = Growth in treatment (mm)

Table 1. List of non systemic fungicides

Treatment No.	Fungicides	Concentrations (ppm)		
		1	2	3
T1	Mancozeb 75 WP	1500	2000	3000
T2	Copper oxychloride 50 WP	1500	2000	3000
T3	Chlorothalonil 75 WP	1500	2000	3000
T4	Captan 50 WP	1500	2000	3000
T5	Propineb 70 WP	1500	2000	3000
T6	Control	-	-	-

Table 2. List of systemic fungicides

Treatment No.	Fungicides	Concentrations (ppm)		
		1	2	3
T1	Carbendazim 50 WP	100	250	500
T2	Fosetyl AL 80 WP	100	250	500
T3	Difenconazole 25 EC	100	250	500
T4	Hexaconazole 5 EC	100	250	500
T5	Propiconazole 25 EC	100	250	500
T6	Control	-	-	-

Table 3. List of combination product of fungicides

Treat. No.	Fungicides	Concentrations (ppm)		
		1	2	3
T1	Pyraclostrobin + Metiram 60 WG	1500	2000	2500
T2	Captan + Hexaconazole 75 WP	1500	2000	2500
T3	Azoxystrobin + Difenconazole 29.6 SC	1500	2000	500
T4	Metalaxyl + Mancozeb 72 WP	1500	2000	500
T5	Fluxapyroxad 167g/l + Pyraclostrobin 333g/l SC	1500	2000	500
T6	Control	-	-	-

Table 4. Inhibitory effect of non systemic fungicides against the *Curvularia lunata* under *in vitro* condition

Sr. No.	Technical name of fungicide	Conc. (ppm)	Average colony diameter of pathogen (mm)	Per cent inhibition over control
T1	Mancozeb (75 WP)	1500	35.00	61.10# (51.82)
		2000	33.00	63.33 (52.92)
		3000	30.00	66.66 (54.35)
T2	Copper oxychloride (50 WP)	1500	56.33	37.42 (37.57)
		2000	43.00	52.22 (46.25)
		3000	39.00	56.66 (48.76)
T3	Chlorothalonil (75 WP)	1500	48.33	46.30 (42.09)
		2000	45.00	50.00 (44.98)
		3000	43.00	52.20 (46.62)
T4	Captan (50 WP)	1500	57.33	36.30 (36.99)
		2000	49.66	44.80 (41.95)
		3000	37.00	58.80 (50.08)
T5	Propineb (70 WP)	1500	54.33	39.60 (38.94)
		2000	51.33	42.90 (40.78)
		3000	50.00	44.40 (41.75)
T6	Control	-	90.00	-
	SEm±	-	0.56	0.38
	CD at 5%	-	1.64	1.10
	CV%	-	2.33	1.65

#Figures outside the parentheses are original values where in parentheses are arc sin transformed values and average of three repetition

Result and discussion

Effect of different non systemic fungicides on the growth inhibition of *Curvularia lunata*

The non systemic fungicides *viz*., mancozeb 75 WP, copper oxychloride 50 WP, chlorothalonil 75 WP, captan 50 WP and propineb 70 WP were evaluated at 1500, 2000 and 3000ppm conc. using the poisoned food technique. The data revealed that as the fungicidal concentration increased the growth of pathogen decreased. The observations regarding the per cent inhibition of linear growth are presented in Table 4 and depicted in Photo 1 with Fig. 1.

Out of five non systemic fungicides tested, the efficacy of mancozeb 75 WP was found the best with the highest mean per cent growth inhibition of *C. lunata* i.e., 61.10, 63.33 and 66.66 at 1500, 2000 and 3000ppm concentration, respectively.

The next best fungicide in order of merit at 3000ppm concentration was captan 50 WP (58.8%), which was followed by copper oxychloride 50 WP (56.66%), chlorothalonil 75 WP (52.20%) and propineb 70 WP

(44.40%). While, in case of the next best fungicide in order of merit at 2000ppm concentration was copper oxychloride 50 WP (52.22%) followed by chlorothalonil 75 WP (50.00%), captan 50 WP (44.80%) and propineb 70 WP (42.90%) and at 1500ppm concentration was mancozeb 75 WP (61.10%) followed by chlorothalonil 75 WP (46.30%), propineb 70 WP (39.60%), copper oxychloride 50 WP (37.42%) and captan 50 WP (36.30%) inhibited the growth of pathogen *C. lunata*.

It is evident from the test results that the growth inhibition of *C. lunata* increased as an increase in the concentration of the respective fungicides.

The present results are in agreement with the research findings obtained by Yan *et al.* (2003) observed that the dithaneM-45, a synthetic fungicide performed significantly superior in the control of *C. lunata* in maize crop. Sumangala *et al*. (2008) evaluated various fungicides, botanicals and bioagents against *C. lunata*, a causal agent of grain discoloration in rice. Among the four systemic fungicides, maximum inhibition of mycelial growth was obtained from difenconazole (98.80%) and propiconazole (98.10%) at 0.1 per cent concentration. Among the non systemic fungicides, mancozeb (98.80%) was found the most effective. Mamun *et al*. (2020) evaluated the efficacy of five fungicides with different active ingredients viz., bavistin DF, capvit 50 WP, dithane M-45, green gel and tilt 250 EC were selected to evaluate their *in vitro* efficacy at 100, 200, 400 and 500ppm concentrations against the *C. lunata* fungus of jute seed. Dithane M-45 and tilt 250 EC completely inhibited the radial growth of *C. lunata* at all the concentrations while, bavistin DF completely inhibited the radial growth of Fusarium oxysporum at all the concentrations. The present investigation revealed that tilt 250 EC, dithane M-45 and capvit were the best fungicide in inhibiting the growth of *C. lunata* and *F. oxysporum*.

Effect of different systemic fungicides on the growth inhibition of *Curvularia lunata*

The systemic fungicides *viz*., carbendazim 50 WP, fosetyl AL 80 WP, difenconazole 25 EC, hexaconazole 5 EC and propiconazole 25 EC were evaluated at 100,250 and 500ppm concentrations using the poisoned food technique. The results revealed that as the fungicidal concentration increased the growth of the pathogen decreased. The observations regarding the per cent growth inhibition of linear growth are presented in Table 5 and depicted in Photo 2 with Fig. 2.

All the five systemic fungicides screened at three concentrations *viz*., 100, 250 and 500ppm were found significantly superior in inhibiting the mycelial growth of *C. lunata*. Out of five systemic fungicides tested, propiconazole 25 EC, difenconazole 25 EC and hexaconazole 5 EC at 500ppm, concentration completely inhibited cent per cent growth of the *C. lunata*.

The next best fungicide in order of merit at 500ppm concentration was fosetyl Al 80 WP(86.66%) and carbendazim 50 WP (62.00%). While, in case of the best fungicide at 250ppm concentration was propiconazole 25 EC (97.77%), hexaconazole 5 EC (97.77%) and difenconazole 25 EC (97.77%) followed by fosetyl Al 80 WP (70.37%) and carbendazim 50 WP (58.88%) and at 100ppm concentration, propiconazole 25 EC (96.66%) and difenconazole 25 EC (96.66%) followed by hexaconazole 5 EC (95.55%), fosetyl AL 80 WP (67.77%) and carbendazim 50 WP (46.55%) respectively.

It is evident from the test results that the growth inhibition of *C. lunata* increased as an increase in the concentration of the fungicides. The fungicide difenconazole 25 EC and propiconazole 25 EC was proved to be the most effective fungicide for the control of the pathogen.

These results are in agreement with the research results obtained by Sumangala *et al*. (2008) evaluated various fungicides, botanicals and bioagents against *Curvularia lunata*, a causal agent of grain discoloration in rice. Among the four systemic fungicides, maximum inhibition of mycelial growth was obtained from difenconazole (98.80%) and propiconazole (98.10%) at 0.1 per cent concentration. Among the non systemic fungicides, mancozeb (98.80%) was found the most effective.

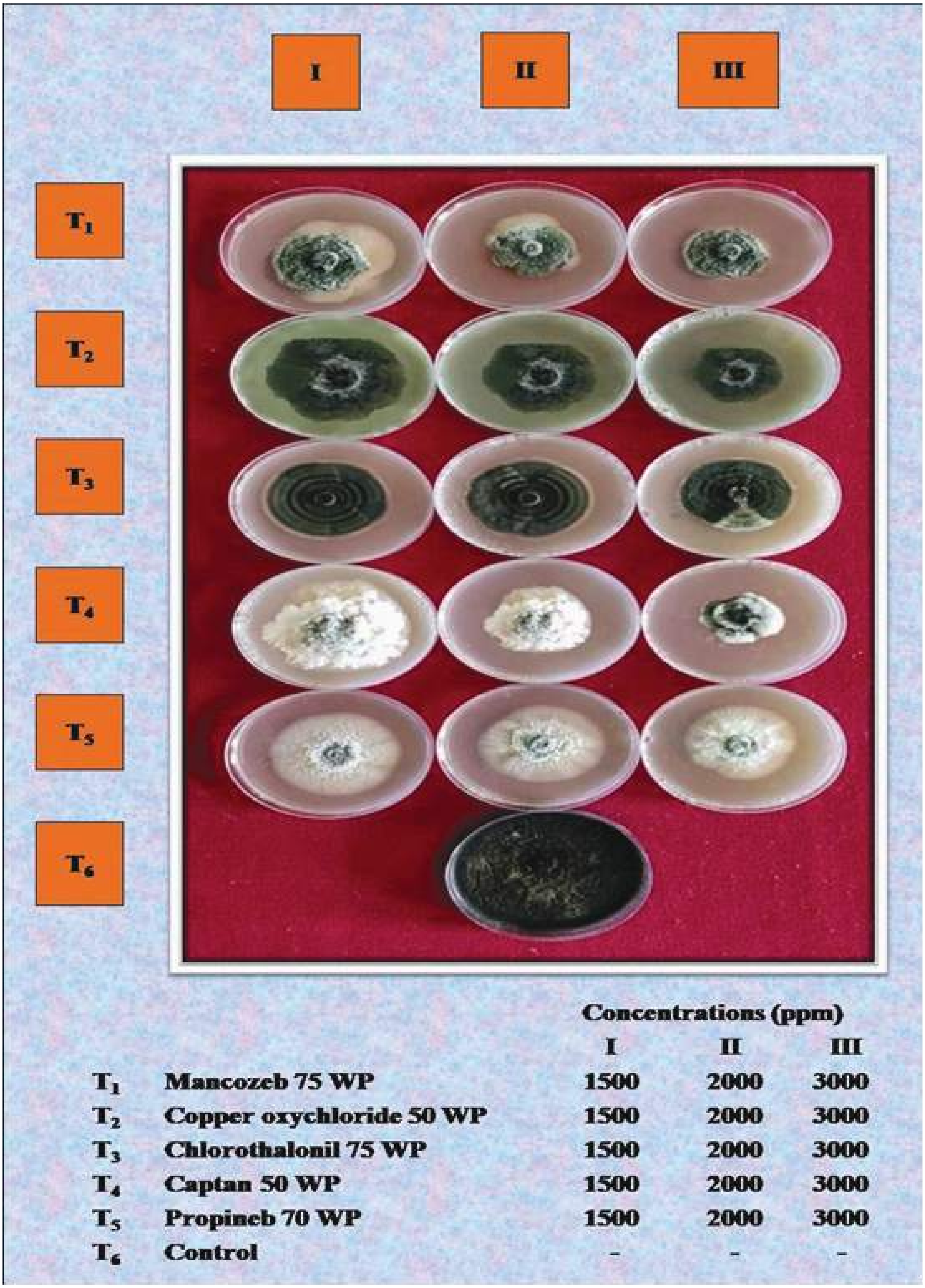

Photo 1. Evaluation of non systemic fungicides against the *Curvularia lunata*

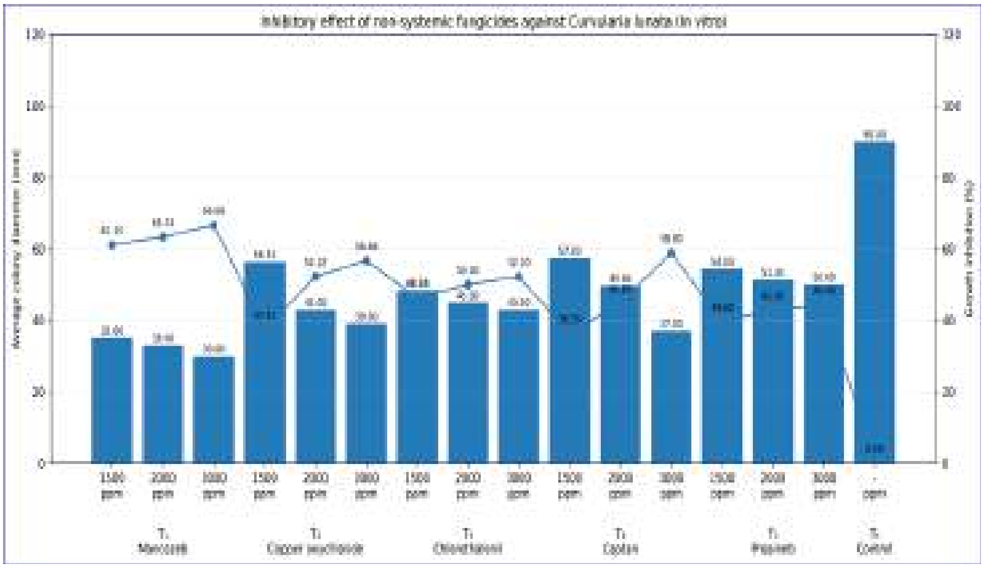

Fig. 1. Inhibitory effect of non systemic fungicides against pathogen under *in vitro* condition

Table 5. Inhibitory effect of systemic fungicides against the *Curvularia lunata* under *in vitro* condition

Sr. No.	Technical name of fungicides	Conc. (ppm)	Average colony diameter of pathogen(mm)	Per cent inhibition over control
T1	Carbendazim (50 WP)	100	48.10	46.55# (42.93)
		250	37.00	58.88 (49.88)
		500	34.20	62.00 (51.72)
T2	Fosetyl AL (80 WP)	100	29.00	67.77 (55.06)
		250	26.66	70.37 (57.61)
		500	12.00	86.66 (68.45)
T3	Difenconazole (25 EC)	100	3.00	96.66 (79.28)
		250	2.00	97.77 (80.89)
		500	0.00	100.00 (90.00)
T4	Hexaconazole (5 EC)	100	4.00	95.55 (77.85)
		250	2.00	97.77 (80.94)
		500	0.00	100. 00 (90.00)
T5	Propiconazole (25 EC)	100	3.00	96.66 (79.28)
		250	2.00	97.77 (80.94)
		500	0.00	100.00 (90.00)
T6	Control	-	90.00	(0.00) 0.40
	SEm±	-	0.43	0.40
	CD at 5 %	-	1.25	1.17
	CV %	-	4.08	1.04

#Figures outside the parentheses are original values where in parentheses are arc sin transformed values and average of three repetition

		Concentrations (ppm)		
		I	II	III
T_1	Carbendazim 50 WP	100	250	500
T_2	Fosetyl AL 80 WP	100	250	500
T_3	Difenconazole 25 EC	100	250	500
T_4	Hexaconazole 5 EC	100	250	500
T_5	Propiconazole 25 EC	100	250	500
T_6	Control	-	-	-

Photo 2. Evaluation of systemic fungicides against the *Curvularia lunata*

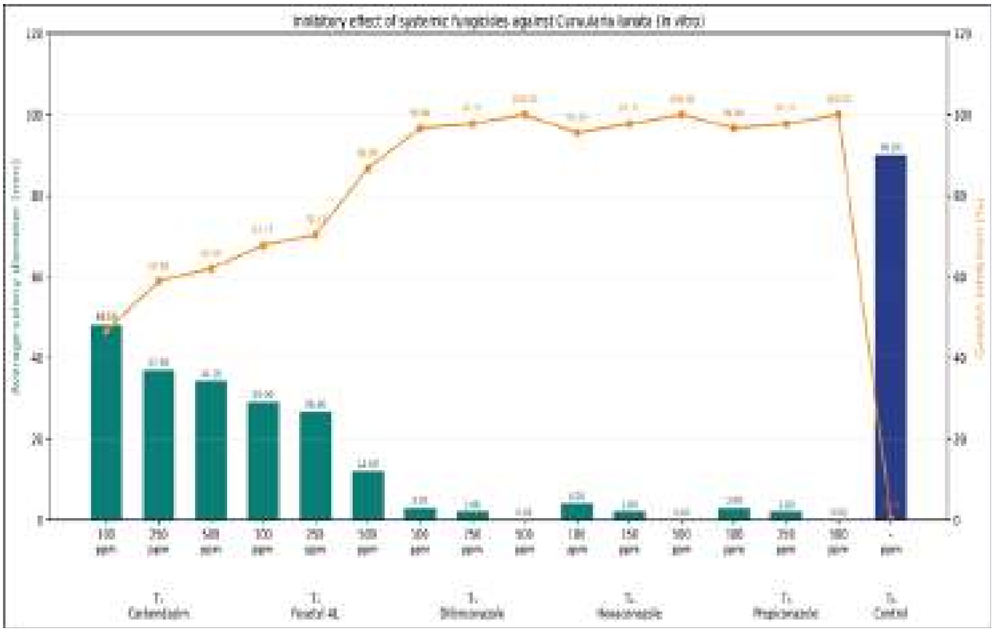

Fig. 2. Inhibitory effect of systemic fungicides against pathogen under *in vitro* condition

Effect of different combination products of fungicides on the growth inhibition of *Curvularia lunata*

The different combinations of fungicides *viz*., pyraclostrobin + metiram 60 WG, captan + hexaconazole 75 WP, azoxystrobin + difenconazole 29.6 SC, metalaxyl + mancozeb 72 WP and fluxapyroxad 167g/l + pyraclostrobin 333g/l SC were evaluated at 1500, 2000 and 2500ppm concentrations using the poisoned food technique. The data revealed that as the fungicidal concentration increased the growth of the pathogen decreased. The regarding per cent inhibition of linear growth is presented in Table 6 and depicted in Photo 3 with Fig.3.

Out of five combination products of the fungicides tested, efficacy of a metalaxyl + mancozeb 72 WP was the best with cent per cent growth inhibition of *C. lunata.* At all the three concentrations inhibited cent per cent growth of the *C. lunata* and proved extremely fungitoxic.

The next best fungicide combination in order of merit at 2500ppm concentration was pyraclostrobin + metiram 60 WG (100%) followed by fluxapyroxad 167g/l + pyraclostrobin 333g/l SC (98.88%), captan + hexaconazole 75 WP (96.66%) and azoxystrobin + difenconazole

29.6 SC (93.33%). The next best fungicide combination at 2000ppm concentration was pyraclostrobin + metiram 60 WG (100%) followed by fluxapyroxad 167g/l + pyraclostrobin 333g/l SC (97.77%), captan + hexaconazole 75 WP (90.00%) and aazoxystrobin + difenconazole 29.6 SC(86.55%) and at 1500ppm concentration was fluxapyroxad 167g/l + pyraclostrobin 333g/l SC (96.66%) followed by pyraclostrobin + metiram 60 WG (95.23%), azoxystrobin + difenconazole 29.6 SC (85.55%) and captan + hexaconazole 75 WP (83.33%).

These results are in agreement with the results obtained by Abrar *et al*. (2020) conducted *in vitro* study to assess the potential of three synthetic fungicides to control *C. lunata*. The fungicides evaluated in this study were thiophanate methyl WP, metalaxyl + mancozeb 72 WP and fosetyl Al 80 WP. The con. of these fungicides used in food poisoning technique were 50, 100, 150, 200 and 250ppm, which was compared with a control having 0ppm concentration. Metalaxyl + mancozeb proved the best fungicide in inhibiting the fungal growth by 26 to 67 per cent over control.

Table 6. Inhibitory effect to combination products of the fungicides against the *Curvularia lunata* under *in vitro* condition

Sr. No.	Technical name of fungicides	Conc. (ppm)	Average colony diameter of pathogen (mm)	Per cent inhibition over control
T_1	Pyraclostrobin + Metiram 60 WG	1500	4.00	95.23[#] (77.50)
		2000	0.00	100.00 (90.00)
		2500	0.00	100.00 (90.00)
T2	Captan+ Hexaconazole 75 WP	1500	15.00	83.33 (66.00)
		2000	9.00	90.00 (71.72)
		2500	3.00	96.66 (79.43)
T_3	Azoxystrobin + Difenconazole 29.6 SC	1500	13.00	85.55 (67.48)
		2000	12.10	86.55 (68.22)
		2500	6.00	93.33 (75.18)
T_4	Metalaxyl + Mancozeb 72 WP	1500	0.00	100.00 (90.00)
		2000	0.00	100.00 (90.00)
		2500	0.00	100.00 (90.00)
T_5	Fluxapyroxad 167g/l+ Pyraclostrobin 333g/l SC	1500	3.00	96.66 (79.62)
		2000	2.00	97.97 (80.64)
		2500	1.00	98.88 (83.03)
T_6	Control	-	90.00	0.00 (0.00)
	SEm±	-	0.204	2.12
	CD at 5%	-	0.591	0.73
	CV %	-	3.62	1.69

#Figures outside the parentheses are original values where in parentheses are arcsine transformed values and average of three repetition

Conclusion

Fifteen fungicides of three different type *viz*., five non systemic, five systemic and five combination products of fungicides were evaluated at three different concentrations by poisoned food technique for evaluating their efficacy against *Curvularia lunata*. Among them, non systemic fungicides mancozeb 75 WP, systemic fungicides propiconazole 25 EC and difenconazole 25 EC, whereas combination products of fungicides, metalaxyl + mancozeb 72 WP significantly inhibited growth of *C. lunata* and proved strongly fungi toxic in nature.

Photo 3. Evalution of combination product of the fungicides against *Curvularia lunata*

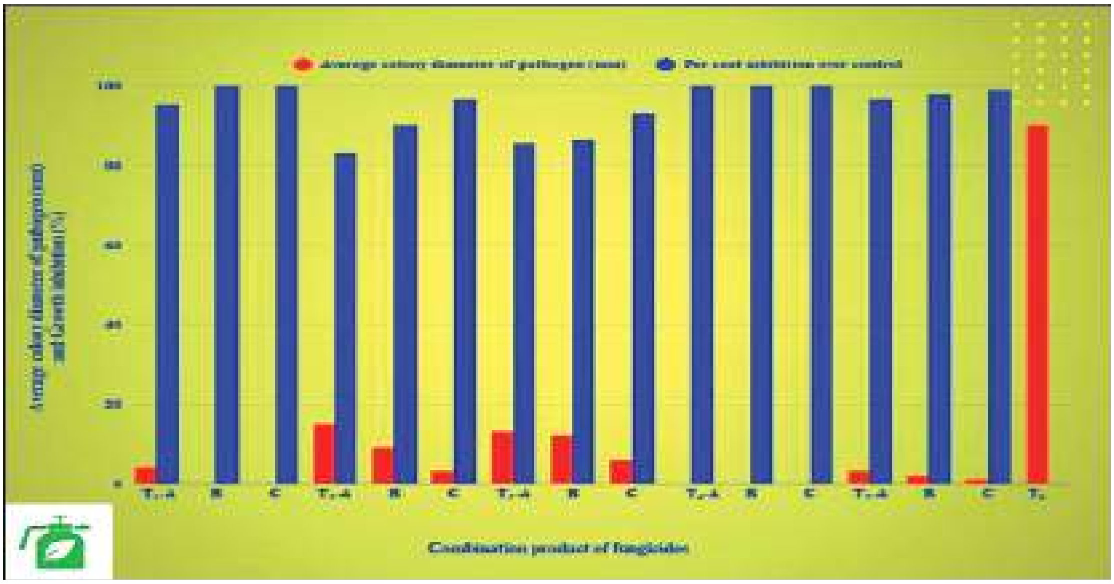

Fig. 3. Inhibitory effect of combination product of fungicides against pathogen

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