Original Research Article

Prospecting the effectiveness of fungicides and bioagents against leaf spot of mango

ABSTRACT

Aims: The leaf spot caused by *Colletotrichum gloeosporioides*(Penz.) Penz &Sacc. is one of the most serious disease in all mango growing regions of the world. *In vitro*bioassay of fungicides and bioagents were performed to evaluate the effectiveness of different fungicides and bioagents against *C. gloeosporioides.*

Study design: Poison food techique for fungicides bioassay and dual culture technique for bioagents bioassay.

Place and Duration of Study: The laboratory studies were conducted in the Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari, Gujarat, India during 2020–2021.

Methodology:*The poison food technique assessed the efficacy of systemic, contact and combination fungicides against C. gloeosporioides. **While the dual culture method was employed to gauge the potency of biocontrol agents, against**C. gloeosporioides.*

Results: In laboratory screening, systemic fungicides such as carbendazim and difenconazole were shown to have 100% growth suppression at 500 ppm. Mancozeb and copper oxychloride, two contact fungicides, demonstrated 100% growth inhibition of *C. gloeosporioides* at 2500 ppm . The combi-product fungicides tricyclazole 18% + mancozeb 62%, hexaconazole 4% + zineb 68%, and carbendazim 12% + mancozeb 63% were shown to exhibit 100% growth inhibition at 2000 ppm. Using the dual culture method, five distinct bioagents were tested *in vitro* for their ability to prevent the development of *C. gleosporiodes.Trichoderma harzianum* showed up as one of them to be a powerful and effective antagonist of *C. gleosporiodes*.

Conclusion:*In vitro* fungicide and bioagent testing provides preliminary data on the effectiveness of fungicides against pathogens, guiding field testing. The study evaluated the effectiveness of fungicides and bioagents in suppressing the leaf spot pathogen of mango.

Keywords: Leaf spot, mango, poison food techniques, dual culture technique

1. INTRODUCTION

Mango (*Mangifera indica* L.) commonly called as king of fruit, is the most important crop among the tropical and sub-tropical fruit crops belonging to the family *Anacardiaceae*, which is grown in more than 110 countries of the world. Mango fruit is a potential source of sugar, pectin, vitamin-A, vitamin-C, and minerals like phosphorous, calcium *etc.* It is also extensively used for medicine and culinary purposes. The availability, acceptability and multipurpose utilization have adorned King's crown on mango. Hence mango has been called as "The First Fruit of India." Although mango is considered to be a hardy plant, it is susceptible to various diseases, insect pests and physiological disorders. Among the various fungal diseases, leaf spot caused by *Colletotrichum gloeosporioides*(Penz.) Penz &Sacc. is one of the most serious disease in all mango growing regions of the world. The disease was first identified in India by McRae [1]. The ubiquitous fungus *C. gloeosporioides*Penz and Sacc. is the anamorph stage (asexual stage of the pathogenic fungus). Itis responsible for many diseases, also referred to as "anthracnose." on many tropical fruits including banana, avocado, papaya, coffee, passion fruit, and others [2].

The pathogen causes black leaf spot, leaf blight, blossom blight, fruit rot and in severe cases die-back [3,4]. In India, it is cultivated in an area over 21,63,470 hectares with a production of 18,52,980 metric tonnes of fruit [5]. It is an important fruit crop in area and production in Gujarat also, where it is cultivated over an area of about 130.1 thousand hectares with annual production of 911.3 thousand tones with productivity of 7.01 tones/ha [6].

During last few years, mango is affected by leaf spot disease at seedling stage and further it causes heavy loss in orchard. Considering the seriousness of the problem and its damage, this research problem was selected for investigation on *in vitro*bioassay of fungicides and bioagents to combat *C. gloeosporioides.*

2. MATERIAL AND METHODS

2.1 *In vitro* evaluation of fungicides against *C. gloeosporioides*

Poisoned food technique was employed to test the *in vitro* efficacy of fungicides against *C. gloeosporioides*belonging to different chemical groups (Systemic, contact and combi-product fungicides) at three different concentrations (Table 1). In all experiments, PDA was used as basal medium. The requisite quantity of the fungicide was mixed in 100 ml PDA medium in 250 ml flask and well shaken to facilitate uniform mixture of fungicides and 20 ml was poured in each sterilized plate.

After 24 hrs a disc of five mm of test fungus was placed in the centre of each poured plate. The discs were cut with the help of a sterilized cork borer from 10 days old culture of *C. gloeosporioides*. Inoculated plates were incubated at 27±1°C. Suitable check was maintained without fungicide and inoculated with *C. gloeosporioides*. The colony growth was measured after 24hrs interval till the entire plate of control treatment was completely covered with mycelium.
The per cent growth inhibition (PGI) over control was calculated using the following formula [7]:

$$\text{Per cent Growth Inhibition} = \frac{C-T}{C} \times 100$$

Where,
C= Colony diameter of control (mm)
T= Colony diameter of treatment (mm)

2.2 *In vitro* evaluation of bioagents against *C. gloeosporioides*

To determine the antagonistic action of various known species of fungal and bacterial bioagents, the dual culture test was carried out. Twenty ml of media poured aseptically in each of the Petri plates and allowed to solidify. A 5 mm mycelial disc of ten-days old culture of *C. gloeosporioides*was placed 10 mm away from the periphery of the Petri plate and on the opposite end (approximately 70 mm away) a 5 mm disc of four days old culture of biocontrol agent was placed at a distance of 10 mm from the periphery. A control having the test pathogen only was kept for comparison. The Petri plates were incubated at 27±1°C till the *C. gloeosporioides*covered the medium surface in control. Radial growth of the pathogens in dual culture was recorded at 24hrs interval. Index of antagonism was determined in each treatment by following standard formula [7]:

$$PGI = \frac{100\ (DC-DT)}{DC}$$

Where,
PGI=Per cent growth inhibition,
DC=Average diameter of mycelial colony of control (mm)
DT=Average diameter of mycelial colony of pathogens in treatment (mm)

2. RESULTS AND DISCUSSION

3.1 *In vitro* evaluation of fungicides against *C. gloeosporioides*

3.1.1 Effect of different systemic fungicides on growth inhibition of *C.gloeosporioides*

The systemic fungicides *viz.,* carbendazim 50 WP, kresoxim methyl 44.3SC, propiconazole 25 EC, difenconazole 25 EC, tebuconazole 25 EC and thiophanate methyl 70 WP were evaluated at 100, 250 and 500 ppm concentrations to check their efficacy against *C. gloeosporioides in vitro*. The observations regarding per cent inhibition of linear growth are presented in table 1 and depicted in figure 1(a), 3(a).

At 100 ppm concentration, significantly highest per cent growth inhibition over control was recorded in carbendazim (91.73%) as compared to rest of fungicides. Next best in order of

merit was propiconazole (90.22%). The remaining fungicides *viz.,* difenconazole (62.02%), kresoxim methyl (50.00%), tebuconazole (38.72%), thiophanate methyl (28.57%) proved comparatively less effective against *C.gloeosporioides*. At 250 ppm concentration, propiconazole recorded significantly highest per cent growth inhibition (93.98%) as compared to rest of fungicides. Next best in order of merit was carbendazim (93.60%), followed by difenconazole (76.68%) was also considerably effective fungicide but tebuconazole (65.79%), kresoxim methyl (63.53%), thiophanate methyl (53.76%) proved comparatively less in their efficacy against *C. gloeosporioides*at 250 ppm. At 500 ppm concentration, carbendazim and difenconazole recorded cent per cent growth inhibition of the *C. gloeosporioides*. Propiconazole (95.10%) and tebuconazole (81.58%) was significantly superior in inhibiting the fungal growth over rest of the fungicides tested. Next best in order of merit was thiophanate methyl (77.44%). kresoxim methyl (71.04%) was comparatively less effective against *C.gloeosporioides*. The growth inhibition per cent positively correlated with increase in concentration for all the chemicals tested. The growth inhibition of fungus increased with the increase in concentrations.

There was no mycelial growth of the *C. gloeosporioides*in carbendazim and difenconazole at 500 ppm and also significantly lesser growth at 100 and 250 ppm. Thus, both the fungicides proved the most effective for *C. gloeosporioides*. Next best fungicide in order of merit was propiconazole. While, the rest of the fungicides were comparatively medium or less effective. Thiophanate methyl was found least effective at all concentration as compared to other fungicides.

Similar findings were also obtained from earlier research work. It was reported that *C. gloeosporioides* completely inhibited by carbendazim at 500 ppm [8]. Under *in vitro* studies carbendenzim (0.1%) was beneficial for inhibiting the growth of *C. gloeosporioides* that caused blossom blight of mango [9]. Complete inhibition of the mycelial growth of the *Colletotrichum* spp. by carbendazim at 0.1% was observed [10, 11]. Carbendazim 0.15 % found to be effective among all the tested chemicals and gave 88.23 % mycelial growth inhibition of *C. gloeosporioides*[12].

3.1.2 Effect of different contact fungicides on growth inhibition of *C. gloeosporioides*

The relative efficacy of contact fungicides *viz*., copper oxychloride (50 WP), mancozeb (75 WP), chlorothalonil (75 WP), propineb 70 (WP), zineb (75 WP), and sulphur (80 WG) were evaluated at 1500, 2000 and 2500ppm concentrations by poisoned food technique. The observations regarding average colony diameter and per cent inhibition of linear growth are presented in Table 1 and depicted in figure 1(b), 3(b).

At 1500 ppm concentration, significantly the highest per cent growth inhibition over control was recorded in mancozeb (76.04%) as compared to rest of fungicides. Next best in order of merit was copper oxychloride (69.20%). The remaining fungicides viz., propineb (58.93%), zineb (57.79%), chlorothalonil (43.34%) were moderately effective. While sulphur (34.21%) recorded least effective in growth inhibition as compared to other fungicides against *C. gloeosporioides.* At 2000 ppm concentration, mancozeb recorded significantly highest per cent growth inhibition (91.25%) as compared to rest of fungicides. Next best in order of merit was copper oxychloride (84.03%). Zineb (77.56%), propineb (69.96%) were also considerably effective fungicides. Chlorothalonil (59.70%) and sulphur (54.75%) proved comparatively less in their efficacy against *C. gloeosporioides.* At 2500 ppm concentration, copper oxychloride and mancozeb recorded 100 per cent growth inhibition of the pathogen. Next best in order of merit was zineb (91.25%) followed by propineb (79.09%), chlorothalonil (74.52%) and sulphur (65.40%) effective against *C. gloeosporioides*.

There was no mycelial growth of the pathogen in mancozeb and copper oxychloride at 2500 ppm and also significantly lesser growth at 2000 and 1500 ppm. Thus, both the fungicides

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

SYSTEMIC FUNGICIDES					CONTACT FUNGICIDES				COMBI-FUNGICIDES			
Sr. No.	**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 (%)**
1	Carbendazim 50 WP	100	7.33	91.73	Copper oxychloride 50 WP	1000	27.00	69.20	Trifloxystrobin 25%+Tebuconazole 50%	100	57.33	34.85
		250	5.67	93.60		2000	14.00	84.03		250	46.67	46.96
		500	0.00	100.00		2500	0.00	100.0		500	32.67	62.87
2	Kresoxim methyl 44.3 SC	100	44.33	50.00	Mancozeb 75 WP	1000	21.00	76.04	Carboxin (37.5%) + Thiram (37.5%)	100	39.33	55.30
		250	32.33	63.53		2000	7.07	91.25		250	24.33	72.35
		500	25.67	71.04		2500	0.00	100.00		500	18.67	78.78
3	Propiconazole 25 EC	100	8.67	90.22	Chlorothalonil 75 WP	1000	49.69	43.34	Tricyclazole 18% + Mancozeb 62%	100	6.33	92.80
		250	5.33	93.98		2000	35.33	59.70		250	5.00	94.31
		500	4.34	95.10		2500	22.33	74.52		500	0.00	100.00
4	Difenconazole 25 EC	100	33.67	62.02	Propineb 70 WP	1000	36.00	58.93	Carbendazim 12% + Mancozeb 63%	100	5.67	93.55
		250	20.67	76.68		2000	26.33	69.96		250	5.00	94.31
		500	0.00	100.00		2500	18.33	79.09		500	0.00	100.00
5	Tebuconazole 25.9 EC	100	54.33	38.72	Zineb 75 WP	1000	37.00	57.79	Hexaconazole 4% + Zineb 68%	100	14.67	83.32
		250	30.33	65.79		2000	19.67	77.56		250	8.00	90.90
		500	16.33	81.58		2500	7.67	91.25		500	0.00	100.00
6	Thiophanate methyl 70 WP	100	63.33	28.57	Sulphur 80 WP	1000	57.67	34.21	Hexaconazole 4% + Zineb 68%	100	20.33	76.89
		250	41.00	53.76		2000	39.67	54.75		250	14.00	84.09
		500	20.00	77.44		2500	30.33	65.40		500	6.00	93.18
7	Absolute control	-	88.67	-	Absolute control		87.67		Absolute control		88.00	
SEm ±		0.46			SEm ±	0.51			SEm ±	0.51		
CD at 5%		1.31			CD at 5%	1.47			CD at 5%	1.47		

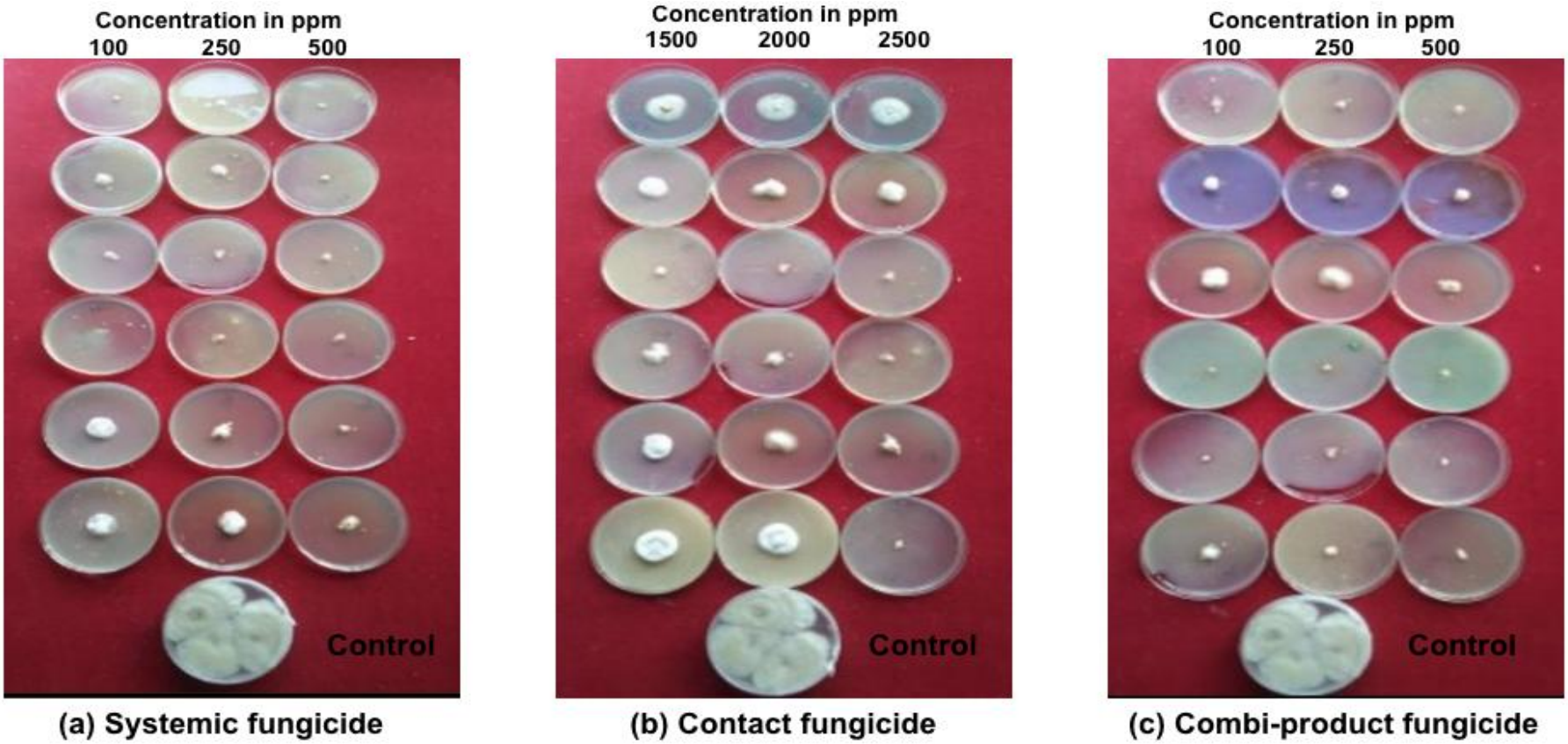

Figure 1: Growth inhibition of *C. gloeosporioides* at different concentration of (a) systemic, (b) contact and (c) combi product fungicides

proved the most effective for *C. gloeosporioides*. Next best fungicide in order of merit was zineb followed by propineb. The rest of the fungicides were comparatively less effective.

Significant growth inhibition of *C. gloeosporioides*in copper oxychloride at 0.1, 0.2 and 0.3 per cent concentrations was observed [10]. It was stated that copper oxychloride (0.3%) cent per cent inhibited growth and sporulation of *C. gloeosporioides*[11]. Poor sporulation of *C. gloeosporioides*was recorded in mancozeb (0.25%) [10]. It was reported that sulphur (0.2%) was least effective against *C. gloeosporioides*under *in vitro* condition [13].

3.1.3 Effect of different combi-product fungicides on growth inhibition of *C. gloeosporioides*

In vitro testing of six combi-product fungicides *viz*., trifloxystrobin 25% + tebuconazole 50%, carboxin 37.5%+ thiram 37.5%, tricyclazole 18% + mancozeb 62%, carbendazim 12%+ mancozeb 63%, hexaconazole4% + zineb 68% and pyraclostrobin 5% + mitiram 55% belonging to different groups with three concentrations viz., 1000, 1500 and 2000 ppm by poisoned food technique for their efficacy against *C.gloeosporioides*. The observations regarding per cent inhibition of linear growth are presented in table 1 and depicted figure 1(c), 3(c).

At 1000 ppm concentration, highest per cent growth inhibition over control was recorded in carbendazim 12% + mancozeb 63% (93.55%) as compared to rest of fungicides. Next best in order of merit was tricyclazole 18% + mancozeb 62% (92.80%). The remaining fungicides *viz.*, hexaconazole 4% + zineb 68% (83.32%) and pyraclostrobin 5% + metiram 55% (76.89%) were moderately effective. While carboxin 37.5% + thiram 37.5% (55.30%) and trifloxystrobin 25% + tebuconazole 50% (34.85%) recorded least effective in growth inhibition as compared to other fungicides against *C. gleosporiodes*. At 1500 ppm concentration, carbendazim 12% + mancozeb 63% and tricyclazole 18% + mancozeb 62% recorded significantly highest per cent growth inhibition (94.31%) as compared to rest of fungicides. Next best in order of merit was hexaconazole 4% + zineb 68% (90.90%). pyraclostrobin 5% + mitiram 55% (84.09%) and carboxin 37.5% + thiram 37.5% (72.35%) were also considerably effective fungicides. Trifloxystrobin 25% + tebuconazole 50% (46.96%) proved comparatively less effective in their efficacy against *C. gloeosporioides*. At 2000 ppm concentration, tricyclazole 18% + mancozeb 62%, carbendazim 12% + mancozeb 63% and hexaconazole 4% + zineb 68% recorded 100 per cent growth inhibition of the pathogen. Next best in order of merit was pyraclostrobin 5% + metiram 55% (93.18%) followed by carboxin 37.5% + thiram 37.5% (78.78%). Trifloxystrobin 25% + tebuconazole 50% was found 62.87% effective against *C. gloeosporioides*.

The growth inhibition per cent positively correlated with increase in concentration for all the fungicides tested. It is inferred from results that there was no mycelial growth of the *C. gleosporiodes*in carbendazim 12% + mancozeb 63% ,tricyclazole 18% + mancozeb 62% and hexaconazole 4% + zineb 68% at 2000 ppm and also significantly lesser growth at 500 and 1500 ppm. Thus, both the fungicides proved the most effective for *C. gleosporiodes*. Next best fungicide in order of merit was hexaconazole 4% + zineb 68% followed by pyraclostrobin 5% + mitiram 55%. The rest of the fungicides were comparatively less effective.

It was investigated that carbendazim 12% + mancozeb 63% was found most effective with mean growth inhibition of 82.34 per cent at 2000 ppm against mango anthracnose caused by *C. gloeosporioides*[14].It was reported that *Colletotrichum spp.* isolates from mango sensitive to MBC fungicides and prochloraz [15]. It was also suggested that spraying with mancozeb along with carbendazim was most effective in disease reduction of 88.0 per cent of pomegranate fruit spot (*C. gloeosporioides*) [16, 17].

3.2 *In vitro* evaluation of bioagents against *C. gloeosporioides*

In vitro evaluation of native isolated antagonists under dual culture revealed growth inhibition of test fungus (*C. gloeosporioides*) by the test antagonists *viz., Trichoderma virens*, *Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis*. An appraisal of data regarding per cent inhibition presented in table 2 and depicted in figure 2, 3(d).

The results revealed that all the antagonists were significantly more effective in checking the growth of the *C. gleosporiodes*. All the antagonists inhibited more than 30 per cent growth of the test fungus. Among them, significantly lower mycelial growth of the pathogen was recorded in *T. harzianum*(22.25 mm) which was at par with *T. viride*(23.88 mm). Next best in order of merit was *P. fluorescens* (28.00 mm), followed by *T. virens (*32.38 mm) and *B. subtilis* (40.75 mm) produced comparatively higher mycelial growth. *T. harzianum*gave maximum per cent growth inhibition (74.53%) and appeared to be most superior over all the antagonists tested followed by *T. viride*(72.67%), *P. fluorescens* (67.95%), *T. virens* (62.94%) and *B. subtilis* (53.36%) were also found moderately effective against the *C. gloeosporioides*.

Similar results were recorded in previous reports. *T.harzianum*completely over grew the *C. gleosporiodes*and covered the entire medium surface [12]. *T. harzinum*was found superior over *T. viride*and *T. virens* against *Colletotrichum gloeosporioides in vitro* [18].

Table 2: *In vitro* evaluation of different bioagents against *C. gloeosporioides*

Sr. No.	Bioagents	Mean diameter of pathogen (mm)	Growth inhibition (%)
1	*Trichoderma harzianum,* Navsari isolate	22.25	74.53
2	*Trichoderma viride,* Navsari isolate	23.88	72.67
3	*Trichoderma virens,* Navsari isolate	32.38	62.94
4	*Bacillus subtilis*, Navsari isolate	40.75	53.36
5	*Pseudomonas fluorescens,* Navsari isolate	28.00	67.95
6	Control	87.38	**-**
	SEm ±	**0.49**	
	CD at 5%	**1.48**	

Figure 2: *In vitro* growth inhibition of *C. gloeosporioides*with different biocontrol agents

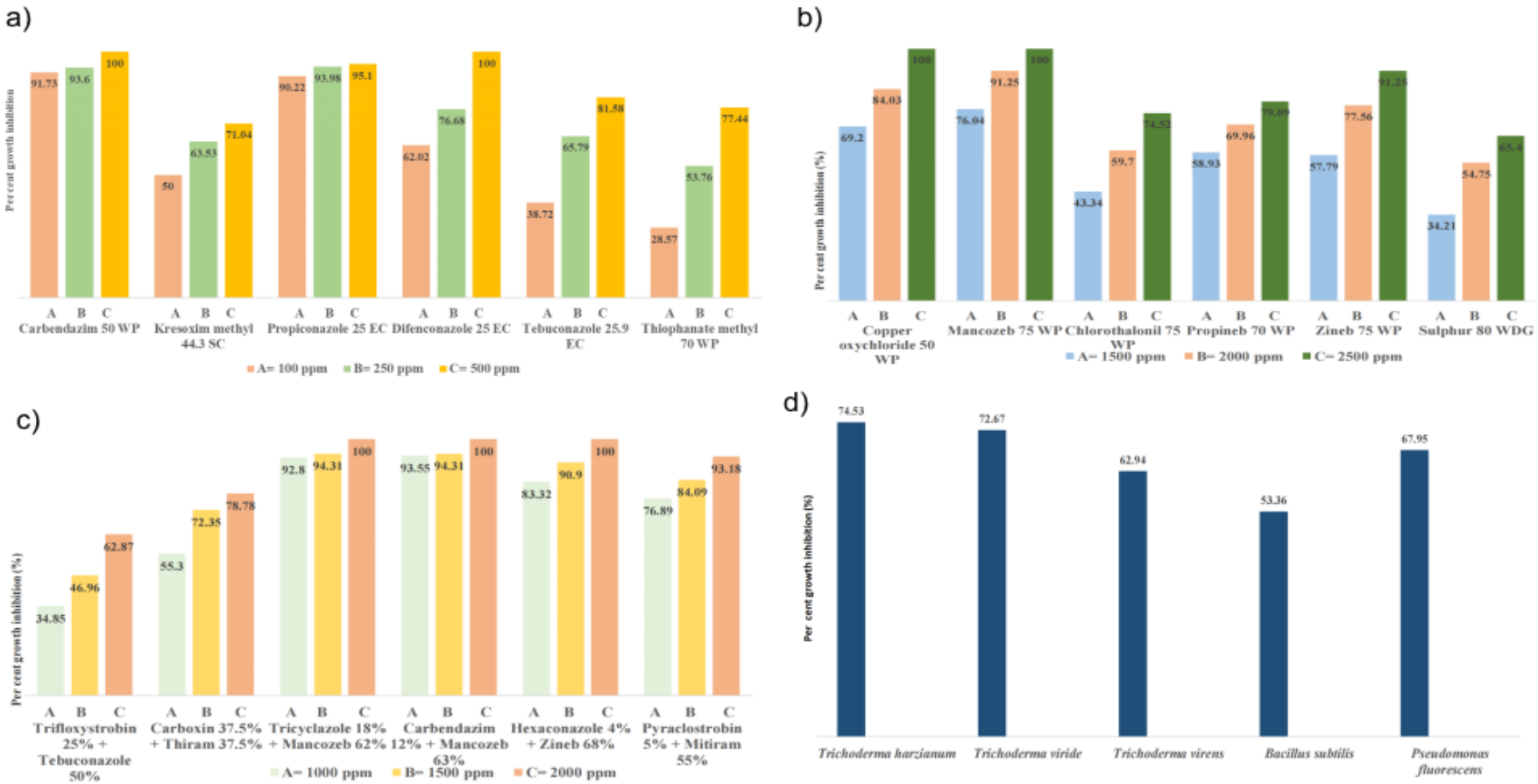

Figure 3: Grapical representation of average colony diameter of pathogen 3(a) systemic fungicides; 3(b) contact fungicides; and 3(c) combi-products fungicides and 3(d) Per cent growth inhibition of bioagents

CONCLUSION:

Fungicide testing *in vitro* acts as a guide for field testing by offering valuable and preliminary information regarding fungicides' ability to combat diseases in a brief amount of time. Cent percent growth inhibition *in vitro* was found in systemic fungicide carbendazim and difenoconazole at 500 ppm, contact fungicides *viz.,* mancozeb and copper oxychloride at 2500 ppm, ready mix fungicides *viz.,* carbendazim 12% + mancozeb 63%, tricyclazole 18% + mancozeb 62% and hexaconazole 4% + zineb 68% at 2000 ppm. During testing of biocontrol agents i*n vitro* condition found that maximum per cent growth inhibition of *C. gloeosporioides*was in *T. harzianum*. Systemic fungicides carry a high risk of developing resistance. When it comes to contact fungicides, the likelihood of resistance developing is minimal because they only protect the plant on which they have been applied, while simultaneously targeting several disease sites. One component systemic fungicide and one part contact fungicide make up combi-fungicides. As a result, while using combination fungicides, there is less chance of resistance developing. Although systemic fungicides effectively suppress the disease, the possibility of fungicide resistance developing cannot be completely ruled out. Combination fungicides are therefore a suitable choice, and they contribute significantly to the IDM of mango leafspot in south Gujarat.

ETHICS APPROVAL

This research is observational. It has been certified by the Navsari Agricultural University Research Ethics Committee that no ethical clearance is needed.

CONSENT TO PARTICIPATE

The study did not involve any human volunteers in its investigation.

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