Int.J.Curr.Microbiol.App.Sci (2019) *8*(10): 518-530

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 8 Number 10 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.810.057

To evaluate *in-vitro* Bio-efficiency of Different Fungicides against *Colletotrichum gloeosporioides* Penz. and Sacc. causing Fruit Rot of Aonla

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Keywords

Aonla (*Emblica officinalis*. Gaertn), *Colletotrichum gloeosporioides* Penz

Article Info

Accepted:
07 September 2019
Available Online:
10 October 2019

A B S T R A C T

Colletotrichum gloeosporioides Penz. and Sacc**.** is associated with aonla anthracnose, and fungicides remain one of the important means to manage the disease. *In-vitro* evaluation of the fungicides revealed that all the treatments significantly inhibited mycelial growth of the test pathogen over untreated control. The pathogen was most sensitive to systemic fungicides, carbendazim + mancozeb (100 % inhibition) followed by the next best fungicide found effective in per cent mycelial inhibition was azoxistrobin (99.50 %), propiconazole (97.51 %). this was followed by fungicides *viz*., carbendazim (96.41 %), among the thirteen fungicides evaluated *in-vitro* at different concentrations. The study provides preliminary information on the types of fungicides that are suitable for managing anthracnose of aonla fruit in Maharashtra.

Introduction

Aonla (*Emblica officinalis.*Gaertn.) is one of the major fruit crop in the State of Maharashtra. The anola is affected by number of fungal pathogens such as *Colletotricum gloesporioides.* Penz. and Sacc. (fruit rot) *Ravenelia emblicae* Styd. (rust), *Fusarium* spp. (wilt), *Penicillium citrinum* Thom. (fruit rot or blue mould), *Phomopsis phyllanthi* Punith (soft rot), *Phoma putaminum* Speg. (dry fruit rot), *Aspergillus terreus* (fruit rot) *etc*. Among them, the fruit rot caused by *Colletotricum gloesporioides.* Penz. and Sacc. is a major disease of aonla fruit and responsible for causing 2- 29 per cent yield loss (Sohi, 1975).

Keeping in view economic importance of aonla and losses incurred due to fruit rot disease, present investigations on the various aspects *viz.*, survey, symptomatology, pathogenicity test, morphological and cultural characteristics, efficacy of different

fungicides, bio-agents, plant extracts were undertaken during the season of *Kharif* 2018-2019 at Department of Plant Pathology, College of Agriculture, Badnapur, V.N.M.K.V. Parbhani. The results obtained on the above aspects during the present investigations are being interpreted and presented in the following paragraphs.

Patil *et al.,* (2009) reported that during *in-vitro* studies of different chemicals, Mancozeb + Carbendazim (0.2 %) was found most effective in inhibiting 96.26 per cent growth of *Colletotrichum gloeosporioides* followed by carbendazim (0.1 %) 68.34 per cent, mancozeb (0.25 %) 67.51 per cent and copper oxychloride (0.3 %) 64.88 per cent.

Materials and Methods

For evaluation of different fungicides "Poisoned Food Technique" developed by Nene and Thapliyal (1971) was followed. Efficacy of thirteen different fungicides was calculated at different concentrations (each @ 500, 1000, 2000 and 2500 ppm) against *Colletotrichum gloeosporioides* Penz. and Sacc. by using Potato Dextrose Agar (PDA) as basal culture medium. Based on active ingredient, the requisite quantity of each test fungicides as calculated and mixed thoroughly with autoclaved and cooled (40 ^{0}C) PDA medium separately in conical flasks (250 ml/cap) to obtain desired concentrations of 500, 1000, 2000 and 2500 ppm. Fungicides amended PDA medium was then poured (20 ml/plate) aseptically in the glass petri plates (90 mm dia.) and allowed to solidify at room temperature. For each of the test fungicides and its test concentrations, a replicate set of petri plates/treatment/replication were maintained. After solidification of the medium, all the plates were inoculated aseptically with a 5 mm culture disc obtained from a one week old actively growing pure culture of *Colletotrichum gloeosporioides* separately. The culture disc was placed on PDA in inverted position in the centre of the petri plate and plates were incubated at 27 $\pm$ 1 ^{0}C. Petri plates filled with plain PDA (without any fungicides) and inoculated separately with the culture disc of *Colletotrichum gloeosporioides* were maintained as untreated control

The bio efficacy of these fungicides was evaluated at different concentrations @ 500, 1000, 2000 and 2500 ppm.

The observations on radial mycelial growth and sporulation of test fungus were recorded at 24 hours intervals and were continued till growth of test pathogen in untreated control plate was fully covered. Per cent inhibition of test pathogen was calculated by applying the formula given by Vincent (1927)

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

Where, **C** = Growth of test fungus in untreated control plates.

T = Growth of test fungus in treated plates.

Results and Discussion

In-vitro evaluation of fungicides against *Colletotrichum gloeosporioides*

A total of thirteen fungicides were evaluated *in-vitro* against *C. gloeosporioides* exhibited a wide range of mycelial growth and inhibition of the test pathogen (Table 1, Plate 1 and Fig. 1, 2, 3).

Radial mycelial growth

At 500 ppm, radial mycelial growth of the test pathogen was recorded from 00 mm

(carbendazim + mancozeb) to 45.60 mm (propineb), as against 90 mm in untreated control. However, least mycelial growth was found with carbendazim + mancozeb (00 mm) followed by azoxystrobin (01.80 mm) and the next fungicides in order of merit were *viz.*, propiconazole (05.26 mm), carbendazim (07.27 mm), thiophanate methyl (10.15 mm), difenoconazole (15.20 mm), mancozeb (16.37 mm), captan (18.20 mm), copper oxy-chloride (19.36 mm), thiram (24.17 mm), copper hydroxide (33.86 mm), chlorothalonil (35.56 mm) and propineb (45.60 mm) over control. Among all the fungicides tested chlorothalonil and propineb were found comparatively less effective against *C. gloeosporioides* with maximum mycelial growth of 35.56 mm and 45.60 mm, respectively (Table 1, Plate 1 and Fig. 1).

At 1000 ppm, radial mycelial growth of the test pathogen was recorded from 00 mm (carbendazim + mancozeb and azoxystrobin), to 39.29 mm (propineb), as against 90 mm in untreated control. However, least mycelial growth was found with carbendazim + mancozeb and azoxystrobin (00 mm) followed by propiconazole (03.70 mm) and the next fungicides in order of merit were *viz.*, propiconazole (03.70 mm), carbendazim (05.66 mm), thiophanate methyl (05.78 mm), difenoconazole and copper oxy-chloride (10.30 mm), mancozeb (12.16 mm), captan (13.90 mm), thiram (18.40 mm), chlorothalonil (29.63 mm), copper hydroxide (29.69 mm), and propineb (39.29 mm) over control. Among all the fungicides tested copper hydroxide and propineb were found comparatively less effective against *C. gloeosporioides* with maximum mycelial growth of 29.69mm and 39.29 mm, respectively (Table 1, Plate 1 and Fig. 1).

At 2000 ppm, radial mycelial growth of the test pathogen was recorded from 00 mm (carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole) to 35.60 mm (propineb), as against 90 mm in untreated control. However, least mycelial growth was found with carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole (00 mm) followed by difenoconazole (05.50 mm), and the next fungicides in order of merit were *viz.* mancozeb (05.77 mm), copper oxy-chloride (06.63), captan (06.63 mm), thiram (14.15 mm), chlorothalonil (25.50 mm), copper hydroxide (25.53 mm), and propineb (35.60 mm) over control. Among all the fungicides tested copper hydroxide and propineb were found comparatively less effective against *C. gloeosporioides* with maximum mycelial growth of 25.53 mm and 35.60 mm, respectively (Table 1, Plate 1 and Fig. 1).

At 2500 ppm, all the fungicides evaluated exhibited similar trend of mycelial growth as that of observed at 500 ppm, 1000 ppm and 2000 ppm but comparatively reduced radial mycelial growth recorded was ranged from 00 mm (carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole) to 30.80 mm (propineb), as against 90 mm in untreated control.

However, least mycelial growth was found with carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole (00.00 mm) followed by copper oxy-chloride (02.60 mm), and the next fungicides in order of merit were viz. difenoconazole and mancozeb (03.80 mm), captan (07.30 mm), thiram (09.10 mm), chlorothalonil (20.30 mm), copper hydroxide (22.60 mm), and propineb (30.40 mm) over control. Among all the fungicides tested Copper hydroxide and Propineb were found comparatively less effective against *C. gloeosporioides* with maximum mycelial growth of 22.60 mm and 30.40 mm, respectively (Table 1, Plate 1 and Fig. 1).

Average radial mycelial growth recorded with all the thirteen fungicides tested ranged from 00 mm (carbendazim + mancozeb) to 37.73 mm (propineb), as against 90 mm in untreated control.

However, least average mycelial growth was found with carbendazim + mancozeb as 00 mm. This was followed by the fungicides *viz*., propineb (02.24 mm), carbendazim (03.23 mm), thiophanate methyl (03.98 mm), difenoconazole (08.70 mm), mancozeb (09.52 mm), copper oxy-chloride (09.72), captan (12.29 mm), thiram (16.45mm), chlorothalonil (27.74 mm), copper hydroxide (27.92 mm) and propineb (37.73 mm), in order of merit. The comparatively maximum radial mycelial growth was recorded with copper oxy-chloride (27.92 mm) and propineb 37.73 mm (Table 1, Plate 1 and Fig. 3).

Per cent mycelial growth inhibition

Results revealed that all the non-systemic and systemic fungicides tested @ 500, 1000, 2000 and 2500 each significantly inhibited mycelial growth of *C. gloeosporioides*, over untreated control (00 %). Further, the percentage mycelial growth inhibition was increased with increase in concentrations of the fungicides tested.

At 500 ppm, mycelial growth inhibition of the test pathogen was ranged from (49.33 %) propineb to (100 %) carbendazim+ mancozeb. However, significantly highest mycelial inhibition was recorded with the fungicide, carbendazim + mancozeb (100 %) over control and it was followed by the fungicides azoxystrobin (97.98 %). The next fungicides in order of merit were propiconazole (94.26 %), carbendazim (91.92 %), thiophanate methyl (88.82 %), difenoconazole (83.11 %), mancozeb (81.80 %) captan (79.77 %) copper oxy-chloride (78.48 %), thiram (73.17 %), copper hydroxide (62.37 %) chlorothalonil (60.81 %) and propineb (49.33 %) were found comparatively least effective with minimum mycelial growth inhibition (Table 1, Plate 1 and Fig. 2).

At 1000 ppm, mycelial growth inhibition of the test pathogen was ranged from (56.17 %) propineb to (100 %) carbendazim+ mancozeb and azoxystrobin.

However, significantly highest mycelial inhibition was recorded with the fungicide, carbendazim + mancozeb and azoxystrobin (100 %) over control and it was followed by the propiconazole (95.88 %), carbendazim (93.70 %), thiophanate methyl (93.57 %), difenoconazole and copper oxy-chloride (88.55 %), mancozeb (86.48 %), captan (84.55 %) thiram (79.55 %), chlorothalonil (67.07 %), copper hydroxide (67.00 %) and propineb (56.17 %) were found comparatively least effective with minimum mycelial growth inhibition (Table 1, Plate 1 and Fig. 2).

At 2000 ppm, mycelial growth inhibition of the test pathogen was ranged from (60.44 %) propineb to (100 %) carbendazim+ mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole. However, significantly highest mycelial inhibition was recorded with the fungicide, carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole (100 %) over control and it was followed by the difenoconazole (93.88 %), mancozeb (93.58 %), copper oxy- chloride (92.63 %), captan (89.15 %), thiram (84.26 %), chlorothalonil (71.66 %), copper hydroxide (71.62 %) and propineb (60.44 %) were found comparatively least effective with minimum mycelial growth inhibition (Table 1, Plate 1 and Fig. 2). At 2500 ppm, mycelial growth inhibition of the test pathogen was ranged from (66.22%) propineb to (100%) carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole.

Table.1 Details of experiment

Non systemic fungicides				
Treatment	**Fungicide**	**Trade name**	**Group of Fungicide**	**Concentration**
T_1	Copper oxy-chloride 50 % WP	Blitox 50%	Non-systemic	2000-2500 ppm
T_2	Chlorothalonil 75 % WP	Kavach	Non-systemic	2000-2500 ppm
T_3	Mancozeb 75 % WP	Dithane M-45	Non-systemic	2000-2500 ppm
T_4	Captan 50 % WP	Captaf	Non-systemic	2000-2500 ppm
T_5	Thiram 75 % WS	Optimus	Non-systemic	2000-2500 ppm
T_6	Propineb 70 % WP	Antracol 70 WP	Non-systemic	2000-2500 ppm
T_7	Copper hydroxide 77 % WP	Kocide 101	Non-systemic	2000-2500 ppm
systemic fungicides				
Treatment	**Fungicide**	**Trade name**	**Group of fungicide**	**Concentration**
T_8	Thiophanate methyl 70 % WP	Topsin M	Systemic	500-1000 ppm
T_9	Azoxystrobin 25 % WP	Amistar	Systemic	500-1000 ppm
T_{10}	Carbendazim 50 % WP	Bavistin	Systemic	500-1000 ppm
T_{11}	Carbendazim 50 % WP + Mancozeb 64 % WP	SAAF	Combi product	500-1000 ppm
T_{12}	Propiconazole 25 % EC	Tilt	Systemic	500-1000 ppm
T_{13}	Difenoconazole 25 % EC	Score 25EC	Systemic	500-1000 ppm
T_{14}	Control (Untreated)	–	–	–

Design : CRD
Replications : Three
Treatments : Fourteen

Table.2 *In-vitro* efficacy of different systemic and non systemic fungicides against radial mycelial growth and per cent inhibition of *Colletotrichum gloeosporioides*

Tr. No.	Fungicides	Colony diameter* (mm)					Per cent inhibition*				
		500 Ppm	1000 ppm	2000 ppm	2500 Ppm	Av. mean (mm)	500 Ppm	Ee 1000 ppm	2000 ppm	2500 ppm	Av. mean % inhibition
T_1	Copper oxy-chloride 50 % WP	19.36	10.30	06.63	02.60	09.72	78.48 (62.36)	88.55 (70.22)	92.63 (74.24)	97.11 (80.21)	89.20 (71.53)
T_2	Chlorothalonil 75 % WP	35.56	29.63	25.50	20.30	27.74	60.81 (51.24)	67.07 (54.98)	71.66 (57.83)	77.44 (61.64)	69.17 (56.27)
T_3	Mancozeb 75 % WP	16.37	12.16	05.77	03.80	09.52	81.80 (64.74)	86.48 (68.42)	93.58 (75.32)	95.77 (78.13)	89.42 (71.01)
T_4	Captan 50 % WP	18.20	13.90	09.76	07.30	12.29	79.77 (63.27)	84.55 (66.85)	89.15 (70.76)	91.88 (73.44)	86.34 (68.30)
T_5	Thiram 75 % WP	24.17	18.40	14.15	09.10	16.45	73.17 (58.80)	79.55 (63.11)	84.26 (66.62)	89.88 (71.45)	81.72 (64.68)
T_6	Propineb 70 % WP	45.60	39.29	35.60	30.40	37.73	49.33 (44.61)	56.17 (48.54)	60.44 (54.46)	66.22 (41.46)	58.07 (49.64)
T_7	Copper hydroxide 77 % WP	33.86	29.69	25.53	22.60	27.92	62.37 (52.16)	67.00 (54.93)	71.62 (57.80)	74.88 (59.92)	68.97 (56.14)

Table to be continue…

Tr. No.	Fungicides	500 Ppm	1000 ppm	2000 ppm	2500 Ppm	Av. mean (mm)	500 ppm	1000 ppm	2000 ppm	2500 ppm	Av. mean % inhibition
T_8	Thiophanate methyl 70 % WP	10.15	05.78	00.00	00.00	03.98	88.72 (70.46)	93.57 (75.31)	100 (90.00)	100 (90.00)	95.57 (77.84)
T_9	Azoxystrobin 25 % WP	01.80	00.00	00.00	00.00	00.45	97.98 (81.82)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	99.50 (85.94)
T_{10}	Carbendazim 50 % WP	07.27	05.66	00.00	00.00	03.23	91.92 (73.48)	93.70 (75.46)	100.00 (90.00)	100.00 (90.00)	96.41 (79.07)
T_{11}	Carbendazim 50 %WP + Mancozeb 64 % WP	00.00	00.00	00.00	00.00	00.00	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
T_{12}	Propiconazole 25 % EC	05.26	03.70	00.00	00.00	02.24	94.26 (76.13)	95.88 (78.28)	100.00 (90.00)	100.00 (90.00)	97.51 (80.92)
T_{13}	Difenoconazole 25 % EC	15.20	10.30	05.50	03.80	08.70	83.11 (65.73)	88.55 (70.22)	93.88 (75.67)	95.77 (78.13)	90.33 (71.88)
T_{14}	Control	90.00	90.00	90.00	90.00	90.00	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	SE ±	**0.48**	**0.33**	**0.38**	**0.41**	**--**	**0.56**	**0.76**	**0.40**	**0.12**	**-**
	C.D. @ 0.01	**1.44**	**1.00**	**1.13**	**1.24**	**--**	**1.67**	**2.06**	**1.20**	**0.36**	**-**

*Mean of three replications, Figures in parenthesis are arc sine transformed value.

Plate - I

500 ppm **1000 ppm**

2000 ppm **2500 ppm**

In-vitro **inhibitory effect of different systemic and non-systemic fungicides at 500, 1000, 2000 and 2500 ppm on radial growth and per cent inhibition of *C. gloeosporioides* Penz. and Sacc.**

T_1 Copper oxy-chloride 50 % WP
T_2 Chlorothalonil 75 %WP
T_3 Mancozeb 75 % WP
T_4 Captan 50 %WP
T_5 Thiram 75 % WP
T_6 Propineb 70 %WP
T_7 Copper hydroxide 77 WP
T_8 Thiophanate methyl 70 % WP
T9 Azoxystrobin 25 % WP
T_{10} Carbendazim 50 %WP
T_{11} Carbendazim 50 %WP+ Mancozeb 64 % WP
T_{12} Propiconazole 25% EC
T_{13} Difenoconazole 25% EC
T_{14} Control

Fig.1 *In-vitro,* effect of different fungicides on radial mycelium growth of *C. gloeosporioides*

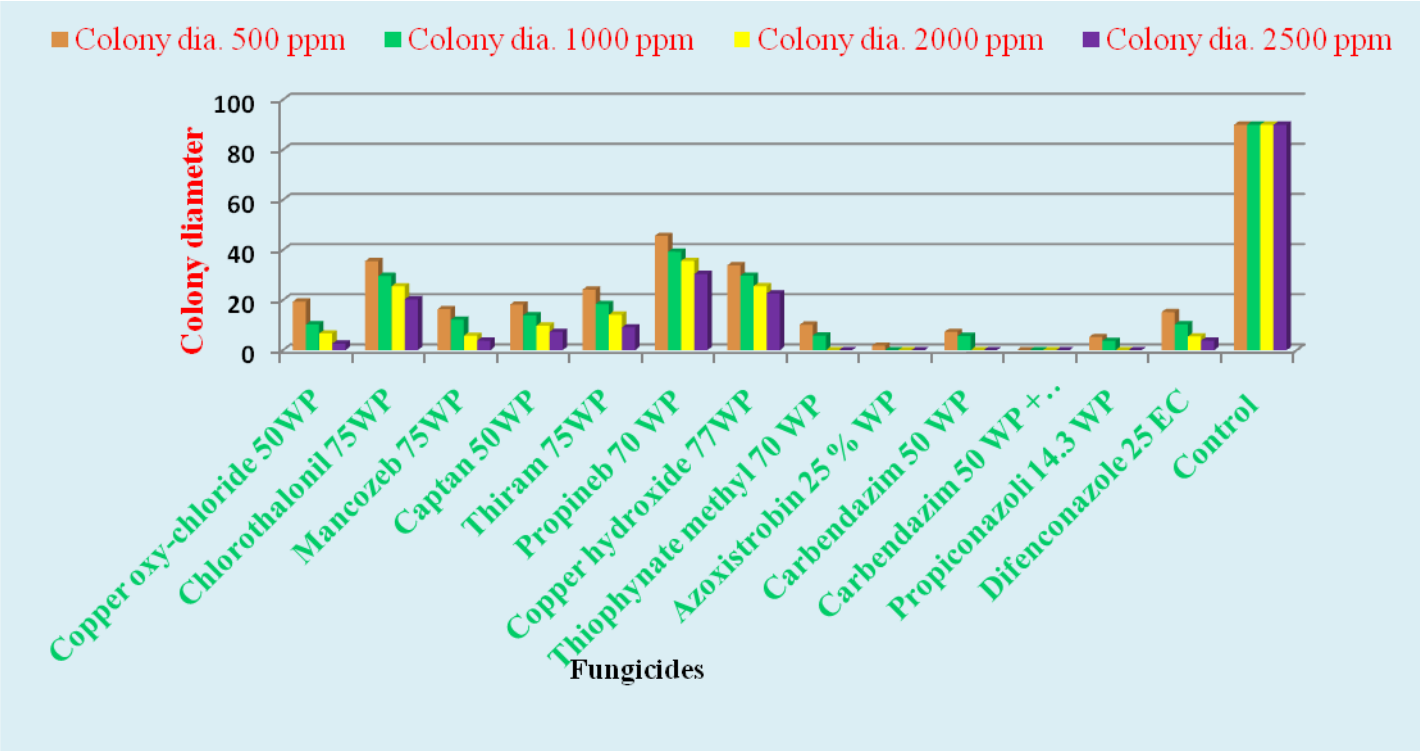


Fig.2 *In-vitro,* effect of different fungicide on per cent inhibition of *C. gloeosporioides*

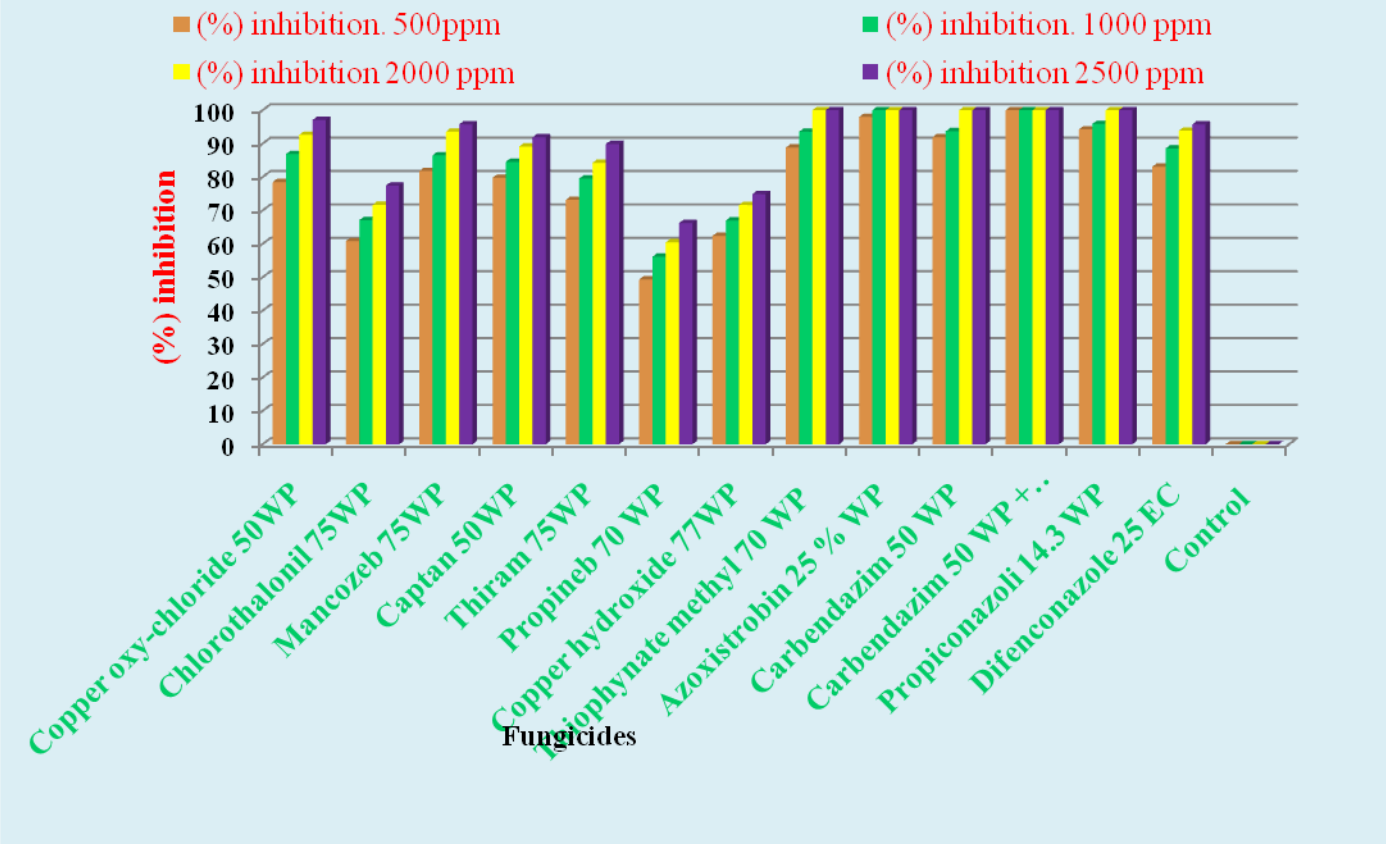
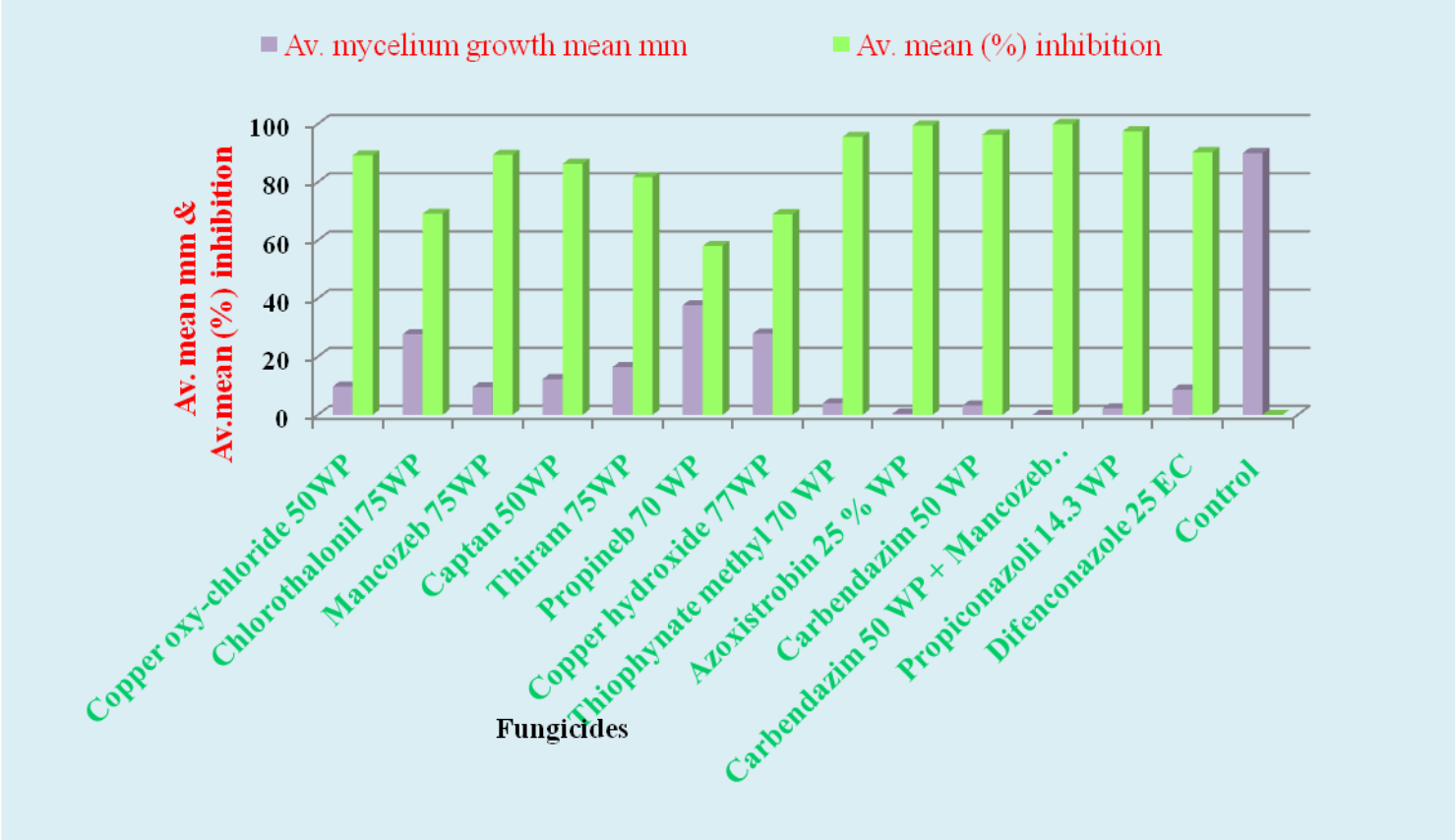


Fig.3 *In-vitro,* effect of different fungicides on mycelium growth mean and per cent inhibition of *C. gloeosporioides*



However, significantly highest mycelial inhibition was recorded with the fungicide, carbendazim + mancozeb, azoxystrobin, carbendazim, thiophanate methyl and propiconazole (100 %) over control and it was followed by the copper oxy- chloride (97.11 %), difenoconazole and mancozeb (95.77 %), captan (91.88 %) thiram (89.88 %), chlorothalonil (77.44 %), copper hydroxide (74.88 %) and propineb (66.22 %) were found comparatively least effective with minimum mycelial growth inhibition (Table 1, Plate 1 and Fig. 2). Average mycelial growth inhibition recorded with the test fungicides was ranged from carbendazim + mancozeb (100 %) to propineb (58.07 %) However, highest average mycelial growth inhibition was recorded with carbendazim + mancozeb (100 %) (Table 2).

The next best fungicide found effective in per cent mycelial inhibition was azoxystrobin (99.50 %), propiconazole (97.51 %). this was followed by fungicides *viz*., carbendazim (96.41 %), thiophanate methyl (95.57 %), difenoconazole (90.33 %), mancozeb (89.42 %), copper oxy- chloride (89.2 %), captan (86.34 %), thiram (81.72 %), chlorothalonil (69.17 %), copper hydroxide (68.97 %) and propineb (58.07 %) in order of merit fungicides, copper hydroxide and propineb were found comparatively less effective with minimum mycelial inhibition of 68.97 % and 58.07 % respectively (Table 1, Plate 1 and Fig. 3). The results of present investigation are in consonance with earlier records of scientist *viz.*, Thind and Kumar (2008), Watve *et al.*, (2009), Vinod *et al.*, (2009), Patil *et al.*, (2009), Kolase *et al.*, (2014), Fitsum *et al.*, (2014), Ingle *et al.*, (2014), Kadam *et al.*, (2014), Kaur *et al.*, (2015), Parvathy and Girija., (2016), Rathva *et al.*, (2017).

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How to cite this article:

Asalkar, U. A., D. G. Hingole and Mete, V. S. 2019. To Evaluate *in-vitro* Bio-efficiency of Different Fungicides against *Colletotrichum gloeosporioides* Penz. and Sacc. causing Fruit Rot of Aonla. *Int.J.Curr.Microbiol.App.Sci.* 8(10): 518-530.
doi: https://doi.org/10.20546/ijcmas.2019.810.057