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In vitro study of cultural character and lower concentration fungicides against anthracnose disease of avocado caused by *Colletotrichum gloeosporioides*

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Abstract
Anthracnose of avocado caused by *Colletotrichum gloeosporioides* is the most widespread and serious disease affecting leaves, flowers and young fruits in wet and humid conditions. In the present experiment, eight different culture media *viz.*, Potato dextrose agar (PDA), Oat meal agar (OMA), Martin's agar medium (MA), Czapek's dox agar (CDA), Richard's agar medium (RA), Sabouraud's dextrose agar (SA), Conns agar medium (CA) and Asthana and Hawkers agar (AHM) medium were used for morphological and cultural studies of the *C. gloeosporioides* and six fungicides *viz.*, Azoxystrobin 23% SC, Propiconazole 25% EC, Tebuconazole 25.9% EC, Mancozeb 50% WP, Chlorothalonil 75% WP and Tebuconazole 50% WG + Trifloxystrobin 25% WG were evaluate against the *C. gloeosporioides* at different concentrations by poisoned food technique in *in vitro*. Among all the media the growth on PDA was the maximum (90.00 mm) followed by OMA (77.67 mm), CA medium (75.33 mm), AHM medium (71.33 mm), MA medium (64.33 mm), SA medium (60.00) and CDA (57 mm). Least growth was observed on RA (55.67 mm) medium. Among the fungicide evaluated against *C. gloeosporioides* it is evident that the combination product (T_6) was numerically superior but statistically at par with T_5 and T_3. They were followed by T_2, T_1 and T_4. The least inhibition of the pathogen (49.63%) was caused by the sole contact fungicide- Mancozeb 50% WP at 0.1% concentration.

Keywords: *C. gloeosporioides*, avocado, anthracnose, fungicides

Introduction
Avocado (*Persea Americana* Mill) is a native of tropical America. Originating from South Central Mexico. In India, the avocado was introduced in 1920s (the early part of the 20th century) from Sri Lanka (Tripathi *et al*., 2014) [9]. The mild tropical humid climate of Konkan region is highly suitable for cultivation of different fruits. Considering the climate suitability and economic returns, organized plantation of Alphanso, Cashewnut, Jackfruit, Banana, Kokum, Sapota and plantation crops like Coconut and Arecanut are well established in the region. These traditionally cultivated fruit crop many times suffer heavily due to biotic and abiotic stresses due to climate change scenario in last decade or so. To overcome this issue University is taking efforts to introduced some exotic fruit crops like Avocado, Rambutan, Star fruits, Mangosteen etc. Probably due to test season, the newly planted avocado orchard at CES Wakawali might have susceptible to infection of *Colletotrichum*. At CES Wakawali an orchard containing 200 grafts exotic variety of avocado and 200 grafts TKD-1 obtained from TNAU Coimbatore is established. Newly planted seedlings exhibited fungal pathogen *Colletotrichum* causing anthracnose, is new and emerging disease in this crop. Anthracnose on avocado is the most widespread and serious disease affecting the leaves, flowers and fruits in wet and humid conditions. On the fruits, the symptoms appear as circular, brown-black, moistened lesions. In the avocado orchard, defoliated leaves and those remaining on the tree exhibited great brown necrotic sections appearing in the midway and on their edges. In order to obtained effective control for *Colletotrichum* causing anthracnose disease. It was important to evaluate noval and effective strategies involving use of fungicides and bio-control agent for management of *C. gloeosporioides.*

Materials and Methods
To study the cultural character of *C. gloeosporioides* on different media.
Eight different culture media were used in the present investigation. Media were prepared with given ingredient as per the required quantity. Other procedure was same as mentioned earlier as preparation of culture media.

Media used and their Ingredients

1) Oat meal agar medium

1. Oat meal: 60 g
2. Agar-agar: 20 g
3. Distilled water: 1000 ml

2) Conn's agar medium

1. Potassium nitrate (KNO_3): 2 g
2. Magnesium sulphate ($MgSO_4.7H_2O$): 1.2 g
3. Monopotassium dihydrogen phosphate (KH_2PO_4): 2.7 g
4. Sucrose ($C_{12}H_{22}O_{11}$): 7.2 g
5. Potato starch: 10 g
6. Agar-agar: 20 g
7. Distilled water: 1000 ml

3) Sabouraud's dextrose agar

1. Sucrose ($C_{12}H_{22}O_{11}$): 40 g
2. Peptic digest of animal tissue: 10 g
3. Agar-agar: 20 g
4. Distilled water: 1000 ml

4) Asthana and Hawker's medium.

1. Potassium nitrate (KNO_3): 3.5 g
2. Monopotassium dihydrogen phosphate (KH_2PO_4): 1.75 g
3. Magnesium sulphate ($MgSO_4.7H_2O$): 0.75 g
4. Glucose ($C_6H_{12}O_6$): 5 g
5. Agar-agar: 20 g
6. Distilled water: 1000 ml

5) Czapek's dox agar medium

1. Sucrose ($C_{12}H_{22}O_{11}$): 30 g
2. Sodium nitrate ($NaNO_3$): 2 g
3. Dipotassium hydrogen phosphate (K_2HPO_4): 1 g
4. Magnesium sulphate ($MgSO_4.7H_2O$: 0.5 g
5. Potassium chloride (KCL): 0.1 g
6. Ferric sulphate ($FeSO_4.7H_2O$): 0.01 g
7. Agar-agar: 20 g
8. Distilled water: 1000 ml

6) Richard's synthetic agar medium

1. Potassium nitrate (KNO_3): 10 g
2. Monopotassium dihydrogen phosphate (KH_2PO_4): 5 g
3. Magnesium sulphate ($MgSO_4.7H_2O$): 2.5 g
4. Ferric chloride: 0.02 g
5. Sucrose ($C_{12}H_{22}O_{11}$): 50 g
6. Agar-agar: 20 g
7. Distilled water: 1000 ml

7) Martin agar medium

1. Peptic digest of animal tissue: 23 g
2. Starch: 1 g
3. Sodium chloride: 5 g
4. Dextrose: 2.5 g
5. Agar-agar: 20 g
6. Distilled water: 1000 ml

8) Potato dextrose agar medium

1. Peeled potato: 200 g
2. Dextrose: 20 g
3. Agar-agar: 20 g
4. Distilled water: 1000 ml

Above listed media was prepared as per the requirement and was poured into sterilized petri plates, separately. After solidification, 5 mm mycelium disc of the test fungus from actively growing 7 days old culture were cut with the help of sterilized cork borer and single disc was placed at the center of each petri plate and incubated at 27±2 °C. each treatment was replicate three time. The measurement of the colony diameter was taken when the maximum mycelium growth was achieved in any of the media tested. The cultural character such as colony diameter, colony colour and colony character were recorded.

Evaluation of lower concentration fungicides against *C. gloeosporioides*

All the laboratory material required for the present investigation are available and experiment were conducted in the laboratory, Department of Plant Pathology, College of Agriculture, Dapoli, Dist. Ratnagiri (M.S). Six fungicides *viz*., Azoxystrobin 23% SC, Propiconazole 25% EC, Tebuconazole 25.9% EC, Mancozeb 50% WP, Chlorothalonil 75% WP and Tebuconazole 50% WG + Trifloxystrobin 25% WG were evaluate to check their *in vitro* efficacy against the *C. gloeosporioides* at different concentrations with three replications in Completely Randomized Designed. The plates were incubated at 27±2 °C temperature for seven days and radial colony growth was measured. The efficacy of fungicides was expressed as per cent inhibition of mycelial growth over the control and that was calculated by using formula given by Vincent (1947) [10].

$$\text{Per cent Inhibition (I)} = \frac{C - T}{C} \times 100$$

C = Growth (mm) of the test fungus in untreated control plate
T = Growth (mm) of the test fungus in treated plate

Result and discussion

The cultural characters were studied on eight different solid media. The radial growth of *C. gloeosporioides* was measured and colony characters such as growth of mycelium, color of colony were recorded.

The data represented in the (Table 1, Plate I and fig 1), revealed that *C. gloeosporioides* exhibited variation in growth on eight different media under study. In terms of redial growth, the growth on PDA was the maximum (90.00 mm) followed by OMA (77.67 mm), CA medium (75.33 mm), AHM medium (71.33 mm), MA medium (64.33 mm), SA medium (60.00), CDA (57mm) and RA medium (55.67 mm). However, the growth on PDA was sparse in the central zone and there after it formed a ring of uneven discontinuous growth. The best, compact, thick white mycelium was formed in AHM (Plate I). The mycelial growth flourished well in OMA and CA media also. Similarly on MA medium colony diameter was better but the growth was sparse and stranded. Least growth was on RA medium (Plate I, Fig 1)

The inference drawn by Jayalakshmi *et al*., (2015) [6] who studied cultural characters *C. gloeosporioides* on seven different media also state that maximum radial growth was on PDA and the least on Czapek Dox agar medium. These conclusions are at variance with present results in terms of Czapek Dox agar.

As per the results of Dev *et al*., (2017) [3] Malt extract agar was at par with PDA and they were followed by OMA. Growth of *C. gloeosporioides* on Sabouraud's dextrose medium was 63.3 mm. These results are congruent with

present conclusions. Many earlier workers also reported that maximum radial growth was found on PDA (Majumdar and Mandal. 2019, Dharbale *et al*., 2019 and Malipatil *et al*., 2021) [7, 4, 8].

Table 1: Cultural characters of *C. gloeosporioides* on different solid media

Tr. No.	Medium Used	Mean Colony Dia.*(mm)	Colony Characters	Colony Color
T_1	OMA	77.67	Compact colony with entire margin	Creamy white
T_2	CA	75.33	Cottony somewhat loose with subaerial hyphae	Grey white
T_3	SA	60.00	Cottony somewhat loose with subaerial hyphae	White to light grey
T_4	AHM	71.33	Cottony somewhat loose with subaerial hyphae	Grey white
T_5	CDA	57.00	Cottony somewhat loose with subaerial hyphae	Grey to dark grey
T_6	RA	55.67	Compact colony with entire margin	Whitish grey
T_7	MA	64.33	Cottony somewhat loose with subaerial hyphae	Grey white
T_8	PDA	90.00	Thick compact colony with elevation	Cottony white with Salmon ring of conidial mass
	SE(m) ±	1.11		
	C. D. at 1%	4.59		

*****Mean of three replications.

In the present study the *in vitro* efficacy of six fungicides was evaluated by poisoned food technique against *C. gloeosporioides*. All the fungicides were tested at one lower and one higher concentration. The results of the efficacy of fungicides at lower concentration are presented in table 2.

It is apparent from the data presented in It is evident from the results presented in (Table. 2, Plate II and fig. 2) that the combination product (T_6) was numerically superior but statistically at par with T_5 and T_3. They were followed by T_2, T_1 and T_4. The least inhibition of the pathogen (49.63%) was caused by the sole contact fungicide- Mancozeb 50% WP at 0.1% concentration.

According to the conclusions of Jayalakshmi *et al*., (2015) [6] and Bhagwat *et al.*, (2016) [1], Propiconazole caused cent per cent mycelial growth inhibition of *C. gloeosporioides* at 0.1% concentration. These results are in proximity with present findings. The conclusions of Dev and Narendrappa (2016) [2] concur with the results of present experiment. Similarly, the earlier worker Golakiya *et al*., (2020) [5] who studied the different fungicides at different concentration *viz*; 100 ppm, 250 ppm, 500 ppm against *C. gloeosporioides*, the finding confirms that Tebuconazole 25.9% EC and Tebuconazole 50% + Trifloxystrobin 25% WG showed maximum per cent inhibition. The minimum per cent inhibition found Mancozeb 75% WP at same concentration. These finding confirm that Tebuconazole cause better mycelial inhibition and Mancozeb cause poor mycelial inhibition of *C. gloeosporioides*.

Table 2: *In vitro* evaluation of different fungicides at lower concentration against *C. gloeosporioides*.

Tr No.	Fungicides and their formulation	Conc. (%)	Colony Dia. (mm)*	Per cent inhibition
T_1	Azoxystrobin 23% SC	0.05%	44.33	50.74
T_2	Propiconazole 25% EC	0.075%	32.33	64.07
T_3	Tebuconazole 25.9% EC	0.075%	24.00	73.33
T_4	Mancozeb 50% WP	0.1%	45.33	49.63
T_5	Chlorothalonil 75% WP	0.1%	22.16	75.37
T_6	Tebuconazole 50% WG + Trifloxystrobin 25% WG	0.1%	20.00	77.77
T_7	Control	-	90.00	-
	SE. (m)±		1.10	
	C. D.at 1%		4.64	

*Mean of three replications.

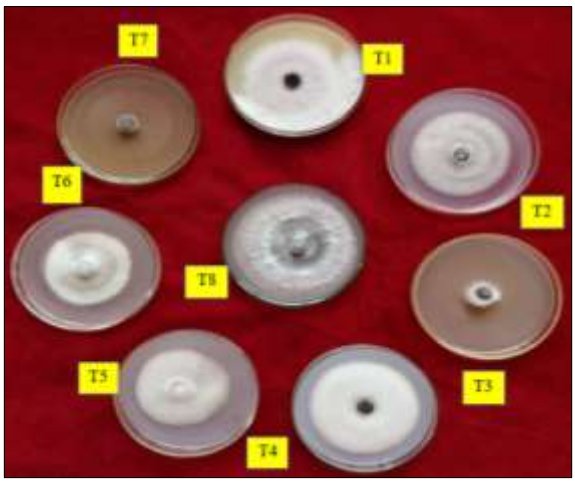

Plate 1: Effect of different cultural media on radial growth of *C. gloeosporioides*

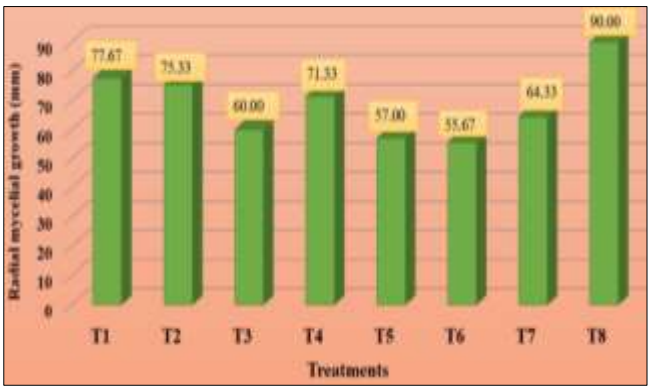

Fig 1: Culture characters of *C. gloeosporioides* on different solid media

Plate 2: *In vitro* evaluation of lower concentration of fungicides against *C. gloeosporioides*

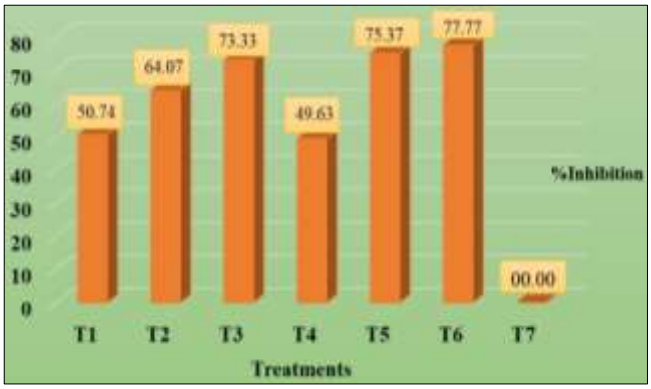

Fig 2: Per cent inhibition of lower concentration of different fungicides against *C. gloeosporioides*

Conclusion

The results obtained from the present experiment it is concluded that anthracnose disease of avocado incited by *C. gloeosporioides* exhibited variability in growth on different solid media. Among the solid media tested, maximum radial growth (90.00 mm) was *C. gloeosporioides* on PDA medium. Whereas on OMA, CA medium, AHM medium, MA medium, SA medium and CDA medium, it was fair. RA medium was the least suitable medium. The *C. gloeosporioides* can be effectively controlled by using Tebuconazole 50% WG + Trifloxystrobin 25% WG @ 0.1%.

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