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In vitro* exploration of fungicides and bio-agents 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, 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 evaluated their efficacy against the *C. gloeosporioides* at different concentrations by poisoned food technique and the antagonistic activity of three *Trichoderma* strains viz., *T. harzianum*, *T. longibrachiatum* and *T. koningii* were tested against *C. gloeosporioides* using dual culture technique in *in vitro*. Among all the fungicides evaluated, Tebuconazole at 0.1% concentration was the most effective as it completely inhibited the mycelial growth of *C. gloeosporioides*. It was followed by Propiconazole (inhibition-89.26%). The combination formulation ranked third (77.22%) which was trailed by Azoxystrobin (59.26%). Mancozeb (54.26%) was superior to Chlorothalonil (48.88%). Among three strains of *Trichoderma* evaluated, *T. harzianum* caused maximum inhibition 66.27% and it was followed by *T. longibrachiatum* (61.51%) and *T. koningii* (57.86%).

Keywords: *C. gloeosporioides*, anthracnose, avocado, *Trichoderma* spp

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) [10]. 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 Alphonso, 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, Mangos teen etc. Probably due to test season, the newly planted Avocado Orchard at CES Wakavali might have susceptible to infection of *Colletotrichum*. At CES Wakavali 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 novel and effective strategies involving use of fungicides and bio-control agent for management of *C. gloeosporioides*.

Materials and Methods

Evaluation of 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%

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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) [12].

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

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

Evaluation of bio-agents against *C. gloeosporioides*

Antagonistic activity of three *Trichoderma* strains against *C. gloeosporioides* was tested on PDA using dual culture technique (Huang and Hoes 1976) [13]. Twenty ml of sterilized and cooled potato dextrose agar was poured into sterile Petri plates and allowed to solidify. For evaluation of fungal bio-control agents, mycelial discs of 5 mm in diameter of test fungus were inoculated at one end of the Petri plates and antagonistic fungus was placed opposite to it on the other end. The experiments were performed thrice with seven replications in Completely Randomize Designed and one plates kept as a control. The plates were incubated at 27 ± 2 °C and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The colony diameter of pathogen in control plate was also recorded. The percentage inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947) [12].

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

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

Result and discussion

It is evident from the results presented in (Table 1, Plate I and fig 1) that T₃- Tebuconazole at 0.1% concentration was the most effective as it completely inhibited the mycelial growth of *C. gloeosporioides*. It was followed by Propiconazole (inhibition-89.26%). The combination formulation ranked third (77.22%) which was trailed by Azoxystrobin (59.26%). Mancozeb (54.26%) was superior to Chlorothalonil (48.88%). According to the conclusions of Jayalakshmi *et al.*, (2015) [7] 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 1: *In vitro* evaluation of different fungicides against *C. gloeosporioides*

Tr No.	Fungicides and their formulation	Conc. (%)	Colony Dia. (mm)*	Percent inhibition
T ₁	Azoxystrobin 23% SC	0.1%	36.66	59.26
T ₂	Propiconazole 25% EC	0.1%	9.66	89.26
T ₃	Tebuconazole 25.9% EC	0.1%	0.00	0.00
T ₄	Mancozeb 50% WP	0.25%	41.16	54.26
T ₅	Chlorothalonil 75% WP	0.2%	46	48.88
T ₆	Tebuconazole 50% WG + Trifloxystrobin 25% WG	0.2%	20.50	77.22
T ₇	Control	-	90	-
	SE. (m)±	0.58		
	C. D.at 1%	2.44		

(*Mean of three replications).

The antagonistic activity of bio-control agents against the test pathogen was determined by dual culture technique. All the treatments were statistically significant. Among the three species of *Trichoderma*, *T. harzianum* caused maximum inhibition 66.27% and it was followed by *T. longibrachiatum* (61.51%) and *T. koningii* (57.86%) (Table 2, Plate II and Fig 2).

These results are in conformity with the results of Hussain *et al.*, (2008) [6]; Tapwal *et al.*, (2015) [11]; Dev *et al.*, (2016) [2]; Musheer and Ashraf. (2017) [8]; Sutarman *et al.*, (2020) [9] and Devamma *et al.*, (2021) [4] as they also reported *T. harzianum* as the best fungal antagonist against *C. gloeosporioides*.

Among the fungal bio-agents studied worldwide, *Trichoderma* species are a leading group of fungi with an innate ability to delimit the growth of harmful plant pathogens. This is mainly due to their capability to colonize all sorts of saprophytic material and profuse sporulation within limited time frame. Out of the more than 25 members of the genus explored so far, *T. harzianum* and *T. viride* have been reported to be the best performing species. The species used in present study were local isolates and the results indicate the *T. harzianum* is superior to rest of the two. This may be due to its ability to quickly colonize the pathogen mycelium and synthesize enzymes and secondary metabolites which degrade the cell wall of the pathogen within short period of time after contacting the pathogen.

Table 2: *In vitro* evaluation of bio-agents against *C. gloeosporioides*

Tr. no.	Name of bio-control agents	Mean colony Dia. (mm)*	Per cent Inhibition
T ₁	<i>Trichoderma harzianum</i>	30.35	66.27
T ₂	<i>Trichoderma longibrachiatum</i>	34.64	61.51
T ₃	<i>Trichoderma koningii</i>	37.92	57.86
T ₄	Control	90	-
	SE. (m)±	0.79	
	C. D.at 1%	3.12	

(*Mean of seven replications)

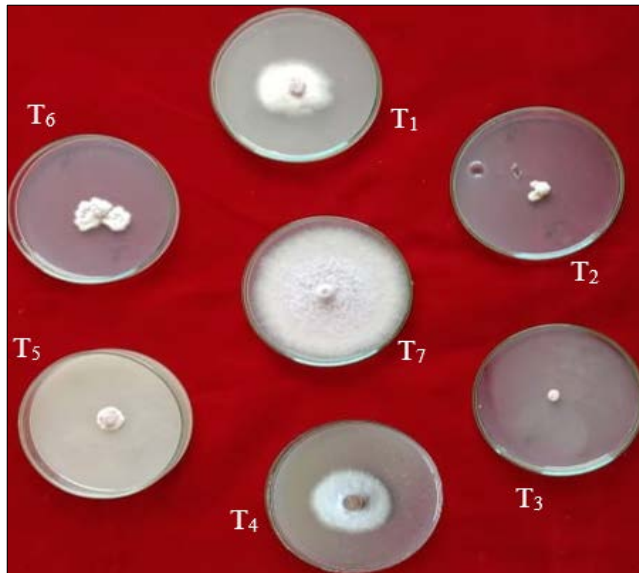


Plate 1: *In vitro* evaluation of fungicides against *C. gloeosporioides*

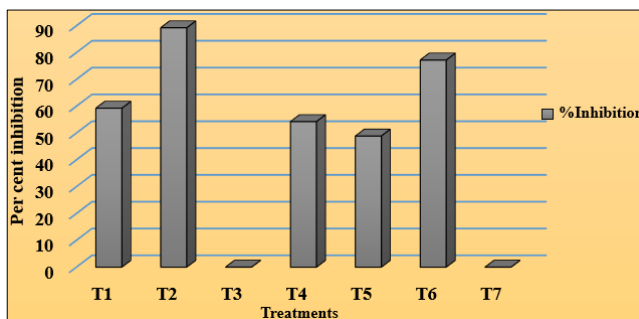


Fig 1: Per cent inhibition of fungicides against *C. gloeosporioides*

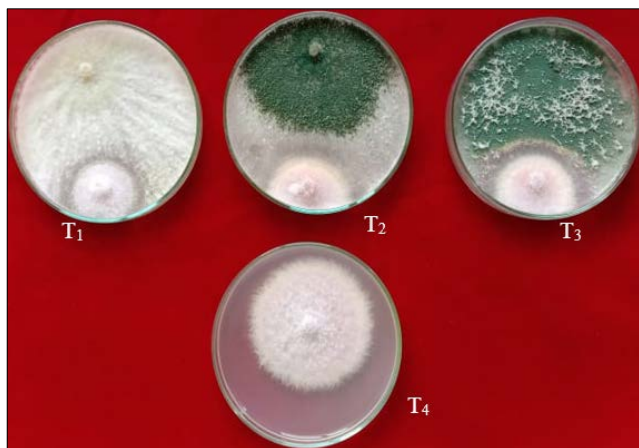


Plate 2: *In vitro* evaluation of bio-agents against *C. gloeosporioides*

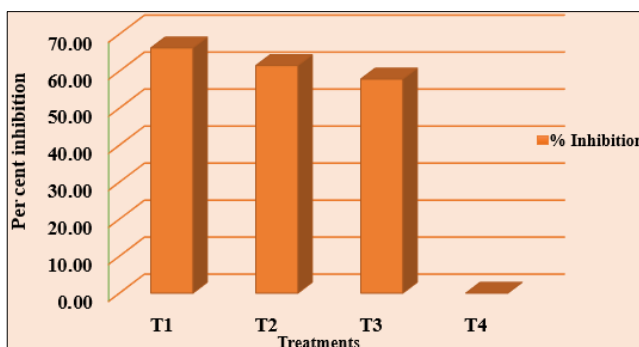


Fig 2: Per cent inhibition of bio-agents against *C. gloeosporioides*

Conclusion

The results obtained from the present experiment it is concluded that anthracnose disease of avocado incited by *Colletotrichum gloeosporioides* can be effectively controlled by Tebuconazole 25.9% EC @ 0.1% and by using *Trichoderma harzianum* as a bio-control agent.

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