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In vitro study of fungicides and biocontrol agents against *Colletotrichum capsici* causing anthracnose of chilli (*Capsicum annuumm* L.)

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Abstract

Nine fungicides namely: carbendazim, mancozeb, captan+hexaconazole, chlorothalonil, propiconazole, difenoconazole, azoxystrobin and bioagents namely: Trichoderma viride and Pseudomonas flourescens were evaluated in vitro by using poisoned food technique and dual culture respectively for studying their effect on the inhibition of mycelial growth of C.capsici. Among these fungicides maximum inhibition on mycelial growth was observed in propiconazole (0.1%) and difenoconazole (0.1%) (100%) followed by captan + hexaconazole 0.1% with 86.66 percent growth inhibition over control. The fungicide carbendazim @ 0.05% recorded 75% inhibition. This was followed by mancozeb @ 0.2% which recorded 70% inhibition. Azoxystrobin @ 0.1% recorded 67.50% inhibition. The least inhibition (63.77%) was recorded in chlorothalonil @ 0.1%. In dual culture mycelial growth inhibition of 55.5% was recorded in T. viride. Pseudomonas flourescens recorded 90% mycelial growth inhibition of the pathogen

Keywords- ***Fungicides, bioagents, C.capsici, dual culture***

I. INTRODUCTION

Chilli (*Capsicum annuum* L.) is one of the most valued spicy vegetable and it has a unique role in human diet. They are nutrient rich with vitamins A, C, E and minerals like pottassium. It is an annual herbaceous vegetable of solanaceae which is cultivated in almost all places. Unfortunately chilli fields are facing many serious threats including viruses, insects, bacterial wilt and anthracnose. Anthracnose is one of the serious diseases in chilli which affects fruits in particular and it is caused by *C. capsici* (Syd.) Butler and Bisby [1]. The disease is characterized by the production of symptoms on leaves, stem and fruits and causes severe damage to mature fruits in the field. Moreover it causes both pre and post harvest fruit decay during storage [2]. Ramachandran *et al* (2007) reported that yield losses accounted for more than fifty per cent [3]. Anthracnose infection on fruits causes lesions and fruits become unmarketable. Several management strategies are used for the control of *Colletotrichum* diseases such as cultural control, the use of resistant cultivars, biological control, and the use of fungicides [4]. Manandhar *et al*. (1995) found that fungicide spraying is the most common and practical method to control anthracnose [5]. Fungicides like bavistin and captan are known to be effective against wide range of fungal pathogen including seed borne *C.capsici*. Biocontrol agents are fast replacing synthetic pesticides owing to their safety aspects as against chemical fungicides. Therefore, in the present investigation, inhibition of mycelial growth of *C.capsici* exposed to different concentrations of some fungicides and bioagents were studied *in vitro*. The objectives of the study were to evaluate different fungicides and bioagents under lab conditions to find out the most effective one for final use. The results of these studies will be helpful to the growers to adopt the most suitable control strategy.

II. MATERIALS AND METHODS

2.1. Isolation and identification of *Colletotrichum capsici*

Chilli fruits having fruit rot symptoms were collected from chilli growing fields of COA, Vellayani. Isolation was done by cutting small pieces from the advancing margin of lesions which

were then immersed in 0.1% mercuric chloride for thirty seconds, washed three times in sterile distilled water, and blotted dry before being placed on PDA. The mycelium coming out of the tissues were sub-cultured to another petriplate incubated in room temperature. *Colletotrichum capsici* identification was done based on morphological characters such as size and shape of conidia and existence of setae.

2.2. Purification of *Colletotrichum capsici* - single spore isolation

Isolated pathogen *Colletotrichum capsici* was purified by single spore isolation [6]. Ten ml of 2% water agar was poured into sterile petridishes and allowed to solidify. Dilute spore suspension was prepared in sterilized distilled water from 7 days old culture. One ml of spore suspension was spread uniformly on agar plate. These plates were incubated at 28±2°C for 12 hrs. The plates were examined under microscope to locate single isolated and germinated conidium and marked with ink on the surface of the dishes.

The growing hyphal tip was cut with the help of cork borer under aseptic conditions and with an inoculation needle it was carefully transferred to PDA slants and incubated at 28±2°C. This culture was used for further *in vitro* studies.

2.3. In vitro evaluation of fungicides - Effect of fungicides on the mycelial growth of *C. capsici*

The *in vitro* chemical evaluation of *Colletotrichum capsici* was done by using Poisoned food technique [7] .Seven fungicides difenoconazole (0.01%, 0.05%, 0.1%), propiconazole (0.05%, 0.1%, 0.2%), chlorothalonil (0.05%, 0.1%, 0.2%), azoxystrobin (0.05%, 0.1%, 0.2%), captan + hexaconazole (0.05%, 0.1%, 0.2%), carbendazim (0.01%, 0.05%, 0.1%), mancozeb (0.1%, 0.2%, 0.3%) were used at three concentrations i.e., at recommended field concentration, higher dose than field concentration.

In order to study this, 50 ml distilled water in three separate 250 ml conical flask and 50 ml double strength PDA medium in another three separate 250 ml conical flask were taken and sterilized. Added desired concentration of fungicide in to the 50 ml sterile distilled water and then mixed with 50 ml molten double strength PDA to get desired concentration. There after 20 ml of the poisoned medium was poured in to sterilized petriplate (9.0 cm diameter) under aseptic conditions in Laminar Air flow inoculation chamber and allowed to solidify. The same procedure was repeated for all fungicides. Each plate was inoculated in the centre with 5mm diameter disc cut from the 7days old test *Colletotrichum capsici* culture individually under aseptic conditions and incubated at room temperature.

Unamended PDA plates inoculated with *Colletotrichum capsici* served as checks. Radial growth of the test isolates was recorded after 24 h and 48 h of incubation. Per cent inhibition of growth over control was calculated using the formula [8]

$$I = \frac{C - T}{C} \times 100$$

Where,

I = per cent inhibition.

C = growth of *Colletotrichum capsici* in unamended medium.

T = growth of *Colletotrichum capsici* in amended medium.

2.4. Effect of biocontrol agents on mycelial growth of *C.capsici* - dual culture technique

Antagonistic activity of *Trichoderma viride* against *C.capsici* causing anthracnose was assessed by dual culture technique [9]. Five mm mycelial discs each of seven days old culture of test pathogen and four days old biocontrol agent were taken with the help of a cork borer and placed on the fresh PDA plates about 2.5 cm apart in 9 cm petriplate and incubated at 25 ± 2°C. The petriplates with pathogen disc only served as control. Three replications for each treatment were laid out in a completely randomized design. Observations with respect to zone of inhibition on growth of the pathogen in dual culture as well as in control plates were recorded 7 days after incubation. The per cent inhibition in mycelial growth of the pathogen over control was calculated using formula given by [10].

$$\text{Percent inhibition in mycelial growth} = \frac{C - T}{C} \times 100$$

C = Mycelial growth of *C.capsici* in control
T = Mycelial growth of *C.capsici* in plates inoculated with biocontrol agent

In case of *Pseudomonas fluorescens* a bacterial biocontrol agent, five mm mycelial discs cut from the seven days old culture of the pathogen *Colletotrichum capsici* were placed on the centre of the PDA plates and two streaks were done with bacterial biocontrol agent *Pseudomonas fluorescens* on the both side perpendicular to the disc 2.5 cm apart as described by [11].

III. RESULTS AND DISCUSSION

Colletotrichum capsici was isolated from infected fruit specimens collected from Kerala Agriculture University, Vellayani.

3.1 Effect of fungicides on the mycelial growth of *C. capsici*

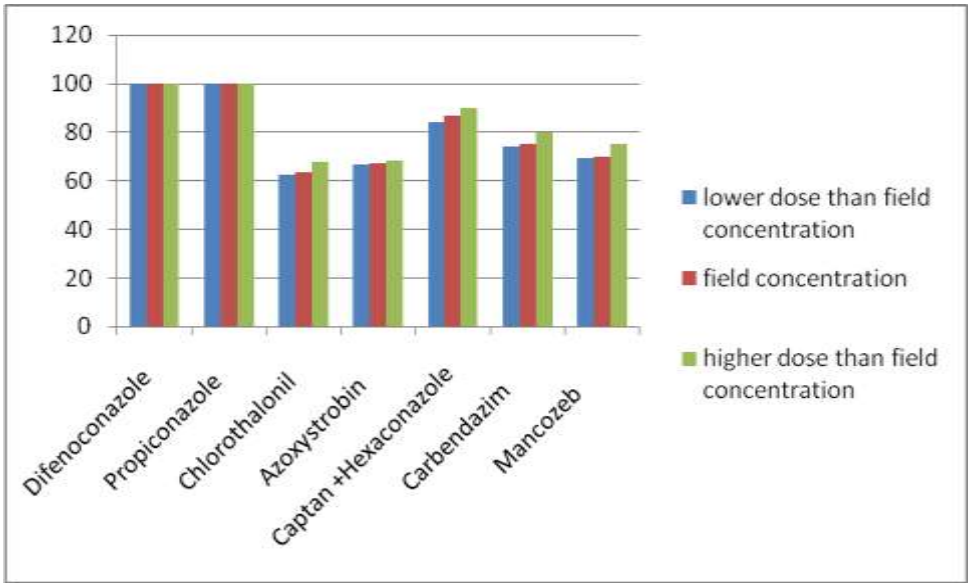
The studies on *in vitro* evaluation of fungicides against *C. capsici* through poisoned food technique revealed that the recommended dose, propiconazole @ 0.05% and difenoconazole @ 0.1% completely inhibited the mycelial growth (100%) of *C. capsici* and was significantly better than all other fungicides. It was followed by captan + hexaconazole 0.1% with 86.66 percent growth inhibition over control. The fungicide carbendazim @ 0.05% recorded 75% inhibition. This was followed by mancozeb @ 0.2% which recorded 70% inhibition. Azoxystrobin @ 0.1% recorded 67.50% inhibition. The least inhibition (63.77%) was recorded in chlorothalonil @ 0.1%.

A similar trend was observed in lower recommended dose also. Propiconazole @ 0.01% and difenoconazole @ 0.05% completely inhibited the mycelial growth (100%) of *C.capsici* and was significantly better than all other fungicides. It was followed by captan + hexaconazole 0.05% with 84.06 percent growth inhibition over control. The fungicide carbendazim @ 0.01% recorded 74% inhibition. This was followed by mancozeb @ 0.1% which recorded 69.55% inhibition. Azoxystrobin @ 0.05% recorded 66.50% inhibition. The least inhibition (62.77%) was recorded in chlorothalonil @ 0.1%.

At higher dose than field concentration, propiconazole @ 0.1% and difenoconazole @ 0.15% completely inhibited the mycelial growth (100%) of *C. capsici* and was significantly better than all

other fungicides. It was followed by captan + hexaconazole 0.15% with 90 percent growth inhibition over control. The fungicide carbendazim @ 0.1% recorded 80 % inhibition. This was followed by mancozeb @ 0.3% which recorded 75% inhibition. Azoxystrobin @ 0.15% recorded 68.50% inhibition. The least inhibition (64.09%) was recorded in chlorothalonil @ 0.15%.

Among the seven fungicides tested, difenoconazole (0.01%, 0.05%, 0.1%), propiconazole (0.05%, 0.1%, 0.2%)was the most effective at all doses, showing strong inhibition of both, mycelial growth and colony development of *C. capsici*. These findings are in conformity with Barhate *et aI* (2012) who reported that the fungicide propiconazole (0.1%) was effective in inhibiting cent percent radial mycelial growth of *C.capsici* followed by difenoconazole (0.05%), captan + hexaconazole (0.1%), mancozeb (0.2%), carbendazim (0.05%) and chlorothalonil (0.2%) with 86.66 , 85.55 , 84.44, 73.33, 68.88 and 65.55 percent growth inhibition over control respectively [12]. Gaikwad *et aI* (2002) reported that the fungicide propiconazole was effective in inhibiting cent percent radial mycelial growth of C. *gloeosporioides* followed by hexaconazole (88.65 %), carbendazim (88.54 %), difenoconazole (77.43 %), mancozeb (69.59 %) and chlorothalonil (67.72%) [13].



Per cent mycelial inhibition of *C. capsici* by various fungicides (Diagaram 1)

3.2 Effect of biocontrol agents on mycelial growth of *C.capsici*

In vitro evaluation of antagonists under dual culture revealed growth inhibition of chilli frui rot pathogen (*C. capsici*) by these test antagonists. Among the bioagents *T. viride* caused mycelial growth inhibition of 55.5% in dual culture (fig.1). These findings are in conformity with Kaur *et al* (2006) who noticed 53.0% inhibition of *C.capsici* by *T. viride* and completely overgrew the host mycelia once in contact with them. The formation of hyphal coils by *T. viride* on pathogenic colonies was also noticed [14].

Pseudomonas flourescens showed 90% of the radial growth inhibition of the test pathogen; *Colletotrichum capsici* (fig.2).

Fig. 1 Antagonistic action of *Trichoderma viride* against *C.capsici*

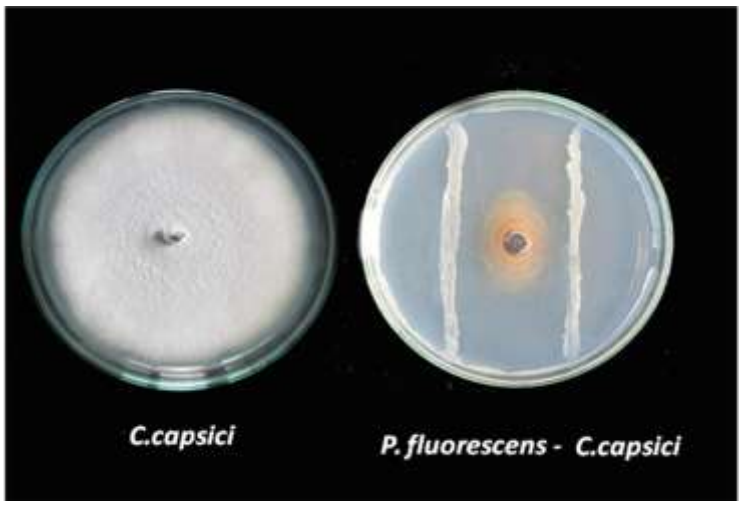


Fig. 2 Antagonistic action of *Pseudomonas flourescens* against *C.capsici*

IV. CONCLUSION

In this study propiconazole (0.05%, 0.1%, and 0.2%) and difenoconazole (0.01%, 0.05%, and 0.1%) at all concentrations were found to be effective in completely inhibiting the mycelial growth of *C. capsici in vitro. Pseudomonas flourescens* (90%) and *T. viride* (55%) also inhibited the mycelial growth of *C. capsici.*

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