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In vitro evaluation of different fungicides in inhibiting the mycelia growth of *Colletotrichum capsici* causing Anthracnose of chili

Monika Kharkwal, JP Mishra and Rajendra Prasad

Abstract

Five fungicides were evaluated *In vitro* namely: difenoconazole, cabrioTop, azoxystrobin, carbendazim, and chlorothalonil by using poisoned food technique against *Colletotrichum capsici* for studying their effect on the inhibition of mycelia growth at different concentration (100, 250, and 500 ppm). Among these fungicides maximum inhibition of mycelia growth was observed in difenoconazole at 500 ppm almost complete inhibition followed by cabrioTop with 91.83% growth inhibition over control at 500 ppm. The fungicide azoxystrobin at 500 ppm recorded 83.04% inhibition. It was followed by carbendazim at 500 ppm recorded 79.21% inhibition. The least inhibition was recorded in chlorothalonil at 500 ppm which was about 71.56%.

Keywords: Fungicides, *Colletotrichum capsici*, anthracnose, poisoned food technique, ppm (parts per million) (100 ppm = 0.01%, 250ppm = 0.025%, and 500 ppm = 0.05%)

Introduction

Chili (*Capsicum annum* L.) belongs to the family Solanaceae, is an important spice, vegetable as well as cash crop cultivated in tropical and sub-tropical climates, at up to 2,000 m altitudes. The most ideal climatic conditions for chili cultivation are 20-25 °C temperature and about 850-1200 mm per annum rain fall. It is rich source of vitamin A, C, E and minerals like potassium and cultivated in all places but unfortunately various threats like viruses, insects, bacterial wilt and anthracnose attack the chilli crop. Among them anthracnose is one of the serious disease cause the yield loss and reduce the quantity and quality of marketable fruits. Disease incidence is recorded from 20 to 80% on fruits of *Capsicum annum* and 5 to 20% on fruits of *C. frutescens* infected in the field conditions. It has been reported that a part of post harvest losses of fruit quality deterioration of chili is due to anthracnose ranges from 21- 47% (Rajapakse *et al.*, 2007). *Colletotrichum capsici* infecting diverse hosts and has a high degree of pathogenic variability (Sharma *et al.*, 1999). Different strategies such as cultural control, growing resistant cultivars, biological control, and fungicides are used to control the *Colletotrichum* diseases. Manandhar *et al.* (1995) found that the most common and practical method to control anthracnose is fungicide spraying. Therefore, in the present study, different concentrations of some fungicides studied *In vitro* to inhibit the mycelial growth of *C. capsici*. The objectives was to evaluate different fungicides 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 fungicides.

Materials and Methods

The present investigation is carried out in the Department of Plant Pathology laboratory, Uttaranchal School of Agriculture, Uttaranchal University, Dehradun, India (2019-2020).

Isolation

First the identification of diseased chilli leaf or fruit was done by visible symptoms. Then with the sterile needle and scalpel cut about 1 cm infected part along with some healthy region. It was then sterilized with 0.1% mercuric chloride (HgCl₂) for 2-3 minutes and washed it for 3 – 4 times in sterile distilled water and blotted dry before being placed on PDA plates. Incubated at 25±2 °C for 3-5 days. After the growth of the pathogen sub culturing was done by transferring 5 mm mycelia disc by sterilized cork borer and to get pure culture it was incubated at 25 ±0°C.

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Identification of pathogen

The fungus was identified by the morphological characters initially of whitish colored mycelia which later turn to greyish black on culture media. Conidiophores are hyaline, cylindrical, unicellular or septate and conidia are one-celled, glutulate, hyaline, fusiform with both ends pointed was observed under trinocular microscope.

In vitro evaluation of fungicides

Five fungicides difenoconazole, cabri Top, azoxystrobin, carbendazim, and chlorothalonil were used and tested at 100, 250 and 500 ppm concentration using poisoned food technique (Nene and Thapliyal, 1993). The desired concentrations were obtained by adding appropriate amount of stock solution of fungicides to PDA medium in petri plates. PDA without fungicide served as control. Each plate was inoculated with a 5 mm mycelia disc of the pathogen i.e. fungus taken from seven day old culture grown on PDA. The inoculated plates were incubated at 25 ± 2 °C till the fungus covered the whole plate in control. The radial growth of colony was recorded and per cent inhibition of each treatment was calculated by using formula given by Vincent

$$I = [C - T] \div C * 100$$

Where

I = Per cent inhibition.

C = Growth of pathogen (mm) in control.

T = Growth of pathogen (mm) in treatment.

Results and Discussion

Inhibition of mycelia growth of *Colletotrichum capsici* by the use of fungicides under *In vitro* condition.

Five fungicides were evaluated at 100 ppm, 250 ppm, and 500 ppm concentrations with four replications to find out the

efficacy of fungicides in inhibition of mycelia growth of *C. capsici* using poisoned food technique. It was observed that all the fungicides significantly inhibited the mycelia growth of *C. capsici*. The mycelia inhibition of fungus increases with the increase in concentrations of fungicides, and maximum inhibition was obtained at highest concentration (500 ppm). The data mentioned in Table 2 revealed that difenoconazole was the most effective fungicide. Followed by cabrio Top, azoxystrobin, carbendazim and chlorothalonil.

Difenoconazole showed 86.99, 88.61, 93.83 per cent inhibition at 100, 250 and 500 ppm respectively, followed by cabrioTop, azoxystrobin showing 85.55 and 83.047% inhibition at 500 ppm, respectively. Next treatment against *C. capsici* was carbendazim which showed inhibition of 59.47, 64.64, and 79.21% respectively at 100, 250 and 500 ppm concentration. However, chlorothalonil showed minimum mycelia growth inhibition 57.03, 63.80, and 71.56% at 100, 250, and 500 ppm, respectively (Fig. 2).

Results for difenoconazole and chlorothalonil according to Barhate *et al.* (2012) [1] who reported that the fungicide difenoconazole (500 ppm) and chlorothalonil exhibited 85.55% and 65.55% growth inhibition whereas in the present study it was showing 93% for difenoconazole and 71% for chlorothalonil. However, results were similar for carbendazim which exhibited 68.88% growth inhibition at 500 ppm. Gaikwad *et al.* (2002) [5] reported that carbendazim (88.54%), difenoconazole (77.43%) and chlorothalonil (67.72%) inhibiting the fungicide growth of *C. gloeosporioides*. The result showed that Carbrio Top effectively controlling the growth at both Concentration viz. 250 and 500 ppm and inhibition percentage was 76% and 84.11% respectively. Madhavan *et al.* (2017) showed Cabrio Top was highly effective in suppressing the growth at 250 ppm and Saha *et al.* (2017) [12] reported the superiority fungicide against anthracnose of chilli.

Table 1: *In vitro* evaluation of fungicides (Mycelial growth of *Colletotrichum capsici* on PDA (colony diameter on fungicides (mm)*).

Concentration (ppm)	Colony diameter (mm)*on fungicides				
	Cabriotop	Difenoconazole	Cholorothalonil	Azoxystrobin	Carbendazim
100	28.71	11.71	39.07	29.573	36.47
250	25.07	10.24	32.58	22.30	31.82
500	6.55	5.55	24.4	15.26	11.25
Control	90.00	90.00	90.00	90.00	90.00
SE(M)	1.33	0.19	0.74	1.14	0.82
CD at 5%	4.33	0.63	2.34	3.69	2.67

Table 2: *In vitro* evaluation of fungicides (Per cent growth inhibition (%) on fungicides.)

Concentration (ppm)	Percentage growth inhibition (%) on fungicides				
	Cabriotop	Difenoconazole	Cholorothalonil	Azoxystrobin	Carbendazim
100	68.10	86.99	57.03	67.14	59.47
250	72.13	88.61	63.80	75.22	64.64
500	91.83	93.83	71.56	83.04	79.21
Control	00.00	00.00	00.00	00.00	00.00
SE (m) ±	1.48	0.22	0.80	1.26	0.91
CD at 5%	4.80	0.69	2.82	4.09	2.97

SE (m) ± - Standard Error mean, CD – Critical Difference.

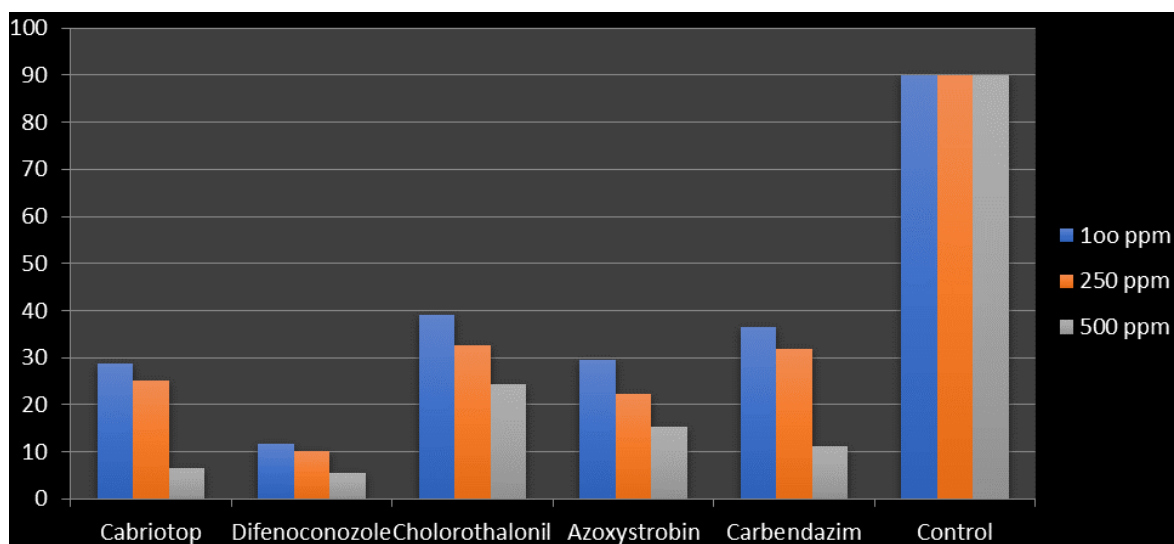


Fig 1: *In vitro* evaluation of fungicides (Mycelial growth of *Colletotrichum capsici* on PDA (colony diameter on fungicides (mm*)).

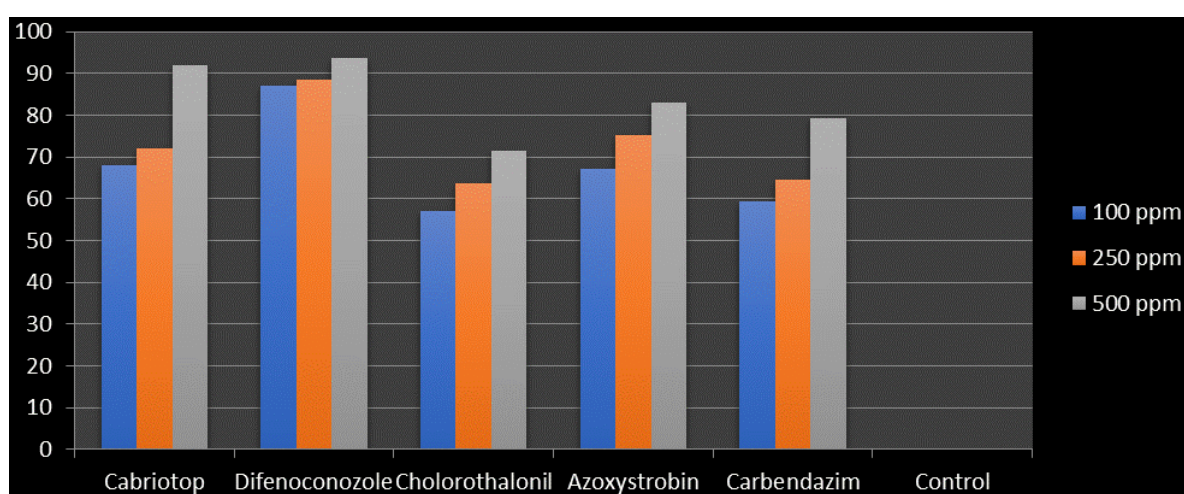


Fig 2: *In vitro* evaluation of fungicides (Per cent growth inhibition (%) on fungicides)

(0.01% = 100 ppm, 0.025% = 250 ppm, and 0.05% = 500 ppm).

Conclusion

Among the five fungicides, difenoconazole and cabrio Top were effectively controlling the mycelia growth in any respect concentration (100, 250 and 500 ppm) but at 500 ppm both showing almost complete inhibition of mycelia growth.

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