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Analytical study on *In vitro* efficacy of fungicides against *Colletotrichum capsici* (Syd.) Butler and Bisby, causing anthracnose of chilli using CRD design

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Abstract
Chilli [*Capsicum annuum* L.] crop is being affected by several fungal, bacterial, viral and nematode induced diseases. However, Anthracnose, caused by *Colletotrichum capsici* (Syd.) Butler and Bisby has been common occurrence, causing quantitative as well as qualitative losses in chilli. Therefore, present *in vitro* study was planned in CRD *in vitro*, in eight treatments replicated thrice, to assess the efficacy of fungicides against *Colletotrichum capsici*, at the Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani (MS).
Results revealed that, all of the eight (03 systemic, 03 contact and 02 combi-products fungicides (each @ 500, 1000 and 1500 ppm), evaluated *in vitro* were found fungistatic and exhibited significant mycelial growth inhibition of *C. capsici*, causing Anthracnose of Chilli. However, the fungicide (T_2) Propiconazole 25% EC (92.62%, 100% and 100%) was found to be most effective there by inhibiting the maximum mycelial growth, followed by fungicide (T_7) Azoxystrobin 18.2% + Difenoconazole 11.4% SC (89.44%, 94.44% and 100%), each @ 500, 1000 and 1500 ppm, respectively.

Keywords: Chilli, *Colletotrichum capsici*, anthracnose, fungicides, *In-vitro*

Introduction
Chilli (*Capsicum annuum* L.), belongs to the family Solanaceae is an important spice cum vegetable crop of the world. Chilli is native of tropical America (Brazil) and has been propagated throughout the world including the tropics, subtropics, and temperate regions. (Pickersgill, 1997) [7]. Chillies are the good sources of vitamin ‘A’, vitamin 'B' and vitamin 'C' and minerals like calcium, phosphorus, ferrous, sodium and copper in trace amounts. Chillies produce alkaloids, capsaicinoids, carotenoids and red pigments (Capsanthin, Capsorubin and Capxanthin) which make chilli hot and pungent (Kumar *et al*., 2019) [5].
India is the world’s largest producer, consumer and exporter of chilli and contributes about 25 percent to total world production. Pungent varieties of chilli contribute about 30 percent of the total production which attract international trade. According to the 2007-08 data, chilli in India occupies an area of 805.8 thousand 2 hectares, with production of 1297.9 thousand tonnes and yield of 1611 kg/hectare, respectively. (Anonymous, 2020) [1].
Chilli crop is attacked with different pests and pathogens in field and during post-harvest, contamination with mycotoxins are major constraints in chilli production. Worldwide, *Capsicum* is vulnerable to various pests, weeds, fungal, bacterial, and viral pathogens; among the fungal diseases, anthracnose/die-back/fruit-rot of chillies is an important disease-causing serious loss in field, transit, transport, and storage (Kiran *et al*., 2020) [4]. Among a number of fungal, bacterial and viral diseases, Anthracnose/fruit rot caused by *Colletotrichum capsici* (Syd.) Butler and Bisby is an economically important disease of chilli, causing yield losses more than 50 percent and is one of the most destructive disease and commonly prevailing in almost all chilli growing major areas of India. Whatever, chilli varieties under cultivation are more or less prone to anthracnose. Therefore, present study on *in vitro* efficacy of fungicides against *C. capsici*, causing Anthracnose of Chilli was planned and conducted at the

Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

Materials and Methods

Collection of samples

Leaves exhibiting typical symptoms of chilli anthracnose disease were collected in the paper bags from chilli fields, in the Parbhani district.

Isolation of the pathogen

Fresh samples of diseased leaves, showing typical symptoms of Anthracnose were collected from the fields, brought to the laboratory, washed thoroughly with distilled water, blot dried and cut with sharp sterilized blade into small bits (5 mm), keeping half healthy and half diseased portion intact. These pieces were surface sterilized with 1 percent Sodium hypochlorite for two minutes and then washed by giving three changes with sterile distilled water and blot dried. This surface sterilized and blot dried leaf bits were then inoculated on the autoclaved, solidified and cooled PDA (Potato dextrose agar) medium in Petri plates under aseptic conditions of Laminar–air-flow cabinet. Inoculated plates were then incubated in BOD incubator at 27±10 C temperature. After a week from incubation, the well-developed mycelial growth of the test pathogen, free from any contaminant was obtained. By following hyphal tip technique test pathogen was transferred aseptically on the PDA slants in test tubes. Through subculturing, the pathogen was purified and its pure culture was maintained on agar slants in test tubes and stored in refrigerator for further studies.

Identification of the pathogen

Based on cultural and morphological characteristics, microscopic observations and pathogenicity test, the pathogen under study was identified as *Colletotrichum capsici* (Syd.) Butler and Bisby.

In vitro efficacy of fungicide

Efficacy of three systemic, three non-systemic and two combi-product fungicides (each @ 500,1000 and 1500 ppm) was evaluated *in vitro* against *C. capsici* by applying Poisoned Food Technique (Nene and Thapliyal,1993)[6]. Three Petri plates / treatment / replication were maintained and also untreated control plates were maintained along with suitable control plates.

The requisite quantity of each fungicide based on active ingredient (a.i.) was calculated and thoroughly mixed with the autoclaved and cooled, (40 to 45 °C) PDA in conical flasks to obtain desired concentrations of 500, 1000 and 1500 ppm. The fungicides amended PDA was then poured (15 to 20 ml/plate) in sterilized Petri dishes (90 mm diameter) under aseptic conditions. For each fungicide and its different concentrations, a triplicate set of Petri dishes was maintained and replicated for three times. On solidification of PDA, all plates were inoculated/ seeded by placing in the centre with 5 mm uniform mycelial disc, obtained from the 7 days old culture of *C. capsici* grown on Potato dextrose agar plates. Plates containing PDA without any fungicide and inoculated with test pathogen were maintained as control (untreated). All these plates were then incubated at room temperature (28±2° C) for a week or until the control plates were fully covered with mycelial growth of the test pathogen. The experimental details were as given below.

Experimental details

Design: CRD

Replications: Three.

Treatments: Nine.

Treatment details

Treatment No.	Treatments
Systemic Fungicides	
T_1	Azoxystrobin 25% EC
T_2	Propiconazole 25% EC
T_3	Hexaconazole 5% EC
Non-Systemic Fungicides	
T_4	Propineb 70% WP
T_5	Chlorothalonil 75% WP
T_6	Mancozeb 75% WP
Combi-products Fungicides	
T_7	Azoxystrobin18.2% + Difenoconazole11.4% SC
T_8	Hexaconazole 4% + zineb 68% WP
T_9	Control

Observations on radial mycelial growth were recorded at 24 hrs. interval and were continued till growth of the test pathogen in untreated control plate is fully covered. Percent inhibition of the test pathogen will be calculated by applying formula given by Vincent, 1927.

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

Where,
C= Growth of the test fungus in untreated control plates
T= Growth of the test fungus in treated plates

Results and Discussion

A total of eight fungicides *viz*., Azoxystrobin, Propiconazole, Hexaconazole, Propineb, Chlorothalonil, Mancozeb, Azoxystrobin + Difenoconazole and Hexaconazole + Zineb with three different concentrations (500, 1000 and 1500 ppm) were tested *in vitro* against *C. capsici*, applying Poisoned food technique (Nene and Thapliyal, 1993) [6] and using Potato dextrose agar (PDA) as basal medium. Effect of these fungicides on radial mycelial growth and inhibition of test pathogen were recorded. The results are presented in Table 1 and 2.

Radial mycelial growth

At 500 ppm concentration the fungicides tested the minimum radial mycelial growth observed in the treatment (T_2) Propiconazole (6.63 mm), followed by (T_7) Azoxystrobin + Difenoconazole (09.49 mm), (T_6) Mancozeb (13.43 mm), (T_3) Hexaconazole (22.93 mm). Among the fungicides tested, the maximum radial mycelial growth was recorded on (T_4) Propineb (75.38 mm) followed by (T_5) Chlorothalonil (72.19 mm), (T_8) Hexaconazole + Zineb (42.37 mm) and (T_1) Azoxystrobin (39.56 mm) as compared to highest growth in (T_0) untreated control (90 mm). (Table- 1, Fig.- 1, Plate- 1).

Table 1: Radial mycelial growth of *Colletotrichum capsici* at different concentrations

Tr. No.	Fungicides	Radial mycelial growth (mm)		
		500 ppm	1000 ppm	1500 ppm
T_1	Azoxystrobin 25%EC	39.56	33.00	27.66
T_2	Propiconazole 25%EC	06.63	00.00	00.00
T_3	Hexaconazole 5%EC	22.93	11.50	05.00
T_4	Propineb 70%WP	75.38	61.00	56.07
T_5	Chlorothalonil 75% WP	72.49	59.00	49.40
T_6	Mancozeb 75%WP	13.43	09.37	07.00
T_7	Azoxystrobin18.2%+Difenoconazole11.4% SC	09.49	05.00	00.00
T_8	Hexaconazole 4% + zineb 68%WP	42.37	36.49	31.56
T_0	Control	90.00	90.00	90.00
	SE±	0.56	0.57	0.58
	CD at 1%	1.68	1.74	1.58

At 1000 ppm concentration all the fungicides restricted the radial mycelial growth, complete inhibition of radial mycelial growth was observed in the treatment (T_2) Propiconazole. The minimum radial mycelial growth was observed in the treatment (T_7) Azoxystrobin + Difenoconazole (05.00 mm), followed by (T_6) Mancozeb (09.37 mm) and (T_3) Hexaconazole (11.50 mm). Among the fungicides tested, the maximum radial mycelial growth was recorded on (T_4) Propineb (61.00 mm), followed by (T_5) Chlorothalonil (59.00 mm), (T_8) Hexaconazole + Zineb (36.49 mm) and (T_1) Azoxystrobin (33.00 mm) as compared to highest growth in (T_0) untreated control (90.00 mm) (Table- 1, Fig.- 1, Plate-2)

At 1500 ppm concentration all the fungicides restricted the radial mycelial growth, complete inhibition of radial mycelial growth was observed in the treatment (T_2) Propiconazole and (T_7) Azoxystrobin + Difenoconazole. The minimum radial mycelial growth was observed in the treatment (T_6) Mancozeb (07.00 mm), (T_3) Hexaconazole (05.00 mm) and (T_1) Azoxystrobin (27.66 mm). Among the fungicides tested, the maximum radial mycelial growth was recorded on (T_4) Propineb (56.07 mm) followed by (T_5) Chlorothalonil (49.40 mm) and (T_8) Hexaconazole + Zineb (31.56 mm) as compared to highest growth in (T_0) untreated control (90.00 mm**).** (Table- 1, Fig.- 1, Plate-3).

Inhibition of mycelial growth

At 500 ppm concentration, the fungicide (T_2) Propiconazole (92.62%) was found to be most effective there by inhibiting the maximum mycelial growth. The second-best fungicide was (T_7) Azoxystrobin + Difenoconazole (89.44%), followed by (T_6) Mancozeb (85.05%) and (T_3) Hexaconazole (76.62%). The minimum inhibition of mycelial growth was observed on (T_4) Propineb (16.23%), followed by (T_5) Chlorothalonil (19.44%), (T_8) Hexaconazole + Zineb (52.91%) and (T_1) Azoxystrobin (56.03%). (Table- 2, Fig.- 2, Plate- 1). At 1000 ppm concentration, the fungicide (T2) propiconazole was found to be most effective there by inhibiting the cent percent mycelial growth of test fungus. The second-best fungicide was (T_7) Azoxystrobin + Difenoconazole (94.44%) followed by (T_6) Mancozeb (89.58%) and (T_3) Hexaconazole (87.20%). (Table- 2, Fig.- 2, Plate- 2). The minimum inhibition of mycelial growth was observed on (T_4) Propineb (32.22%), followed by (T_5) Chlorothalonil (33.70%), (T_8) Hexaconazole + Zineb (59.44%) and (T_1) Azoxystrobin (62.95%). The treatment (T_4) Propineb (32.22%) is at par with treatment (T_5) Chlorothalonil (33.70%).

Table 2: Percent inhibition of Radial mycelial growth at different concentrations

Tr. No.	Fungicides	Percent inhibition of Radial mycelial growth		
		500 ppm	1000 ppm	1500 ppm
T_1	Azoxystrobin 25% EC	56.03 (48.44)*	62.55 (52.49)	69.26 (56.31)
T_2	Propiconazole 25% EC	92.62 (74.25)	100.00 (90.00)	100.00 (90.00)
T_3	Hexaconazole 5% EC	74.62 (59.72)	87.20 (69.02)	94.44 (76.37)
T_4	Propineb 70% WP	16.23 (23.74)	32.22 (34.56)	37.76 (37.90)
T_5	Chlorothalonil 75% WP	19.44 (26.15)	33.70 (35.46)	45.10 (42.17)
T_6	Mancozeb 75% WP	85.05 (67.24)	89.58 (71.15)	92.22 (73.80)
T_7	Azoxystrobin18.2%+Difenoconazole11.4% SC	89.44 (71.03)	94.44 (76.37)	100.00 (90.00)
T_8	Hexaconazole 4% + zineb 68% WP	52.91 (46.65)	59.44 (50.42)	64.93 (53.66)
T_0	Control	00.00 (00.00)	00.00 (00.00)	00.00 (00.00)
	SE±	0.58	0.65	0.67
	CD at 1%	1.75	1.98	2.03

* Mean of three replications

Figures in parentheses are arcsine transformed values

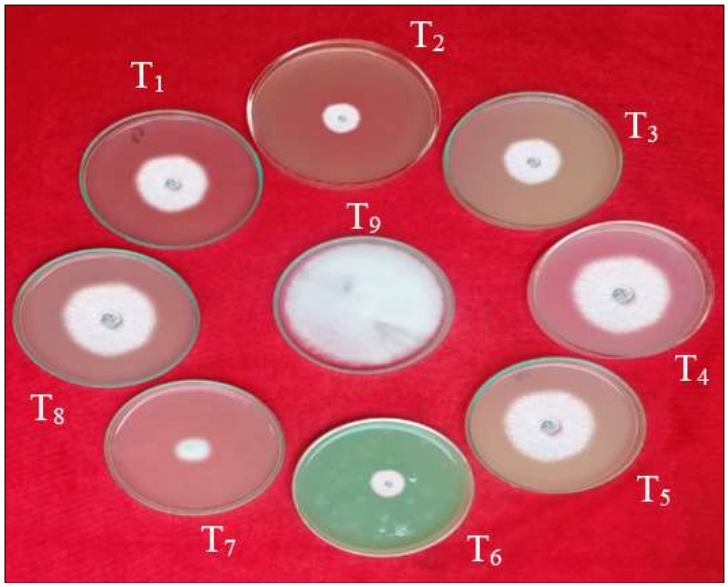

Plate 1: Radial mycelial growth and percent inhibition of *C. capsici* at 500 ppm concentration

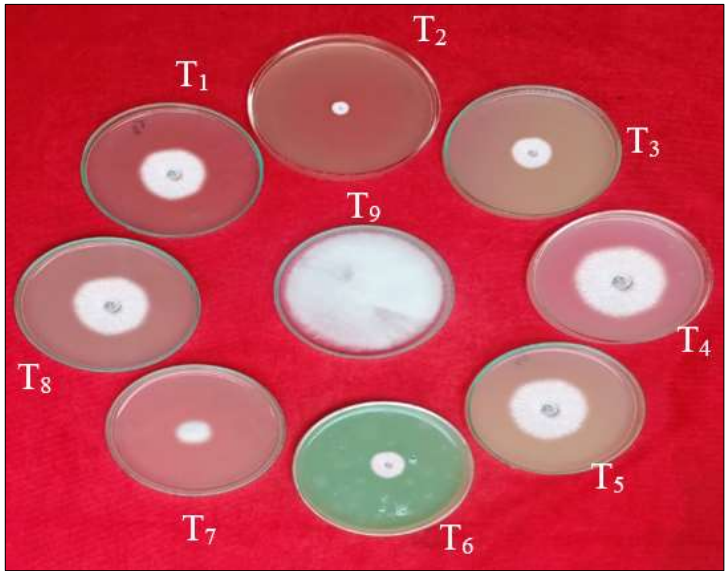

Plate 2: Radial mycelial growth and percent inhibition of C. capsici at 1000 ppm concentration

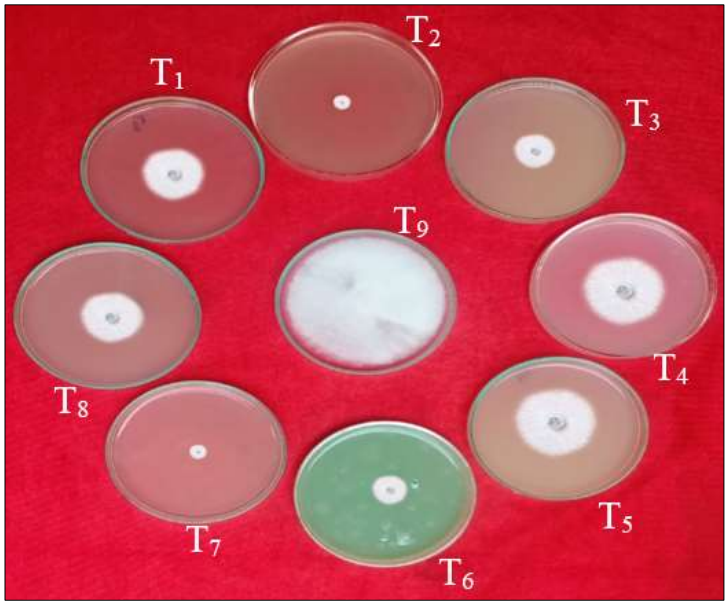

Plate 3: Radial mycelial growth and percent inhibition of *C. capsici* at 1500 ppm concentration

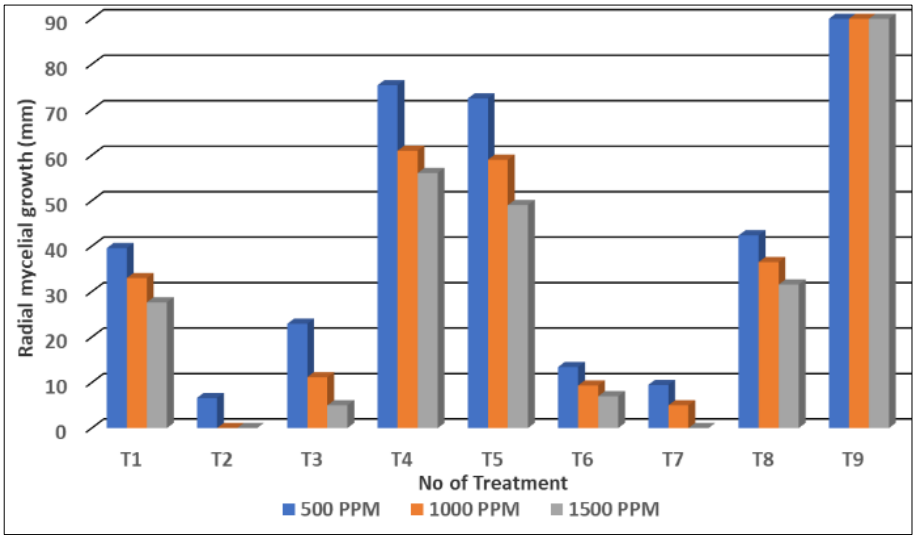

Fig 1: Effect of Different Concentrations (500 PPM, 1000 PPM, 1500 PPM) on Radial Mycelial Growth (mm) for Various Treatments (T_1-T_9)

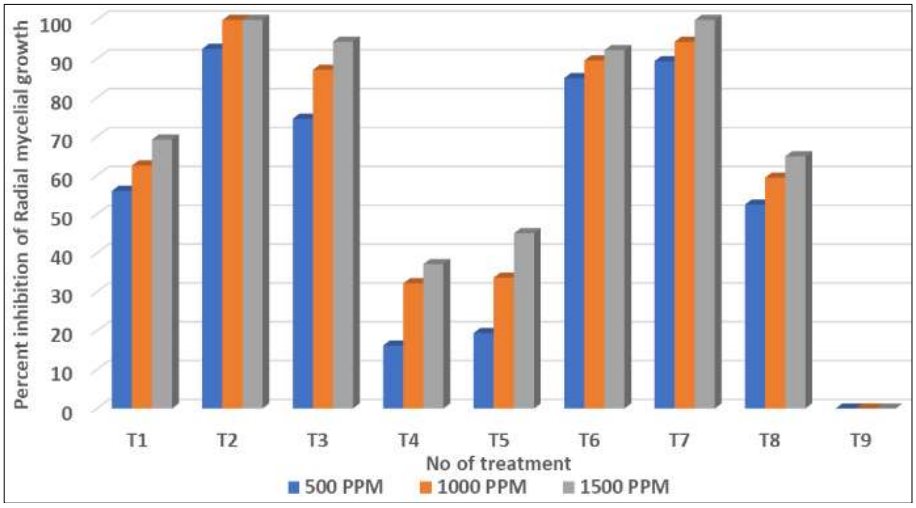

Fig 2: Percent inhibition of C. capsici at different concentrations

At 1500 ppm concentration, the fungicide (T_2) propiconazole and (T_7) Azoxystrobin + Difenoconazole was found to be most effective there by inhibiting the cent percent mycelial growth of test fungus. The next best fungicide was (T_6) Mancozeb (92.22%) followed by (T_3) Hexaconazole (94.44%). The minimum inhibition of mycelial growth was observed on (T_4) Propineb (37.76%) followed by (T_5) Chlorothalonil (45.10%), (T_8) Hexaconazole + Zineb (64.93%) and (T_1) Azoxystrobin (69.26%). (Table- 2, Fig.- 2, Plate- 3).

These results of the present study are in consonance with the findings of many earlier workers, Chacko *et al.* (2015) [2], Rao *et al*. (2020) [9], Rajashree *et al*. (2020) [8] and Gurjar *et al*. (2021) [3].

Conclusion

Hence, from ongoing results and discussion, it is concluded that, the systemic fungicide Propiconazole 25% EC and combi-product fungicide Azoxystrobin 18.2% + Difenoconazole 11.4% SC were found most effective against the test pathogen.

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