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IN VITRO EFFICACY OF DIFFERENT FUNGICIDES AGAINST *COLLETOTRICHUM CAPSICI* CAUSING ANTHRACNOSE OF CHILLI

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ABSTRACT

Chilli (*Capsicum annuum* L.) is a significant crop that is widely grown around the world as vegetable and a spice. Anthracnose is a common and destructive disease of the chilli with major constraint resulting into significant losses of up to 10-60% in both primary yield and fruit quality. Most of the fungicides has a fungistatic and fungicidal activity. Hence it is essential to recommend the appropriate fungicide and its concentration to reduce the yield loss caused by them. So, in this study thirteen fungicides have been tested at different concentrations against *C. capsici,* all the fungicides exhibited a wide range of growth pattern from 0.00 to 65.67 mm radial mycelial growth of *C. capsici* over untreated control. All the tested fungicides proved to be efficient and statistically significant to reduce the radial mycelial growth. Among the 13 fungicides the propiconazole 25% EC and tebuconazole 25.9 % EC (each @ 0.05 and 0.15 % conc.) and the combi-product i.e. trifloxystrobin 25 % + tebuconazole 50 % WG at 0.05 % conc. was highly effective as cent per cent inhibition was achieved followed by azoxystrobin 18.2% + difenoconazole 11.4 % SC (92.85 %) and pyraclostrobin 133 g/L+ epoxiconazole 50 g/L SE (88.52 %) as they were equal level of significance in inhibiting the *C. capsici* causing anthracnose of chilli. The minimum inhibition at 0.05 % was recorded in Propineb 70% WP (27.03 %) followed by Myclobutanil 10% WP (44.81 %). Though these products were less inhibitory, but the combi-product with these systemic chemicals was highly efficient to reduce the growth as it might be due to synergistic effect of the combination of these fungicides. This practice provides initial information on the types of fungicide that are suitable for managing anthracnose of chilli. The chemical ingredients are inhibitory to fungus as it is interacting with the metabolism of the fungus resulting in highly efficient systemic fungicidal activity.
Keywords **:** *Colletotrichum capsici*, systemic fungicides, *in vitro*, combi-product, synergistic effect.

Introduction

Chilli (*Capsicum annuum* L.) is a globally significant crop, extensively cultivated for its dual roles as both a culinary staple and a valuable medicinal resource. It is known as the "miracle spice" because it is the most widely used spice in the world. India, in particular, holds a pivotal position in the global chilli market, being a major exporter, producer, and consumer of this versatile crop. Several Indian states, including Andhra Pradesh, Karnataka, Maharashtra, Odisha, and Tamil Nadu, are renowned for their substantial contributions to chilli production.

Chilli cultivation faces a myriad of challenges, primarily stemming from diseases caused by bacteria, viruses, and fungi. Rangaswami (1979) reports over 40 fungal diseases affecting chilli crops. Among these, anthracnose (also known as die-back) stands out as a destructive force, causing substantial losses, ranging from 10% to 60%, in both yield and fruit quality, contingent on the chilli variety. Anthracnose is pervasive in tropical and subtropical regions, afflicting countries such as India, Thailand, China, and Indonesia. In India, anthracnose is primarily caused by three species of *Colletotrichum*: *Colletotrichum capsici*, *Colletotrichum acutatum*, and *Colletotrichum gleosporoides*, with *Colletotrichum capsici* Syd. Butler and Bisby being responsible for significant fruit losses during the plant's mature stage (Saxena *et al*., 2016). This disease is particularly devastating, resulting in substantial economic losses.

Anthracnose of chilli is now an important disease found to be the major constraint in chilli production for both profitable cultivation and seed production. Mostly the chemical fungicides were recommended for the management of this disease. Chemical protection is a primary strategy for plant disease control. In the absence of a resistant source, the most common way to protect plants from diseases is to use fungicides. According to Morsy and Elshahawy (2016) and Abdel-Rahman *et al*. (2020), the systemic fungicide carbendazim is effective *in vitro* against *Colletotrichum spp*. Thiophanate methyl, difenoconazole + azoxytrobin,

iprodione, tebuconazole, flusllazole, and cyprodinil + fludioxonil have all shown promise in the control of Colletotrichum in other crops Chaudhari *et. al.,* (2021), Elshahawy *et. al*., (2016), Nuraini, and Latiffah (2019) and Ishii (2022)

Materials and Methods

The present experiment was conducted at Post Graduate institute, Dr. PDKV Akola (M.S.) during 2022-23. The thirteen fungicides were evaluated *in vitro* for their antifungal activities against chilli anthracnose, was done at different concentrations. The efficacy of fungicides was tested by using Poisoned Food Technique described by Nene and Thapliyal (1993) on potato dextrose agar (PDA) medium.

Isolation and identification of *Colletotrichum capsici*

Chilli fruits having anthracnose symptoms were collected from the fields. Isolation was done by cutting small pieces from the margin of lesions which were then surface sterilized by immersing in 1 per cent Sodium Hypochlorite solution for 30 seconds and washed with sterilized distilled water. To remove excess moisture from the samples the pieces were transferred on to sterilized blotter paper. These pieces were then transferred to Petri plates containing PDA medium under aseptic conditions followed by incubation at 26±2ºC.

Purification of *Colletotrichum capsici*

To obtain pure cultures, the single hyphal tip technique was employed. The resulting pure cultures were identified as *C. capsici* based on their morphological characteristics, and these purified cultures were subsequently maintained on PDA slants for further investigations.

Identification of Pathogen

The cultural and morphological nature of fungi, such as the colour of the colony, growth pattern and acervulus, were examined and confirmed. A hyphal tip culture of *C. capsici* was picked up from the 10-day-old culture to establish their identity. The culture was kept on the microscopic slide and mixed thoroughly with cotton blue to obtain clear stained spores. A coverslip was placed over the culture drop and observed under a compound microscope (Singh, 1978; Brayford and Samuels, 1993; Subhani, 2015).

Pathogenicity Test

Under in vitro conditions, the healthy chilli fruits were treated with fungus for pathogenicity. Pathogenicity test was the pin prick inoculation method suggested by Sanders and Korsten (2003). The healthy fruits were first decontaminated by 70% (v/v) ethanol, followed by sterile distilled water wash. *C. capsici* spore suspension of 10 µL spores was taken from the 10-day- old culture and inoculated on chilli fruits. A control was separately maintained in which all the operations were similar except the addition of the fungal culture. Symptom appearance was observed at regular intervals. Koch's postulates were confirmed by reisolating the fungus from diseased fruits and compared with the original test fungus.

The PDA medium amended with fungicides was poured separately @ 20ml per Petri plate. After solidification of poisoned medium, the plates were inoculated with 0.5 mm mycelium disc of *C. capsici* obtained from seven days old culture of pathogen. Plates containing um-amended medium served as control. The inoculated plates were incubated in B.O.D incubator at 26±2˚C. The colony diameter of culture was recorded when plates under control were fully covered. The efficacy of fungicides was expressed as per cent inhibition of mycelial growth over control, which was calculated by using the following formula (Vincent, 1947).

$$\text{Per cent Growth} = \frac{\text{Colony growth in Control plate} - \text{Colony growth in treated plate}}{\text{Inhibition colony growth in control plate}} \times 100$$

Results and Discussion

Most fungicides exhibit both fungistatic and fungicidal activities. Thirteen fungicides were tested at different concentrations against *C. capsici*, the data on the percentage inhibition of radial growth of the fungus are presented in Table 1.

The results, as shown in Table 1, Plate 1 and fig 1, indicated that all tested fungicides displayed a wide range of inhibition of radial mycelial growth of *C. capsici*, ranging from 0.00 to 65.67 mm when compared to the untreated control. All tested fungicides were effective and statistically significant in reducing radial mycelial growth. Among the 13 fungicides, Propiconazole 25% EC and Tebuconazole 25.9 % EC (each at 0.05% and 0.15%) in combination with Trifloxystrobin 25% + Tebuconazole 50% WG at 0.05% concentration exhibited the highest efficiency, achieving cent percent inhibition. Azoxystrobin 18.2% + Difenoconazole 11.4 % SC (92.85%) and Pyraclostrobin 133 g/L + Epoxiconazole 50 g/L SE (88.52%) also proved highly effective, with statistically significant inhibition of the pathogen *C. capsici*, responsible for anthracnose in chilli. The lowest inhibition, indicating the maximum radial growth, was observed with Propineb 70% WP, followed by Myclobutanil 10% WP. However, these products, when combined with systemic chemicals, demonstrated high efficiency in reducing growth, possibly due to a synergistic effect of the combination of these fungicides. The chemical ingredients inhibited the fungus by interacting with its metabolism, resulting in a higher level of systemic fungicidal activity.

Present results of *in vitro* evaluation of fungicide are in line with previous findings. Barhate *et al* (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.

Table 1 : *In vitro* efficacy of different fungicides against *C. capsici*

Tr. No.	Treatments	Conc. (%)	Colony Diameter* (mm)	% inhibition
T_1	Propiconazole 25% EC	0.05	0.00	100.00
T_2	Penconazole 10% EC	0.1	36.33	59.63
T_3	Hexaconazole 5% EC	0.3	28.67	68.14
T_4	Tebuconazole 25.9 % EC	0.15	0.00	100.00
T_5	Difenoconazole 25% EC	0.05	0.00	100.00
T_6	Azoxystrobin 18.2% + Difenoconazole 11.4 % SC	0.3	7.33	91.85
T_7	Trifloxystrobin 25 % + Tebuconazole 50 % WG	0.05	0.00	100.00
T_8	Azoxystrobin 23% SC	0.025	33.33	62.96
T_9	Myclobutanil 10% WP	0.05	49.67	44.81
T_{10}	Propineb 70% WP	0.05	65.67	27.03
T_{11}	Pyraclostrobin 133 g/L+ Epoxiconazole 50 g/L SE	0.1	10.33	88.52
T_{12}	Hexaconazole 4% + Zineb 68 % WP	0.25	22.00	75.55
T_{13}	Captan 70 % Hexaconazole 5% WP	0.1	25.33	71.85
T_{14}	Control (untreated)	-	90.00	0.00
	SE±		**0.45**	**-**
	CD P= 0.01%		**2.18**	**-**

*Average of three replications.

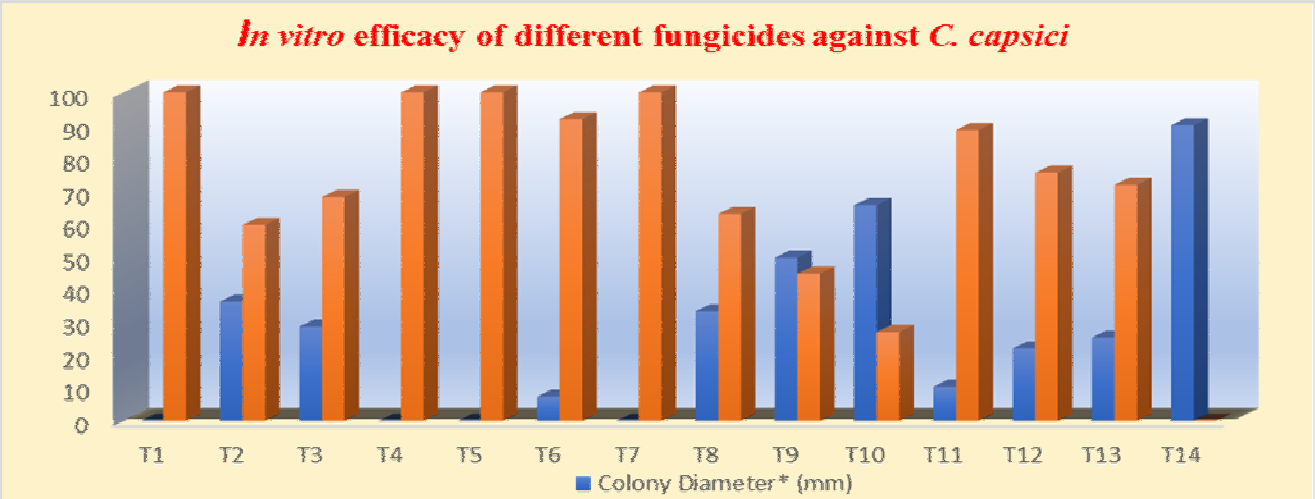

Fig. 1 : *In vitro* efficacy of different fungicides against *C. capsici*

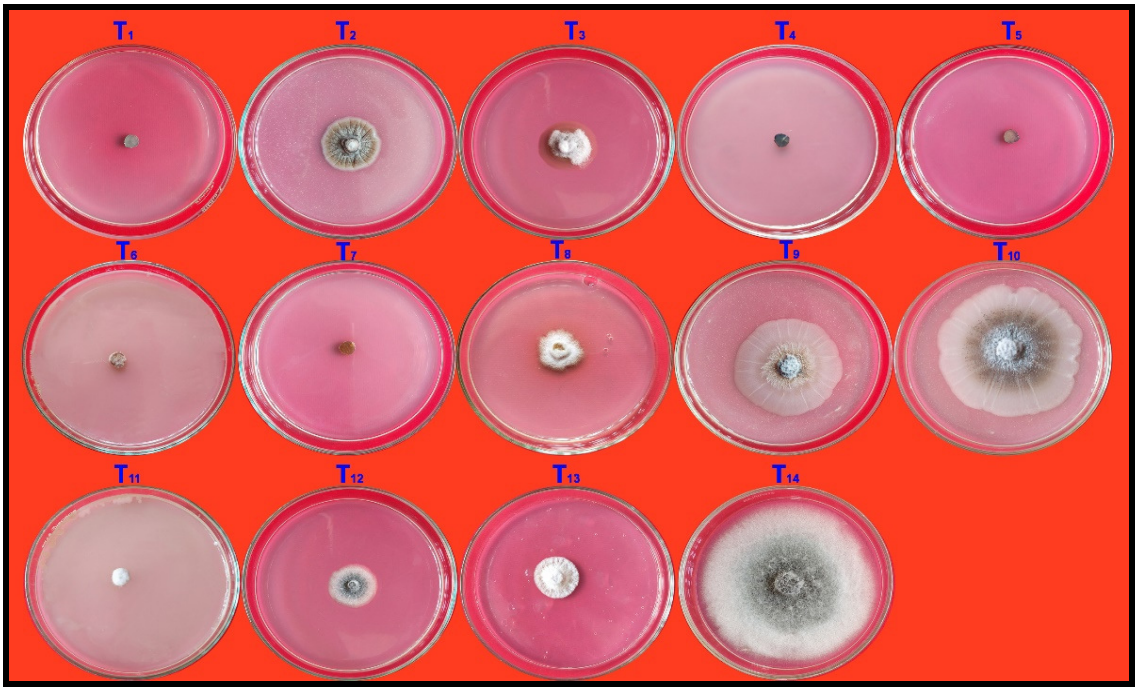

Plate 1 : *In vitro* efficacy of different fungicides against *C. capsici*

Conclusion

In this study thirteen fungicides have been tested at different concentrations against *C. capsici,* all the fungicides exhibited a wide range of growth pattern. Among them propiconazole 25% EC and tebuconazole 25.9 % EC (each @ 0.05 and 0.15 % conc.) and the combi-product i.e. trifloxystrobin 25 % + tebuconazole 50 % WG at 0.05 % conc. was highly effective as cent per cent inhibition was achieved. The minimum inhibition at 0.05 % was recorded in Propineb 70% WP (27.03 %) followed by Myclobutanil 10% WP (44.81 %). Though these products were less inhibitory, but the combi-product with these systemic chemicals was highly efficient to reduce the growth as it might be due to synergistic effect of the combination of these fungicides.

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