

***In vitro* evaluation of some botanical extracts and fungicides against the mycelial growth of *Colletotrichum capsici* causing Anthracnose disease of chilli (*Capsicum annuum* L.)**

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Chilli (*Capsicum annuum* L.) is an important spice crop belonging to the family Solanaceae. It is suffered many diseases of which Anthracnose of chilli caused by *Colletotrichum capsici* is one of the major disease causing severe yield loss of all chilli growing fields. Therefore an investigation was carried out to test the efficacy of some botanical extracts and fungicides for the management of the pathogen. Botanical extracts (*Allium sativum*, *Azadirachta indica*, *Cassia alata*, *Lantana camera* and *Terminalia arjuna*) and fungicides (Hexaconazole, Carbendazim, Mancozeb, Copper oxychloride and Ridomil) were evaluated against the pathogen under *in vitro* condition by poisoned food technique. Amongst the botanical extracts tested *Allium sativum* @ 15% concentration was found most effective 100% inhibition in reducing the mycelial growth of the pathogen. On the other hand the fungicides tested Hexaconazole was found most effective 98.5% inhibition @ .2% concentration of mycelial growth of *C. capsici* followed by Carbendazim (90%) inhibition.

Keywords: Anthracnose, botanical, chilli, fungicides, mycelial growth

INTRODUCTION

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is an important vegetable spice crops grown in Assam and all parts of the world. India is the second largest exporter of chilli in the world which is grown commercially in Bihar, Madhya Pradesh, Uttar Pradesh, Tamil Nadu, Karnataka, Andhra Pradesh and Rajasthan. It has many medicinal properties which reduces the risk of cancer by preventing carcinogens from binding to DNA and reduce calorie intake by increasing thermo genesis (Saxena *et al.* 2014). *Colletotrichum* causes Anthracnose disease and post-harvest decay on a wide range of tropical, subtropical and temperate fruits, crops and ornamental plants reported by many researchers (Sharma *et al.* 2011, Damm *et al.* 2012, Saxena *et al.* 2016).

Anthracnose disease of chilli caused by *Colletotrichum capsici* is one of the most prevalent, serious diseases of chilli. The Symptoms appeared as small, circular, yellowish to pinkish sunken spots on fruits. Ripe fruits are more vulnerable to attack than green ones. The affected fruits may fall off subsequently. In India this disease causes severe damage to fruits in the field as well as in storage up to 84 per cent (Saxena *et al.* 2014). Different species of *Colletotrichum* infects chilli plants at different growth stages. *Colletotrichum capsici* mostly affects red chilli fruits (Kim *et al.* 2014, Than *et al.*, 2008, Rashid *et al.* 2015). Though biological and other methods of controlling plant disease are effective, the use of chemical fungicides is considered as most effective method. Control of plant diseases typically depends upon the application of chemical fungicides, despite their potentiality toxic effects on non target organisms and the environment (Santos *et al.* 2008; Ferrer Alcon *et al.* 2009). Hence, there is an urgent need to look for an alternative safer means for managing

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plant diseases. Effective and efficient management of crop disease is generally achieved by the use of synthetic pesticides (Kiran *et al.* 2006). Use of botanicals for prevention of plant diseases has been recorded in ancient Indian agriculture. Several botanicals viz. leaves and oil of neem (*Azadirachta indica*) leaves of garlic (*Allium sativum*), leaves and fruits of datura (*Datura stramonium*), seeds of babool (*Acacia nilotica*) and fruits of brihati (*Solanum indicum*) have antimicrobial activities against some plant pathogens (Kumar and Yadav, 2007; Meena *et al.* 2007; Menaria *et al.* 2012). Hence the present study was aims to evaluate with some phytotoxic extracts and fungicides to manage the Anthracnose disease of chilli in *in vitro*.

MATERIALS AND METHODS

The fungus *Colletotrichum capsici* was isolated from the infected fruit of chilli plants collected from different fields of Barpeta district (Assam) following tissue segmentation method. The pure culture was maintained on Potato Dextrose Agar slants at $4\pm 1^\circ\text{C}$.

In vitro bio assay of plant extracts

Fresh leaves of *Azadirachta indica*, *Cassia alata*, *Lantana camera*, bulb of *Allium sativum* and bark extract of *Terminalia arjun* were collected for preparing crude extracts for their antifungal property against *Colletotrichum capsici* by 'Poisoned Food Technique' (Nene and Thapthial, 1982) in *in vitro* condition. They were thoroughly washed with water and fine slurry was prepared by taking 100g leaves with 100 ml of distilled water. The resultant slurry was filtered through muslin cloth and then through what man No.1 filter paper and the extracts were used as stock solution. From the stock solution different concentrations of aqueous extracts like 5ml, 10ml and 15 ml was added with 95ml, 90ml and 85 ml of PDA medium to make 5%, 10% and 15% concentrations respectively. The medium was thoroughly shaken for uniform mixing of extract. Twenty ml of medium was poured into sterile petriplates. Mycelium of five mm size discs from periphery of actively growing culture were cut out

by sterile cork borer and one such disc was placed at the centre of each agar plate. Control was also maintained by growing the pathogen on PDA plates. The plates were then incubated at $28\pm 1^\circ\text{C}$ for 7 days. The radial growth of the test fungus in the treated plates was measured when the fungal growth of the control treatment touched the periphery of the petridish. The percent inhibition of fungal growth was estimated as per (Vincent, 1947).

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

where, I= Percent inhibition over control ; C= Radial growth in control; T= Radial growth in treatments.

In vitro effect of different fungicides

The comparative toxicity of fungicides on the growth of the fungus under *in vitro* condition was evaluated by poisoned food technique Nene and Thapthial (1982). Fungicides like Hexaconazole, Carbendazim, Copper oxychloride, Mancozeb and Ridomil at different concentration (0.1, 0.15 and 0.2 percent) were used for *in vitro* assay. The fungicides were incorporated into the sterilized PDA medium. The sterilized petriplates containing amended medium were inoculated with 5 mm disc of freshly prepared culture of the test fungus and incubated at $28\pm 1^\circ\text{C}$ for 7 days. The efficacy of fungicides was expressed as percent of radial growth over control which was calculated by using the formula Vincent (1947).

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

where, I= Percent inhibition over control ; C= Radial growth in control; T= Radial growth in treatments.

RESULTS AND DISCUSSION

In vitro evaluation of fungicides against *Colletotrichum capsici*.

The results presented in (Table.1), revealed that all the botanical extracts were evaluated under *in vitro* condition against *Colletotrichum capsici* significantly inhibited mycelial growth over control. Among them *Allium sativum* extracts was found most

Table 1: Effect of different concentrations of plant extracts on mycelial growth of *Colletotrichum capsici*.

Botanical extracts	Percent inhibition of mycelial growth			Mean ± SE
	5%	10%	15%	
<i>Allium sativum</i> L	69.88	84	100	84.62±8.44
<i>Azadirachta indica</i> L	51.7	56	80	62.56±3.09
<i>Cassia alata</i> L	38.5	53	55	48.83±3.29
<i>Lantana camera</i> L	38	49.7	53.3	47.00 ± 2.89
<i>Terminalia arjuna</i> L	12	22.2	27.6	20.6 ± 4.13

Values shown are the mean ± SE of 3 replicates.

Table 2 : Effect of different concentrations of fungicides on mycelial growth of *Colletotrichum capsici*.

Fungicides	Percent inhibition of mycelial growth			Mean ± SE
	Concentration (%)			
	0.1	.15	0.2	
Hexaconazole	94.2	97.4	98.5	96.70 ± .83
Carbendazim	73	87.4	90	83.46 ± 4.56
Copper oxychloride	78.4	82.6	84.5	81.83 ± .66
Mancozeb	73.5	79	80.8	77.76± 1.70
Ridomil	38	41.7	44.6	41.43

Values shown are the mean ± SE of 3 replicates.

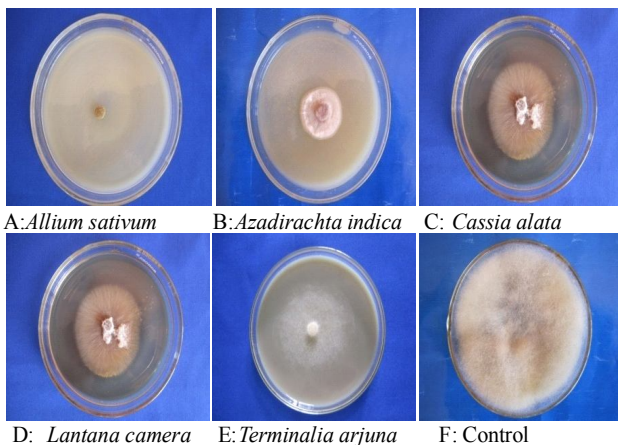


Fig.1: (A-E): Effect of plant extracts on mycelial growth of *Colletotrichum capsici* at 15% concentration

effective (100%) followed by *Azadirachta indica* (80%), *Cassia alata* (55%), *Lantana camera* (53.3%). Least inhibition of mycelial growth was observed by *Terminalia arjuna* with 27.6 percent inhibition at the equal concentration (Fig.1). Among the tested plants extracts were found to be

inhibitory against the fungus and the rate of inhibition increased generally by increasing the concentration. The plant extracts at 15% concentration were significantly superior over the other two concentrations i.e. 10 and 5 percent. The

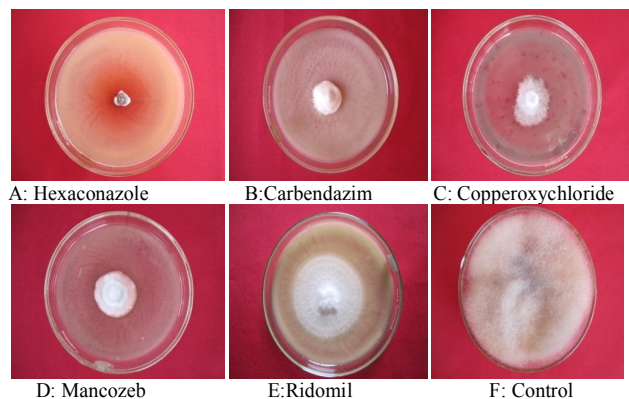


Fig.2: A-E): Effect of fungicides on mycelial growth of *Colletotrichum capsici* at .2% concentration.

findings are in agreement with that of Gomathi and Kannabiran (2000). They reported that botanicals extract viz. Garlic, Neem, Tulsi, etc. were most effective for inhibiting the mycelial growth of *Colletotrichum capsici* under *in vitro* evaluation.

***In vitro* evaluation of fungicides against Colletotrichum capsici**

The result showed in (Table2) revealed that all the fungicides were significantly superior in reducing the mycelial growth of *C.capsici* when compared in control. Among the fungicides tested during the present study, Hexaconazole was found significantly superior (98.5%) inhibition, followed by Carbendazim (90% inhibition), Coppe-

roxychloride (84.5% inhibition) and Mancozeb (80.8% inhibition) at the highest concentration i.e. 0.2%. On the other hand Ridomil was found least effective 44.6 percent inhibition at the same concentration (Fig.2). Similar result was reported by Sharma *et al.* (2004). They found that Carbendazim and Mancozeb were highly effective against the pathogen causing die back and anthracnose disease of chilli *in vitro*.

CONCLUSION

From the above study the result indicate that among the botanical extracts of *Allium sativum* and Hexaconazole among the fungicides showed most effective in respect of reducing mycelial growth of the pathogen. Thus these may be used in field against the pathogen for management of the disease.

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