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Evaluation of Plant Extracts, Biocontrol Agents and Fungicides Against the Growth of Turmeric Leaf Spot Pathogen, *Colletotrichum capsici* under *In Vitro* Condition

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Abstract Leaf spot incited by *Colletotrichum capsici* is one of the most serious foliar disease of turmeric and becoming a major limiting factor for production and quality of turmeric which causes 15–60% losses in India. An experiment was conducted to study the *in vitro* effect of various botanicals, biocontrol agents (BCAs) and fungicides on the mycelial growth of *C. capsici*. Among the plant extracts, garlic clove extract at concentration of 10% and 25% was found most effective with complete inhibition of *C. capsici*. Effect of biocontrol agents against *C. capsici* revealed that *Trichoderma harzianum* was found most effective with maximum inhibition of 74.89% followed by *T. viride* (66.67%). Among the fungicides tested, Carbendazim + Mancozeb, Propiconazole and Hexaconazole were found best with complete inhibition in all the concentrations tested. Even though fungicides acts as effective controlling agent of fungal pathogen, Meghalaya being an organic state, fungicides could be replaced with biocontrol agents and plant extracts.

Keywords *Colletotrichum capsici*, Plant extracts, Biocontrol agents, Fungicides.

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Introduction

Turmeric (*Curcuma longa* L.) is one of the most important spices of the world which is commercially cultivated for rhizome. It is traditionally used in India for medicinal, religious and culinary purposes. Turmeric ranks second in terms of spice production with 7% share in area and 20% share in India's production [1]. Among North Eastern states of India, Meghalaya ranks second and first in area and production of turmeric respectively [2]. Some of the varieties grown in this region have superior yield and quality rhizomes which are worldwide popular due to their rich curcumin content (7.5%) [3].

Table 1. *In-vitro* efficacy of plant extracts against mycelial growth of *C. capsici*. *Mean of three replications. '*F*' test= at 5% level.

Sl. No.	Plant extract	Radial growth (cm) 5%	10%	25%	Mean	Per cent inhibition of mycelial growth over control (%) 5%	10%	25%
2	*D. stramonium*	4.11	4.37	3.23	3.90	54.33	51.44	64.11
2	*L. camera*	4.58	4.36	0.54	3.16	49.11	51.56	94.00
3	*P. longifolia*	4.43	4.49	3.73	4.22	50.78	50.11	58.56
4	*A. cepa* L.	3.48	3.12	1.86	2.82	61.33	65.33	79.33
5	*A. indica*	5.01	4.87	4.70	4.86	44.33	45.89	47.78
6	*D. repens* L.	0.43	0.23	0.14	0.27	95.22	97.44	98.44
7	*A. sativum* L	0.34	0.00	0.00	0.11	96.22	100.00	100.00
8	*O. sanctum*	3.93	3.73	3.30	3.65	56.33	58.56	63.33
9	Control	9.00	9.00	9.00	9.00	0.00	0.00	0.00
	SE (d)	1.39	1.40	1.16				
	CD (p=0.05)	3.50	3.51	2.91				

Turmeric plant is highly prone to several fungal diseases. Among them, leaf spot caused by *Colletotrichum capsici* Syd. Butler and Bisby appear more severe and considered as important limiting factor for its production. The disease appears in severe form and causes yield losses in the range of 15–60% [4]. The disease has also been found to occur frequently in the turmeric fields of North Eastern Hill (NEH) states of India and major losses incurred due to poor development of rhizomes from diseased plants which ultimately leads to heavy reduction in yield. Although the disease appears in turmeric regularly in the region, managing the disease involves the use of fungicides only [5—7]. However, many other workers have also started usage of biocontrol agents [8] and a number of plant species which possess natural substances that are toxic to many fungal plant pathogens [9]. Since the disease occurs regularly in North East India especially in Meghalaya, the present work was carried out to find out the efficacy of fungicides, biocontrol agents and plant extracts against the pathogen.

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Materials and Methods

Isolation of turmeric leaf spot pathogen

Turmeric leaves showing the typical leaf spot symptoms were collected from the turmeric field and used for the isolation of the pathogen. Infected leaf portion was cut into small pieces and isolation was carried out in potato dextrose agar (PDA) using standard method. Subsequently, sub-culturing of the pathogen was done once in a month and was stored at 4°C.

Preparation of plant extracts

Locally available plants like Neem (*Azadirachta indica* A. Juss), Onion (*Allium cepa* L.), Garlic (*Allium sativum*L.), Duranta (*Duranta repens* L.), Ashok (*Polyalthia longifolia*), Tulsi (*Ocimum sanctum*), Datura (*Datura stramonium*) and Lantana (*Lantana camara*) were used in the present study. 100 grams of fresh plant parts (leaf, bulb) were collected and washed with distilled water then crushed in a sterilized pestle

Table 2. *In-vitro* efficacy of BCAs against mycelial growth of *C. capsici*. *Mean of three replications. '*F*' test- at 5% level.

Sl. No.	BCAs	Radial growth of mycelium (cm)	Per cent inhibition (%)
1	*T. harzianum*	2.26	74.89
2	*T. viride*	3.00	66.67
3	*P. fluorescens*	3.24	64.00
4	*P. putida*	3.94	56.22
5	*B. subtilis*	3.68	59.11
6	Control	9.00	0.00
	SE (D)	0.13	
	CD (p=0.05)	0.24	

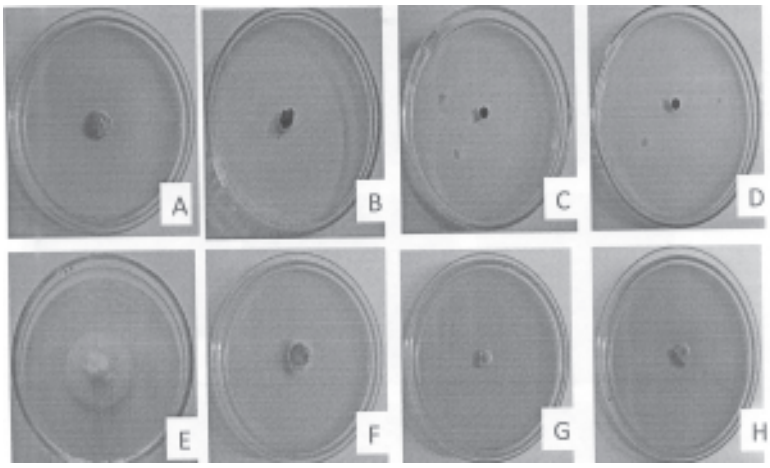

Fig. 1. Plant extracts showing maximum inhibition of *C. capsici*by A) *Lantana camara* (25%); B) *Allium sativum* (5%); C) *Allium sativum* (10%); D) *Allium sativum* (25%); E) *Allium cepa* (25%); F) *Duranta repens* (5%), G) *Duranta repens* (10%); H) *Duranta repens* (25%).

and mortar under aseptic condition. Then extracts were collected and filtered through muslin cloth and kept overnight for sedimentation of the crude materials. The supernatants were filtered through bacteria proof membrane syringe filter (0.22 µ pore size). Finally these extracts (supernatants) were stored at 4°C in refrigerator as stock solution.

Preparation of bacterial and fungal bioagents

Commercial formulations of bacteria (*Pseudomonas putida* and *P. fluorescens*) and fungal bioagents (*Trichoderma harzianum* and *T. viride*) from Agrilife Company were used for the study. Dilutions up to 10^{-4} prepared from the commercial formulations were plated in media and incubate. The plates were observed to pick up single colonies which were later transferred to their respective media (NA/PDA plates) and stored for further use. *Bacillus subtilis* culture was obtained from IARI, New Delhi, India.

Seven fungicides viz., Carbendazim, Carbendazim + Mancozeb, Copper oxy chloride, Captan, Mancozeb, Propiconazole and Hexaconazole were used in the current study.

In-vitro experiments were carried out by dual culture and poisoned food technique on respective media. In dual culture experiment, bacterial bioagents were streaked in opposite side with pathogen in the centre. As for the fungal bioagents, 5 mm disc from 7 day old pathogen and the bioagent were inoculated in opposite direction 1.5 cm away from the edge of the plates. Inhibition of mycelial growth of the pathogen was recorded on the basis of radial growth in dual culture and compared with the control plate. In poisoned food technique, PDA media mixed with plant extracts (10%, 15% and 25%) and fungicides (0.1%,

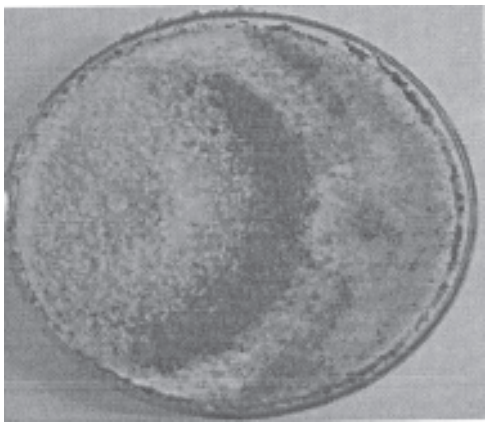

Fig. 2. Inhibition of *C. capsici* by *Trichoderma harzianum.*

Table 3. *In-vitro* efficacy of fungicides against mycelial growth of *C. capsici.* After 7 days incubation at 27± 1°C. *Mean of three replications. Figures in parentheses indicate inverse sine transformed values. Means having common letter are not significantly different. '*F*' test- at 5% level.

Sl. No.	Fungicide	Radial growth (cm) 0.1%	0.2%	0.3%	Mean	Per cent inhibition of radial growth over control (%) 0.3%	0.2%	0.3%
	Carbendazim+Mancoze		0.00	0.00	0.00			
1	b	0.00 (0.00)*	(0.00)	(0.00)	(0.00)*f	100	100	100
			0.59	0.55	0.58			
2	Captan	0.59 (4.41)	(4.41)	(4.25)	(4.35)d	93.44	93.44	93.89
			0.00	0.00	0.00			
3	Propiconazole	0.00 (0.00)	(0.00)	(0.00)	(0.00)f	100	100	100
			0.65	0.76	0.72			
4	Copper oxy chloride	0.74 (4.93)	(4.62)	(5.00)	(4.85)c	91.78	92.78	91.56
				3.21	3.18			
5	Mancozeb	3.09 (10.12)	3.24 (10.37)	(10.32)	(10.27)b	65.67	64	64.33
				0.00				
			0.00		0.00			
6	Hexaconazole	0.00 (0.00)			(0.00)	100	100	100
			(0.00)		(0.00)f			
			0.50	0.50	0.49			
7	Carbendazim	0.48 (3.97)	(4.05)	(4.05)	(4.03)e	94.67	94.44	94.67
				9.00	9.00			
8	Control	9.00 (17.46)	9.00 (17.46)	(17.46)	(17.46)a	0.00	0.00	0.00
	SE (d)	0.43	0.50	0.48				
	CD (p=0.05)	0.90	1.07	1.02				

0.2% and 0.3%) were served as growing media for the pathogen. A mycelial disc (5mm) from 7 day old actively growing culture of the pathogen was inoculated in center into each petri plate. Plates grown only with the pathogen served as control plates. All the plates were incubated at 27 ± 1°C and radial growth of mycelia were recorded till control plates reached full growth. Mean colony diameter for each treatment was recorded after the mycelia in control plate attained full growth. Three replications were maintained for all the experiment including control plates. The per cent inhibition of mycelial growth of the pathogen was calculated by using the formula [10]

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

where, I=Per cent inhibition, C = Radial growth in control, T = Radial growth in treatment.

Statistical analysis

The experiment was planned as under complete randomized design (CRD). The data generated from various experiments of this study were analyzed statistically by one way analysis of variance (ANOVA) and the means compared by Duncan's Multiple Range. Test (p<0.05) and further angular transformations were made for the data.

Results and Discussion

In-vitro efficacy of plant extracts on fungal growth

The results of *in vitro* efficacy of 8 plant extracts

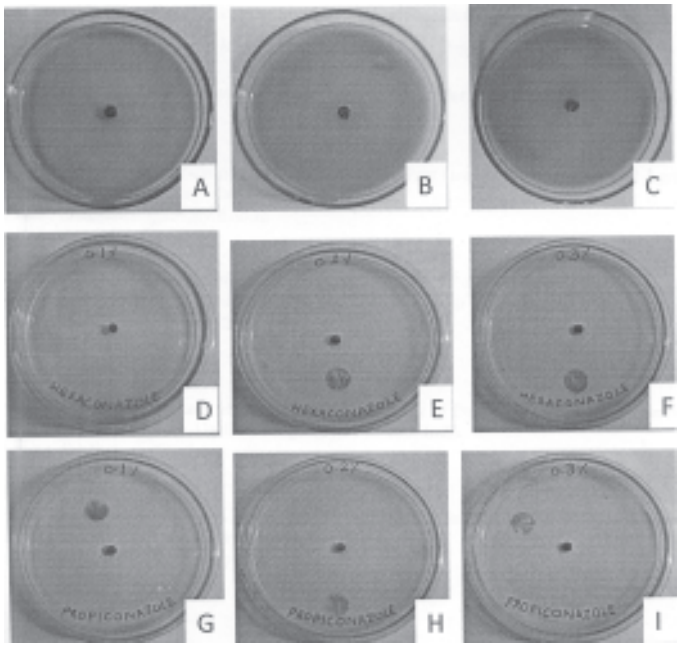

Fig. 3. Fungicides showing maximum inhibition of *C. capsici* by A) Carbendazim + mancozeb (0.1%); B) Carbendazim + mancozeb (0.2%), C) Carbendazim + mancozeb (0.3%); D) Hexaconazole (0.1%); E) Hexaconazole (0.2%); F) Hexaconazole (0.3%); G) Propiconazole (0.1%); H) Propiconazole (0.2%); I) Propiconazole (0.3%).

showed significant differences in per cent inhibition on mycelial growth of *C. capsici* (Table 1). The experiment revealed that *A. sativum* clove extract at 10% and 25% were found to be highly effective with complete inhibition of radial growth of *C. capsici*. At a concentration of 25%, leaf extracts of *D. repens* with 98.44% inhibition was found as second highest effective. At 25% concentration of *L. camara* and *A. cepa* inhibited mycelial growth with 94.00% and 79.33% respectively. *A. sativum* extract at 5% was also found to be effective in inhibiting the radial growth of the pathogen by 96.22% inhibition (Fig. 1). However, *P. longifolia, O. sanctum* and *D. stramonium* exhibited comparatively lesser inhibition of radial growth of *C. capsici* over control. Minimum inhibition percent was found in leaf extract of *A. indica*. Extract of *L. camara* at 5% was also found less effective in inhibiting (49.11%) the radial growth of *C. capsici*. Several workers have already found the effect of plant extracts such as garlic [11] and *L. camara* [12] in inhibiting the radial growth of *C. capsici*.

In-vitro efficacy of biocontrol agent

The results of the BCAs tested shows that there was significant effect on per cent inhibition of radial growth of *C. capsici* (Table 2 and Fig. 2). The highest per cent inhibition of mycelial growth was found in *T. harzianum* with 79.89% followed by *T. viride* (66.67%), *P. fluorescens* (64.00%). *P. putida* (56.22%) exhibited the least inhibition percentage. Many researchers have used biocontrol agents against *C. capsici* and their effectiveness have also been well documented [13]. Fungal bioagents were found better performance than bacterial bioagents in inhibiting the radial growth of *C. capsici* in turmeric [8]. The same result was also obtained in the present study. It was reported that *T. viride* and *P. fluorescens* were found very effective in inhibiting the radial growth of *C. capsici* [11]. *Trichoderma* excreted extracellular hydrolytic enzymes such as chitinases, β-1, 3-gluconases, proteases and lipases which acted on the cell walls of the pathogen and caused lysis [14].

In-vitro efficacy of fungicides

The results showed effectiveness in inhibition of radial growth of *C. capsici* by different fungicides (Table 3 and Fig. 3). The per cent inhibition of radial growth of *C. capsici* was found maximum (100% inhibition) in 3 fungicides viz., Carbendazim + Mancozeb, Propiconazole and Hexaconazole in all conc tested. It was found that these three fungicides were significantly superior then rest of the fungicides rested. This was followed by Carbendazim, Captan and Copper oxy chloride respectively. Use of Carbendazim in controlling *C. capsici* was reported already [8]. Propiconazole was reported effective in inhibiting the mycelial growth of *C. capsici* even in very low concentration which was followed by carbendazim [5]. Carbendazim (0.1%) and a mixture of two fungicides i.e. Carbendazim + Mancozeb (0.25%) were found effective in controlling the leaf spot [7—15].

From the study, it can be concluded that plant extracts as well as biocontrol agents which performed well against the pathogen *in vitro* can be further used for field trials along with the fungicides so as to give an integrated approach to pant disease management and this in turn might help in reducing the load of hazardous chemicals to the environment.

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