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Eco-friendly management of anthracnose of chilli caused by *Colletotrichum capsici*

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Abstract: The anthracnose of chilli caused by *Colletotrichum capsici* is a wide spread problem limiting the profitable cultivation and seed production of chilli throughout the major chilli growing regions of India. Four isolates (SCC_1, SCC_2, SCC_3 and SCC_4) of *C. capsici* were collected from different chilli growing areas of West Bengal. An experiment on efficacy of four botanical oils *viz.,* Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Polyalthia (*Polyalthia longifolia*) and Citronella(*Cymbopogon nardus*) at concentrations of 0.05%, 0.1% and 0.2% and two isolates each of three biocontrol agents (*Trichoderma harzianum, T. viride* and *Pseudomonas flourescens*) were carried out against these isolates of *C. capsici in vitro*. The result revealed significant (5% level of significance) antifungal activities of these botanicals and biocontrol agents. Garlic gave cent per cent inhibition of mycelial growth of all the four isolates of *C. capsici* at all the concentrations tested. However, neem showed cent per cent inhibition of mycelial growth of *C. capsici* at 0.1%. Citronella was least effective among the botanical oils. Among the biocontrol agents tested *T. harzianum* isolate Th-2 was found most effective giving 77.78%, 100%, 83.33 % and 88.89% inhibition on the mycelial growth of SCC_1, SCC_2, SCC_3 and SCC_4 respectively followed by *T. harzianum* isolate Th-1 inhibiting 74.00%, 78.9% 81.7% and 80.90% of the mycelial growth of SCC_1, SCC_2, SCC_3 and SCC_4 respectively. *P. fluorescens* was least effective. Thus, garlic, neem, polyalthia, *T. harzianum* and *T. viride* can be utilized for management of anthracnose of chilli.

Keywords: Anthracnose, Biocontrol Agents, Botanical Oils, *Colletotrichum capsici*, Isolates

INTRODUCTION

India accounts for 25% of the world's total production of chilli. The crop is a significant source of income making in India, the world's single largest producer and exporter to the USA, Canada, UK, Saudi Arabia, Singapore, Malaysia, Germany and many other countries across the world (Ashwini and Srividya, 2014). The sustainability of chilli-based agriculture is threatened by a number of factors. Anthracnose disease is a major problem in India and one of the most significant economic constraints to chilli production worldwide, especially in tropical and subtropical regions (Than *et al*., 2008). Economic losses caused by the disease are mainly attributed to lower fruit quality and marketability. The control of chilli anthracnose fruit rot has, for many years, relied on chemicals and resulted in many undesirable problems. There are numerous reports of negative effects of using chemicals on farmers' income and health, and toxic contamination to the environment, particularly in developing countries (Voorrips *et al*., 2004). Thus, there is a need to incorporate alternative control components that are effective in field. The use of botanical oils and biocontrol agents is the best alternative for management of chilli anthracnose. There is little work done on exploitation of botanicals and biocontrol agents. As chilli is an edible crop and large quantity of pesticides are being used, there is growing demand for chemical pesticide free organic chilli world over. Hence, the present investigation was undertaken to find the eco-friendly management of anthracnose of chilli caused by *Colletotrichum capsici*.

MATERIALS AND METHODS

The present investigation was conducted in the laboratory of the Department of Plant Pathology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, West Bengal during 2013-14.

Collection, isolation and identification of *C. capsici:* Chilli fruits showing typical fruit rot symptoms include sunken necrotic tissues, with concentric rings of acervuli were collected from different localities of West Bengal. Infected fruit bits were surface sterilized in 0.1% mercuric chloride for 30 seconds and repeatedly washed with sterilized distilled water to remove traces of mercury and then transferred to water agar media and incubated at 27 ± 1° C. Fungal mycelium developed from the infected tissue in water agar media was finally transferred to Potato Dextrose Agar (PDA) slants and incubated at 27 ± 1° C to obtain pure culture of *C. capsici*. Four isolates of the fungus were collected from different areas of West Bengal. Identification of the fungus was done with microscope.

***In vitro* effect of botanical oils:** Four botanical oils *viz.,* Garlic (*Allium sativum*), Neem (*Azadirachta indica*), Polyalthia (*Polyalthia longifolia*) and Citronella (*Cymbopogon nardus*) were tested for their efficacy against four isolates (SCC_1, SCC_2, SCC_3 and SCC_4) of *C. capsici* of at concentrations 0.05%, 0.1% and 0.2%. The poisoned food technique (Shervelle, 1979) was followed to evaluate the efficacy of essential oils in laboratory against the test fungus. Required amount of oil extracts were dissolved in 5% tween 80 (5g of

Table 1. *In vitro* efficacy of botanical oils on the mycelial growth of *C. capsici.*

Botanical oil	**% inhibition of mycelial growth**		
	Concentration		
	0.05%	**0.10%**	**0.20%**
Isolate SCC_1			
Garlic	100 (90.00)	100 (90.00)	100 (90.00)
Neem	84.45 (66.78)	100 (90.00)	100 (90.00)
Polyalthia	73.89 (59.27)	78.89 (62.65)	84.45 (66.78)
Citronella	8.27 (16.71)	8.36 (16.81)	13.84 (21.84)
Solvent	7.80 (16.22)	8.00 (16.43)	8.14 (16.58)
	Botanical oil (B)	Concentration (C)	B x C
CD at 5%	0.62	0.48	1.08
SEm±	0.21	0.17	0.37
Isolate SCC_2			
Garlic	100 (90.00)	100 (90.00)	100 (90.00)
Neem	82.78 (65.48)	100 (90.00)	100 (90.00)
Polyalthia	58.34 (49.80)	76.67 (61.12)	85.00 (67.21)
Citronella	8.29 (16.73)	8.44 (16.89)	13.76 21.77)
Solvent	7.80 (16.22)	8.00 (16.43)	8.14 (16.58)
	Botanical oil (B)	Concentration (C)	B x C
CD at 5%	0.58	0.45	1.00
SEm±	0.20	0.16	0.35
Isolate SCC_3			
Garlic	100 (90.00)	100 (90.00)	100 (90.00)
Neem	82.22 (65.06)	100 (90.00)	100 (90.00)
Polyalthia	75.00 (60.00)	86.11 (68.12)	100 (90)
Citronella	8.30 (16.74)	8.44 (16.89)	12.31 (20.54)
Solvent	7.80 (16.22)	8.00 (16.43)	8.14 (16.58)
	Botanical oil (B)	Concentration (C)	B x C
CD at 5%	0.56	0.43	0.96
SEm±	0.19	0.15	0.33
Isolate SCC_4			
Garlic	100 (90.00)	100 (90.00)	100 (90.00)
Neem	83.33 (65.90)	100 (90.00)	100 (90.00)
Polyalthia	62.22 (52.07)	80.00 (63.43)	85.00 (67.21)
Citronella	8.23 (16.67)	8.33 (16.67)	10.23 (18.65)
Solvent	7.80 (16.22)	8.00 (16.43)	8.14 (16.58)
	Botanical oil (B)	Concentration (C)	B x C
CD at 5%	0.60	0.46	1.04
SEm±	0.21	0.16	0.36

* Figures in parenthesis are arcsine transformed values.

Table 2. *In vitro* efficacy of biocontrol agents on the mycelial growth of *C. capsici.*

Biocontrol agents	Isolate	Per cent inhibition of mycelial growth
Isolate SCC_1		
T. harzianum	Th-1	74.00 (59.34)
T. harzianum	Th-2	77.78 (61.88)
T. viride	Tv-1	73.90 (59.28)
T. viride	Tv-2	72.22 (58.19)
P. fluorescence	Ps-1	31.44 (34.11)
P. fluorescence	Ps-2	50.00 (45.00)
Control		0 (0.00)
CD at 5%		1.88
S.Em±		0.62
Isolate SCC_2		
T. harzianum	Th-1	78.90 (62.65)
T. harzianum	Th-2	100 (90.00)
T. viride	Tv-1	72.90 (58.63)
T. viride	Tv-2	66.67 (54.74)
P. fluorescence	Ps-1	29.67 (33.00)
P. fluorescence	Ps-2	44.44 (41.81)
Control		0 (0.00)
CD at 5%		1.65
S.Em±		0.54
Isolate SCC_3		
T. harzianum	Th-1	81.70 (64.67)
T. harzianum	Th-2	83.33 (65.90)
T. viride	Tv-1	75.40 (60.27)
T. viride	Tv-2	77.78 61.88)
P. fluorescence	Ps-1	16.67 (24.10)
P. fluorescence	Ps-2	42.22 (40.52)
Control		0 (0.00)
CD at 5%		2.18
S.Em±		0.72
Isolate SCC_4		
T. harzianum	Th-1	80.90 (60.09)
T. harzianum	Th-2	88.89 70.53)
T. viride	Tv-1	76.20 (60.80)
T. viride	Tv-2	72.22 (58.19)
P. fluorescence	Ps-1	22.22 (28.12)
P. fluorescence	Ps-2	38.89 (38.58)
Control		0 (0.00)
CD at 5%		2.15
S.Em±		0.71

***** Figures in parenthesis are arcsine transformed values.

tween 80 + 95g of xylene) and thoroughly mixed with melted PDA to provide concentrations of 0.05, 0.1 and 0.2 % with three replications each of different botanical oils. The actively growing periphery of seven days old

culture of *C. capsici* was aseptically transferred to the centre of each petri plates containing the poisoned solid media. Suitable control was maintained by growing the cultures on PDA without botanical oils. Inoculated plates were incubated at 27 ± 1º C for twelve days and colony diameter was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) as given below:

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

Where, I = per cent inhibition; C = linear growth of the fungus in control (cm) and T = linear growth of the fungus in treatment (cm)

***In vitro* effect of biocontrol agents :** Two isolates each of three bioagents *viz*., *Trichoderma harzianum*, *T. viride* and *Pseudomonas fluorescens* were evaluated for their antagonistic properties against *C. capsici* through dual culture technique (Faheem *et al*., 2010). The bioagents and the test fungus were inoculated side by side on a single petridish containing solidified PDA medium. Three replications were maintained for each treatment with one control by maintaining only pathogen and bioagent separately. Inoculated plates were incubated at 27 ± 1º C for twelve days. The diameter of the colony of both bioagents and the pathogen was measured in two directions and average was recorded. Per cent inhibition of growth of the test fungus was calculated by using the formula of Vincent (1947) as described above.

Statistical analysis: Statistical analysis of the data was performed with SPSS Statistical software version 16.00 (2001).

RESULTS AND DISCUSSION

It is evident from table 1 that garlic gave cent per cent inhibition of mycelial growth of all the four isolates (SCC_1, SCC_2, SCC_3 and SCC_4) of *C. capsici* at all concentrations. However, neem showed cent per cent inhibition of mycelial growth of *C. capsici* at 0.1%. Citronella oil was least effective among the botanical oils. The tested botanical oils caused a significant reduction in growth of *C. capsici*. This reduction was gradually increased by increasing the concentration of extracts in the growth medium. The present finding is in accordance with the findings of Tiwari *et al*. (2008) reported that plant extracts datura leaf, onion and garlic bulb extracts completely inhibited the growth and sporulation of *C. capsici*. Many researchers have reported the antimicrobial activity of essential oils against different fungal species (Mishra and Dubey, 1994; Tzortzakis, 2007; Viudamartos *et al*., 2007). The biological activity of essential oils and their components are generally active against a broad spectrum of pest; interspecic toxicity of individual oils and compounds are highly idiosyncratic. Perhaps the most attractive aspect of using essential oils and their constituents in pest management is thei favourable mammalian toxicity and their non-persistence in the environment (Isman, 2000).

Results summarized in table 2 indicates that among the biocontrol agents tested *T. harzianum* isolate Th-2 was found most effective giving 77.78%, 100%, 83.33 % and 88.89% inhibition on the mycelial growth of SCC_1, SCC_2, SCC_3 and SCC_4 respectively followed by *T. harzianum* isolate Th-1 inhibiting 74.00%, 78.9% 81.7% and 80.90% of the mycelial growth of SCC_1, SCC_2, SCC_3 and SCC_4 respectively while *P. fluorescens* was least effective. Ushakiran *et al.* (2006) observed that in dual culture technique with six biocontrol agents (*T. harzianum, T. hamatum, T. viride, Verticillium lecanii, Beauberia bassiana* and *Metarhizium anisopliae*) *T.harzianum, T. hamatum* and *T. viride* could induce maximum percent inhibition on the linear growth of *C. capsici.* Bailey *et al*., (2004) had reported biological control by antagonism as a potential, non-chemical and eco-friendly approach for managing plant diseases. Statistical analysis of the results revealed signicance of the data at 5% level of significance.

Conclusion

It was concluded that Garlic, Neem, *Trichoderma harzianum* and *Trichoderma viride* can give promising results in controlling the growth of *C. capsici* under laboratory conditions. Recently there has been great interest in essential oils and biocontrol agents for controlling plant pathogens. The present study shows that botanical oils possess antifungal activity and can be exploited for effective management of plant diseases. Potential antagonistic organisms should be screened and formulated for eco-friendly management plant diseases.

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