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In vitro Evaluation of Plant Products and Bio-control Agents against *Colletotrichum capsici* Causing Fruit Rot of Chilli (*Capsicum annum* L.)

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An experiment was conducted *in vitro* to study the effect of different oils [palmarosa oil (*Cymbopogon martinii*), citronella oil (*Cymbopogon citratus*), karanj oil (*Pongamia glabra*), neem oil (*Azadiracta indica*) and mahua oil (*Madhuca longifolia*)], plant extracts (mahua, neem, ginelly, castor, groundnut, coconut and karanj) and antagonistic micro organisms (*Trichoderma* sp*, Pseudomonas* sp *and Bacillus* sp) on fruit rot of chilli. It was noticed that citronella oil (0.05% and 0.1%) and palmarosa oil (0.05% and 0.1%) showed absolute inhibition of mycelial growth while karanj plant extracts showed 38.6% inhibition over control. Among the bio-control agents *Trichoderma viride* showed 58.1% inhibition of mycelial growth over control.

Key words: *Colletotrichum capsici,* chilli, biological control, plant products, fungicides

Chilli (*Capsicum annum L.*) cultivation has existed for several hundred years as a sustainable form of agriculture in India and in many other countries. India is the world's single largest producer and exporter to USA, Canada, UK and many more countries across the world. Anthracnose/ die-back or ripe fruit rot caused by *Colletotrichum capsici* is the most destructive disease in chilli production causing huge yield losses all over the world[1]. The indiscriminatory uses of synthetic chemicals against fruit rot leads to cause undesirable effects. Plant derived extracts with toxic properties against the phytopathogen are now being explored for the management of plant disease. Due to their easy decomposition, lack of environmental pollution, non-residual toxicity and non-phytotoxic properties plant derived toxins are more useful than synthetic chemicals[2]. Bioagents are known to induce systemic resistance against several plant diseases[3,4,5]. The ethanolic root extracts of *Abrus precatorius* and *Rauvolfia tetraphylla* showed significant inhibitory effects on both the conidial germination and radial growth of *C. capsici*[6]. The antifungal activity of *Acorus calamus, Aegle marmelos, Allium sativum, Abrus precatorius, Ocimum sanctum, Prosopis juliflora, Coleus aromaticus and Cymbopogan martini* were proved against *Colletotrichum* sp[7]. Keeping in view the importance of crop and the disease, present study was conducted to evaluate the efficacy of different plant oil, oil cake, bio-control agents and fungicides against the pathogen thereby increasing crop yield.

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MATERIALS AND METHODS

Pathogen: Infected chilli fruit collected from farm fields of melur, Madurai district were used for the present investigation. *C. capsici* was isolated from rotted chilli fruits using potato dextrose agar (PDA) medium, which was previously plated and solidified in sterilized Petri plates, there after they were incubated at 28±2 ºC. After three days of incubation, the hyphal tip of the fungus radiating from the infected tissue was transferred on to PDA slants. The pathogen was maintained on PDA slants by sub-culturing periodically. To avoid loss of virulence, fresh isolations were made as and when required and ascertained the pathogenicity of the isolates on chilli plants.

Bioagents: The fungal and bacterial antagonists *viz., Trichoderma viride, Trichoderma harzianum, Trichoderma longibrachiatum, Trichoderma reesi*, *Trichoderma virens, Pseudomonas fluorescens* isolate 1*, P. fluorescens* isolate 2 and *Bacillus subtilis* obtained from Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore and were tested against the growth of *C. capsici in vitro* by dual culture technique[8].

Plant oils and oil cakes: The plant oil from palmarosa (*Cymbopogon martinii*) (0.05 and 0.1%), citronella (0.05 and 0.1%), *Pongamia glabra* (3%), *Azadiracta indica* (3%) and *Madhuca longifolia* (3%) and the oil cakes of mahua, neem, gingelly, castor, groundnut, coconut and karanj were obtained from market and then the oil cakes were soaked in sterile distilled water individually @ 2 g/ml and kept overnight for further study.

Efficacy of plant oils against *C. capsici in vitro*: Pre-standardizing experiment was carried out with different concentrations for all the plant oils and finalized the concentration of oil for testing the efficacy. The appropriate concentration of oil after emulsifying with teepol at one ml per liter mixed with sterilized potato dextrose agar medium (PDA) and thoroughly mixed just before plating so as to get the specified concentration of the plant oils. Twenty ml of this mixture was poured into a sterilized Petri dish (10 cm di-ameter) in three replications and allowed to solidify. A 6 mm culture disc of the pathogen was taken and placed onto the center of the medium. The plates were incubated at room temperature at 28±2 °C. The radial growth of the colony in each plate was measured when the control plate showed full growth.

Efficacy of oilcakes against *C. capsici in vitro*: The oil cakes of mahua, neem, gingelly, castor, groundnut, coconut and karanj were soaked in sterile distilled water individually @ 2 g/ml and kept for overnight. The material was then blended and filtered to get the standard cold water extracts. The 10 ml of the oil cake extract was added to 90 ml of PDA medium and poured into sterile Petri dish and were inoculated with 6 mm disc of *C. capsici* at the centre of the medium. Control plates without oilcake extracts served as control and incubated at room temperature at 28±2 °C. Three replications were maintained for each test. The result was expressed as per cent growth inhibition over control.

Efficacy of fungal antagonists against *C. capsici*: Six mm actively growing culture disc of *C. capsici* was placed onto sterilized Petri dish containing previously plated and solidified Czapek's Dox medium at 1.5 cm away from the edge of the plate. Another 8 mm fresh culture disc of antagonistic organism was placed opposite to *C. capsici* disc. Czapek's Dox medium inoculated with pathogen alone served as control. Three replications were maintained for each test. The plates were incubated at room temperature at 28±2 °C. The radial growth of pathogen in each plate was measured when the control plate showed full growth. The results were expressed as per cent inhibition of the mycelial growth of pathogen over control as mentioned below.

$$I = \frac{100\ (C - T)}{C}$$

where,

I = Per cent inhibition over control

C = Growth of pathogen in control

T = Growth of pathogen in treatment

Efficacy of bacterial antagonists against *C. capsici:* A 6 mm actively growing PDA culture disc of the pathogen was placed on a PDA Petri dish at one side, 1.5 cm away from the edge of the plate and incubated at room temperature 27±2°C. Forty-eight hours later, actively growing cultures of the respective test bacteria were separately streaked onto the medium at the opposite side of the plate, 1.5 cm away from the edge. The plates were incubated at room temperature. The plates inoculated with the pathogen alone served as control. Three replications were maintained for each antagonist. The radial growth of the pathogen was measured four days after inoculation and the results were expressed as per cent growth inhibition over control.

***In vitro* evaluation of the fungicides against C. *capsici*:** The appropriate quantity of the fungicide was weighed and added into 100 ml Erlenmeyer flask containing 20 ml sterilized and melted potato dextrose agar medium (PDA) and thoroughly mixed by gently swirling, poured into Petridish and allowed to solidify. A 6 mm actively growing mycelial disc of the pathogen was placed on the medium. Three replications were maintained for each test. The plates were incubated at room temperature at 28±2 °C. The potato dextrose agar medium without incorporating the fungicides and inoculated with the pathogen served as control. The radial growth of pathogen in each plate was measured when the control plate showed full growth. The data were subjected to statistical analysis using INDOSTAT package developed by Indostat service Hyderabad, India.

RESULTS AND DISCUSSION

In vitro* assay of plant oils against *C. capsici: Among the five different plant oils tested against *C. capsici*, palmarosa oil and citronella oil @ 0.05 and 0.1% resulted 100% inhibition of mycelial growth of the pathogen than

Table 1. *In vitro* assay of plant oils against *C. capsici*

S.No.	Plant oil	Mycelial growth (cm)	Per cent inhibition over control*
1.	Palmarosa oil (0.1%)	0.00	100.00
2.	Palmarosa oil (0.05%)	0.00	100.00
3.	Citronella oil (0.1%)	0.00	100.00
4.	Citronella oil (0.05%)	0.00	100.00
5.	Pongamia glabra oil (3%)	4.80	45.45
6.	Neem oil (3%)	5.40	38.60
7.	Madhuca longifolia oil (3%)	6.70	25.18
8.	Control	8.80	
	CD (P=0.05)	0.146	

*Mean of three replications

control followed by *Pongamia glabra* oil @ 3% produced 45.5% inhibition of mycelial growth (Table 1). Use of plant oils, *Nigella sativa*[9], *Ocimum, Palamarosa* and *neem*[10,11] for the management *C. capsici* could restrict the growth of fungus significantly and there by reduction in the disease intensity.

Effect of oil cakes extracts against *C. capsici*: The minimum diameter of mycelial growth of 5.3 cm and the maximum per cent inhibition of 38.6% was recorded in karanj oil cake extract followed by mahua oil cake extract (6.4 cm and 26.9%). The maximum diameter of mycelial growth of 8.5 cm and the minimum per cent inhibition of 1.2% was recorded in coconut and groundnut oil cakes respectively. Soil application of neem oil cake extract @10% showed the best performance to reduce the disease intensity of chilli fruit rot[10].

***In vitro* assay of bio-control agents against *C. capsici*:** Among the bio-control agents *Pseudomonas fluorescens* isolate 1 and *Trichoderma viride* showed significantly highest mycelial inhibition of *C. capsici* in the dual culture. The mycelial inhibitions in the above plates were 61.2 and 58.1% respectively, over control. The bacterial antagonist *Bacillus subtilis* was the next best antagonists in inhibiting 57.6% growth of *C. capsici*. Isolate *T. harzianum* and *P. fluorescens* isolate 2 were the other two antagonists, which inhibited the pathogen growth to 43.0 and 40.9% respectively. The mycelial growth inhibition in all the other antagonists was less than 30%. The antagonist *T. virens* was identified as a poor inhibitor against *C. capsici* with the growth inhibition of only 19.6% over control. *Trichoderma harzianum* and *Trichoderma virens* significantly decreased the severity of disease and increased the grain yield[12]. *Trichoderma harzianum* and *Trichoderma viride* were most effective in controlling the anthracnose fungus of *C. capsici* in chilli under *in vitro* condition[13] and *Pseudomonas fluorescens* as seed treatment effectively inhibited the mycelial growth of the pathogen and decreased the fruit rot incidence under green house condition[14]. The *in vitro* antagonism of *T. viride, T. harzianum* and other bacterial and fungal antagonists against *C. capsici*[10,15,16] known to reduce the fruit rot of chilli under greenhouse and field conditions, with an increase in yield[17].

***In vitro* assay of fungicides against *C.capsici*:** Among the treatments, mancozeb @ 0.2% recorded absolute inhibition of mycelial growth of the pathogen followed by copper oxy chloride @ 0.25% produced 75.66%, carbendazim @ 0.1% resulted 74.15% and this was on par

Table 2. *In vitro* assay of oil cake against *C. capsici*

S.No.	Oil cake	Mycelial growth (cm)	Per cent inhibition over control*
1.	Pongamia glabra oil cake	5.30	38.6
2.	Neem oil cake	8.20	4.65
3.	Gingelly oil cake	8.40	2.32
4.	Castor oil cake	7.30	15.11
5.	Groundnut oil cake	8.50	1.16
6.	Coconut oil cake	8.50	1.16
7.	Madhuca longifolia oil cake	6.36	26.88
8.	Control	8.60	
	CD (P=0.05)	0.146	

*Mean of three replications

Table 3. Effect of antagonists on the growth of *C. capsici*

S.No.	Antagonistic organisms	Mycelial growth (cm)	Per cent inhibition over control*
1.	*Trichoderma viride*	3.73	58.05
2.	*T. harzianum*	5.00	43.00
3.	*T. longibrachiatum*	6.60	25.80
4.	*T. reesi*	7.00	21.30
5.	*T. virens*	7.16	19.55
6.	*Pseudomonas fluorescens* isolate 1	3.30	61.17
7.	*P. fluorescens* isolate 2	5.00	40.94
8.	*Bacillus subtilis*	3.60	57.64
9.	Control	8.50	
	CD (P=0.05)	0.49	

*Mean of three replications

Table 4. *In vitro* assay of fungicides against *C. capsici*

S.No.	Fungicides	Mycelial growth (cm)	Per cent inhibition over control*
1.	Mancozeb (0.2%)	0.00	100.00
2.	Carbendazim (0.1%)	2.33	74.15
3.	Captan (0.2%)	2.50	71.90
4.	Ziram (0.1%)	4.20	52.80
5.	Chlorothalonil (0.2%)	5.80	34.80
6.	Chlorothalonil (0.3%)	4.30	51.66
7.	Copper oxy chloride (0.25%)	2.20	75.66
8.	Control	8.90	
	CD (P=0.05)	0.755	

*Mean of three replications

with each other (Table 4). Chlorothalonil @ 0.2% showed the maximum mycelial growth of 5.80 cm and least per cent inhibition over control (34.80%) followed by ziram @ 0.1% which recorded 52.80% inhibition over control. Mancozeb at 0.2% showed cent per cent inhibition of the pathogen in poison food technique. This is in agreement with the report of earlier studies[18,19,20] . Three spray of 0.25% mancozeb recorded best control of *C.capsici*[21].

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