



Effect of Plant Extracts and Bio-control Agents on Radial Growth of *Colletotrichum capsici* and Development of Chilli Fruit Rot Disease

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ABSTRACT

Bulb extract of *Allium sativum* and leaf extract of *Calotropis gigantean* were found to inhibit radial growth of *Colletotrichum capsici* where as the leaf extract of *Azadirachta indica* and bulb extract of *A. sativum* and *C. gigantean* were found to be the most effective in inhibiting the chilli fruit rot development. *Trichoderma harzianum* isolate 4 and *T. longibrachiatum* isolate 1 were found to be the most effective in inhibiting the radial growth of *C. capsici* where as *T. harzianum* isolate 3 and *T. virens* isolate 6 were found to be the most effective in inhibiting the chilli fruit rot development.

Key words : Bio-control agents, Chilli, *Colletotrichum capsici*, Plant extracts.

Chilli (*Capsicum annum* L.), one of the spice crops belonging to the family, Solanaceae, is a well known commercial crop used both as condiment or culinary supplement and vegetable. Chillies are excellent sources of Vitamin A, B, C and E with minerals like molybdenum, manganese, folate, potassium, thiamine and copper. Chillies produce alkaloids, capsaicinoids and carotenoids which make chilli hot and pungent. Many of the diseases have been reported to affect chilli crop. Among these diseases, dieback and fruit rot caused by *Colletotrichum capsici* (Syd) Butler and Bisby is prevalent in all chilli growing states of the country and causes losses ranging from 10 to 60 %. Although, only ripe fruits turning red are most frequently affected. In Assam, 12-32% fruits are found to be affected by this disease (Chowdhury, 1957).

MATERIAL AND METHODS

Preparation of plant extracts:

The plant tissue was ground with sterile water at the rate of 1g plant material/1 ml of water using a pestle and mortar, and the macerate was filtered through a muslin cloth to get the crude extract. Efficacy of extracts in inhibiting growth of *Colletotrichum capsici* was determined by poisoned food technique at 5, 10 and 15 per cent concentrations.

Effect of plant extracts on chilli fruit rot infection:

The crude extract of each plant species obtained was diluted with sterilized distilled water

to prepare 0.5, 1.0 and 2.0 per cent concentrations. Surface sterilized chilli fruits were inoculated with spore suspension of *Colletotrichum capsici* (10^6 conidia ml⁻¹), and then injected with the extract of each plant species at three concentrations immediately. The inoculated fruits were incubated at $25 \pm 1^\circ\text{C}$ for five days. Inoculated fruits with pathogen alone served as control. Four replications were maintained for each treatment and each replication consisted of ten fruits. At the end of incubation, the size of lesion on inoculated fruits was measured and per cent disease severity was calculated and expressed as per cent disease index (PDI).

$$\text{PDI} = \frac{\text{sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

The per cent disease index (PDI) was computed using the 0-9 scale (Wheeler, 1969) according to the formula given below.

Effect of bio-control agents on radial growth of *Colletotrichum capsici*:

Efficacy of *Trichoderma* isolates was determined by dual culture plate technique.

Effect of bio-control agents on chilli fruit rot infection:

Spore suspension (10^8 conidia ml⁻¹) of two isolates each of three species of *Trichoderma* viz., *T. longibrachiatum*, *T. harzianum* and *T. virens* was

Table 1. Effect of leaf/bulb extracts on radial growth of *C. capsici*.

Plant extract	Concentrations (%)			
	5.0 %Inhibition of radial growth *	10.0 %Inhibition of radial growth *	15.0 %Inhibition of radial growth *	Mean % inhibition of radial growth *
Leaf extract of <i>Ocimum sanctum</i>	18.13 (20.88)	20.65 (23.03)	40.63 (37.29)	26.47 (30.69)
Leaf extract of <i>Datura stramonium</i>	24.00 (25.77)	39.63 (36.69)	43.50 (39.11)	35.71 (36.69)
Leaf extract of <i>Azadirachta indica</i>	26.25 (25.77)	44.38 (39.64)	47.50 (41.55)	39.37 (38.88)
Bulb extract of <i>Allium sativum</i>	50.63 (43.45)	70.63 (55.98)	100.00 (80.90)	73.75 (59.21)
Leaf extract of <i>Calotropis gigantean</i>	31.25 (25.77)	46.00 (39.11)	57.30 (47.52)	44.85 (42.07)
Control	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Mean	36.25 (36.99)	49.28 (44.60)	61.38 (51.59)	48.97 (44.43)
CD at 5% level	Plant extract 0.19	Concentration 0.14	Interaction 0.32	

*Mean of four replications

Figures in parentheses are angular transformed values

prepared. Surface sterilized chilli fruits were injected separately with spore suspension of the *Colletotrichum capsici* and then immediately inoculated by spore injection method with the spore suspension of each *Trichoderma* isolate. Inoculated fruits with pathogen alone served as control. Inoculated fruits were incubated for five days at 25±1°C temperature. At the end of incubation, the size of lesion on inoculated fruits was measured and per cent disease severity was calculated and expressed as per cent disease index (PDI).

RESULTS AND DISCUSSION

Effect of plant extracts on radial growth of *Colletotrichum capsici*:

Bulb extract of *Allium sativum* was most effective in inhibiting the growth of *Colletotrichum capsici* (73.75%) than other extracts irrespective of the concentration. Irrespective of plant species, concentration of the extracts at 15 per cent brought about the highest inhibition of the growth (61.38%), followed by the concentration of extracts at 10% (49.28%) and 5% (36.25%), indicating that increase in the concentration of the extract increased the

inhibitory effect on the growth of the *Colletotrichum capsici* (Table 1).

The differences among the extracts in inhibiting the growth of the *Colletotrichum capsici* were affected by concentration at which they were used. Extract of the *Allium sativum* at 15% completely inhibited the growth of *Colletotrichum capsici* and it was significantly superior at all other concentrations. *Ocimum sanctum* at 5% was the least effective against the growth of *Colletotrichum capsici*.

Singh et al., (1997) reported that extracts of *Allium sativum* at 3% concentration completely inhibited the growth and spore germination of *Colletotrichum capsici*, whereas, extracts of onion bulbs, *Azadirachta indica*, *Pongamia pinnata* and *Tagetes erecta* each at 4% concentration gave complete inhibition of mycelial growth and spore germination of *Colletotrichum capsici*. Shovan et al., (2008) reported that garlic extract at 20% concentration to be the best in inhibiting the radial growth and mycelial dryweight of the *Colletotrichum dematium* followed by onion, ginger and neem extracts.

Table 2. Effect of plant extracts on chilli fruit rot infection

Plant extract	Concentration of extract						Mean	
	2% concentration		1% concentration		0.5% concentration		Lesion size (mm)	PDI
	Lesion size (mm)	PDI	Lesion size (mm)	PDI	Lesion size (mm)	PDI		
Leaf extract of <i>Azadirachta indica</i>	5.67	21.17 (27.42)	7.34	27.41 (31.56)	8.07	36.30 (37.05)	7.03	28.29 (32.13)
Bulb extract of <i>Allium sativum</i>	7.50	31.11 (33.90)	8.78	40.00 (39.23)	9.83	45.67 (42.53)	8.70	38.93 (38.60)
Leaf extract of <i>Calotropis gigantean</i>	8.20	36.67 (37.29)	9.80	37.50 (37.76)	11.00	52.27 (46.32)	9.67	42.15 (40.48)
Leaf extract of <i>Datura stramonium</i>	10.00	38.67 (38.47)	11.50	50.78 (45.46)	13.00	54.50 (47.58)	11.50	47.98 (43.84)
Leaf extract of <i>Ocimum sanctum</i>	13.00	43.42 (41.21)	15.10	64.45 (53.53)	17.00	68.92 (56.10)	15.03	58.93 (50.14)
Control	25.00	75.10 (60.07)	25.00	75.10 (60.07)	25.00	75.10 (60.07)	25.00	75.10 (60.07)
Mean	11.56	41.02 (39.83)	12.92	49.21 (44.55)	13.98	55.46 (48.13)		
	CD (P=0.05)		Lesion size		PDI			
	Plant extract		0.05		0.32			
	Concentration		0.07		0.42			
	Interaction		0.11		0.72			

Values in parentheses are angular transformed values

The different antimicrobial components, like allicin, E-and Z-ajoene and iso-E-10-devinylajoene present in the bulbs were effective against bacteria, yeasts, and phytopathogenic fungi (Prithiviraj *et al.*, 1998). Fungitoxicity exhibited by the extracts of *Azadirachta indica* and the extracts of *Ocimum gratissimum* was attributed to their chemical constituents including tannins, glycosides, alkaloids, saponins and flavonoids (Nduagu *et al.*, 2008).

Effect of plant extracts on chilli fruit rot infection:

Among the plant extracts, irrespective of concentration, extract of *Azadirachta indica* was found to be the most effective in reducing fruit rot infection in terms of lesion size, recording the lowest mean lesion size of 7.03 mm, and it was significantly superior to the other plant extracts. It was followed by the extracts of *Allium sativum* (8.70 mm) and *Calotropis gigantean* (9.67 mm). Highest lesion development was found in respect of the extract of *Ocimum sanctum* (15.03 mm), indicating that it was the least inhibitory on the development of chilli fruit rot (Table 2).

Leaf extract of *Azadirachta indica* was found to be the most effective against fruit rot development with the lowest per cent disease index (28.29), and was significantly superior to the other leaf/bulb extracts followed by *Allium sativum* (38.93) and *Calotropis gigantean* (42.15). The extract of *Ocimum sanctum* was found to be the least effective recording the highest per cent disease index (58.93).

Irrespective of the plant species, concentration of the extracts at 2.0% was found to be the most effective against fruit rot development, recording the lowest lesion development (11.56 mm), followed by the concentration at 1.0% (12.92 mm) and concentration at 0.5% (13.98 mm).

Singh *et al.*, (1999) reported that *Allium sativum* extract performed well under room humidity, while tagak-tagak extract showed good control of chilli anthracnose under high moisture conditions. Neem extract minimized fruit rot in ripe chilli. Alam *et al.*, (2002) reported that *Tagetes erecta* leaf and *Azadirachta indica* bark extracts in 5:1.25 (w/v) concentrations were the most effective in inhibiting *Colletotrichum gloeosporioides*.

Table 3. Effect of bio-control agents on radial growth of *Colletotrichum capsici*.

Bio-control agent	Inhibition of radial growth (%)
<i>Trichoderma longibrachiatum</i> isolate 1	81.25 (64.38)
<i>Trichoderma longibrachiatum</i> isolate 2	71.25 (57.61)
<i>Trichoderma harzianum</i> isolate 3	78.75 (62.58)
<i>Trichoderma harzianum</i> isolate 4	82.50 (65.27)
<i>Trichoderma virens</i> isolate 5	70.00 (56.79)
<i>Trichoderma virens</i> isolate 6	71.25 (57.61)
Control	0.00 (0.00)
SEm ±	0.05
CD at 5% level	0.16

Table 4. Effect of biocontrol agents on chilli fruit rot infection.

Bio-control agent	Lesion Size (mm)	PDI
<i>Trichoderma longibrachiatum</i> isolate 1	5.92	25.19 (23.81)
<i>Trichoderma longibrachiatum</i> isolate 2	6.76	31.85 (34.39)
<i>Trichoderma harzianum</i> isolate 3	3.65	16.30 (23.81)
<i>Trichoderma harzianum</i> isolate 4	5.20	24.44 (29.60)
<i>Trichoderma virens</i> isolate 5	6.75	31.85 (34.39)
<i>Trichoderma virens</i> isolate 6	4.10	21.48 (27.62)
Check (Pathogen inoculated)	13.60	55.60 (48.22)
CD at 5% level	0.16	0.71

Values in parentheses are angular transformed values.

Effect of bio-control agents on radial growth of *Colletotrichum capsici*:

All the bio-control agents significantly inhibited the radial growth of *Colletotrichum capsici*. *T. harzianum* isolate 4 was found to be the most effective in inhibiting the growth of *Colletotrichum capsici* (82.50 %) followed by *T. longibrachiatum* isolate 1 which caused 81.25 per cent inhibition of growth of the fungus. The lowest per cent inhibition was found in treatment of *T. vires* isolate 5 (70.00 %) (Table 3).

It was also found that the colonies of *T. harzianum* grew faster than those of *Colletotrichum capsici* in agar plates in the present study. It may be assumed that the rapid growth of *T. harzianum* may give it an advantage in the competition with pathogenic fungi for space and nutrients.

A number of antibiotics, such as trichodermin, trichodermol, trichotoxin, harzianum A and harzianolide produced by *Trichoderma* spp. were reported to be responsible for inhibition of *Colletotrichum* isolates (Dennis and Webster, 1971).

Effect of biocontrol agents on chilli fruit rot infection:

Trichoderma isolates significantly inhibited the development of chilli fruit rot relative to control. *T. harzianum* isolate 3 was found to be the most effective, recording the smallest mean lesion size of 3.65 mm and PDI (16.30) and was significantly superior to all other biocontrol agents. Largest lesion (6.76 mm) development and PDI (31.85) were found in respect of *T. longibrachiatum* isolate 2, indicating that it was least inhibitory on the development of chilli fruit rot (Table 4).

Ngullie *et al.*, (2010) reported that *T. viride* and *T. hamatum* showed maximum reduction of chilli fruit rot caused by *Colletotrichum gloeosporioides* in field evaluation. Oanh *et al.*, (2006) reported that *T. harzianum* induced host resistance against anthracnose disease of chilli. Anand and Bhaskaran (2009) reported *T. viride* reduced the fruit rot disease incidence in chilli and increased the per cent yield over control.

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