EXPLOITATION OF PLANT PRODUCTS AND BIOAGENTS FOR ECOFRIENDLY MANAGEMENT OF CHILLI FRUIT ROT DISEASE

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Received: April 1, 2008
Accepted: May 15, 2009

Abstract: Forty four plant species and eight antagonistic organisms were tested against *Colletotrichum capsici* and *Alternaria alternata*, the causal agents of fruit rot disease of chilli. *In vitro* studies indicated that leaf extracts (10%) of *Abrus precatorius* (Gundumuthu) and *Aegle marmelos* (vilvum), demonstrated the highest inhibition of spore germination and mycelial growth of these two pathogens. Among the fungal and bacterial antagonists tested, *Trichoderma viride* isolate 3 and *Pseudomonas fluorescens* were very effective in inhibiting mycelial growth of the pathogens *in vitro*. In the pot culture experiment, two sprays with leaf extract of *A. precatorius* (10%), first spray 20 days after fruit set and the second spray 2 days after inoculation with the pathogens, resulted in the lowest disease incidence (23.95%) and intensity (27.60 PDI – Per cent Disease Index) as compared to 71.50% incidence and PDI of 78.20 in the control. Among the antagonistic microorganisms two sprays of talc-based formulation of *P. fluorescens* (2%) were very effective in reducing the disease intensity (35.70 PDI). However, the leaf extracts and antagonistic organisms only ranked next to the fungicide (carbendazim 0.1%) (18.05 PDI). Field evaluation of the effective plant extracts, antagonistic microorganisms and fungicide revealed that spraying with *A. precatorius* leaf extract (10%) twice, the first spray at the time of fruit set and the second spray 20 days after fruit set caused the maximum disease reduction (25.53 PDI) followed by a single spray of the same leaf extract (10%) on 20th day after fruit set (28.50 PDI).

Key words: *Alternaria alternata*, antagonistic microorganisms, plant species, biocontrol, *Colletotrichum capsici*, fruit rot, Per cent Disease Index (PDI)

INTRODUCTION

Chilli (*Capsicum annuum* L.) is considered an important tropical and subtropical crop on the basis of its high consumption, nutritional and cash value to farmers and consumers both in developed and developing countries. Among them, India is the largest consumer and exporter of chilli in the international market and exports dry chilli, chilli powder and olioresins to over 90 countries (Singhal 1999). India produces 1.08 million tonnes of dry chilli on the area of 0.97 million ha (Anonymous 2004). The major constraint to chilli production in India is fruit rot diseases, caused by *Colletotrichum capsici* (Syd.) Butler and Bisby and *Alternaria alternata* (Fr.) Keissler. Losses varying from 10–60% have been reported in India (Patil *et al*. 1993; Pandey and Pandey 2003). Although chemicals are available for the management of the disease, a continuous, inappropriate, non-discriminative use of chemicals is known to cause undesirable effects such as residual toxicity, development of resistance, environmental pollution, health hazards to humans and animals. In an attempt to modify this condition some alternative methods of control have been adopted. The plant pathologists of late have focused their attention on developing environmentally safe, long-lasting and effective biocontrol methods by gradually replacing chemicals for the management of plant diseases. A number of plant species have been reported to possess natural substances that are toxic to many fungi causing plant diseases (Kazmi *et al*. 1993; Jeyalakshmi and Seetharaman 1998; Amadioha 2000; Sateesh *et al*. 2004). Bioagents of late have been known to induce systemic resistance against several plant diseases (Ramamoorthy *et al*. 2001; Radjacommare *et al*. 2002). With this background, in the present study plant species and antagonistic microorganisms were exploited for the effective management of fruit rot incidence in glasshouse and field conditions.

MATERIALS AND METHODS

Plant materials, pathogens and bioagents

Chilli cv K2, which is susceptible to fruit rot disease, was obtained from Agricultural Research Station, Kovilpatti, Tamil Nadu Agricultural University (TNAU), Tamil Nadu, India. *C. capsici* and *A. alternata* were isolated from rotted chilli fruits by using potato dextrose agar (PDA) medium. The fungi were purified by single spore isolation technique (Ricker and Ricker 1936) and identified based on the description given by Rangaswamy (1972). Four isolates of *T. viride* 2, 3, 4 and 5 were isolated from rhizosphere region of chilli plants using *Trichoderma* special medium (Elad and Chet 1983). These four isolates,

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along with *T. viride* isolate 1, *T. harzianum*, *T. hamatum*, *T. longibrachiatum* and *T. reesei*, *Chaetomium globosum* and *Gliocladium virens*, *P. fluorescens* (Pf1) and *Bacillus subtilis* obtained from the Department of Plant Pathology, TNAU, Coimbatore, were tested against the growth of *C. capsici* and *A. alternata in vitro* by dual culture technique. *P. fluorescens* was multiplied on King's B medium and *B. subtilis* on nutrient agar medium.

Preparation of plant extracts

Used fresh plant materials (leaf, bulb or rhizome) were washed separately with fresh water and finally with sterilized water. They were ground with a pestle in mortar with sterile water at the rate of one ml/g. The extract was obtained by squeezing the macerate with cotton wool. It was strained through muslin cloth, finally through Whatman No. 1 filter paper and passed through Zeitz filter to free it from bacterial contaminants. This formed a standard plant extract solution (100%). This extract was further diluted with sterilized distilled water to the required concentrations (Shekhawat and Prasada 1971).

Efficacy of leaf extracts on spore germination

One drop of 10% plant extract was placed in a cavity slide and allowed to air dry. A drop of the spore suspension (5 x 10^5 spores/ml) of *C. capsici* or *A. alternata* prepared in sterile distilled water was added to dried plant extract and thoroughly mixed. The cavity slide was incubated in a Petri dish glass bridge moist chamber. Three replications were prepared for each treatment. The spore germination was observed and recorded after 48 h and the per cent germination was calculated. The spore suspension in sterile distilled water served as the control (Anonymous 1943).

Efficacy of leaf extracts against the growth of *C. capsici* and *A. alternata*

The efficacy of leaf extracts in relation to the growth of pathogens was determined by the method of Schmitz (1930). An appropriate amount of leaf extract was added to sterilized warm Czapek-Dox medium and thoroughly mixed just before plating to form 10% concentration. Twenty ml of this mixture was immediately poured into a sterilized Petri plate (10 cm diameter) in three replications and allowed to solidify. A 10–mm culture disc of *C. capsici* or *A. alternata* from PDA culture was removed and placed onto the center of the medium. The plates were incubated at 28±2°C for 10 days. Czapek- Dox medium without plant extract served as the control. The radial growth of the colony was measured. The per cent inhibition of the growth was calculated.

Efficacy of fungal antagonists against *C. capsici* and *A. alternata in vitro*

A 10-mm disc of actively growing on PDA culture of the pathogen was placed on Czapek-Dox medium 1.5 cm away from the edge of each Petri dish. On the opposite side of Petri dish a 10-mm culture disc of the fungal antagonist was placed. Czapek-Dox medium inoculated with the pathogen alone served as the control. The plates were incubated at room temperature (28±2ºC). When the control plate showed full growth of the pathogen the radial growth of the mycelium was measured. The results were expressed as per cent inhibition of growth over control (Dennis and Webster 1971).

Preparation of talc based formulation of *Trichoderma* sp.

Trichoderma was multiplied in the molasses yeast medium (30 g molasses, 5 g yeast, 1000 ml distilled water, pH 7.0) for five days. After multiplication, the biomass of the flask was homogenized and mixed with talc at 1:2 ratio (v/w). To the mixture 5 g of carboxy methyl cellulose (CMC) was added as sticker and dried in shade for 72 h, powdered and stored in polypropylene bags (Jeyarajan *et al.* 1994). The concentration of *Trichoderma* during application was 3.0 x 10^7 colony forming units (cfu)/g.

Efficacy of bacterial antagonists against *C. capsici* and *A. alternata in vitro*

A 10-mm actively growing PDA culture disc of the pathogen was placed on PDA Petri dish at one side, 1.5 cm away from the edge of the plate and incubated at room temperature (28±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 in three replications for each treatment and incubated at room temperature. PDA medium inoculated with the pathogen alone served as control. After 8 days the radial growth of the pathogen was measured. The results were expressed as per cent growth inhibition over control.

Preparation of talc-based formulation of bacterial (Pf1) strain

A loopful of bacterial suspension was inoculated into King's B broth (KBB) and incubated on a rotary shaker at 150 rpm for 48 h at room temperature (28±2°C). After 48 h, the broth containing 9×10^8 cfu/ml was used for the preparation of talc based formulation. To the 400 ml of bacterial suspension 1 kg of the talc powder (sterilized at 105°C for 12h), calcium carbonate 15 g (to adjust the pH to neutral) and CMC 10 g (adhesive) were mixed under sterile conditions, following the method described by Vidhyasekaran and Muthamilan (1995). After shade drying overnight under sterile conditions, it was packed in a polypropylene bag and sealed. At the time of application, the population of bacteria in talc formulation was 2.5 to 3.0×10^8 cfu/g.

Efficacy of plant extracts and antagonists against fruit rot disease of chilli in pot culture

The effective plant extracts and antagonistic organisms against the pathogen *in vitro* [*A. precatorius* leaf extract, *A. marmelos* leaf extract, talc-based *T. viride* isolate 3 (20 g/l) and *P. fluorescens* (20 g/l)] were tested against fruit rot disease in a pot culture experiment. Carbendazim (0.1%) was used for comparison. Chilli plants of susceptible cv. K2 were raised in mud (clay) pots (30 cm) in the glasshouse. Each pot contained 3 kg of pot mixture. The plant extracts and antagonistic organisms were sprayed on 105-day-old plants 20 days after fruit set. A spore suspension of *C. capsici* and *A. alternata* containing 5x10^5 spores/ml of each pathogen was prepared and sprayed on ripe fruits on the plants *in situ* 5 days after application of the antagonist or plant extract.

Two days after inoculation, a second spray with the antagonist or plant extract was performed. Water congestion was provided both 24 h prior and after inoculation by covering the plants with polythene bags and spraying plants with sterile distilled water. Two days after inoculation, the plants were sprayed with the plant extracts and antagonistic organisms. The plants inoculated with the pathogens alone served as control. Three replications were performed. The plants were maintained in the glasshouse conditions. The intensity of fruit rot and number of diseased fruits were recorded 15 days after the last spray. To accomplish this total number of diseased fruits and were counted from each 10 randomly selected plants for each treatment and per cent diseased was calculated. The disease intensity was recorded on fruits selected at random in each treatment following the score chart 0 to 5 scale proposed by Ravinder Reddy (1982). The per cent disease index (PDI) was calculated using Mckinney (1923) infection index.

$$PDI = \frac{\text{Sum of numerical ratings}}{\text{Total number of fruit observed}} \times \frac{100}{\text{Maximum category value}}$$

Evaluation of plant extracts and antagonists for the management of fruit rot disease under field conditions

A plot experiment in randomized block design and three replications was conducted during February–June 2002 at Agricultural College and Research Institute, Madurai, Tamil Nadu to assess the efficacy of plant products, biocontrol agents and fungicide that were effective *in vitro* and in pot culture. The susceptible chilli cv. K2 was used. The plants were raised at spacing of 45x30 cm in plots of 30 m^2 size. The plant extracts and antagonistic organisms were sprayed at fruit set stage and 20 days after fruit set.

The incidence and intensity of fruit rot was recorded 15 days after the last spray. The total number and number of diseased fruits were counted on 10 randomly selected plants for each replication and the per cent fruit rot was calculated. The intensity of fruit rot disease was recorded on 25 fruits selected at random in each plot by following the score chart of 0–5 scale. The per cent disease index (PDI) was calculated by using Mckinney formula. The yield data were also recorded in terms of dry chilli fruits.

Another field trail was conducted during July–November 2002 at Agricultural College and Research Institute, Madurai, Tamil Nadu using the same variety in the same way to confirm the results obtained in the field experiment I.

Statistical analysis

The data generated from various experiments of this study were statistically analyzed following the procedure described by Gomez and Gomez (1984). The package used for analysis was IRRI STAT version 92-1 developed by the International Rice Research Institute Biometrics Unit, the Phillippines.

RESULTS

In vitro* assay of plant extracts against *C. capsici* and *A. alternata

Spore germination

Among the 44 plant extracts, leaf extract of *A. precatorius* (10%) and *A. marmelos* (10%) were effective as only 23.2 and 26.4% spores germinated, respectively, accounting for 74.5 and 71.0% inhibition of spore germination. The rhizome extract of *Curcuma longa* and *Prosopis juliflora* ranked next showing 30.3 and 32.3% spore germination, respectively (Table 1).

The spore germination of *A. alternata* in the presence of various plant extracts ranged from 22.3 to 85.9% as compared to 92.0% in the control. The leaf extracts of *A. marmelos* and *A. precatorius* were significantly superior to other plant extracts in inhibiting the spore germination of the pathogen recording 22.3 and 24.2% spore germination, respectively. Rhizome extract of turmeric ranked next with 25.2% spore germination. The other plant extracts showed varying degrees of inhibition of spore germination ranging from 14.7 to 68.0 % (Table 1).

Growth on solid medium

The results of the *in vitro* screening of the extracts of 44 plants species carried out against *C. capsici* revealed that 10% leaf extract of *A. precatorius* and *A. marmelos* exerted 64.3 and 62.3% inhibition on mycelial growth of the pathogen. This was followed by rhizome extract of *C. longa* (10%) and leaf extract of *P. juliflora* (10%). After 10 days, they had showed significantly less colony diameter of 4.2 cm as compared to 8.8 cm in the control. Other plant extracts also markedly reduced the growth of *C. capsici* (Table 1).

With regard to *A. alternata*, the results revealed that the leaf extract of *A. marmelos* (colony diameter 3.2 cm) and *A. precatorius* (colony diameter 3.2 cm) were significantly superior in inhibiting the mycelial growth of the pathogen when compared to other plant extracts. This was followed by the rhizome extract of turmeric (*C. longa*), rhizome extract of ginger (*Z. officinale*) and leaf extract of velikaruvel (*P. juliflora*) which were on par having the colony diameter of 3.8, 3.9 and 4.0 cm, respectively. Extracts of other plant species caused significantly marked reduction in the mycelial growth of the pathogen (Table 1).

Efficacy of fungal and bacterial antagonists against *C. capsici* and *A. alternata in vitro*

Among the 10 fungal antagonists, *T. viride* isolate 3 exerted significantly the highest inhibition (63.7%) of mycelial growth of the pathogen (3.10 cm as against 8.55 cm in control) followed by *T. viride* isolate 1 and 4 (3.82 cm). Among the bacterial antagonists tested *P. fluorescens* exerted the maximum inhibition (70.8%) on the mycelial growth resulting in 2.50 cm colony diameter of the pathogen as compared to 8.55 cm in the control (Fig. 1).

Similarly, *T. viride* isolate 3 significantly exerted the maximum inhibition (63.2%) on the mycelial growth (3.15 cm) of *A. alternata* as compared to 8.55 cm colony diameter in the control. This was followed by *T. viride*

Table 1. Effect of plant extracts on spore germination and mycelial growth of *C. capsici* and *A. alternata* (Poisoned food technique)

Serial. No.	Plant extracts	*C. capsici**				*A. alternata**			
		spore germination [%]	per cent inhibition over control	colony diameter [cm]	per cent inhibition over control	spore germination [%]	per cent inhibition over control	colony diameter [cm]	per cent inhibition over control
1.	*Adhatoda vasica* Nees.	83.1	9.1	8.1	7.6	70.5	23.4	7.8	10.5
2.	*Abrus precatorius* L.	23.2	74.5	3.1	64.3	24.2	73.7	3.2	63.5
3.	*Abutilon indicum* (L.) Sweet	51.1	43.9	5.5	37.1	53.5	41.9	5.7	34.4
4.	*Achyranthes aspera* L.	70.3	22.7	7.4	15.5	73.2	20.5	7.5	14.0
5.	*Acorus calamus* L.	66.8	26.6	7.2	17.4	71.2	22.6	7.1	18.3
6.	*Aegle marmelos* Corr.	26.4	71.0	3.3	62.3	22.3	75.8	3.2	63.9
7.	*Agave angustifolia* Haw.	44.6	51.0	4.7	46.8	40.7	55.7	4.1	52.5
8.	*Allium cepa* L.	38.5	57.7	5.4	36.5	36.0	60.9	5.0	42.7
9.	*Allium sativum* L.	34.1	62.5	4.7	46.3	35.2	61.7	5.3	39.8
10.	*Asparagus racemosus* Willd.	74.3	18.4	7.8	10.5	85.9	6.6	8.0	8.0
11.	*Azadirachta indica* Adv. Juss.	64.6	29.0	6.2	29.5	49.7	45.9	5.3	39.2
12.	*Basella rubra* L.	83.2	8.6	7.9	9.4	78.4	14.8	7.7	11.7
13.	*Bassia latifolia* Roxb.	50.3	44.7	7.0	19.7	48.2	47.6	5.3	38.5
14.	*Carica papaya* L.	45.9	49.6	5.0	42.8	43.4	52.8	5.3	42.3
15.	*Carum roxburghianum* Benth.	72.3	20.5	7.7	12.0	76.7	16.6	7.70	11.7
16.	*Casuarina equisetifolia* L.	40.8	55.2	5.0	42.8	43.2	53.1	5.70	34.6
17.	*Catheranthus roseus* Don.	68.3	25.0	7.1	18.6	60.7	34.0	6.42	26.4
18.	*Cissus quadrangularis* L.	60.1	33.9	6.5	25.7	55.6	39.6	6.00	31.2
19.	*Curcuma longa* L.	30.3	66.7	4.2	52.6	25.2	72.6	3.80	56.4
20.	*Cymbopogan martinii* (Roxb.) Watson	60.2	33.8	6.5	25.8	59.8	35.0	6.27	28.1
21.	*Datura stramonium* L.	42.1	53.7	5.8	33.7	40.2	56.3	5.60	35.7
22.	*Delonix regia* Raf.	39.8	56.3	4.4	49.3	44.7	51.4	5.3	39.8
23.	*Euphorbia hirta* L.	57.5	36.8	6.3	28.0	68.9	25.1	7.1	18.9
24.	*Ficus bengalensis* L.	62.7	31.1	6.5	27.7	40.8	55.7	4.6	47.8
25.	*Hibiscus rosa-sinensis* L.	60.3	33.8	5.8	33.7	54.9	40.4	4.6	47.3
26.	*Ipomoea cornea* L.	64.8	28.8	6.9	20.6	67.4	26.8	7.1	19.2
27.	Karuathalai	51.4	43.5	7.3	16.1	41.8	54.6	5.4	38.6
28.	*Lantana camara* L.	46.7	48.7	6.0	31.4	41.8	54.6	5.2	40.7
29.	*Lawsonia alba* L.	63.4	30.3	6.5	26.1	61.5	33.2	6.6	24.3
30.	*Mimosa pudica* L.	69.8	23.3	7.0	19.5	68.7	25.3	6.9	20.6
31.	*Nerium oleander* L.	76.4	16.1	7.8	10.3	78.5	14.7	8.1	7.1
32.	*Ocimum basilicum* L.	68.8	24.5	7.2	17.4	57.4	37.6	6.2	28.3
33.	*Ocimum sanctum* L.	54.2	40.4	7.8	10.6	52.1	43.4	7.8	10.7
34.	*Pithecolobium dulce* L.	70.8	22.3	7.3	15.5	58.4	36.5	6.0	31.2
35.	*Polyalthia longifolia* Thw.	49.9	45.2	5.4	38.9	48.1	47.7	5.3	39.8
36.	*Pongamia pinnata* (L.) Pierre.	48.9	46.3	6.7	22.9	45.5	50.5	5.8	33.5
37.	*Prosopis juliflora* DC	32.3	64.5	4.2	51.9	37.6	59.1	4.0	54.2
38.	*Solanum trilobatum* L.	69.3	23.9	7.6	13.2	85.7	6.9	8.0	8.3
39.	*Thespesia populnea* Corr.	47.3	48.1	5.4	38.9	45.8	50.3	5.2	40.9
40.	*Tinospera cordifolia* Miers.	59.7	34.4	6.6	24.9	61.3	33.4	6.8	22.6
41.	*Vitex negundo* L.	48.1	47.1	7.2	16.3	44.1	52.0	5.6	36.1
42.	*Vitex negundo* L. (Ienthelai)	57.3	37.0	6.2	28.7	50.3	45.3	5.3	39.0
43.	*Wedelia chinensis* Merrill.	71.5	21.5	7.6	13.4	75.4	18.1	7.9	10.0
44.	*Zingiber officinale* Rosc.	39.3	56.8	5.4	37.7	29.4	68.0	3.9	55.3
45.	Control	91.0		8.8		92.0		8.7	
	CD (p = 0.05)	1.30		0.40		1.25		0.40	
	SD	0.61		0.18		0.56		0.19	

* mean of three replications

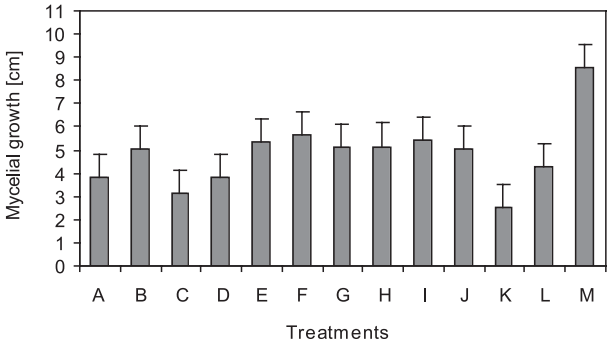

Fig. 1. Efficacy of antagonistic microorganisms on mycelial growth of *C. capsici*

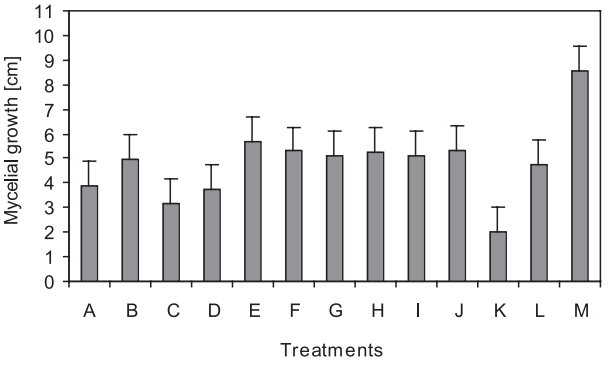

Fig. 2. Efficacy of antagonistic microorganisms on mycelial growth of *A. alternata*

Legends

A – *T. viride* isolate 1; B – *T. viride* isolate 2; C – *T. viride* isolate 3; D – *T. viride* isolate 4; E – *T. viride* isolate 5; F – *T. harzianum*; G – *T. reesei*; H – *T. longibrachiatum*; I – *C. globosum*; J – *G. virens*; K – *P. fluorescens*; L – *B. subtilis*; M – Control

Error bars indicates ± SED

isolate 4 and 1 recording colony diameter to 3.71 and 3.91 cm, respectively. Among the bacterial antagonists *P. fluorescens* exerted the maximum inhibition (76.6%) as the colony diameter of *A. alternata* was only 2.00 cm compared to 8.55 cm in the control (Fig. 2).

Efficacy of plant extracts and antagonists against fruit rot of chilli under pot culture conditions

The results of the pot culture experiment conducted to assess the efficacy of the selected plant extracts and the antagonistic organisms which were found effective against the pathogen under *in vitro* conditions and revealed that the leaf extract of *A. precatorius* (10%) was the most effective in reducing the incidence and intensity of fruit rot to 23.95 and 27.60%, respectively. This was followed by leaf extract of vilvum (10%). In case of antagonistic organisms, *P. fluorescens* was the most effective in reducing the fruit rot incidence and intensity to 33.32 and 35.70%, respectively. This was followed by *T. viride* isolate 3 which caused 52.12% reduction in disease intensity (40.76 PDI). A single spray of the leaf extract or antagonistic organisms was less effective than two sprays. Carbendazim (0.1%) spray was the most effective in reducing the fruit rot resulting in 14.65% fruit rot incidence with 18.05 PDI and in the control the maximum fruit rot incidence was 71.50% (78.20 PDI) (Table 2).

Evaluation of plant extracts and antagonists against fruit rot of chilli under field conditions

The results of fruit rot incidence, its intensity and dry chilli yield are shown in table 3.

Fruit rot incidence

In the field trials, all treatments were found significantly superior to the control in reducing the incidence of fruit rot in both the seasons. Among the plant extracts *A. precatoius*

Table 2. Evaluation of plant extracts and antagonistic organisms against fruit rot of chilli under pot culture conditions

Treatments		Sprayed 20 days after fruit set	Sprayed two days after inoculation	Fruit rot*			
				Disease incidence [%]	Per cent reduction over control	PDI	Per cent reduction over control
T_1	Leaf extract of *A. precatorius* (10%)	+	+	23.95 (29.30)	66.5	27.60 (31.69)	91.99
T_2	Leaf extract of *A. precatorius* (10%)	–	+	24.86 (29.91)	65.2	30.93 (33.79)	72.62
T_3	Leaf extract of *A. marmelos* (10%)	+	+	29.79 (33.08)	58.3	32.64 (34.84)	65.89
T_4	Leaf extract of *A. marmelos* (10%)	–	+	34.26 (35.83)	52.1	37.45 (37.73)	54.78
T_5	*T. viride* isolate 3 (2%)	+	+	37.52 (37.77)	47.5	40.76 (39.68)	44.22
T_6	*T. viride* isolate 3 (2%)	–	+	41.83 (40.30)	41.5	45.26 (42.28)	30.23
T_7	*P. fluorescens* (2%)	+	+	33.32 (35.26)	53.4	35.70 (36.69)	60.26
T_8	*P. fluorescens* (2%)	–	+	36.22 (37.00)	49.3	39.95 (39.20)	34.98
T_9	Carbendazim (0.1%)	+	+	14.65 (22.50)	79.5	18.05 (25.14)	139.78
T_{10}	Control			71.50 (57.74)		78.20 (62.17)	
	CD (p = 0.05)			1.20		1.10	
	SD			0.51		0.42	

+ sprayed, – no spray; PDI – Per cent Disease Index

*mean of three replications; Data in parentheses are arc sine transformed values

Table 3. Efficacy of plant extracts and antagonistic organisms against fruit rot of chilli under field conditions (Pooled mean of two seasons)

Treatments		Sprayed at fruit set stage	Sprayed at 20 days after fruit set	Disease incidence*	PDI*	Yield*	
						[kg/ha]	% increase over control
T_1	*A. precatorius* leaf extract (10%)	+	+	21.18 (27.40)	25.53 (30.35)	1238	92.0
T_2	*A. precatorius* leaf extract (10%)	–	+	24.85 (29.90)	28.50 (32.27)	1113	72.6
T_3	*A. marmelos* leaf extract (10%)	+	+	26.80 (31.18)	30.35 (33.43)	1070	65.9
T_4	*A. marmelos* leaf extract (10%)	–	+	31.73 (34.28)	35.10 (36.33)	998	54.8
T_5	*T. viride* isolate 3 (2%)	+	+	33.80 (35.55)	36.90 (37.41)	930	44.2
T_6	*T. viride* isolate 3 (2%)	–	+	35.89 (36.81)	40.25 (39.38)	840	30.2
T_7	*P. fluorescens* (2%)	+	+	29.70 (33.02)	33.46 (35.34)	1034	60.3
T_8	*P. fluorescens* (2%)	–	+	34.40 (35.91)	37.80 (37.94)	871	35.0
T_9	Carbendazim (0.1%)	+	+	12.73 (20.90)	15.30 (27.03)	1547	139.8
T_{10}	Control			49.86 (44.92)	52.59 (46.49)	645	
	CD (p = 0.05)			2.03	2.13	1.84	
	SD			0.92	0.96	0.89	

+ sprayed, – no spray; PDI – Per cent Disease Index
*mean of three replications; Data in parentheses are arc sine transformed values

leaf extract (10%) sprayed twice significantly reduced the disease whose incidence was 21.18% compared to 49.86% in the control. The same leaf extract sprayed only once was on par with leaf extract of vilvum (10%) sprayed twice. Among the antagonistic organisms tested, two sprays with *P. fluorescens* confined least fruit rot incidence (29.70%).

Fruit rot intensity

In plots sprayed twice with *A. precatorius* leaf extract, the fruit rot intensity was significantly very low (25.53 PDI) as compared to the control (52.59 PDI), followed by single spray with the same leaf extract (28.50 PDI). This was on par with two sprays of 10% leaf extract of vilvum (30.35 PDI). Two sprays with *P. fluorescens* (33.46 PDI) and a single spray with leaf extract of vilvum (35.10) were equally effective in controlling fruit rot intensity. Among all the treatments carbendazim sprayed plots recorded the lowest disease intensity (15.30 PDI).

Yield

Carbendazim sprayed plot recorded the highest fruit yield of 1547 kg/ha; among the plant products and antagonists, two sprays with *A. precatorius* leaf extract, resulted in significantly the highest yield (dry chilli) of 1238 kg/ha as compared to 645 kg/ha in the control. Single spray with the same leaf extract ranked next giving fruit yield of 1113 kg/ha, this was followed by two sprays of vilvum leaf extract (1070 kg/ha) and *P. fluorescens* (1034 kg/ha).

DISCUSSION

Using of the plant products for the management of plant diseases has a special significance in the context of environmental pollution, accumulation of toxic substances in the produce and development of resistance by plant pathogens. Screening of plant extracts for their antifungal properties against *C. capsici* revealed that certain plant species showed a very high antifungal activity. Of the tested 44 plant species, 10% leaf extract of *A. precatorius* and *A. marmelos* effectively inhibited the spore germination and mycelial growth of *C. capsici*. However, leaf extract of vilvum and *A. precatorius* were most effective in inhibiting the mycelial growth of *A. alternata*. Natural toxic substances and the antifungal activity of plant products have been exploited in relation to several plant diseases (Wilson *et al*. 1987; Cutler *et al*. 1996; Wilson *et al*. 1997; Al-Mughrabi *et al*. 2001). Garlic bulb extract is known to have antifungal activity against *C. capsici* (Murthy and Amonker 1974) and Singh *et al*. (1990) reported that a compound ajoene, derived from garlic inhibited *Colletotrichum* spp. Oil extracted from leaves of vilvum has been reported to be effective against *Rhizoctonia solani* (Renu 1981). In this laboratory, Senthilnathan (1988) has found that leaf extract of vilvum inhibited mycelial growth and spore germination of *A. tenuissima* infecting onion. Muthulakshmi (1990) and Sujatha Bai (1992) also reported that leaf extract of vilvum effectively inhibited mycelial growth of the pathogen.

Certain weeds found commonly in various crop fields have been reported to exhibit fungicidal properties

(Qasem and Abu-Blan 1996). The antifungal activity of *Lawsonia inermis* leaf extract on *Drechslera oryzae* caused a gradual decrease in growth, total DNA, RNA, protein synthesis and oxygen uptake of the pathogen. The inhibition might be due to inhibition of respiration. The antifungal activity in the leaf was identified as 2-hydroxy-1,4 naphthoquinone (Natarajan and Lalithakumari 1987). Plant extracts as potential antifungal agents have been exploited in relation to several plant diseases (Chaudhuri 1982; Asthana *et al*. 1986; Harish *et al.* 2004; Sateesh *et al.* 2004). In our study, the inhibition of *C. capsici* and *A. alternata* by *A. precatorius* and *A. marmelos* might be due to the fungitoxic principle present in plants. The active principle responsible for the fungitoxic activity in plants needs to be isolated and formulated for large scale application.

The results of the present investigation revealed that among 10 fungal antagonists tested, *T. viride* isolate 3 and among two bacterial antagonists, *P. fluorescens* significantly reduced the mycelial growth of the pathogens. The review of literature indicated that the present investigation could provide some scope for further studies on *Trichoderma* spp. and *P. fluorescens* for the management of the disease. *Trichoderma* spp. grew over the pathogen and caused hyphal coiling, hyphal abnormalities, reduction in sclerotial production, lysis of hyphae and sclerotia (Malathi 1996). Jeyalaksmi and Seetharaman (1998) reported that *T. viride* reduced the mycelial growth of *C. capsici*. Among the bacterial antagonists *P. fluorescens* and *B. subtilis* exhibited the maximum inhibition of mycelial growth.

Based on the *in vitro* effectiveness of plant products and antagonistic organisms, the leaf extracts of *A. precatorius* (10%), *A. marmelos* (10%), *P. fluorescens* (2%) and *T. viride* isolate 3 (2%) were tested for the management of fruit rot in pot culture. The results revealed that two sprays (one at 20 days after fruit set and second at two days after pathogen inoculation) with *A. precatorius* leaf extract, a single spray (2 days after inoculation) with the same leaf extract, two sprays with *A. marmelos* leaf extract and two sprays with *P. fluorescens* (2%) resulted in the minimum fruit rot incidence. The efficacy of *A. precatorius* leaf extract and *T. viride* isolate 3 for the management of fruit rot of chilli in pot culture is reported for the first time. The leaf extracts of vilvum and prosopis sprayed 100 and 115 days after sowing effectively reduced chilli fruit rot caused by *A. tenuis* as reported by Muthulakshmi (1990) and Sujatha Bai (1992). Babu (1994) also found that vilvum leaf extract effectively reduced the leaf blight disease incidence of tomato. In the present study, however, carbendazim (0.1%) spray was significantly superior to the plant products and antagonists. This is in agreement with the report of earlier studies (Jagannathan and Narasimhan 1987; Sujatha Bai 1992; Jeyalakshmi 1996). The results obtained in field trial followed the same trend as those obtained in the pot culture.

Direct antimicrobial activity of plants and biocontrol organisms may not be the sole mode of action. Spraying with spinach and rhubarb leaf extracts induced systemic resistance (ISR) in cucumber to anthracnose and resistance appeared to be a host-mediated response (Daubrava *et al*. 1988). Narwal *et al*. (2000) observed the induction of systemic resistance by an antiviral protein from *Bougainvillea xbuttiana* in *Nicotiana glutinosa* and *Cyamopsis tatragonoloba* against tobacco mosaic virus (TMV) and sunhemp rosette virus (SRV). The efficacy of plant products and bioagents against *C. capsici* and *A. alternata* may be attributed to the induction of systemic resistance. The reduction in disease intensity might be due to induction of resistance in the plant system by the plant extract apart from the antifungal activity. Plant products have been considered as one of the major groups of compounds that induce ISR. However, induced resistance is not the only mode of action but a direct action of a plant extract on the pathogen is also involved by producing toxic substances which is necessary for protecting the crop against fruit rot disease.

Even though spraying with leaf extracts and an antagonistic microorganism were effective against *C. capsici* and *A. alternata in vitro* and against the disease in pot culture and field experiments, all these only ranked next to spraying with carbendazim. The efficacy of carbendazim against the fruit rot pathogen was reported by several workers (Raju and Rao 1989; Biswas 1992; Datar 1996). Although carbendazim was observed to be the most effective treatment, the easy availability of a plant species coupled with its less phytotoxicity makes it a potential alternative. Fungitoxicity of plant products was considered to be the safe means of plant disease control. Furthermore, the combined studies with plants and biocontrol agents need to be tested for a better protection of the chilli crop. Thus plant products and bioagents can be well exploited in the future and active principles from the plant extracts can also be isolated and formulated for the effective management of various plant diseases.

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POLISH SUMMARY

WYKORZYSTANIE PRODUKTÓW ROŚLINNYCH I CZYNNIKÓW BIOLOGICZNEGO ZWALCZANIA W PRZYJAZNYM DLA ŚRODOWISKA ZWALCZANIU ANTRAKNOZY CHILLI

Badano wpływ czterdziestu czterech gatunków roślin oraz ośmiu antagonistycznych organizmów na *Colletotrichum capsici* i *Alternaria alternata*, które powodują zgniliznę owoców chilli. Badania *in vitro* wskazały, że ekstrakty z liści (10%) *Abrus precatorius* i *Aegle marmelos* najsilniej inhibitowały kiełkowanie zarodników i wzrost grzybni tych patogenów. Spośród testowanych, grzybowych i bakteryjnych antagonistów *Trichoderma viride* izolat 3 i *Pseudomonas fluorescens* inhibitowały bardzo efektywnie wzrost grzybni patogenów *in vitro*. W doświadczeniu wazonowym, dwa zabiegi opryskiwania ekstraktem z liści *A. precatorius* (10%) – pierwszy zabieg 20 dni po pierwszym zawiązaniu owoców i drugi zabieg dwa dni po inokulacji patogenami, spowodowały najniższe wystąpienie choroby (23,95%), o najniższym nasileniu (indeks porażenia 27,60), odpowiednio w porównaniu do kontroli (71,50% i 78,20). Spośród antagonistycznych mikroorganizmów dwa opryski formulacją *P. fluorescens* opartą na talku, były bardzo efektywne w ograniczeniu nasilenia choroby (indeks porażenia 35,70). Jednak ekstrakty z liści i antagonistyczne mikroorganizmy były mniej efektywne niż fungicyd (karbendazym 0,1%) (indeks porażenia 18,05). Polowa ocena efektywnych wyciągów roślinnych, antagonistycznych mikroorganizmów i fungicydu wykazała, że dwa opryski wyciągiem z liści *A. precatorius* (10%) – pierwszy w czasie zawiązywania owoców i drugi 20 dni po zawiązaniu owoców, spowodowały maksymalne ograniczenie choroby (indeks porażenia 25,53), a na następnym miejscu był jeden oprysk tym samym ekstraktem z liści (10%), wykonanym dwudziestego dnia po zawiązaniu owoców (indeks porażenia 28,50).