BIOLOGICAL MANAGEMENT OF FRUIT ROT IN THE WORLD'S HOTTEST CHILLI (*CAPSICUM CHINENSE* JACQ.)

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Abstract: Nine plant species and 7 antagonists were tested against *Colletotrichum gloeosporioides* which is the causal agent of fruit rot disease in the Naga king chilli. *In vitro* studies indicated that *Trichoderma viride* and *Pseudomonas fluorescens* were very effective in inhibiting mycelial growth of the pathogen. Among the plant extracts, *Allium sativum* (10%) and *Azadirachta indica* (10%) demonstrated the highest inhibition of mycelial growth of *C. gloeosporioides*. Field evaluation of effective plant extracts and antagonists and fungicide, revealed that spraying with *T. viride* (2%) showed a maximum disease reduction of 61.41% followed by *P. fluorescens,* (58.10%). However, the fungicide (Bavistin 0.1%) with 80.84% disease reduction ranked first.

Key words: Naga king chilli, fruit rot, *Colletotrichum gloeosporioides*, antagonists, plants extracts

INTRODUCTION

Naga king chilli (*Capsicum chinense* Jacq.) also called Bhut jolokia in Assam, is the world's hottest chilli. It is in the Guinness book of world records: measuring at 855 000 Scoville units. Due to its extra-ordinary pungency level, oleoresin powder extracted from Naga king chilli is predicted to dominate the world market in the coming years as the mainstay for riot control. The general concept is that lachrymatory compounds from a natural product will be most acceptable from the human rights point of view and more environmentally-friendly than synthetically produced compounds (Ritesh *et al*. 2000). Anthracnose causes extensive pre- and post-harvest damage to chilli fruits causing anthracnose lesions. Even small anthracnose lesions on chilli fruits reduce their market value (Manandhar *et al*. 1995). *Colletotrichum gloeosporioides* (Penz.) Penz & Sacc. Penz [teleomorph, *Glomerella cingulata* (Stoneman) Spauld & H. Schrenk] has been found to be associated with this disease for the first time in this state. The symptoms of the disease appear mostly on unripe fruits. Bleaching symptoms and lesions in concentric rings can be seen on the fruit. The infected tissue forms a depression and the fruit shrinks. The spots on the tissue measure 20–40 mm in diameter. The world hottest chilli cultivated in Nagaland suffers great loss due to fruit rot disease caused by *C. gloeosporioides*. Although chemicals are available for the management of fruit rot, a continuous, inappropriate, non-discriminatory use of chemicals is known to cause undesirable effects. Such effects are residual toxicity, resistance, environmental pollution, and health hazards to humans and animals. In an attempt to modify this condition some alternative methods of control have been adopted. Plant extracts with toxic properties against the phytopathogen are now being explored for use. They are being considered for controlling the phytopathogen because of their easy decomposition, lack of environmental pollution, non-residual toxicity and non-phytotoxic properties (Dixit *et al*. 1979). Bioagents of late have been known to induce systemic resistance against several plant diseases (Radajacommare *et al*. 2000; Ramamoorthy *et al*. 2001). The above conditions prompted the present study of plant species and antagonistic microorganisms to be tested for effective management of fruit rot incidence under field condition.

MATERIALS AND METHODS

Plant materials, pathogen and bioagents

Naga king chilli collected from farm fields were used for the present investigation. *C. gloeosporioides* were isolated from rotted chilli fruits using a potato dextrose agar (PDA) medium. The culture of *C. gloeosporioides* was identified and deposited at the Indian Type Culture Collection, (ITCC-6442.06) IARI, New Delhi. Antagonists *Trichoderma viride*, *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. hamatum*, *Pseudomonas fluorescens* and *Bacillus subtilis* obtained from the Biological Control laboratory, in Medziphema, Nagaland were tested against the growth of *C. gloeosporioides in vitro* by the dual culture technique.

Preparation of plant extracts

Fresh plant materials (leaf, bulb or shoots) were washed separately with fresh water and finally with sterilized water. They were ground with a pestle in mortar

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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, and then through Whatman No.1 filter paper and passed through a Zeitz filter to free it from bacterial contaminants. This formed the standard plant extract solution (100%). This extract was further diluted with sterilized distilled water to the required concentrations (Shekhawat and Prasada 1971).

Efficacy of plant extracts and fungicide against *C. gloeosporioides in vitro* growth

The efficacy of plant extracts and fungicide in relation to the growth of pathogens was determined by the Schmitz method (1930). An appropriate amount of plant extract was added to sterilized warm Czapek-Dox medium and thoroughly mixed just before plating to form a 10% concentration. Twenty ml of this mixture was immediately poured into a sterilized Petri dish (10 cm diameter) in three replications and allowed to solidify. A 10 mm culture disc of *C. gloeosporioides* from PDA culture was taken and placed onto the center of the medium. The chemical fungicide Bavistin at 0.1% was added to a sterilized Czapek-Dox medium, mixed and poured on plates. The plates were incubated at 27±2°C for 10 days. Czapek-Dox medium without plant extract served as the control. The radial growth of the colony was measured. The percent inhibition of the growth was calculated.

Efficacy of fungal antagonists against *C. gloeosporioides* in *vitro*

A 10 mm disc of actively growing PDA culture of 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 (27±2°C). The radial growth of the pathogen in the control was measured. The results were expressed as, percent inhibition of growth above that of the control (Dennis and Webster 1971).

Preparation of talc-based formulation of *T. viride*

Trichoderma was multiplied in a molasses yeast medium (30 g molasses, 5 g yeast, 1 000 ml distilled water, pH 6.5) for 7 days. The fungal mats were extracted and mixed with talc at 1 : 3 ratio (v/w). Five g of ethyl cellulose (CMC) was added to the mixture to hold it together. The mixture was dried in the shade for 72 h, powdered and stored in polypropylene bags (Jeyarajan *et al*. 1994). The cell count during application was 5.0×10^6 colony forming units (cfu)/g.

Efficacy of bacterial antagonists against *C. gloeosporioides in vitro*

A 10 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 site of the plate. 1.5 cm away from the edge. This was done in three replications for each treatment and incubated. The medium with the pathogen alone, served as a control. After 6 days the radial growth of the pathogen was measured. The results were expressed as percent growth inhibition on the control.

Preparation of talc-based formulation of bacterial bioagents

A loopful of bacterial suspension was inoculated into Nutrient medium and incubated on a rotary shaker at 150 rpm for 48 h at room temperature. A bacterial suspension of 400 ml was mixed in 1 kg of talc powder (sterilized at 110°C for 24 h), then calcium carbonate 15 g (to adjust the pH to neutral) and CMC 10 g (adhesive) were mixed under sterile conditions, according to the method described by Vidhyasekaran and Muthamilan (1995). After shade drying overnight under sterile conditions, it was packed in a polypropylene bag and sealed. The cell count at application was 4.0×10^8 cfu/g.

Evaluation of plant extracts, antagonists and chemical fungicide for management of fruit rot disease under field conditions

A plot experiment in randomized block design and three replications was conducted from April–August in 2006–2007 at the School of Agricultural Sciences and Rural Development, in Medziphema, Nagaland. The experiment was conducted to evaluate the efficacy of plant products, biocontrol agents and fungicide that were effective under *in vitro* conditions. The altitude of the experimental area is 310 m above sea level, at 25° 45′ 43″ N latitude and 93° 53′ 04″ E longitude. Rainfall varies from 2 000 mm to 2 700 mm and temperature ranges from 21°C to 32°C during the summer. The soil of the experimental plot was sandy loam with nitrogen – 40.76 kg/ha, phosphorus – 23.59 kg/ha, potassium – 201.6 kg/ha and pH 4.6. Naga king chilli were raised at spacings of 50x30 cm in plots of 2 m^2 size. The plant extracts, antagonists and fungicide were sprayed at the fruit set stage and 20 days after fruit set.

The occurrence of fruit rot was recorded 15 days after the last spray. The total number and number of diseased fruit were counted on 5 randomly selected plants for each replication and the percent of fruit rot was calculated.

Statistical analysis

The data generated from our experiment were statistically analyzed following the procedure described Gomez and Gomez (1984). The package used for analysis was the INDOSTAT version 7.01.0028. Developed by Indostat service Hyderabad, India.

$$\text{Fruit rot incidence } [\%] = \frac{\text{No. of fruit infected in the sample population}}{\text{total No. of fruit in the sample population}} \times 100$$

RESULTS

In vitro* assay of plant extracts and fungicide against *C. gloeosporioides

The results of the *in vitro* screening of nine plant extracts and one chemical fungicide were carried out against *C. gloeosporioides*. Bavistin (0.1%) showed maximum growth inhibition at 83.40%. Among the plant extracts, a 10% bulb extract of *Allium sativum* (colony diameter 3.0 cm) exerted a 54.75% inhibition on mycelial growth of the pathogen. A 10% leaf extract of *Azadirachta indica* and a 10% shoot extract of *Dendrocalamus hemiltonii* showed an inhibition of 42.23 and 40.72% respectively. Other plant extracts showed varying degree of inhibition (Table 1).

Efficacy of fungal and bacterial antagonists against *C. gloeosporioides*

Among the 7 antagonists tested, *Pseudomonas fluorescens* exerted the maximum inhibition (67.42%) of mycelial growth of the pathogen (2.16 cm compared to 6.63 cm in the control) followed by *T. viride* and *Bacillus subtilis* with an inhibition of 63.34 and 56.86% on mycelial growth resulting in a 2.48 and 2.86 cm colony diameter of the pathogen as compared to 6.63 cm in the control. Other antagonists showed significant inhibition on mycelial growth (Table 2).

Table 1. Effect of plant extracts and Bavistin on radial growth of *C. gloeosporioides*

No.	Treatments	Colony growth after 10 days diameter [cm]	Percent inhibition of the growth in the control
1.	*A. indica* (10%)	3.83	42.23
2.	*A. sativum* (10%)	3.00	54.75
3.	*D. hemiltonii* (10%)	3.93	40.72
4.	*Ocimum sanctum* (10%)	4.00	39.66
5.	*Lantana camara* (10%)	4.33	34.69
6.	*Cymobopogan flexuosus* (10%)	4.23	36.19
7.	*Eucalyptus globules* (10%)	4.06	38.76
8.	*Datura stramonium* (10%)	5.03	24.13
9.	*Ipomoea cornea* (10%)	4.10	38.15
10.	Bavistin (0.1%)	1.10	83.40
11.	Control	6.63	0.0
	CD (p = 0.05%)	1.31	
	SD	0.63	

Values are the mean of three replications. Significant at (p = 0.05)

Table 2. Effect of antagonists on *C. gloeosporioides*

No.	Treatments	Average growth diameter [cm]	Percent inhibition of the growth in the control
1.	*T. viride*	2.43	63.34
2.	*T. harzianum*	3.33	49.77
3.	*T. koningii*	3.50	47.20
4.	*T. longibrachiatum*	3.53	46.75
5.	*T. hamatum*	3.63	45.24
6.	*P. fluorescens*	2.16	67.42
7.	Bacillus subtilis	2.86	56.86
8.	Control	6.63	0.0
	CD (p = 0.05)	0.92	
	SD	0.43	

Values are the mean of three replications. Significant at (p = 0.05)

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

The results of fruit rot incidence and fresh chilli yield are shown in table 3.

Fruit rot incidence

In the field trials, all the treatments were found significantly superior to the control in reducing the incidence of fruit rot in both years. The minimum disease incidence was recorded with Bavistin (0.1%) with 9.26%. Among the antagonists *T. viride* significantly reduced the disease whose incidence was 18.65% compared to 48.33% in the control. Among the plant extracts tested, *A. sativum* (10%) confined the least fruit rot incidence (25.62%).

Yield

The Bavistin (0.1%) sprayed plot recorded the highest fruit yield of 282.5 q/ha, among the antagonists. A spray with *T. viride* resulted in the highest yield (fresh chilli) of 225.5 q/ha as compared to 90.4 q/ha in the control. It was followed by *P. fluorescens* (205 q/ha). Among the plant extracts, *A. sativum* recorded 143 q/ha.

Table 3. Efficacy of plant extracts, Bavistin and antagonistic organisms against fruit rot of chilli under field conditions (pooled data of two years)

No.	Treatments	Disease incidence* [%]	Percent reduction of growth in the control	Yield* [fresh]	
				[q/ha]	% increase above that of the control
1.	*T. viride* (2%)	18.65 (25.58)	61.41	225.5	149.4
2.	*P. fluorescens* (2%)	20.25 (26.74)	58.10	205.0	126.7
3.	*B. subtilis* (2%)	23.22 (28.80)	51.90	186.0	105.7
4.	*T. harzianum* (2%)	22.10 (28.03)	54.27	194.3	114.9
5.	*A. indica* (10%)	27.34 (31.50)	43.43	157.5	74.2
6.	*A. sativum* (10%)	25.62 (30.38)	46.98	143.0	58.1
7.	*D. hemiltonii* (10%)	29.22 (32.70)	39.54	122.6	35.6
8.	*Ocimum sanctum* (10%)	30.50 (33.52)	36.89	118.5	31.1
9.	Bavistin (0.1%)	9.26 (17.66)	80.84	282.5	212.5
10.	Control	48.33 (44.04)		90.4	
	CD (p = 0.05)	2.12		85.53	
	SD	1.01		40.71	

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

DISCUSSION

Control of anthracnose fruit rot, has for many years relied on chemical control and resulted in many undesirable problems, environmental pollution, accumulation of toxic substances and development of resistance in plant pathogens. Screening of plant extracts and antagonists against *C. gloeosporioides* revealed that certain plant extracts and biological agents showed very high inhibiting properties. Among the tested 9 plant species, a 10% extract of *A. sativum* and *A. indica* effectively inhibited mycelial growth of *C. gloeosporioides*. Murthy and Amonker (1974) and Singh *et al*. (1990) reported that a compound ajoene, derived from garlic inhibited *Colletotrichum* spp. Leaf extracts and neem (*A. indica*) oil could restrict growth of the anthracnose fungus (Jeyalakshmi and Seetharaman 1998).

The results of the present investigation revealed that from among the 7 antagonists tested, *P. fluorescens* and *T. viride* significantly reduced the mycelial growth of the pathogen. Jeyalakshmi and Seetharaman (1999) reported that *T. viride* reduced the mycelial growth of *Colletotrichum* spp. *Trichoderma* spp, grew over the pathogen and caused hyphal coiling, hyphal abnormalities, reduction in sclerotial production, lysis of hyphae and sclerotia (Malathi 1996).

Based on the *in vitro* effectiveness of the plant products and antagonistic organisms, the plant extracts of *A. indica* (10%), *A. sativum* (10%), *D. hemiltonii* (10%), *Ocimum sanctum* (10%), *T. viride* (2%), *T. harzianum* (2%), *P. fluorescens* (2%), *B. subtilis* (2%) and Bavistin (0.1%) were tested for management of fruit rot in field. Among the biological agents *T. viride* (2%) and *P. fluorescens* (2%) resulted in the minimum of fruit rot incidence. In the present study, however, Bavistin (0.1%) was significantly superior to the plant products and antagonists. This is in agreement with the report of earlier studies (Sujatha Bai 1992; Jeyalakshmi 1999. *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success (Maymon *et al*. 2004). Ramamoorthy *et al*. (2001) reported that *P. fluorescens* isolate Pf1 was found to reduce the fruit rot of chilli both under greenhouse and field conditions, with an increase in yield. Fluorescent pseudomonads produce plant growth promoting substances such as auxins and gibberellins and enhance plant growth and yield (Dubeikovsky *et al*. 1993).

Spraying with plant extracts and antagonistic microorganisms were effective against *C. gloeosporioides in vitro* and against the disease in field experiments. But all these ranked behind Bavistin. The efficacy of Bavistin against the fruit rot pathogen was reported by several workers (Mishra 1988; Raju and Rao 1989; Azad *et al*. 1992). Although Bavistin was observed to be the most effective treatment, there are numerous reports of the negative effects of using chemicals on farm income and the health of farm workers. Toxic contamination to the environment, particularly in developing countries (Voorrips *et al*. 2004) has also been reported. There is a need to incorporate alternative control components that are effective in controlling the disease. Furthermore, combined studies with plant extracts and bioagents need to be tested to better protect the Naga king chilli.

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POLISH SUMMARY

BIOLOGICZNE ZWALCZANIE ZGNILIZNY OWOCÓW NAJOSTRZEJSZEGO NA ŚWIECIE CHILLI (*CAPSICUM CHINENSE* JACQ.)

Badano dziewięć gatunków roślin i 7 ich antagonistów przeciw *Colletotrichum gloeosporioides*, sprawcy zgnilizny owoców Naga king chilli. Badania *in vitro* wykazały, że *Trichoderma viride* i *Pseudomonas fluorescens* skutecznie hamowały wzrost grzybni patogena. Spośród wyciągów roślinnych, *Allium sativum* (10%) i *Azadirachta indica* (10%) w najwyższym stopniu hamowały wzrost grzybni *C. gloeosporioides*. Ocena skuteczności wyciągów roślinnych, antagonistów i środków grzybobójczych wykazała, że oprysk *T. viride* (2%) redukował chorobę w 61.41%, *P. fluorescens* w 58.10%. Największą skuteczność wykazał fungicyd Bavistin (0,1%), który redukował chorobę w 80,84%.