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In vitro evaluation of biocontrol agents, botanicals and fungicides against *Pestalotiopsis* sp. infecting large cardamom (*Amomum subulatum* Roxb.)

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Abstract

Commercially available biological control agents (*Trichoderma harzianum, T. viride, Pseudomonas fluorescens* and *Bacillus subtilis*), aqueous extracts of plant species (*Artemisia vulgaris* and *Schima wallichi*) and four fungicides were screened *in vitro* at Tadong (Sikkim), against *Pestalotiopsis* sp. infecting large cardamom (*Amomum subulatum*). *B. subtilis* showed significantly high inhibition (62.6%) of the pathogen followed by *T. viride* (50.9%). Inhibition of mycelial growth of *Pestalotiopsis* sp. by combined aqueous extract of *A. vulgaris* and *S. wallichi* varied from 12.2% to 74.5% and was significantly higher at 15% concentration. Inhibition of the pathogen increased with increase in concentration of the extract. The growth inhibition of the pathogen increased with increase in concentration of all fungicides tested. Carbendazim 50WP was significantly effective at all concentrations tested (0.05%-0.15%). The study indicated the potential of using biocontrol agents and botanicals for eco-friendly management of *Pestalotiopsis* sp. infecting large cardamom and fungicides in case the incidence is severe.

Keywords: *Amomum subulatum*, bioagent, botanical, fungicide, large cardamom, *Pestalotiopsis.*

Large cardamom (*Amomum subulatum* Roxb.) is traditionally grown in the moist deciduous and evergreen forests of sub-Himalayan tract, especially in Sikkim and Darjeeling District of West Bengal. Leaf streak caused by *Pestalotiopsis* sp. often results in severe foliar damage to the crop. The disease is characterized by rectangular spots running parallel to the veins and elongated rectangular translucent streaks appearing on young leaves along the veins. The streaks turn reddish brown within 3-4 days with a central straw colored necrotic area surrounded by a prominent dark brown margin (Srivastava & Verma 1989a; 1989b). This study reports the *in vitro* antagonism of commercially available biocontrol agents (*Trichoderma harzianum* Rifai, *T. viride* Pers., *Pseudomonas fluorescens* Migula and *Bacillus subtilis* (Ehrenberg) Cohn) and sensitivity of aqueous extracts of young twigs of *Artemisia vulgaris* L. (Indian wormwood, *titopati* in Nepali) and *Schima wallichi* Choisy (*chilaune* in Nepali) and fungicides (copper oxychloride, mancozeb, combined formulation of carbendazim + mancozeb, and carbendazim) against *Pestalotiopsis* sp. The plant species are being maintained randomly in large cardamom plantations by local farming communities based

on the belief that they can repel insects. Biocontrol agents and botanicals would be useful for eco-friendly management of the disease in view of the organic status of Sikkim. Fungicides would be required in case the disease incidence is severe and especially in other large cardamom growing states wherever applicable.

The study was conducted during September-October 2007 at Indian Cardamom Research Institute, Regional Station, Tadong, Sikkim. *Pestalotiopsis* sp. was isolated from the infected leaves of large cardamom collected from the research farms located at Pangthang (2160 m MSL) and Kabi (1650 m MSL). The isolation was done on potato dextrose agar (PDA) and the culture was maintained on slants of the same medium. The biological control agents were obtained from Indian Cardamom Research Institute, Myladumpara, Kerala. The cultures of *T. harzianum* and *T. viride* were maintained on PDA and the bacterial antagonists namely, *P. fluorescens* and *B. subtilis* were maintained on King's B Agar (KBA) and Nutrient Agar (NA), respectively. *A. vulgaris* and *S. wallichi* were collected from Sikkim.

In vitro evaluation of biocontrol agents was carried out on PDA medium by following dual culture technique. Petri plates containing PDA were inoculated with 7 day old culture of *Pestalotiopsis* sp. (5 mm diameter disc). The antagonistic fungi (5 mm diameter disc) were inoculated 60 mm away from the pathogen disc. For bacterial antagonists, streaking was done in parallel line on both the sides of the pathogen discs (at 15 mm distance) which was kept at the centre. Plates inoculated with pathogen alone served as control. Six replications were maintained in all cases. The plates were incubated at room temperature (22-23^{0}C). Radius of the pathogen colony was measured after 5 and 7 days of incubation and per cent inhibition of the pathogen by the antagonist was calculated.

For *in vitro* evaluation of aqueous extracts of plant species, fresh twigs of *A. vulgaris* and *S. wallichi* were collected and combined aqueous extract was prepared by grinding 0.5 kg each in 1 l of water. The resultant filtered solution served as the stock. The *in vitro* toxicity of the extract was determined by poisoned food technique (Nene & Thapliyal 1993) at concentrations ranging from 1% to 15% (of the stock). There were three replications for each treatment. The diameter of the colony was measured 5 days after inhibition (at 22-23^{0}C) and inhibition of the pathogen was calculated.

The sensitivity of *Pestalotiopsis* sp. to four fungicides (copper oxychloride 50WP, mancozeb 75WP, combined formulation of carbendazim + mancozeb (12 + 63) 75WP and carbendazim 50WP) was evaluated by poisoned food technique (Nene & Thapliyal 1993). Each fungicide was evaluated at 0.1, 0.2, 0.3 and 0.4% concentrations based on active ingredient except carbendazim which was evaluated at 0.05, 0.1 and 0.15% concentrations. Seven replicates of each concentration were maintained. After 5 days of incubation at 22-23^{o}C, the diameter of the colony was measured for each plate and inhibition of growth was calculated. The fungicide was considered to be toxic to *Pestalotiopsis* sp. at a particular concentration when 50% or more growth of the fungus was inhibited. All the data was analysed by ANOVA after transforming the per cent values to corresponding angular values.

Evaluation of biocontrol agents

All the four biocontrol agents tested were effective in inhibiting the pathogen in dual culture to varying degrees. Observations on mycelial growth of the pathogen after 7 days of incubation indicated that *B. subtilis* showed highest inhibition (62.6%) against *Pestalotiopsis* sp. followed by *T. viride* (50.9%), *P. fluorescens* (41.3%) and *T. harzianum* (30.4%). There were significant differences among the biocontrol agents in inhibiting the pathogen (Table 1). The fungal biocontrol agents overgrew the pathogen colony after 7 days. The bacterial antagonists inhibited the pathogen by forming an inhibition zone which is indicative of antibiotic production (Mech *et al.* 2008).

Evaluation of plant extracts

Inhibition of mycelial growth of *Pestalotiopsis* sp. by combined aqueous extract of *A. vulgaris*

Table 1. *In vitro* inhibition of *Pestalotiopsis* sp. by biocontrol agents

Biocontrol agent	Colony radius (mm)		Per cent inhibition	
	5 DAI	7 DAI	5 DAI	7 DAI
Trichoderma harzianum	13.8	16.0	18.8 (25.70)	30.4 (33.46)
Trichoderma viride	9.3	11.3	45.3 (42.30)	50.9 (45.52)
Pseudomonas fluorescens	12.8	13.5	24.7 (29.80)	41.3 (39.99)
Bacillus subtilis	8.3	8.6	51.2 (45.69)	62.6 (52.30)
Control	17.0	23.0	-	-
CD (P=0.05)	1.96	1.51	8.66	5.62

DAI=Days after inoculation; Values in parentheses are angular transformations

Table 2. *In vitro* inhibition of *Pestalotiopsis* sp. by combined aqueous extract of *Artemesia vulgaris* and *Schima wallichi*

Conc. (%) of aqueous extract	Colony diameter (mm), 5 DAI	Per cent inhibition
1	18.7	12.2 (20.44)
2	17.9	16.0 (23.58)
3	16.9	20.8 (27.13)
4	15.4	27.6 (31.69)
5	13.7	35.9 (36.81)
6	8.8	58.8 (50.07)
7	8.0	62.6 (52.30)
8	7.8	63.3 (52.71)
9	7.4	65.1 (53.73)
10	7.1	66.6 (54.70)
11	6.6	68.8 (56.04)
12	6.5	69.3 (56.35)
13	5.8	72.6 (58.44)
14	5.7	73.1 (58.76)
15	5.4	74.5 (69.67)
Control	21.3	-
CD (P=0.05)	0.56	1.98

DAI=Days after inoculation; Values in parentheses are angular transformations

and *S. wallichi* varied from 12.2% to 74.5%. Inhibition of pathogen increased with increase in concentration of the extract. There was no significant difference in the inhibition of the pathogen recorded at 7%, 8% and 9% concentrations of the extract. Among 11% and 12% concentrations too, there was no significant difference in the inhibition of the pathogen. The mycelial growth of the pathogen in treated plates showed deformation with more cottony and upward growth as compared to the control plate probably due to the anti microbial properties of the extract. The fungi-toxic properties of the extract are an added advantage in integrated disease and pest management systems (Mech *et al.* 2008).

Evaluation of fungicides

All the fungicides inhibited the growth of the pathogen at all the test concentrations to varying degrees. At 0.05% concentration, only carbendazim was evaluated and there was 88.6% inhibition of *Pestalotiopsis* sp. All the fungicides were evaluated at 0.1% concentration and carbendazim showed significantly high inhibition of the pathogen (91.4%) followed by carbendazim + mancozeb (79.8%). At 0.15%, carbendazim showed 91.9% inhibition of the pathogen (other fungicides were not evaluated at this concentration). All the fungicides except carbendazim were evaluated at 0.2, 0.3 and 0.4% concentrations. At 0.2% concentration, carbendazim + mancozeb showed significantly high inhibition of the pathogen (87.8%) followed by mancozeb (53.1%). Carbendazim + mancozeb showed 91.9% inhibition of the pathogen followed by copper oxychloride (86.4%) at 0.3% concentration and there was a significant difference among the values. All the fungicides tested at 0.4% were highly sensitive to the pathogen and showed 91.9% inhibition (Table 3). The per cent growth inhibition

Table 3. *In vitro* inhibition of *Pestalotiopsis* sp. by fungicides

Fungicide	Colony diameter (mm), 5 DAI at conc. (% a.i.)					Per cent inhibition, 5 DAI at conc. (% a.i.)					
	0.05	0.1	0.15	0.2	0.3	0.4	0.05	0.1	0.15	0.2	0.3	0.4
Copper oxychloride	NT	54.2	NT	50.4	8.4	5.0	NT	12.7 (20.88)	NT	18.7 (25.62)	86.4 (68.36)	91.9 (73.46)
Mancozeb	NT	32.1	NT	29.1	30.9	5.0	NT	48.3 (44.03)	NT	53.1 (46.78)	50.2 (45.11)	91.9 (73.46)
Carbendazim + Mancozeb	NT	12.5	NT	7.6	5.0	5.0	NT	79.8 (63.29)	NT	87.8 (69.56)	91.9 (73.46)	91.9 (73.46)
Carbendazim	7.1	5.3	5.0	NT	NT	NT	88.6 (70.27)	91.4 (72.95)	91.9 (73.46)	NT	NT	NT
Control	62.1	62.1	62.1	62.1	62.1	62.1	NT	NT	NT	NT	NT	NT
CD (P=0.05)	-	2.63	-	2.56	3.19	2.39	-	2.39	-	2.16	2.37	0.00

DAI=Days after inoculation; NT=Not tested; Values in parentheses are angular transformations

increased with increase in concentration of all fungicides tested. Thus carbendazim 50WP was most effective at all the concentrations tested followed by carbendazim + mancozeb 75WP.

The study indicated the potential of using biological control agents and botanicals for the eco-friendly management of leaf streak disease of large cardamom and fungicides in case the incidence of the disease is severe. However, further evaluation in the field is required before the biocontrol agents, plant extracts and fungicides are recommended for disease management.

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