Research Article

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In vitro studies on management of bud rot disease of arecanut caused by *Phytophthora* sp.

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Abstract

Bud rot is one of the fatal diseases of areca palm caused by *Phytophthora* sp. The present study was carried out to test the efficacy of antagonist, fungicides and plant extracts in inhibiting the pathogen, *Phytophthora*, under *in vitro* condition. A single isolate of *Phytophthora* was collected and isolated from deeper down the basal cut end of bud rot affected areca palm of CPCRI, RC, Kahikuchi, Guwahati. Among the three species of *Trichoderma* tested, *T. viride* was found to be most effective *i.e.* 90.44% inhibition followed by *T. virens* and *T. harzianum* with 89.44 and 76.29 per cent inhibition, respectively, over control after 96 h of incubation period. The fungicides, akomin (salt of Phosphonic acid) and contaf (Hexaconazol) each @ 0.3% showed 100 per cent inhibition over control whereas dithane M-45 (Mancozeb) and antracol (Propineb) showed 82.22 and 76.22 per cent inhibition, respectively, over control. Among the twenty five plant extracts tested, the aqueous extract (10%) of *Allium sativum, Cleome viscosa, Melastoma malabathricum* and *Oxalis corniculata* exerted 100 per cent inhibition of the pathogen over control after 48 h, 72 h and 96 h of incubation compared to other extracts.

Keywords: Arecanut, antagonists, botanicals, fungicides, *Phytophthora* sp.

Introduction

Arecanut palm (*Areca catechu* L.) is one of the important plantation crops in India and is mainly grown in the states of Assam, Karnataka, Kerala, Tamil Nadu and West Bengal. Bud rot, caused by *Phytophthora* sp. is one of the fatal diseases of areca palm. The disease is characterized by the rotting of the growing bud and the surrounding tissues. Due to colonization of secondary organisms on the rotten spindle, the infected palm emits a disagreeable odour. Young palms in the age group of 5-20 years are more susceptible to infection.

Bud rot or rotting of growing bud of arecanut is one of the major diseases observed in heavy rainfall areas of Meghalaya. In Assam, the disease is very sporadic in nature and occurs in closely planted gardens under unhygienic conditions during the monsoon season (May-August). In endemic areas such as Panchotiya, Laplang, Nohwet, Rimasar under the Meghalaya state, the disease also appears during the post monsoon period (October-December). A survey was conducted during the year 2002-04 in Kamrup, Darrang, Nagaon and Morigaon districts of Assam to assess the intensity of the disease in these regions. The prevalence of the disease was more in Bahupara (9.75%), Baruabari (6.66%) villages of Kamrup district and Salmara village (6.66%) of Darrang district and low in Agsia village (0.08%) of Kamrup district. In Nagoan and Morigaon districts, the incidence of bud rot was not observed. Crop loss to a considerable extent (> 50% incidence) has been reported in the endemic areas of Meghalaya (East Khasi and Jaintia hills) where both dry rot and wet bud rot are found to be severe (Anon., 2005).

With the increased awareness on the market driven economy, safe and wholesome products are in demand in the recent years. Hence, disease management has to be achieved through integrated management practices involving cultural and biological means with minimum

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dependence on chemical control measures. Considering the above factors, the present investigation was undertaken to evaluate the efficacy of some common fungicides, bio-agents and botanicals on the *in vitro* inhibition of *Phytophthora* sp.

Materials and Methods

Three species of the antagonistic fungus *Trichoderma*, two systemic and two contact fungicides and extracts of 25 locally available plant species were evaluated *in vitro* for their inhibitory efficacy against *Phytophthora*. Three plates per treatment were maintained and the incubation period was uniform *i.e.*, 96 h for all the treatments. At the end of incubation period mean of three values in each case was compared with that of control. *Phytophthora* was isolated from samples of diseased tissues collected from deeper down the basal cut end of bud rot affected areca palm. Small tissue pieces were plated on PDA (potato dextrose agar) after surface sterilization with 0.1% mercuric chloride, and incubated at 22 ± 2 °C. The pure cultures of the pathogen was prepared and maintained in PDA medium.

Trichoderma spp. were isolated from the rhizosphere soils of arecanut palms of CPCRI, RC, Kahikuchi farm, Guwahati on *Trichoderma* specific medium with captan (TSMC) (Elad and Chet, 1983). These isolates were purified and tested for their antagonism against the pathogen by 'dual culture technique' on potato dextrose agar medium (Dhingra and Sinclair, 1985).

The comparative toxicity of fungicides on the growth of the fungus under *in vitro* condition was evaluated by poisoned food technique (Bhaskaran *et al.*, 1988). Fungicides used were akomin (salt of Phosphonic acid), contaf (Hexaconazol), dithane M-45 (Mancozeb) and antracol (Propineb) @ 0.3%. Stock solutions of the fungicides were prepared in sterile distilled water and added aseptically to sterilized PDA medium to get the required concentrations and then poured into petri plates. The plates prepared without any fungicides served as control. These plates were inoculated with 7 mm disc of four day old culture of the test fungus and incubated at 22 ± 2 °C for 4 days.

The extracts of twenty five locally available plants were tested for their antifungal property against *Phytophthora* sp. by poisoned food technique (Bhaskaran *et al.*, 1988) under *in vitro* condition. Leaves of test plants except *Allium sativum* (corm) and *Terminalia arjuna* (bark) were taken for preparing crude extracts. The leaves/corm/bark were thoroughly washed with sterile water and a fine slurry was prepared by taking 100 g sample with 100 ml of sterile distilled water. The resultant slurry was filtered though muslin cloth and then through Whatman No.1 filter paper and the extracts collected in sterile glassware were used as stock solution. From the stock solution, 10 ml was added to 90 ml of PDA medium to make 10% concentration.

Results and Discussion

Three isolates of the antagonistic fungus, *Trichoderma,* were isolated from the rhizosphere soils of arecanut palms. These isolates were identified as *T. harzianum* (AF 1), *T. viride* (AF 2) and *T. virens* (AF 3) and the identity was further confirmed according to the identification key (Rifai, 1969) based on the branching of conidiophores, shape of phialides, emergence of phialospores and shape of the phialospores.

Among the three antagonists tested, *T. viride* was found to be the most effective, *i.e.* 90.44 per cent followed by *T. virens* and *T. harzianum* with 89.44 and 76.29 per cent inhibition, respectively, over control after 96 h of incubation (Table1). However, *T. viride* and *T. virens* did not show any significant difference in their inhibitory property against the pathogen. In coconut, the inhibitory property of *T.harzianum* and two isolates of *T.viride* against the fungus, *Ganoderma lucidum* was reported by Bhaskaran *et al.* (1988). Gunasekaran *et al.* (1986) and Bhaskaran (1990) also reported the effectiveness of *T.viride* and *T. harzianum* in suppressing the growth of *G. lucidum* in coconut.

Table 1. Effect of different antagonists on growth of *Phytophthora* sp.

Treatments	Per cent inhibition over control		
	48 h	72 h	96 h
Trichoderma harzianum	11.44 (19.77)	52.11 (46.21)	76.29 (60.86)
T. viride	13.78 (21.79)	57.33 (49.22)	90.44 (71.99)
T. virens	13.55 (21.60)	56.55 (48.76)	89.44 (71.04)
SEd±	0.45	0.41	0.48
CD (P=0.05)	1.12	1.00	1.16

Figures in parentheses are angular transformed values

The effect of fungicides on the growth of the fungus is presented in Table 2. Among the tested fungicides, akomin and contaf @ 0.3% showed 100 per cent inhibition over control. The other fungicides viz., dithane M-45 and antracol @ 0.3 per cent recorded 82.22 and 76.22 per cent inhibition, respectively, over control after 96 h of incubation. The inhibitory effect of dithane M-45 on respiration and mycelial growth of *Phytophthora colocasiae* was reported in colocassia by Aggarwal and Mehrotra (1988).

Table 2. Effect of different fungicides on growth of *Phytophthora* sp.

Treatments	Per cent inhibition over control		
	48 h	72 h	96 h
Akomin(Phosphonic acid) @0.3%	100 (89.17)	100 (89.17)	100 (89.17)
Contaf(Hexaconazol) @0.3%	100 (89.17)	100 (89.17)	100 (89.17)
Dithane M-45 (Mancozeb) @0.3%	85.00 (67.21)	83.00 (65.65)	82.22 (65.06)
Antracol(Propineb) @0.3%	78.89 (62.65)	76.78 (61.19)	76.22 (60.81)
SEd±	0.84	0.80	1.06
CD (P=0.05)	1.94	1.85	2.45

Figures in parentheses are angular transformed values

Among the 25 plant extracts tested against *Phytophthora* sp., *Allium sativum, Cleome viscosa, Melastoma malabathricum* and *Oxalis corniculata* showed 100 per cent inhibition (Table 3) over control after 48 h, 72 h and 96 h of incubation compared to other botanicals. Complete inhibition on the growth of the pathogen *G. lucidum* by the aqueous leaf extracts of *Allium sativum* was reported by Iyer *et al.* (2004) in arecanut. The aqueous extract of *Bidens pilosa* also showed higher inhibition (81.01%) over control after 96 h of incubation. Powell and Wen-hsiung Ko (1986) reported that the root extract of garlic inhibited germination of chlamydospores and encysted zoospores of *Phytophthora palmivora* in soil and reduced damping off of papaya seedlings caused by this pathogen.

Among the three species of *Trichoderma* tested, *T. viride* was found to be the most effective, followed by *T. virens* and *T. harzianum* in inhibiting the growth of the fungus. Out of the four fungicides, akomin and contaf (@ 0.3%) showed 100 per cent inhibition over control. The study revealed that the aqueous extracts (10%) of *Allium sativum, Cleome viscosa, Melastoma malabathricum* and *Oxalis corniculata* can be used against the bud rot disease of arecanut. The technique adopted in this experiment for the suppression of growth of the fungus is cost-effective, user-friendly and does not require high technical skills. Moreover, the extracts of the plants showing better result are available in plenty. The results of this study, therefore, could be used to find out a suitable alternative for environmentally unsafe fungicides and may form an important element in Integrated Disease Management.

Table 3. Efficacy of botanicals on mycelial growth of *Phytophthora* sp.

Sl. No.	Botanicals	Per cent inhibition over control		
		48 h	72 h	96 h
1.	*Allium sativum**	100 (89.67)	100 (89.67)	100 (89.67)
2.	*Azadirachta indica*	24.67 (29.78)	0.00 (0.33)	0.00 (0.33)
3.	*Bidens pilosa*	96.00 (78.46)	80.89 (64.08)	81.01 (64.17)
4.	*Bryophyllum pinnatum*	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
5.	*Carthamus oxycantha*	10.78 (19.17)	0.00 (0.33)	0.00 (0.33)
6.	*Centella asiatica*	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
7.	*Cleome viscosa*	100.00 (89.67)	100.00 (89.67)	100.00 (89.67)
8.	*Clerodendron infortunatum*	14.22 (22.11)	0.00 (0.33)	0.00 (0.33)
9.	*Curcuma longa*	75.67 (60.45)	23.11 (28.73)	0.00 (0.33)
10.	*Dryopteris flix-mas*	65.67 (54.13)	23.11 (28.73)	0.00 (0.33)
11.	*Gliricidia sepium*	38.55 (38.38)	0.00 (0.33)	0.00 (0.33)
12.	*Lippia geminata*	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
13.	*Melastoma malabathricum*	100.00 (89.67)	100.00 (89.67)	100.00 (89.67)
14.	*Momordica charantia*	68.44 (55.82)	28.67 (32.37)	0.00 (0.33)
15.	*Ocimum basilicum*	68.33 (55.75)	39.44 (38.90)	0.00 (0.33)
16.	*Ocimum sanctum*	34.78 (36.14)	0.00 (0.33)	0.00 (0.33)
17.	*Oxalis corniculata*	100.00 (89.67)	100.00 (89.67)	100.00 (89.67)
18.	*Paeberia foetida*	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
19.	*Polyalthia longifolia*	17.89 (25.02)	0.00 (0.33)	0.00 (0.33)
20.	*Pouzolzia zeylenica*	58.22 (49.73)	36.55 (37.20)	0.00 (0.33)
21.	*Psidium guajava*	67.00 (54.94)	44.00 (41.55)	0.00 (0.33)
22.	*Rauwolffia tetraphyla*	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
23.	*Solanum nigrum*	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
24.	*Terminalia arjuna***	64.55 (53.46)	34.00 (35.67)	0.00 (0.33)
25.	*Typhonium trilobatum*	31.22 (33.97)	0.00 (0.33)	0.00 (0.33)
	SEd±	0.48	0.30	0.03
	CD (P=0.05)	0.97	0.60	0.06

Figures in parentheses are angular transformed values, * Corm extracts, ** Bark extracts

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