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Effect of plant extracts on *Phytophthora palmivora* causing bud rot disease of coconut

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Bud rot is a fatal disease of the coconut palm, characterized by the rotting of the terminal bud and surrounding tissues. Butler (1906) first reported bud rot disease from India. It is quite common along the West and East Coast tracts. Radha and Joseph (1974) reported a disease incidence of 1.2 to 10.9 per cent in Kerala, and 35 to 40 per cent in gardens having large number of palms in certain locations.

Studies have clearly shown the existence of two dominant *Phytophthora* spp. viz., *P. palmivora* Butl. and *P. katsurae* Ko & Chang. in relation to bud rot disease of coconut. If the disease is detected when the central shoot begins to wither, removal of affected portions followed by the application of 10% Bordeaux paste on the affected portion can check the disease. As a prophylactic measure, adjacent healthy palms should also be sprayed with 1% Bordeaux mixture. Rohini Iyer (1997) reported that stem injection / root feeding with systemic fungicides can protect the coconut crown from *Phytophthora* attack for a period of 8 weeks. In copper sensitive palms, like Chowghat Orange Dwarf (COD) and Malayan Yellow Dwarf (MYD), keeping perforated sachets containing Mancozeb (3g of Indofil M-45 or Dithane M-45) in the leaf axils during rainy season is found to be useful. Continuous use of specific systemic fungicides is not recommended, because, it is possible that systemic fungicides when incorporated into the plant through stem and root injections can leave residues in copra, nut-water and oil and there could also be the danger of the pathogen acquiring resistance against these chemicals. Under these circumstances, environment-friendly, and less expensive methods like that of plant extracts has great relevance.

Coconut tissues showing typical symptom of bud rot were thoroughly washed in running water. Tissues from the advancing margins of the lesions showing initial stage of infection were used for the isolation of the fungus. Pieces measuring 5 x 5 mm of infected tissues were cut with sterile scalpel from the advancing margin of the lesion and surface sterilized in 70 % ethyl alcohol, and inoculated in 90 mm dia. petriplates containing P_{10}ARP medium (Kannwischer and Mitchell, 1978). These plates were incubated in the dark at 24±1°C. Characteristic growth of *P. palmivora* obtained on the third day was sub cultured and maintained on carrot agar slopes.

The effect of plant extracts on the mycelial growth of *P. palmivora* was done by 'poisoned food technique' by incorporating the plant extract in carrot agar medium. *P. palmivora* isolate Pp21 was used for the study. Plant leaves (100 g) were extracted with 100 ml of water and filtered through a double layer of muslin cloth to obtain the standard extract. This was added to carrot agar medium to make up the volume to one litre and was autoclaved for 20 min. (Bhaskaran *et al*., 1988). Mycelial discs of 5mm dia were centrally inoculated on the poured media in 90 mm petri plates. Radial growth of *P. palmivora* isolate was measured after 24, 48, and 72 h. Mycelial growth in media without plant extracts was taken as control. Growth inhibition was calculated based on the growth of *P. palmivora* in control plate.

To produce sporangia, the fungal isolates were grown on carrot agar medium for the first three days in dark at 24±1°C. These cultures were then incubated under continuous light for three days. Sporangium bearing mycelium was washed three times by decanting the water from the mycelial mat in the plate and by replacing with 10 ml distilled water. The plates were incubated at 15°C for 20 min. After 20 min, 10 ml of the zoospore suspension was transferred to test tubes and treated with

1 ml of each plant extract, and the number of germinated zoospores were counted at different intervals of time using a hemocytometer. The effect on colony forming units (CFU) was studied by plating the zoospore suspension in P_{10}ARP medium.

The effectiveness of plant extracts to suppress bud rot disease was tested on detached COD (Chowghat Orange Dwarf) spear leaf pieces of about 12 cm length. Promising plant extracts which showed more inhibition in dual culture were used for this assay. For comparing the effect of plant extracts with commonly used fungicide, 1% Bordeaux mixture was also included in the treatments. Various treatments and the pathogen were inoculated in the following sequence.

1. *P. palmivora* was inoculated two days prior to plant extract application
2. Both *P. palmivora* and plant extracts were introduced simultaneously
3. Plant extracts were applied two days prior to *P. palmivora* inoculation.

Plant extracts and 1% bordeaux mixture was sprayed on both sides of the leaf pieces (1 ml/ leaf pieces). Leaf pieces sprayed with sterile distilled water served as control. All the treatments were placed in petri plates lined with moist filter paper to maintain high humidity. Discs of 5mm dia were taken from 5-day-old culture of *P. palmivora* and inoculated in the center of each leaf piece by wound inoculation. These leaf pieces were maintained at 20°C and lesion area was calculated from all treatments.

Out of the 40 plant extracts studied, extracts of *Andrographis paniculata*, *Lawsonia inermis* and *Hyptis suaveolens* showed greater inhibition when compared to all other extracts (Table 1). *Andrographis paniculata* leaf extract exerted 100 % inhibition after 24, 48 and 72 h. *Lawsonia inermis* leaf extract showed 100.0, 86.7 and 74.4 % inhibition after 24, 48 and 72 h., respectively. *Hyptis suaveolens* leaf extract showed 100.0, 75.0, 70.9 and 82 % inhibition after 24, 48 and 72 h., respectively. Results of evaluation of different plant extracts against *P. palmivora* are given in Table 1. Leaf extracts (10 %) of *Ocimum sanctum*, *Eucalyptus citriodora* and *Piper betle* were highly inhibitory (84.8–86.5 % inhibition), whereas, extracts of *Clerodendron inerme*, *Lantana camara* and *Nicotiana nudicaulis* were moderate inhibitors (55-62.7 % inhibition) of *Phytophthora parasitica* var. *nicotianae*, the causal agent of black shank disease of tobacco (Patel and Patel, 1999). Leaf extracts of *Azadirachta indica* (2%) *Pongamia pinnata* (3 %) and

Table 1. *In vitro* evaluation of different plant extracts against *P. palmivora*

Plant extracts	Inhibition % after			Mean
	24 h	48h	72h	
Adathoda vasica	10.0	10.0	6.7	8.9
Andrographis paniculata	100.0	100.0	100.0	100.0
Azadirachta indica	16. 7	8.3	2.9	9.3
Biophytum reinwarditti	10.0	5.0	3.3	6.1
Bougainvillea spectabilis	10.0	20.0	30.0	20
Calotropis indica	20.0	25.0	26.6	23.8
Cardiospermum halicacabum	0	0	0	0
Cassia marginata	20	24.1	38.8	27.6
Clerodendron infortunatum	5.0	5.0	6.7	5.6
Cympopogon citratus	30.0	25.0	24.7	26.5
Cynodon dactylon	11.7	7.9	5.5	8.3
Cyperus rotundus	20.0	20.0	23.0	21.0
Datura fastuosa	0	0	0	0
Datura metel	90.0	70.0	60.0	73.3
Eclipta alba	10.0	10.0	10.0	10.0
Eupatorium odoratum	11.7	14.5	26.5	17.6
Gloriosa superba	25.0	25.0	20.0	23.3
Glycosmis pentaphylla	0	0.	0	0
Heliotropium scabrum	10.0	3.3	3.9	5.7
Hemidesmus indicus	10.0	5.0	3.0	6.0
Hyptis suaveolens	100.0	75.0	70.9	82.0
Jatropha glandilifera	0	0	0	0
Lantana camara	8.3	18.0	27.0	17.8
Lawsonia inermis	100.0	86. 7	74.4	87.0
Leucas aspera	8.8	5.0	3.3	5.7
Nerium odoratum	10.0	5.0	1.1	5.4
Ocimum canum	20.0	15.0	13.3	16.1
Ocimum sanctum	20.0	20.0	25.0	21.7
Phyllanthus niruri	1.7	2.3	2.8	2.3
Physalis minima	20.0	15.0	10	15
Plumbago zeylanica	36.7	43.3	48.9	43.0
Polyalthia longifolia	30.0	40.0	40.0	36.7
Polycarpaea aurea	13.3	4.3	4.3	7.3
Pongamia glabra	20.0	15	16.7	17.2
Scoparia dulcis	20.0	39.5	40	33.1
Strychnos- nux-vomica	30.0	33.3	38.9	34.1
Tinospora cordifolia	20.0	24.3	38.3	27.6
Tridax procumbens	0	5.0	28.0	11.0
Vernonia cinaria	9.00	6.7	5.2	7.0
Catharanthus roseus	20.0	24.0	38.0	27.3

C.V. % = 7.11

CD (P = 0.05) for plant extracts = 1.60

CD (P = 0.05) for time intervals = 0.44

CD (P = 0.05) for plant extracts x time intervals = 2.77

Transformation used: $\sin^{-1}\sqrt{p}$ where 'p' is the inhibition per cent

Tagetes erecta (3 %) completely inhibited sporulation of *Phytophthora nicotiane* var. *parasitica* (Yadav *et al.*, 1998). When incorporated into PDA medium used for culturing the fungi, leaf extract of *Adathoda vasica* suppressed mycelial growth of *Phytophthora* and *Pythium* spp. and inhibited sexual reproduction

(Sivakadadcham, 1988). Water extracts of 'Kiriath' (*Andrographis paniculata*) showed reduced radial growth of *P. meadii* causing immature nut fall in arecanut (Anonymous, 2000).

When zoospore suspension of *P. palmivora* was treated with promising plant extracts, germination of zoospores was inhibited. Differences due to treatments were found to be significant, with control recording minimum number of un-germinated zoospores than all the other treatments. Treatments with plant extracts of *Andrographis paniculata, Lawsonia inermis* and *Hyptis suaveolens* recorded maximum number of ungerminated zoospores after 3, 10 and 24 h. than the control. Maximum mean for ungerminated zoospores was recorded for *Andrographis paniculata* followed by *Lawsonia inermis* (Table 2).

Table 2. Effect of plant extracts on *P. palmivora* zoospore germination

Treatments	Number of zoospores x 10^4 per cm^3 /ml Ungerminated after			
	3h	10h	24h	Mean
Andrographis paniculata	1.3	1.1	0.9	1.1
Lawsonia inermis	1.1	1.0	0.9	1.0
Hyptis suaveolens	1.0	0.9	0.8	0.9
Datura metel	0.9	0.9	0.8	0.8
Control	0.16	0.00	0.00	0.05

C.V% = 16.37

CD (P = 0.05) for treatments = 0.19

Colony forming units of *P. palmivora* produced from zoospore suspension treated with plant extracts were found to be reduced compared to that of control. This shows that the plant extract treated *P. palmivora* zoospores could not sustain their ability upon plating. Mean colony forming units formed from zoospores by treatment with plant extracts after 1 h. was found to be highest when compared to that recorded after 3 and 10 h. After 24 h. treatment of zoospore suspension with extracts of selected plants, resulted in the complete inhibition of colony forming units of *P. palmivora* (Table 3). Among the plant extracts, *Andrographis paniculata* exerted maximum mean inhibition of CFU (1.1 CFU) followed by *Lawsonia inermis* (1.2 CFU) and *Hyptis suaveolens*. (1.3 CFU) All these *in vitro* studies showed that the above plant extracts have different degrees of inhibition of *P. palmivora* zoospores, however, *Andrographis paniculata* showed the maximum inhibition.

Lesion area was found to be minimum, when leaf pieces were treated with plant extracts compared to that

Table 3. Effect of plant extracts on *P. palmivora* colony forming units (CFU)

Treatments	CFU X 10^{-2}/ ml from zoospore suspension after				Mean
	1h	3h	10h	24h	
Andrographis paniculata	2.5	1.2	0.7	0.00	1.1
Lawsonia inermis	2.2	1.7	1.0	0.0	1.2
Hyptis suaveolens	2.5	1.7	1.0	0.00	1.3
Datura metel	3.2	2.4	1.3	0.0	1.7
Control	3.5	3.6	3.9	4.2	3.8

C.V % 16.71

CD (P = 0.05) for isolates = 0.25

CD (P = 0.05) for time intervals = 0.22

CD (P = 0.05) for isolates x time intervals = 0.50

of untreated control. Lesion area on spear leaves was minimum when treatments were introduced prior to pathogen. Variations due to sequences, treatments and time intervals were significant. On an average sequence C (application of plant extracts prior to pathogen inoculation) has resulted in the minimum lesion area after 5^{th} and 10^{th} days. The lesion size in all the treatments were found to be high after 10^{th} day compared to that on the 5^{th} day. Lesion area was very low for the treatment 1 % Bordeaux mixture compared to all the other treatments in sequence A, B and C followed by *Andrographis paniculata* (Table 4).

Table 4. *In vivo* effect of plant extracts on *P. palmivora* in the spear leaf of coconut

Sequence	Treatments	Lesion area in cm2 after	
		5 days	10 days
A (Simultaneous)	*Andrographis paniculata*	1.3	2.1
	Lawsonia inermis	1.5	2.7
	Hyptis suaveolens	4.2	6.8
	Datura metel	4.6	8.5
	1 % Bordeaux mixture	1.2	1.6
B (Pp first)	*Andrographis paniculata*	1.7	2.8
	Lawsonia inermis	2.4	3.6
	Hyptis suaveolens	5.5	7.8
	Datura metel	5.6	9.1
	1 % Bordeaux mixture	1.6	1.9
C (Plant extract first)	*Andrographis paniculata*	0.2	1.4
	Lawsonia inermis	0.8	2.1
	Hyptis suaveolens	3.4	7.4
	Datura metel	4.4	8.1
	1 % Bordeaux mixture	0.1	1.2
	Control	13.9	26.4

CV% = 5.05

CD (P = 0.05) for sequence = 0.09

CD (P = 0.05) for treatments = 0.16

CD (P = 0.05) for time intervals =0.08

CD (P = 0.05) for sequence x treatments = 0.29

CD (P = 0.05) for treatments x time intervals =0.23

Our result indicates that these plant extracts can be developed as yet another component for the coconut bud rot disease management.

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