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Emerging trends in the management of bud rot disease of coconut

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Abstract

In recent times there has been a resurgence of bud rot incidence more or less everywhere in the coconut-growing zones. Though application of Bordeaux mixture is very effective, there are practical difficulties in its implementation. These reasons necessitate the formulation of an alternative IDM strategy for bud rot management. For bud rot management to be effective, a sound forecasting model(s) is an essential one. Considering this, attempts were made to develop forecasting models, based on macro and micro temperature, relative humidity (in plains and hilly areas), and rate of survival of the inoculum in endemic areas during off-season. As an alternative to Bordeaux mixture, systemic fungicides were evaluated; Aureofungin – sol (36.4 g / palm) followed by Calixin (21 ml / palm) as stem injection was found effective. The protection offered against bud rot pathogen by these systemic chemicals extended up to 8 weeks, thus safeguarding the palms during vulnerable period. No residues could be detected in nut water and kernal up to six months after application in the case of Calixin. Hence, the systemics can be administered in advance, well before the onset of the monsoon and there is no need to climb the tree since the chemical can be administered from the ground. An endophytic bacterium (*Bacillus amyloliquefaciens*), a fungus (*Trichoderma harzianum*) and botanicals (*Andrographis paniculata* and *Lawsonia inermis*) were found very effective against *P. palmivora*. As these botanicals are not compatible with the antagonist, *T. harzianum* sequential application of botanicals and *T. harzianum* by maintaining a suitable interval between the applications is suggested. With the use of standardized *ex vivo* varietal screening technique, a tall cultivar, Laccadive Ordinary (LO) and a dwarf cultivar, Ganga Bondam (GB) were identified as bud rot tolerant varieties. Formulating an IDM strategy combining all the above practices, *viz.*, planting resistant / tolerant varieties, bud rot forecasting based on weather parameters, application of systemic fungicides as prophylactic and application of antagonists (bacterium and fungus) and botanicals for long term sustenance of disease suppression is discussed in this paper.

Key words: Bud rot, *Phytophthora palmivora*, integrated disease management (IDM), disease forecasting, systemic fungicides, antagonistic organisms, biological control

Introduction

Bud rot, (*Phytophthora palmivora* Butler), a fatal disease of the coconut palm *Cocos nucifera* L. of all ages, commonly occurs in almost all coconut growing countries, especially in the humid regions. The disease is widely prevalent in the East and West coasts of India (Menon and Pandalai, 1958). An incidence of 0.1 to 6.5 per cent in Kerala and 0.4 to 6.7 per cent in Tamil Nadu has been reported by Radha and Joseph (1974).

In India the disease does not assume epiphytotic proportions though in some years certain pockets suffer severely. According to Renard and Darwis (1993) there has been a resurgence of the disease incidence more or less everywhere in the coconut-growing zone in recent times. In certain regions in the Ivory Coast, up to 50 per cent of the coconuts initially planted are killed by bud rot, and a 20 to 25 per cent nut fall can be recorded each year. Extent of damage depends on climatic conditions and type of planting material (Quillec *et al.*, 1984.).

The first symptom of the disease is yellowing and withering of the unopened tender leaf or spindle caused by rotting of the base of the spindle. This leads to rotting of the central bud, resulting in the death of the palm. Temperature range of 21-24° C and relative humidity of 98-100 per cent are congenial for the disease development and such 'favourable days' determined the disease

incidence and intensity (Radha and Joseph, 1974; Joseph and Radha, 1975).

The causal agent of bud rot is *Phytophthora palmivora* Butler. Two dominant *Phytophthora* species viz., *P. palmivora* in Indonesia and the Philippines and *P. katsurae* Ko & Chang in the Ivory Coast have been reported.

Though application of 1% Bordeaux mixture as prophylactic is very effective, there are difficulties in practicing the same. Since the disease occurs during the rainy season, the chemical application in the crown is difficult, as it involves climbing the trees during inclement weather and also there is only a short span of time available to undertake the spraying operations to cover extensive areas. Bordeaux mixture toxicity causes blisters on nuts and other tissues of dwarf palms. These reasons necessitated the formulation of an alternative IDM strategy for bud rot management - combining application of systemic fungicides as prophylactic, application of antagonist(s) and botanicals for long-term sustenance of disease suppression - and evaluation of forecasting models to predict disease outbreak. The results of various experiments conducted to achieve this end has been consolidated and presented in this paper.

A. Studies on the epidemiology - the inoculum hibernation, buildup and dissemination mechanism of bud rot pathogen

Survey was undertaken to study the weather parameters that influence inoculum buildup and agents of transmission. Survey undertaken in Kasaragod, Kannur, Kozhikode, Thrissur, Malappuram, Palakkad, Ernakulam and Wynad districts (Kerala State), revealed a significant variation in bud rot incidence with respect to the location of the garden. Bud rot incidence was higher in hilly tracts (> 300 M above MSL) as compared to plains. In these places, more than 50% of the gardens in certain localities suffered heavy losses due to the disease. In a few gardens in these areas more than 60% of the palms were found destroyed by bud rot and the disease was found to be spreading to neighbouring gardens during the successive years (data not presented). Observations recorded on the disease incidence in the hilly tracts (300 M- 1350 M above MSL) and plain areas (150 M - 300 M above MSL) of Kozhikode, Kannur and Kasaragod districts showed that that the proportion of the diseased palms high in the hilly tracts compared to that of plains (Table 1) (Rasmi, 2003).

Dantre *et al.*, (1997) reported that bud rot incidence was high in the plateau region than in the plains of eastern Madhya Pradesh, a non- traditional coconut growing area.

Bud rot was found to occur during the monsoon season when there is high humidity and low temperature in the atmosphere. In the plains, the disease incidence was recorded only up to the month of September whereas in the hilly areas of Kozhikode, Kannur and Kasaragod districts the disease continued to occur up to the month of January. Initial incidence of bud rot was always dependent upon the monsoon showers. However, its occurrence in the hilly tracts in subsequent months i.e., from October - January, could be attributed to the favourable microclimatic conditions inside the crown with consistent high humidity, low night temperature and the presence of water droplets from dew. Bambawale *et al.*, (1991) reported the occurrence of late blight of potato (*Phytophthora infestans*), even in the absence of rain, where dew appeared to be the alternative source of moisture.

Table 1. Proportion of diseased palms in plains and hilly tracts in different districts

Location	Plains (< 300M MSL)	Hilly tracts (> 300M MSL)	Z value
Kasaragod	0.0060	0.0699	15.78*
Kozhikode	0.0054	0.0644	18.23*
Kannur	0.0039	0.0763	22.78*
Pooled data	0.0049	0.0709	33.00*

B. Survival of the pathogen

To find out the status of moisture content of the crown debris of the infected palms in different locations, samples were collected from Kasaragod, Mandapam (Kasaragod Dist) and Josegiri (Kannur Dist) during April, July, October and January. The survival of propagules was found to be higher in diseased palms than in the healthy. In endemic areas, Mandapam, Josegiri, Udayagiri (Kannur Dist), and Kuttiyadi, (Kozhikode Dist) where bud rot incidence was found to be increasing year after year, the survival percentage of *Phytophthora* propagules was very high (Table 2). The wet samples, which were collected from the axils of the leaves, readily yielded *Phytophthora* without any moistening, thus reveal the survival of the active inoculum in the wet organic matter. The dry samples yielded *Phytophthora* only after moistening with sterile water and incubation at 21± 1Ú C for 6 - 12 days (Rasmi, 2003).

Present study showed that *Phytophthora* propagules were surviving in the axils of diseased and healthy palms even during the off-season. *Phytophthora* infects the soft spear leaf of coconut and the close proximity of spear leaf with leaf axils gives a strong clue that the presence of inoculum in the leaf axils is a crucial

factor in the initiation of infection in the spear leaf. This also points towards the importance of crown cleaning for reducing the source of inoculum.

Table 2. Survival of *P. palmivora* in the crown of coconut palms

Location	Healthy palms		Bud rot infected palms	
	Total no. of samples observed	% isolations obtained	Total no. of samples observed	% isolations obtained
CPCRI, Kasaragod (Kerala)	50	18 (9)*	5	40 (2)
CPCRI-RC, Kidu, (South Kannara Dist, Karnataka)	50	16 (8)	10	80 (8)
Mandapam, Kasaragod (Kerala)	50	42 (21)	20	90 (18)
Josegiri (Kannur Dist, Kerala)	50	38 (19)	20	95 (19)
Udayagiri (Kannur Dist, Kerala)	50	30 (15)	20	90 (18)
Kuttiyadi (Kozhikode Dist, Kerala)	50	24 (12)	20	80 (16)

** Figures in parentheses are number of palms from isolations obtained*

The per cent moisture content of crown debris collected from different places during April, July, October and January revealed that differences due to places, months, and their interactions are highly significant. On an overall basis, the samples from Mandapam was found to have highest moisture content closely followed by those from Josegiri, whereas Kasaragod samples recorded the lowest moisture content (Table 3) (Rasmi, 2003). As regards season, the minimum was recorded in April (pre-monsoon period) and July (rainy season) had the highest. It was also seen that during April, October and January, Kasaragod samples had significantly lower moisture content than those both at Mandapam and Josegiri, while in July the moisture content in all places was on par. Due to cooler temperature at high altitudes and also dense vegetation, condensation was quite high in endemic plots situated in hilly areas even during the post monsoon season. Relative humidity in the endemic plots is highly suitable for the survival of the pathogen in the crown. This also proves why the highest rates of isolation were obtained from the crown debris in endemic plots and also the reason for the occurrence of fresh disease incidence in the endemic plots in hilly areas during post monsoon period even long after the cessation of rain.

Results of the *in vitro* experiment on the effect temperature and moisture levels on the survival of *Phytophthora* in the crown debris indicated that the temperature and moisture had a profound effect on the rate of survival of *Phytophthora*. The maximum colony forming units (cfu) were found at 100% moisture level and the minimum was at 20 %. As regards to temperature, the maximum number of cfu was recorded at 20°C. Frequency of cfu at 20ÚC was significantly higher than that recorded at 10°C and 35°C. This may be due to the loss of viability of *Phytophthora* propagules at very high and very low temperatures. Radha and Joseph (1976) observed that temperature range of 21 - 24°C and relative humidity of 98 - 100 % were found to be optimum for the multiplication and development of the bud rot pathogen. Opoku and Wheeler (1998) reported that *P. palmivora* and *P. megakarya* persisted in association with roots of cacao for at least six months. However, the recovery of both fungi from these sources declined with time; maximum number was observed at the first month and then there has been a significant gradual decline in recovery in subsequent months. This experiment proved that *P. palmivora* could survive in the crown debris for more than six months in the hilly areas where the macro and microclimate are congenial for the survival. One of the reasons behind the higher rate of disease incidence in hilly areas is due to the abundance and availability of viable inoculum in the crown before the monsoon season.

C. Trapping *Phytophthora* propagules from rainwater

Study of disseminating agents of the pathogen from initial source of inoculum assumes a lot of importance in the epidemiology. Hence to study the role of rainwater in disseminating the bud rot pathogen, rainwater was collected in trays fitted to poles at different heights (at 15 cm and 35 cm above ground level and at base of the crown) in the gardens.

Table 3. Moisture content in the crown debris of coconut palms in different months of the year

Location	Moisture content in the crown debris during different months*				
	April	July	October	January	Mean
Mandapam	26.2 (20.0)	62.3 (77.3)	51.0 (60.4)	44.6 (49.3)	46.0
Kasaragod	19.0 (11.3)	60.7 (75.6)	40.8 (42.9)	29.9 (24.9)	37.7
Mandapam	26.2 (20.0)	62.3 (77.3)	51.0 (60.4)	44.6 (49.3)	46.0
Josegiri	27.2 (21.4)	61.6 (76.4)	49.9 (58.5)	43.6 (47.5)	45.0
Mean	24.2	61.5	47.2	39.3	

** Figures in parentheses are the actual per cent values*

Trapping *Phytophthora* propagules from the rainwater collected from the trays fitted to bamboo poles in the garden was higher for the trays fitted at the base of the crown than for the trays fitted to the poles at 15 cm and 35 cm above the ground (Table 4). This shows that the inoculum present in the crown was transported as far as the tray at the base of the crown through rainwater. Statistical analysis revealed that the differences due to location, disease status and sampling height were found to be significant for trapping of *Phytophthora* propagules from rainwater. Rainwater samples collected from Mandapam were found to contain significantly higher propagules than that of CPCRI samples. Rainwater from diseased palms had significantly higher trapping than from the healthy. At 35 cm the trapping was minimum. Higher number of *Phytophthora* propagules was isolated from the trays fitted above 15 cm from the ground. This may be due to the rainwater splashing which has projected soil particles and sometimes soil-borne *Phytophthora* propagules to the tray. At Mandapam, it was also possible to trap *Phytophthora* propagules from healthy trees, from tray fitted to the crown and also 15 cm above the ground. However, the percentage of isolation was high for the trays fitted to diseased palms compared to healthy palms. This indicates that rain water acts as carrier for the infectious propagules and plays an important role in the spread of the disease (Thevenin, 1992; Brahmana *et al.*, 1992).

Table 4. Trapping *P. palmivora* propagules in rain water

Location	Diseased palm			Healthy palm		
	15 cm above ground level	35 cm above ground level	Base of the crown	15 cm above ground	35 cm above ground level	Base of the crown level
Kasaragod	3.69 (2.0)*	0.00 (0.0)	5.31 (4.0)	0.00 (0.0)	0.00 (0.0)	3.68 (2.0)
Mandapam	11.06 (6.0)	3.68 (2.0)	25.10 (22.0)	3.68 (2.0)	0.00 (0.0)	7.37 (4.0)

** Figures in parentheses are actual percentage*

D. Interrelationship between bud rot incidence and macro and microclimate:

Phytophthora infection on coconut occurs when the relative humidity is higher than 94 per cent and the temperature lower than 24 °C. in open spaces it is hard too find humidity higher than 94 percent (Darwis, 1992). Usually the agroclimate data are recorded at the open air stations. This study of the air humidity in the crown aids in understanding better the microclimatic conditions, which favours the disease incidence and further development. With very limited solar radiation reaching under the canopy and large amounts of evaporation from broad leaf cover crops releasing water vapour in the atmosphere can create ideal conditions for fungal development. Macro and micro humidity and temperature of three plots recorded over period of two years revealed that the microclimate in the crown is directly influenced by the macroclimate in the field. In all the fields studied. the micro humidity was high and temperature was low when it was compared with that of the ambient. The mean macro and micro humidity and temperature recorded in the two endemic plots (Mandapam and Josegiri) showed variation compared with that of Bowikanam (plains) with sparse disease.

Study on the interrelation between temperature, macro, micro – humidity and disease incidence in Mandapam, Josegiri, and Bowikanam revealed that there is a high degree of correlation between disease incidence, micro and macro – humidity in Mandapam and Josegiri. However, in the case of Bowikanam the correlation was not significant. Disease incidence was highly correlated with the macro and micro humidity of the same month and also that of the previous two months.

E. Evolving an equation for forecasting bud rot disease in endemic locations:

Forecasting systems based on weather parameters can result in more efficient management of resources. Farmers can take up prophylactic measures and thereby save their crop from this lethal disease. Forecasting can also decrease the risk of heavy crop loss, since additional control measures can be taken up if the forecast indicates the probability of the disease acquiring epidemic proposition within a reasonable time.

Efforts were made to develop an equation to forecast disease incidence in endemic areas (Mandapam and Josegiri) by using relative humidity level of the previous months. Regression equation ($Y = ae^{bx}$) was fitted for forecasting the number of disease palms in each location in different months (July, August, September, October, November, December and January) the compound growth rate showed that, it was higher for the locations 300 - 1350 M above MSL (Mandapam, Josegiri, Udayagiri and Kuttiyadi) when compared to Bovikanam, which is 150 - 300 M above MSL. Out of the models studied (same month, after 1, 2, 3, 4, 5, and 6 months), the one using both macro and micro - humidities of previous moth and another using the same variables of the previous two months, gave the prediction equation with high R^2 at Mandapam. This suggests that *Phytophthora* inoculum development was intense during this wet period and the incubation period was the time taken by the fungus for penetration followed by its

development inside the coconut crown up to the bud and the appearance of the first external symptoms.

The forecasting equations for Mandapam and Josegiri plots:

Location		Y (percent disease incidence of a particular month)	R^2
A.	**Mandapam (300 - 1350 M above MSL)**		
i.	Based on the average relative humidity of the previous month	$(82.2) - 2.764\ RH_{MACRO} + 2.065\ RH_{MICRO}$	0.756
ii.	Based on the average relative humidity of the previous two month	$(72.455) - 4.315\ RH_{MACRO} + 3.699 RH_{MICRO}$	0.759
B.	**Josegiri (300 - 1350 M above MSL)**		
i.	Based on the average relative humidity of the previous month	$(36.5) - RH_{MACRO} + 0.2578\ RH_{MICRO}$	0.623
ii.	Based on the average relative month humidity of the previous two	$(37.9) - 0.2629 RH_{MACRO} + 3.699 RH_{MICRO}$	0.634

The number of palms with bud rot incidence in different locations was estimated using the regression equation. It is clear from the table 5 that the difference between the predicted and actual disease incidence has been insignificant. Rillo and Ploma (1989) found that in the Philippines, higher incidence of *Phytophthora* was always predicted by high rainfall during the previous months. From these studies, it is clear that the microclimate is directly influenced by the macroclimate and the equation based on this model has a distinct advantage that the onset of the disease can be predicted (by using either of the two equations) with reasonable accuracy.

Table 5. Predicted and observed values for bud rot incidence in different locations

Location October	November	July December	August January	September
Mandapam		57.35	65.45	65.85
70.56	75.61	81.02	86.81	
(300 - 1350 M		(55.0)*	(58.0)	(62.0)
(68.0)	(73.0)	(79.0)	(84.0)	
above MSL)				
Josegiri		31.02	33.55	36.28
39.24	42.44	45.89	49.63	
(300 - 1350 M		(26.0)	(28.0)	(35.0)
(38.0)	(39.0)	(42.0)	(46.0)	
above MSL)				
Udayagiri		26.87	28.55	30.35
32.25	34.28	36.44	38.72	(300 - 1350 M
	(23.0)	(25.0)	(28.0)	(31.0)
(34.0)	(35.0)	(36.0)		
above MSL)				
Kuttiyadi		22.97	24.31	25.72
27.2	28.78	30.45	32.21	(300 - 1350 M
	(22.0)	(22.0)	(23.0)	(26.0)
(27.0)	(30.0)	(31.0)		
above MSL)				
Bowikanam		4.33	4.5	4.69
4.88	5.07	5.28	5.49	
(150 - 300 M		(4.0)	(5.0)	(6.0)
(6.0)	(6.0)	(6.0)	(6.0)	
above MSL)				

** Figures in parentheses are observed values*

F. Evaluation of systemic fungicides

To find out an alternative to Bordeaux mixture, systemic fungicides were evaluated for the management of bud rot since dwarf coconut palms are sensitive to copper and also spraying the crown with Bordeaux mixture is labour intensive and difficult during rainy monsoon.

Three systemic fungicides, Akomin (Phosphonic acid), 16.8 ml/palm; Aliete (Fosetyl-Al 80% WP) 21g/palm, Aureofungin - sol (46.5%) 36.4 g/palm and Calixin (Tridemorph 80 %), 21ml/palm were administrated to west Coast Tall (WCT) palms (30 - 35 yr) through root (feeding) or stem (injection using tree injectors).

Tissues from the spear leaf base (3 palms / sampling) were collected at 1, 2, 7, 14, 21, 28 and 90 days after the imposition of treatments. Surface sterilized tissue bits were challenge inoculated with *P. palmivora* and was observed for signs of *Phytophthora* infection 48 hr after incubation.

Aureofungin - sol followed by Calixin was found to be most effective as stem injection. The fungicides provided protection after first week and were effective up to 8 weeks after application (Iyer, 1999).

In order to study whether any harmful residues are lurking in the edible portions, coconut water and kernal samples of 5 and 10 month-old nuts collected from palms administered with Tridemorph (21 ml of 5% Calixin) as root feeding and stem injection were studied for over six months, taking samples at 0, 1, 2, 15, 30, 90 and 180 days with the help of BASF, Mumbai employing the colorimetric method. The residues were below detectable limit in all the samples. Thus, this measure has a distinct

advantage in that the systemics can be administered well before the on set of monsoon. Protection afforded by the systemics extends to two months, thus safe guarding the palms during the critical period. Moreover, this does away with climbing the trees since the chemical can be administered from ground itself.

G. Biological Control

Biocontrol of disease helps to reduce the use of chemical-based fungicides. They help to reduce the risk of developing pathogen resistance to traditional chemicals. In most cases, they are safer to use.

i. Identification of native antagonistic bacteria

A total of 471 endophytic bacteria, 998 epiphytic and 1028 rhizosphere bacteria were isolated. All were screened against *P. palmivora* in dual culture. Out of these three endophytic bacteria, *B. amyloliquefaciens, B. megaterium* and *B. subtilis* showed more than 35 % inhibition against *P. palmivora.*

All the bacteria produced metabolites in the medium and inhibited the growth of *P. palmivora.* The percent inhibition ranged from 59.4 % to 73.5 %. *B. amyloliquefaciens* was found to exhibit maximum mycelial inhibition (73.5%) (Table 6). Hence a study was undertaken to assess the efficiency of translocation and multiplication of naturally occurring rifampicin-resistant isolate of *B. amyloliquefaciens* introduced into the plant system by drenching and root feeding. Spindle leaf samples were collected at interval of 1, 3, 6, 24, 48, 72 and 96 hr after the inoculation and plated on nutrient agar (NA) medium, as well as in the rifampicin – nutrient agar (RNA) medium simultaneously. Of the two methods tried, drenching was found to be a better method of introducing the bacterium. Bacterium was taken up to the tip of the spindle leaves one hour after inoculation.

Table 6. ***In vitro*** **inhibition of** ***P. palmivora*** **by different antagonistic bacteria by dual culture after 96 hours of incubation**

Test bacteria	Growth of *P. palmivora* (mm) and % inhibition		
	Towards opposing bacteria	Towards periphery	% inhibition
Bacillus macerans	20.0	46.0	56.5
Bacillus sp.	17.5	46.0	61.9
B. amyloliquefaciens	15.0	46.0	65.2
Bacillus sp.	21.5	46.0	53.2
B. subtilis	19.0	46.0	58.6
Actinobacillus lignieresii.	25.0	46.0	45.7
Bacillus sp.	20.0	46.0	56.5
Bacillus sp.	19.5	46.0	57.6
Kebsilla terragena	28.0	46.0	39.1
B. megatarium	17.5	46.0	61.9
Bacillus sp.	19.0	46.0	58.6
Bacillus sp.	25.0	46.0	45.7
Bacillus sp.	24.0	46.0	47.8
Bacillus sp.	19.0	46.0	58.6
Mean	20.7	46.0	54.8

The population of *B. amyloliquefaciens* started increasing from 1hr and continued up to 48 hr, to reach 54.4 x 10^{-2} cfu / gm tissue as against the background population of 4.7 x 10^{-2} cfu / gm tissue, *i.e.*, nearly a ten-fold increase in population (Table 7). However, the population started declining from then onwards slowly up to a time lapse of 72 hr and after that registered a sharp decline, reaching 5.3 x 10^{-2} cfu / gm tissue at 96 hr, a level similar to that at 1hr. In the case of root feeding also a similar situation was noticed.

Table 7. Population of bacteria, ***Bacillus amyloliquefaciens*** **in seedlings after inoculation**

Hour (s) after inoculation	Number of bacterial colonies (x 10^{-2})/gm of leaf tissue*			
	Drenching		Rot feeding	
	NA	RNA	NA	RNA
1	5.7	5.1	6.3	5.6
3	6.8	7.0	8.6	8.3
6	11.9	10.5	10.2	10.6
24	51.1	46.0	42.5	41.9
48	60.1	54.4	39.1	38.6
72	54.6	47.0	44.7	27.3
96	43.2	5.30	18.1	5.6

**Observations average of ten seedlings*
NA - Nutrient Agar *RNA – Rifampicin Nutrient Agar*

Another approach was therefore to deliver the endophyte at the actual site of infection. This was effected by keeping sachets containing *B. amyloliquefaciens,* in maroti cake as a carrier, in the top axils. The bacterium was found to survive up to seven months in the axils (Fig. 1). The advantages of maroti cake, as carrier is that it is an insect repellant too (unpublished).

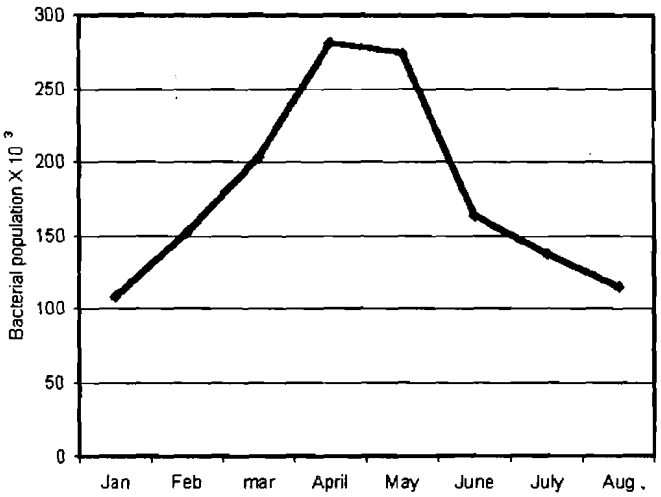

Fig. 1 Survival of Bacillus amyloliquefaciens on axil filling

ii. Identification of fungal antagonists

Similarly a number of fungi exhibiting antagonism to *P. palmivora* were isolated from the rhizospere soil of endemic areas. These were tested under pot culture and under field conditions in sick nursery soil. Soil samples

were collected air dried and used for isolation of fungi by dilution plating. *Trichoderma* selective medium (TSM) and the Potato Dextrose Agar (PDA) were used for the isolation of the fungi.

The fungi obtained were purified were identified based on reference books 'A revision of the genus *Trichoderma*', by Rifai (1969) and Compendium of soil fungi by Domasch *et al.*, (1981). Confirmation of the fungal identification was done with the help of ITCC (Indian Type Culture Collection) IARI New Delhi.

For the rapid screening of the test fungi against the pathogen and to select potential antagonists, dual culture method was adopted. Radial growth of *Phytophthora* isolate was measured towards test fungi at 48 and 72 hr interval. Growth inhibition was calculated based on the growth of *Phytophthora* isolate (Pp21) in the control plate. Among the 17 native fungal antagonists, *T. harzianum* (isolates Th1) and *T. viride* (isolate Tv3), showed maximum inhibition of 68.5% and 75.9% respectively (Table 8).

Table 8. *In vitro* evaluation of fungi antagonistic to *P. palmivora*

Isolates	Inhibition % after 48 h	72 h	Mean
T. harzianum (Th1)	56.00	68.58	62.24
T.harzianum (Th2)	47.54	63.15	55.54
T.harzianum (Th3)	52.79	65.47	59.03
T.harzianum (Th4)	41.95	57.15	49.55
T.harzianum (Th5)	51.79	66.18	58.19
T.harzianum (Th6)	45.78	62.71	54.25
T.harzianum (Th7)	55.44	66.59	61.01
T.harzianum (Th8)	41.63	58.48	50.05
T.harzianum (Th9)	45.91	61.29	53.05
Mean	**48.90**	**63.26**	
T.viride (Tv1)	63.17	74.52	68.84
T.viride (Tv2)	62.04	72.87	67.40
T.viride (Tv3)	65.80	75.85	70.82
Mean	**63.67**	**74.38**	
T. hamatum (Tham1)	54.99	66.69	60.84
Penicillium corylophylum (Pc1)	53.11	61.44	57.71
T. aureoviride (Tav1)	43.83	60.73	52.28
Aspergillus flavus (Af1)	42.47	57.16	50.21
Paecilomyces flavescens (Pf1)	40.73	54.70	47.71

Under pot culture *T. harzianum* recorded maximum cfu (363.9 x 10-6 cfu / g soil), compared to other fungi tested. The population of fungi was significantly influenced by the duration of growing period (Table 9). It is found that *T. harzianum* is highly adapted to survival in soil, compared to other antagonistic fungi tested. This is a highly desirable character for a biocontrol agent to be successful one. Even though *T. viride* proved to be a better antagonist under *in vitro* conditions, its poor adaptability to soil environs relegates it to an inferior position in comparison with that of *T. harzianum* for field use.

Table 9. Interaction of antagonistic fungi with *P. palmivora* in soil (Pot experiment) - Population of antagonistic fungi in soil

Treatments	Population of antagonistic fungi in soil x 10^{-6} Initial	15 d	30 d	Mean
T. hamatum (5 g) + 50g neem cake + 5 g rice bran	83.39	76.070	65.28	74.91
T. hamatum (10g) + 50 g neem cake + 5 g rice bran	163.37	148.24	118.31	143.30
T. viride (5 g) + 50 g neem cake + 5 g rice bran	95.48	85.18	54.27	78.31
T. viride (10g) + 50 g neem cake + 5 g rice bran	125.56	106.98	85.53	106.69
T. auroviride (5 g) + 50g neem cake + 5 g rice bran	85.30	74.93	63.70	74.64
T. auroviride (10g) + 50g neem cake + 5 g rice bran	100.06	82.30	75.30	85.88
T. harzianum (5 g) + 50 g neem cake + 5 g rice bran	255.84	251.41	147.93	218.39
T. harzianum (10g) + 50 g neem cake + 5 g rice bran	373.34	362.59	355.64	363.86
A. flavus (5 g) + 50 g neem cake + 5 g rice bran	72.64	62.10	55.23	63.32
A. flavus (10g) + 50 g neem cake + 5 g rice bran	117.92	104.10	96.10	106.04
P. corylophylum (5 g) + 50 g neem cake + 5 g rice bran	73.62	56.29	45.85	58.59
P. corylophylum (10g) + 50 g neem cake + 5 g rice bran	95.32	84.18	66.74	82.08
P. palmivora + 50 g neem cake + 5 g rice bran	0.22	0.91	0.69	0.61
P. palmivora + 1 kg soil	0.22	0.22	0.22	0.22
Mean	117.31	106.82	88.05	

The ability of *T. harzianum* to suppress the pathogen is inversely proportional to the population of *T. harzianum* in soil. This means that the inhibitory effect of *T. harzianum* is felt only when there is adequate population in soil (Table 10). The reduction in the population of antagonistic fungi was due to the depletion of carrier materials in soil. Adding organic amendments like neem cake into soil can possibly change this situation.

Table 10. Reduction in the population of *P. palmivora* with antagonistic fungi *T. harzianum* (Th1) in pot experiment

Treatments	Reduction % of *P. palmivora*		
	15 d	30 d	Mean
T. hamatum (5 g) + 50 g neem cake + 5 g rice bran	86.33	84.01	85.17
T. hamatum (10g) + 50 g neem cake + 5 g rice bran	89.97	87.20	88.58
T. viride (5 g) + 50 g neem cake + 5 g rice bran	87.20	74.41	80.80
T. viride (10g) + 50 g neem cake + 5 g rice bran	93.60	79.50	86.55
T. auroviride (5 g) + 50 g neem cake + 5 g rice bran	87.20	74.41	80.80
T. auroviride (10g) + 50 g neem cake + 5 g rice bran	93.60	79.50	86.55
T. harzianum (5 g) + 50 g neem cake + 5 g rice bran	87.20	83.20	85.20
T. harzianum (10g) + 50 g neem cake + 5 g rice bran	93.60	91.20	92.40
A. flavus (5 g) + 50 g neem cake + 5 g rice bran	74.40	62.11	68.25
A. flavus (10g) + 50 g neem cake + 5 g rice bran	80.81	72.67	76.74
P. corylophylum (5 g) + 50 g neem cake + 5 g rice bran	74.41	48.44	61.42
P. corylophylum (10g) + 50 g neem cake + 5 g rice bran	80.81	68.94	74.87
P. palmivora + 50 g neem cake + 5 g rice bran	36.44	25.87	31.15
Mean	81.96	71.80	76.80

In bud rot disease management trial in seedlings, disease incidence was less in treatments where aerial spraying of 1% Bordeaux mixture was combined with soil application of *Trichoderma* isolate Th1 (Table 11). In Brazil, application of *Trichoderma* to control *P. cactorum* on apple significantly reduced mortality of the tree after natural or artificial inoculation (Valdebenito Sanhueza, 1987). In glass house trials root rot of apple (*P. cactorum*) was significantly reduced in pots treated with *T. koningii* and *T. harzianum* (Alexander and Stewart, 2001). This underlines the importance of suitably combining more than one method for disease control. Here the combined effect was significantly superior (synergetic) to that of the individual effects. *T. harzianum* sprayed alone or in combination with a commercial fungicide (Dichlofluanid) was reported to control natural infections of *Botrytis cinerea* on apple (Tronsmo and Ystaas, 1980).

Table 11. Disease incidence in seedling plot after the application of *T. harzianum* (Th1)

Treatments	Total seedlings	Diseased	Healthy
Trichoderma soil application	75	6	69
1% Bordeaux mixture spraying	75	4	71
Trichoderma soil application + 1% Bordeaux mixture spraying	75	2	73
Control (with out any treatment)	75	14	61

Multiple interactions involving bacteria and fungi in the rhizosphere are shown to provide enhanced biocontrol in many cases in comparison with biocontrol agents used singly (Whipps, 2003). This points towards a control measure where we can use both antagonistic fungi and bacteria to control bud rot disease of coconut

J. Effect of plant extracts on *Phytophthora*

In tropical country like India where there is rich source of plant diversity available, it is possible to find, many of the plant species that posses the ability to suppress plant pathogens. Hence, some of the commonly available plants were tested for their ability to suppress *P. palmivora*.

Aqueous leaf extracts of *Andrographis paniculata* ('Kiriath'), *Lawsonia inermis* and *Hyptis suaveolens* showed greater inhibition compared to others (Table 12). *Andrographis paniculata* leaf extract showed 89.8 (100.0) % inhibition after 24, 48 and 72 hr incubation. *Lawsonia inermis* leaf extract showed 89.8 (100.0), 68.6 (86.7), 59.6 (74.4) and 59.6 (74.4) % inhibition after 24, 48 and 72 hr respectively. *Hyptis suaveolens* leaf extract showed 89.8 (100.0), 60.0 (75.0), 57.4 (70.9) and 69.0 (82.0) % inhibition after 24, 48 and 72 hr respectively.

P. palmivora is a wet weather pathogen and maximum build up of the population occurs during monsoon season. For the control of this pythiaceous fungus, solubility of the chemical in the water is an added advantage, since free water is required to complete the life cycle of these organisms. The water-soluble nature of the toxic principle present in *Andrographis paniculata, Lawsonia inermis* and *Hyptis suaveolens* is ideal for developing them into botanical pesticides. In the present study, aqueous extracts were tested because this is a farmer friendly procedure compared to the use of solvents or hot water, which can prove to be expensive. As these

plants are present abundantly in Kerala, and their availability does not pose a problem as far as the farmers are concerned. Since the aqueous extracts of these plants were found to affect the vegetative phase of the *P. palmivora*, further efforts are needed to identify and characterize the active principle. Chemical characterization of the compounds present in their extracts may further help their large-scale synthesis / production for widespread use in bud rot management.

Table 12. Evaluation of different plant extracts against *Phytophthora*

Plant extracts	Per cent inhibition			
	24 hrs.	48hrs.	72hrs.	Mean
Adathoda vasica	18.4 (10.0) *	18.4 (10.0)	14.9 (6.6)	17.2 (8.8)
Andrographis paniculata	89.7 (100)	89.7 (100)	89.7 (100)	89.7 (100)
Azadirachta indica	23.8 (16.6)	16.5 (8.33)	9.7 (2.8)	16.7 (9.3)
Biophytum reinwarditi	18.4 (10.0)	12.9 (5.0)	10.5 (3.3)	13.9 (6.6)
Bougainvillea spectabilis	18.4 (10.0)	26.5 (20.0)	33.1 (30.0)	26.0 (20.0)
Calotropis indica	26.5 (20.0)	29.9 (25.0)	31.0 (26.6)	29.2 (23.8)
Cardiospermum halicacabum	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Cassia marginata	26.5 (20.0)	29.3 (24.1)	38.4 (38.7)	31.4 (27.6)
Clerodendron infortunatum	12.9 (5.0)	12.9 (5.0)	14.9 (6.6)	13.5 (5.5)
Cympopogus citratus	33.1 (30.0)	29.9 (25.0)	26.5 (24.6)	28.8 (26.5)
Cynodon dactylon	19.2 (11.6)	16.0 (7.8)	13.1 (5.5)	16.1 (8.2)
Cyperus rotundus	26.5 (20.0)	26.5 (20.0)	28.6 (23.0)	27.2 (21.0)
Datura fastuosa	0.0 (0.0)	0.0 (5.0)	0.0 (6.6)	0.00 (3.8)
Datura metel	71.5 (90.0)	56.7 (70.0)	50.7 (60.0)	59.6 (73.3)
Eclipta alba	18.4 (10.0)	18.4 (10.0)	18.4 (10.0)	18.4 (10.0)
Eupatorium odoratum	19.8 (11.6)	22.3 (14.5)	31.0 (26.5)	24.4 (17.5)
Gloriosa superba	29.9 (25.0)	29.9 (25.0)	26.5 (20.0)	28.8 (23.3)
Glycosmis pentaphylla	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Heliotropium scabrum	18.4 (10.0)	10.3 (3.33)	11.3 (3.8)	13.3 (5.7)
Hemidesmus indicus	18.4 (10.0)	12.9 (5.0)	10.5 (3.3)	13.9 (6.6)
Hyptis suaveolens	89.7 (100)	59.9 (75.0)	57.3 (70.8)	69.0 (81.9)
Jatropha glandilifera	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Lantana camara	16.5 (8.3)	25.0 (18.0)	31.2 (27.0)	24.3 (17.7)
Lawsonia inermis	89.7 (100)	68.6 (86.6)	59.6 (74.4)	72.6 (87.0)
Leucas aspera	16.5 (8.8)	12.9 (5.0)	10.5 (3.3)	13.3 (5.5)
Nerium odoratum	18.4 (10.0)	12.9 (5.0)	3.5 (1.1)	11.6 (5.3)
Ocimum canum	26.5 (20.0)	22.7 (15.0)	21.4 (13.3)	23.5 16.1)
Ocimum sanctum	26.5 (20.0)	26.5 (20.0)	29.9 (25.0)	27.6 (21.6)
Phyllanthus niruri	4.3 (1.6)	8.5 (2.3)	9.5 (2.7)	7.4 (2.2)
Physalis minima	26.5 (20.0)	22.7 (15.0)	18.4 (10.0)	22.5 (15.0)
Plumbago zeylanica	37.2 (36.6)	41.1 (43.3)	44.3 (48.8)	40.8 (42.9)
Polyalthia longifolia	33.1 (30.0)	39.2 (40.0)	39.2 (40.0)	37.2 (36.6)
Polycarpaea aurea	21.1 (13.3)	11.8 (4.3)	11.9 (4.3)	14.9 (7.3)
Pongamia glabra	26.5 (20.0)	22.7 (15.0)	24.0 (16.6)	24.4 (17.2)
Scoparia dulcis	26.5 (20.0)	38.9 (39.5)	39.2 (40.0)	34.8 (33.1)
Strychnos- nux-vomica	33.1 (30.0)	35.2 (33.3)	38.5 (38.8)	35.6 (34.0)
Tinospora cordifolia	26.5 (20.0)	29.5 (24.3)	38.2 (38.3)	31.4 (27.5)
Tridax pocumbens	0.0 (0.0)	12.9 (5.0)	31.9 (28.0)	14.9 (11.0)
Vernonia cinaria	17.2 (9.0)	14.9 (6.6)	13.1 (5.2)	15.1 (6.9)
Vinca rosea (*Catharanthus roseus*)	26.5 (20.0)	29.3 (24.1)	38.2 (38.3)	31.3 (27.5)

*Figure in parentheses are actual %

K. Compatibility of promising plant extracts with antagonistic fungus *T. harzianum*

Compatibility of effective antagonistic fungus *T. harzianum* (Th1) with the most promising plant extracts *viz. Andrographis paniculata* and *Lawsonia inermis* showed that the plant extracts, which suppress *P. palmivora*, also inhibited the growth of antagonist. *A. paniculata* leaf extract showed 58.1 - 60.8% inhibition and *L. inermis* showed 57.9% - 64.4% inhibition of *T. harzianum*.

These results suggest that we cannot apply *T. harzianum* (Th1) and the promising plant extracts simultaneously in soil to suppress the population of *P. palmivora*. However, sequential application of these two can be done i.e. by maintaining a suitable interval between the application of plant extracts and the promising *Trichoderma* isolate Th1. Addition of organic amendments to the soil, after two - three week intervals after the application of plant extracts also help to increase the native population of antagonists. A higher population density of the antagonists can be sustained in soil by regularly fortifying the soil with organic amendments.

L. Identification of resistant cultivars:

The importance of finding a resistant cultivar in disease management needs no mention. Field testing is not feasible in the case of bud rot since it can lead to spread of the disease and death of test palms. Hence a suitable lab test has been developed where the unopened spindle leaf of the test cultivar is inoculated with *Phytophthora*.

Testing *Phytophthora* isolates on different coconut varieties showed that isolates exhibited significant variation with regards to lesion area on spear leaf pieces. Among the five tall varieties Laccadive Ordinary (LO) showed the least mean lesion area (9.7 cm^2) compared to other tall varieties, West Coast Tall (WCT), Philippine ordinary (PO), Andaman Ordinary (AO) and Tiptur Tall (TT). Among the seven dwarf varieties, Ganga Bondam (GB), Chowghat Green Dwarf (CGD), Chowghat Orange Dwarf (COD), Malayan Green Dwarf (MGD), Malayan Orange Dwarf (MOD) and Malayan Yellow Dwarf (MYD) and the minimum mean lesion area was observed on GB (Table 13). Not only these varieties can be recommended for growing in the endemic areas to create a mosaic of tolerant types within the already existing susceptible local cultivars, but also these tolerant sources can effectively be utilized for incorporating the tolerant genes into high yielders.

Conclusion

In recent times there has been a resurgence of bud rot incidence more or less everywhere in the coconut growing zones. In India bud rot does not assume epiphytotic proportions though in some years certain pockets suffer severely. It is imperative therefore to have proper management strategy.

Table 13. Testing *Phytophthora* isolates on different coconut cultivars

Genotype	Coconut cultivars	Lesion area (cm^2) produced by *Phytophthora*isolates 8 days after inoculation				
		Pp21	Pp19	Pp16	Pp24	Mean
Tall	West Coast Tall (WCT)	24.33	13.54	23.98	15.22	19.27
	Philippine Ordinary (PO)	41.60	28.49	39.18	33.32	35.65
	Andaman Ordinary (AO)	22.32	15.39	19.40	16.37	18.37
	Tiptur Tall (TT)	19.64	15.53	17.47	16.58	17.31
	Laccadive Ordinary (LO)	11.59	7.57	10.23	9.46	9.71
	Mean	23.59	16.10	22.05	21.46	
Dwarf	Ganga bondam (GB)	14.47	10.35	13.32	10.49	12.16
	Chowghat Green Dwarf (CGD)	37.58	17.76	21.40	16.54	23.32
	Chowghat Orange Dwarf (COD)	52.85	36.58	47.92	39.57	44.23
	Malayan Green Dwarf (MGD)	40.52	28.84	35.95	31.18	34.12
	Malayan Orange Dwarf (MOD)	51.32	22.18	38.14	23.99	33.19
	Malayan Yellow Dwarf (MYD)	28.31	18.56	24.30	20.47	22.91
	Mean	37.50	22.37	30.17	23.70	

Bud rot disease is amenable for management measures. Planting resistant / tolerant cultivars and proper spacing are important for the management of the disease. Tolerant varieties are identified both in tall and dwarf cultivars. These varieties can be recommended for growing in the endemic areas. Further, these tolerant sources can effectively be utilized for incorporating the tolerant genes into high yielders. Too close a planting encourages spread of the disease. Wider spacing between palms and between rows favours air movement and a consequent dissipation of excess humidity that may accumulate in the garden. The low lands with generally high humidity are very favourable for the development of the disease, especially when the drainage is poor.

Phytophthora propagules are surviving in the crown debris of both diseased and healthy palms in the off-season. This is a potential inoculum that can serve as the source for the subsequent season. Hence phytosanitation/crown cleaning is strictly advised.

Forecasting systems based on weather parameters can result in more efficient management of resources. Disease incidence in the endemic areas now can be predicted with reasonable accuracy based on the temperature and relative humidity (both macro and micro temperature and humidity levels) at least two months in advance. These models can be further fine tuned to evolve a blanket forecasting system fore endemic areas. This would forewarn farmers to take-up need based plant protection measures. Forecasting can also decrease the risk of heavy crop loss, since additional control measures can be taken up if the forecast indicates the probability of the disease acquiring epidemic proposition within a reasonable time

Control measures are effective only when they are adopted in the initial stage of the disease. Spraying Bordeaux mixture as prophylactic though effective, difficulties are encountered during inclement weather. Spurious copper sulphate often sold in the market and Bordeaux mixture toxicity on nuts and other tissues of dwarf palms often makes it impracticable to adopt this measure satisfactorily. Now there are at least two / three alternative measures for effective management of bud rot. The application of systemic fungicides Aureofungin – sol or Calixin can be applied to protect the palms up to eight weeks after application. Systemic fungicides can be applied as prophylactic well before the onset of monsoon so that they can protect the palms during the critical period.

Biocontrol agents were very effective against *P. palmivora* and can be one of the components of management measure. Sequential application of botanicals and biocontrol agents like *T. harzianum* in combination with antagonistic bacterium (*B. amyloliquefaciens*) with suitable interval can effectively suppress the disease.

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