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BUD ROT DISEASE OF COCONUT

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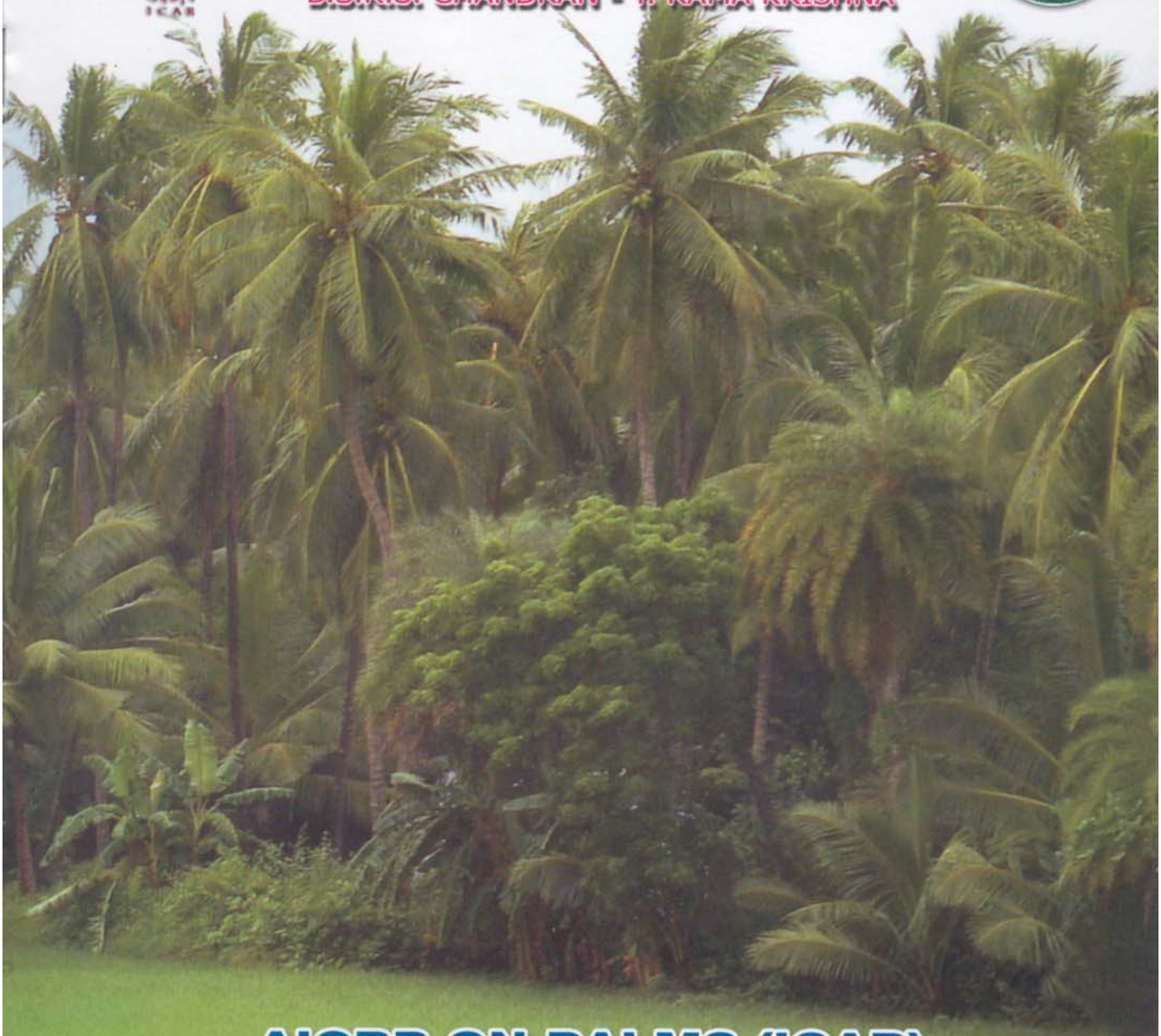

AICRP ON PALMS (ICAR)
ANDHRA PRADESH HORTICULTURAL UNIVERSITY
HORTICULTURAL RESEARCH STATION
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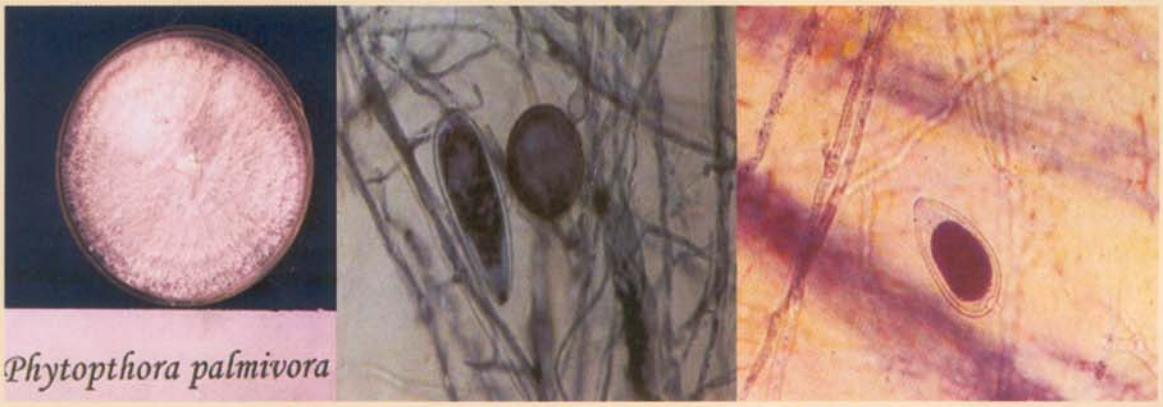
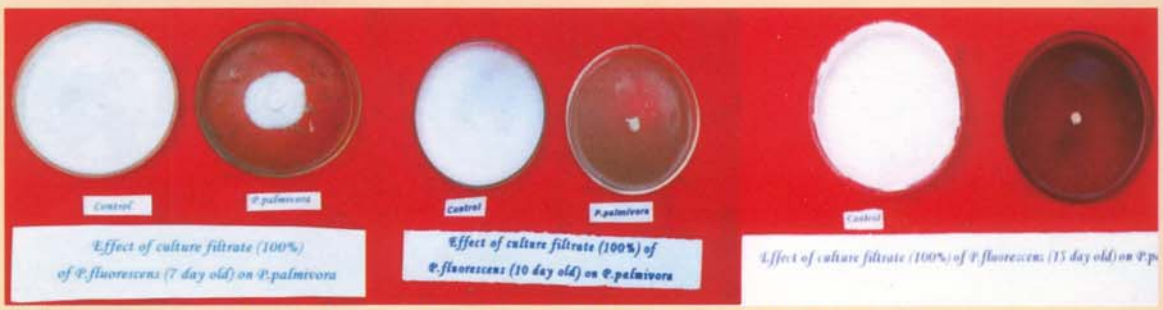


Phytophthora palmivora, causal organism of bud rot

Trichoderma spp on *P. palmivora* | *P. Fluorescens* on P. Palmivora | Trichoderma Spp & *P. Fluorescens* on P. Palmivora

Effect of volatile metabolites *of P. Fluorescens* on P. Palmivora



Effect of non-volatile metabolites *of P. Fluorescens* on P. Palmivora

BUD ROT DISEASE OF COCONUT

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FOREWORD

Coconut, Cocos nucifera L., is deeply rooted in the culture, religion, environment, social status and diet to millions of people of tropics. The coconut palm is cultivated in more than 93 countries allover the world with an area of 12.19 million hectares with an annual production of 61,165 million nuts.India is the third largest producer of coconut in the world, mainly grown in four southern state viz., kerala, Tamil nadu, Karnataka and Andhra Pradesh. Coconut diseases, a major biotic stress, playa great havoc causing considerable reduction in yield level. Among the coconut diseases reported from our country, bud rot caused by Phytophthora palmivora is the severe disease of coconut palm. Palms of all ages 'are susceptible to the disease, but the young palms are more.

Scientists from Central Plantation Crops Research Institute and from the centres of Research Project on Palm located at Ambajipeta [Andhra Pradesh], Arsikere [Karnataka], and Veppankulam [Tamil Nadu] are working on Etiology, Epidemiology and Management strategies for bud rot disease in coconut. Progress achieved by the Horticultural Research Station, Ambajipeta on the biological control of bud rot disease is laudable. The biointensive based disease management package developed against bud rot disease of coconut is a success story and this technology is gaining popularity among the farming community.

There is a need to disseminate the new information generated on bud rot disease management in coconut to researchers, policy makers and extension workers through a simple and comprehensive publication. I congratulate Dr. B. Srinivasulu, Principal Scientist (Plant Pathology) and his team members for their achievements and also for bringing out such an useful technical bulletin.

Date: 07-08-2008
Place: Venkataramannagudem

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FOREWORD

Coconut industry provides sustenance to over 10 million people in India. Though the area under coconut cultivation is expanding in the recent years, from the existing coastal and peninsular regions to North and North-Eastern states in our country, the crop production is limited by various bottlenecks. Of them, bud rot disease is a fatal disease of coconut palm. Palms of all ages are susceptible to the disease, but it is more frequent on young palms.

The research work carried out at AICRP on palms, Ambajipeta Centre on bud rot disease relating to aspects on etiology, epidemiology and biocontrol of the disease has led to new dimensions in the management of the disease. Major research findings from the recent programmes include information on the occurrence of bud rot disease in coconut with relation to soil type, intercrops, age of coconut seedlings and epidemiology, supplemented with useful findings on biological control of the disease through potential biocontrol agents like *Pseudomonas fluorescens* and *Trichoderma viride*. Biocontrol based Integrated Disease Management package for the disease was also evolved.

This technical bulletin from Horticultural Research Station, Ambajipeta provides highly useful information on bud rot disease and its management. I am confident that the bulletin would serve as a guide book for both Researchers and Extension personnel.

I congratulate Dr.B.Srinivasulu, Principal Scientist (Plant pathology) and his team members for their achievements and also for bringing out such a worthwhile publication.

Date: 01-07-08
Place: Kasaragod

(S. ARUL RAJ)

INTRODUCTION :

Bud rot is a fatal disease of coconut palm, characterized by the rotting of the terminal bud and surrounding tissues. Even though it affects the palms of all ages, young palms in low lying and moist situations are more susceptible to the disease. It is generally a sporadic disease, but some times appear in epidemics forms. The disease commonly occurs in West and East Coasts of India (Menon and Pandalai, 1958; Ohler, 1984; Srinivasan, 2001). Bud rot disease incidence on coconut recorded upto 15% in Andhra pradesh (Srinivasulu, 2008).

SYMPTOMATOLOGY:

Palms of all ages are susceptible to the disease, but it is more frequent on young palms. Briton- Jones (1940) described the disease symptoms first. The first visible symptom is the withering of the spindle marked by pale colour. The spear leaf or spindle turns brown and bends over. Basal tissues of the leaf rot quickly and can be easily separated from the crown. Spindle withers and droops down and one by one, the inner leaves also fall away, leaving only fully matured leaves in the crown. A foul smell is emitted by the rotting tissue (Nambiar, 1994). The palms succumb to the disease with the death of the spindle (Briton-Jones, 1940; Menon and Pandalai, 1958; Lingaraj, 1972). Later, infection spreads to the older leaves, causing sunken leaf spots covering the entire leaf blade spreading both up and down. Spot margins are irregular and water soaked, and when the leaves are unfolded, the characteristic irregular spots are conspicuous on the blade. In severely affected trees, the entire crown may rot and in few months the trees wilt. Bud rot and premature nut fall diseases are produced on coconut. The heart leaf becomes chlorotic, wilts and collapses. The disease may spread to older, adjacent leaves and spathes, producing a dead centre with a fringe of living leaves. Light brown to yellow, oily, sunken lesions may be found on leaf bases, stipules or pinnae. Internally, the tissues beneath the bud rot discolored pink to purple with a dark brown border. Infected nuts show brown to black necrotic areas with a yellow border developing on the surface. Internally, they have a mottled appearance. Young nuts are highly susceptible and fail to mature and then fall off from the tree. Older, infected nuts ripen normally (Anon, 2000, Srinivasulu, 2007). The disease is rampant during the monsoon when the atmospheric temperature is low and the humidity is high (Muliyar et.al., 1982).

The disease is called nut fall or Mahali in Kerala (Sundararaman and Ramakrishnan, 1924). The fungus also infects nuts, causing decay of immature nuts and their fall during the rainy season (Nambiar, 1994). Water soaked greyish green area develops at the stalk end of

the nuts against dark green healthy area around. The lesions later turn brown and become sunken due to decay of underlying tissues. The rot extends into the husk and sometimes deep into the endosperm cavity (Nambiar, 1994).

Occurrence & distribution of *Phyfophfhora* bud rot on coconut.

Coconut plantations in East Godavari and West Godavari districts of Andhra Pradesh were surveyed by adopting roving survey and assessed the occurrence and spread of coconut of bud rot disease. During survey, bud rot disease incidence on coconut palms ranged from 2.5% in sandy soils (West Godavari) to 23.3% in black soils (East Godavari). During survey, it was observed that the coconut gardens with intercrops recorded more incidence of bud rot (4.9 to 23.3%) when compared to sole coconut (2.5 to 20.5%). Presence of palmyrah in coconut gardens also influenced the occurrence of bud rot disease in coconut. Bud rot incidence being ranged from 5.8 to 23.3% and 2.5 to 15.6% in coconut gardens with palmyrah and without palmyrah respectively [Table-1].

Table-1: Occurrence of bud rot disease (%) on coconut in AP

Name of the district	Type of soil	Sole cocount		Intercrop	
		Palmyrah Present	Palmyrah Absent	Palmyrah Present	Palmyrah Absent
East Godavari	Black	20.5	13.4	23.3	15.0
	Red	6.0	2.5	6.6	4.9
	Sandy	18.3	15.4	18.3	15.6
	Mean	**13.3**			
West Godavari	Black	12.2	8.7	13.3	10.8
	Red	10.7	6.6	11.2	7.4
	Sandy	5.8	2.5	7.2	6.0
	Mean	**8.5**			
Grand mean		**10.9**			

Occurrence of bud rot with relation to different soil types:

Maximum bud rot disease incidence of 14.65 percent was recorded in coconut gardens existing in black soil followed by sandy soil (11.14%) and red soil (6.99%) [Table-2].

Table-2: Bud rot disease incidence [%] in coconut in different soils of AP

Type of soil	Disease incidence [%]
Black soil	14.65
Red soil	6.99
Sandy soil	11.14

Occurrence of bud rot with relation to intercrops:

Maximum bud rot disease incidence of 13.32 percent was recorded in coconut with intercrops and existence of palmyrah palm in coconut gardens followed by coconut with palmyrah (12.25%), coconut with intercrops (11.63%) and sole coconut without palmyrah (8.18%) [Table-3].

Table-3: Bud rot disease incidence [%] in different cropping systems in AP

Cropping system	Disease incidence [%]
Sole coconut with out Palmyrah	8.18
Coconut with Intercrops	11.63
Coconut with Palmyrah	12.25
Coconut with Intercrops + Palmyrah	13.32

Occurrence of bud rot with relation to age of seedlings:

Bud rot disease incidence was found to be more common with maximum incidence (15.87%) in coconut seedlings with the age of below 5 years followed by 5 to 10 years age (10,63%); 11 to 15 years (7.13%); 16 to 25 years (5.73%) and above 25 years (5.31%) [Table-4].

Table-4: Bud rot disease incidence [%] in relation to age of coconut palms

Age of palm	Disease incidence [%]
Below 5 years	15.87
5 to 10 years	10.63
11 to 15 years	7.13
16 to 25 years	5.73
Above 25 years	5.31

Isolation of *Phytophthora palmivora* :

Phytophthora palmivora E.J.Butler (E.J.Butler) was isolated from bud rot diseased crown & nut tissue of coconut and established pathogenicity.

Effect of media on mycelial growth of *P.palmivora* :

Eight mm diameter discs of 3 days old cultures of *P.palmivora* was inoculated on Potato Dextrose Agar, Carrot Agar and V8 Juice Agar media. The petri plates were incubated at 37°c temperature. No difference in mycelial growth of *Phytophthora palmivora* was recorded at 37°c temperature on PDA, CA and V8 under in vitro condition.

Effect of temperature on mycelial growth of *P.palmivora* :

Eight mm diameter discs of 3 days old cultures of *P.palmivora* was inoculated on Potato Dextrose Agar, Carrot Agar and V8 Juice Agar media. The petriplates were incubated at 25°c, and 28°c temperatures. No difference in mycelial growth of *P.palmivora* was recorded at different temperatures on PDA, CA and V8 under in vitro condition.

Effect of pH on mycelial growth of *P.Palmivora* on PDA, CA & V8 juice agar:

Wide range of p^H viz., 4.0, 5.0,6.0,7.0 and 8.0 was tested against *Phytophthora palmivora* by adjusting p^H of the media viz., PDA, CA and V8 juice agar before autoclaving by using 0.1 N Hcl or 0.1 N NaoH as the case may be Eight mm diameter discs of 3 days old cultures of *P.palmivora* was inoculated on media plates at p^H -4.0, 5.0, 6.0, 6.5, 7.0 and 8.0 and incubated at 26°C temperature. The mycelial growth of *P.palmivora* was found to grow normally at wide range of p^H on different media viz., PDA, CA and V8 juice agar under in vitro condition. A moderate sporangia was found at p^H ranges viz., 4.0,5.0, 6.0,6.5,7.0 and 8.0 where as no growth of *P.palmivora* was recorded at pH 7.0 & 8.0 on V8 juice agar media.

Effect of Light on mycelial growth of *P.palmivora* on PDA, CA & V8 juice agar:

Eight mm diameter discs of 3 days old cultures of *P.palmivora* was inoculated on media viz., PDA, V8 juice agar and Carrot Agar, and exposed to complete light and darkness. The mycelial growth of *P.palmivora* was found to grow normally and a moderate papillate sporangia was observed when exposed to complete lightness and darkness.

Survival and spread of bud rot disease in coconut:

Epidemiology of bud rot disease in coconut indicated that *Phytophthora palmivora*, a pathogen of bud rot is found to survive on infected palmyrah and coconut palms as dormant mycelium. Whenever the seasonal conditions favourable, the dormant mycelium germinates and spreads to the nearby palms. The primary source of inoculum of *Phytophthora palmivora*

was found to exist on dead palms of palmyrah existing in coconut gardens. This finding highlights the important role of palmyrah in occurrence and spread of bud rot in coconut.

Occurrence of bud rot disease in coconut:

Studies in relation to occurrence of bud rot disease in coconut revealed that the disease appears mostly during first fortnight of June of every year i.e., with the onset of the monsoon and maximum disease incidence was recorded during the months of July to October, with maximum disease of 68.9% during October..

Bud rot occurrence with relation to age of seedlings:

Studies conducted on occurrence of bud rot disease with relation to age of palm highlighted that bud rot disease incidence was found to be more common with maximum incidence (15.87%) in coconut seedlings with the age of below 5 years followed by 5 to 10 years age (10.63%); 11 to 15 years (7.13%); 16 to 25 years (5.73%) and above 25 years (5.31%). This result indicates the more susceptibility of young coconut seedlings to the bud rot disease.

Bud rot occurrence with relation to intercrops:

Maximum bud rot disease incidence of 13.32 percent was recorded in coconut with intercrops and existence of palmyrah palm in coconut gardens followed by coconut with palmyrah (12.25%), coconut and palmyrah with intercrops (11.63%) and sole coconut without palmyrah (8.18%). This finding shows the importance of existence of palmyrah palm in coconut gardens, besides the role of intercrops in coconut based cropping system in occurrence and spread of bud rot disease in coconut.

Interrelationship between bud rot disease of coconut & palmyrah:

Presence of palmyrah in coconut gardens also influenced the occurrence of bud rot disease in coconut [Table-5]. Bud rot incidence was recorded upto 14.9% in coconut gardens where palmyrah palms are present where as the disease incidence is only 10.8% in coconut gardens without palmyrah palms.

Table-5: Interrelationship between bud rot disease of coconut & palmyrah

Type of soil	Palmyrah present	Palmyrah absent
Black soil	21.9	14.2
Red soil	8.6	7.0
Sandy soil	14.1	11.2

Bud rot occurrence with relation to different soil types:

Studies on bud rot disease incidence in coconut in different soils viz., black soil, sandy soil and red soil indicated that maximum bud rot disease incidence of 14.65 percent was recorded in coconut gardens existing in black soil followed by sandy soil (11.14%) and red soil (6.99%) (Table 2). This results highlight the importance of soil type in relation to occurrence of bud rot disease in coconut.

Effect of weather parameters on bud rot disease in coconut:

Studies on effect of weather parameters on bud rot disease in coconut with a different age group was studied for a period of 2 years and correlations and regressions was carried out between disease incidence and weather parameters viz., maximum temperature, minimum temperature, relative humidity, rain fall and rainy days.

Effect of temperatures on bud rot disease:

Maximum bud rot disease incidence [68.9%] was recorded during the month of October, when the maximum and minimum temperatures were 33.20°c and 25.27°c respectively. From the perusal of the data [Table-6], it is observed that the differences in day-wise maximum and minimum temperatures are not influencing the occurrence and spread of bud rot disease in coconut.

Effect of relative humidity on bud rot disease:

Maximum bud rot disease incidence [68.9%] was recorded during the month of October, when the maximum and minimum relative humidity were 96.52 and 60.69 respectively. From the perusal of the data [Table-6], it is observed that the differences in day-wise maximum and minimum relative humidity are influencing the occurrence and spread of bud rot disease in coconut. However, further studies are required to confirm the observations.

Effect of rainfall and rainy days on bud rot disease:

Rainfall and number of rainy days were found to have a positive correlation with the occurrence and spread of bud rot disease in coconut. This study highlights that continuous rainfall is more important factor for the occurrence and spread of bud rot disease in coconut. Hence, continuous monitoring of coconut palms immediately after the rainfall is must to manage the bud rot disease in coconut. The developed prophylactic approach i.e., application of talc formulation of Pseudomonas fiuorescens in the crown region of the coconut seedlings in bud rot prone areas can be recommended to manage this disease.

Developed bud rot disease forecasting model in coconut:

A) Bud rot forecasting model in coconut based on field observation:

Based on the studies conducted under this ICAR Adhoc Scheme, the following critical parameters are identified to forecast the occurrence of bud rot disease in coconut.

1. Soil type: Coconut gardens existing in black soils are more prone to bud rot disease.
2. Existence of palmyrah palms in coconut gardens: There is a positive correlation between number of palmyrah palms existing in coconut garden to the occurrence and spread of bud rot disease in coconut.
3. Age of coconut seedlings: Young i.e., below 5 years age coconut seedlings are more susceptible to bud rot.
4. Spacing and existence of number of coconut palms/acre: Closer spacing and existence of more number of coconut palms i.e., more than 60 palms/acre are the favourable conditions for the occurrence of bud rot.
5. Existence of intercrops in coconut: Regular monitoring of coconut palms is required when the intercrops are grown in coconut cropping system. Especially intercrops such as banana and betelvine grown coconut gardens are more prone to bud rot compare to other intercrops such as cocoa, cinnamon, pineapple, black pepper, turmeric, elephant foot yam, colocasia, etc.
6. Existence of coconut nursery in coconut gardens: Farmers are having the practice of raising nursery in coconut gardens. Initial occurrence of bud rot disease is more common in nurseries. Regular monitoring of nurseries existing in coconut gardens is a best method of bud rot forecasting in the garden.
7. Rainfall and Rainy days: Rainfall and rainy days are having positive correlation with the occurrence and spread of bud rot disease in coconut irrespective of the season. Hence, regular monitoring of the coconut gardens, immediately after the rainfall is must to forecast bud rot in coconut.

Based on the above observations, it is suggested to establish a coconut nursery in the middle of the coconut garden to monitor bud rot disease in coconut.

B) Bud rot forecasting model in coconut based on prediction equation:

$$Y = 8.6544 + 1.1244_{x1} - 1.1134_{X2} + 0.2441_{X3} - 0.3035_{x4} - 0.0032_{x5} + 1.5275_{x6}$$

Table.6: Effect of weather parameters on bud rot disease of coconut in different soil situations (Mean of 2005 & 2006)

Month		Temperature		Relative Humidity		Rain fall	Rainy days	Disease [%]
		Min.	Max.	Min.	Max.			
January	1st FN	18.30	28.47	94.15	53.45	0.0	0	0.0
	2nd FN	20.02	30.09	93.37	55.44	0.0	0	0.0
February	1st FN	19.25	30.88	93.57	56.09	0.0	0	0.0
	2nd FN	21.98	33.10	91.21	48.65	0.0	0	0.6
March	1st FN	23.34	32.47	92.59	52.17	3.6	0	0.0
	2nd FN	24.99	34.20	91.00	53.27	13.5	0	0.0
April	1st FN	27.42	34.45	86.53	54.84	1 .3	0	0.6
	2nd FN	27.15	35.27	86.87	54.15	31.7	1	0.6
May	1st FN	28.40	36.43	86.72	57.10	5.1	1	0.0
	2nd FN	29.07	35.90	86.37	58.75	44.0	2	1.7
June	1st FN	29.37	36.90	83.12	54.47	40.5	4	15.0
	2nd FN	29.59	36.94	82.28	55.32	159.3	9	27.8
July	1st FN	27.60	31.77	88.78	69.79	156.9	14	38.4
	2nd FN	27.14	32.37	88.30	65.77	237.7	14	52.8
August	1st FN	25.82	30.92	89.10	71.82	261.5	15	50.5
	2nd FN	26.40	32.23	89.29	70.44	138.0	12	37.8
September	1st FN	25.65	31.10	93.54	73.05	188.9	12	55.0
	2nd FN	24.54	30.22	96.23	73.00	455.4	13	30.6
October	1st FN	25.27	33.20	96.52	60.69	58.8	3	68.9
	2nd FN	23.58	30.30	96.60	70.20	583.1	15	60.6
November	1st FN	22.20	29.43	97.13	64.85	82.8	7	32.2
	2nd FN	19.19	30.23	96.48	47.09	1.9	0	27.8
December	1st FN	18.82	29.25	90.67	42.99	1.9	0	12.2
	2nd FN	18.07	28.38	94.10	50.07	2.4	1	23.3

Management of bud rot disease:

Isolation of native biocontrol agents:

Soil samples were collected during survey from rhizosphere region of coconut gardens. *Trichoderma spp* were isolated by adopting serial dilution technique from the rhizosphere soil samples collected during survey & identified as *Trichoderma viride*, *T.harzianum*, *T.hamatum*, *T.longibrachiatum*, *T.virens* & *T.polysporum* based on their cultural characters and *Pseudomonas fluorescens*.

Efficiency of *Trichoderma spp* & *Pseudomonas fluorescens* on *P.palmivora* :

Dual culture technique was employed to test the efficiency of native fungal bioagents viz., Trichoderma spp and bacterial bioagent *P.fluorescens* on *P.palmivora* under in vitro conditions. All the *Trichoderma spp*viz., *T.viride*, *T.harzianum*, *T.hamatum*, *T.longibrachiatum*, *T.virens* & *T.polysproum* and *P.fluorescens* were found to inhibit the mycelial growth of *P.palmivora* with percent inhibition ranging from 46% to 88%. Among the six species of' *Trichoderma* tested maximum inhibition of pathogen was recorded with *T.Polysporum* with percent inhibition of 87.50%. This is followed by *T.viride*, *T.hamatum* and *T.harzianum* with percent inhibition of 82.03%, 75.39% and 71.37% respectively. Where as *T.longibrachiatum* and *T.virens* were found to inhibit the mycelial growth .of *P.palmivora* up to 51.56% and 46.8% respectively only. However, 7 days after incubation over growth of *P.palmivora* mycelium on all the *Trichoderma spp* were recorded except in *T.longibrachiatum* under dual culture plate. *P.fluorescens* inhibited the mycelial growth of *P.palmivora* up to 50% [Table-7].

Table-7: Antagonistic effect of *Trichoderma spp* & *P.fluorescens* on *P.palmivora*

Bioagent	Mycelial growth of *P.palmivora*	Remarks
T.viride	11.5 (82.03)	Ohfver growth of *P.palmivora* mycelium was observed after 7 days of incubation period
T.harzianum	18.0 (71.37)	
T.hamatum	15.75 (75.39)	
T.virens	30.0 (46.88)	
T.polysproum	56.0 (87.50)	
T.longibrachiatum	33.0 (51.56)	Inhibition effect continued
P.fluorescens	32.0 (50)	
Control	64	

Figures in parenthesis are percent inhibition of mycelial growth over control

In vitro evaluation of *Trichoderma spp* for production of volatile and non-volatile metabolites against *P.palmivora*

The mycelial growth of *P.palmivora* was suppressed when exposed to 0, 15, 25 days old cultures of *Trichoderma spp*. Among the *Trichoderma spp* tested for the production of volatile metabolites, maximum percent inhibition was recorded with *T.harzianum* (58.00%) followed. by *T.viride* (56.15%) and *T.hamatum* (54.00%) [Table-8]. A positive correlation was obtained for all the *Trichoderma spp* between an increase in age of the antagonist culture before being exposed to the bud rot pathogen with percent inhibition of the pathogen.

In case of non-volatile metabolites, all the *Trichoderma spp* were found to inhibit the mycelial growth of *Phytophthora palmivora*. A positive correlation was observed between an increase in concentration of culture filtrate of *T.viride*, *T.harzianum* & *T.hamatum* and the percent inhibition of mycelial growth of *P.palmivora*. The maximum percent inhibition was recorded with *T.viride* (52.00%) followed by *T.hamatum* (51.00%). The culture filtrate of *T.harzianum* was found to inhibit the mycelial growth of *P.palmivora* to an extent of 41.00% only at 100% concentration of the culture filtrate [Table-8].

Table - 8 : Efficacy of volatile & non-volatile metabolites of *Trichoderma spp* on *P.palmivora*

Bioagent	Per cent inhibition of *P.palmivora*						
	Volatile metabolites of *Trichoderma* [Days before exposure]			Non-volatile metabolites of *Trichoderma* [Conc. of culture filtrate (%)]			
	0	15	25	10	50	70	100
T.viride	16.25^{b}	25.00^{b}	56.15^{b}	8.32^{b}	23.56^{b}	49.28^{b}	52.00^{a}
T.harzianum	16.00^{b}	26.25^{b}	58.00^{a}	6.86^{c}	20.00^{c}	48.00^{c}	49.00^{b}
T.hamatum	18.00^{a}	27.00^{a}	54.00^{c}	12.36^{a}	35.25^{a}	50.25^{a}	51.00^{a}

Number in each column followed by the same letters are not significantly different. Values represents the mean of six replicates.

Effect of volatile metabolites of P.fluorescens on P.palmivora :

The mycelial growth of P.palmivora was suppressed when exposed to 0, 2, 4, 6, 10, 15 days old culture of P.fluorescens. The bacteria tested for the production of volatile metabolites, hundred percent inhibition was recorded with 4 day and 6 day old culture. The percent inhibition was decreased when the age of antagonist increased [Table-9].

Table-9: Effect of volatile metabolites of *P.fluorescens* on *P.palmivora*

Treatments	Mycelial growth at 100% concentration of *P.palmivora*	% inhibition
0 days	90	0
2 days	45	50
4 days	0	100
6 days	0	100
10 days	60	33.3
15 days	60	33.3
Control	90	--

Effect of non-volatile metabolites of P.fluorescens on P.palmivora.

Non-volatile metabolites or culture filtrate of P.fluorescens was tested against P.palmivora on PDA under invitro conditions. A positive correlation was observed between an increase in age of culture filtrate of P.fluorescens and the percent inhibition of mycelial growth of P.palmivora. The maximum percent inhibition was recorded with 10 day old and 15 day old culture filtrate of P.fluorescens (100%) at 100% concentration [Table-10].

Table- 10: Effect of culture filtrate of *P.fluorescens* on *P.palmivora*

Culture filtrate of *P.fluorescens*	Mycelial growth at 100% conc. of *P.palmivora*	% inhibition
3 day	38	57.77
6 day	21.6	76
10 day	0	100
15 day	0	100
20 day	0	160

Studies on compatibility between *Trichoderma spp* & *P.fluorescens*:

Studies conducted on compatibility between *Trichoderma spp* and *P.fluorescens* indicated that *P.fluorescens* was not inhibitory to the tested *Trichoderma spp* except around 30-40% inhibition in mycelial growth of *T.longibrachiatum* & *T.virens* was noted.

Combined effect of *Trichoderma spp* & *P.fluorescens* on *P.palmivora*:

The inhibitory action of *Trichoderma spp* and *P.fluorescens* on *P.palmivora* reduced considerably (about 50%) when used in combination than alone.

Mass multiplication of biocontrol agent, *Trichoderma spp*:

Studies conducted on identification of suitable substrate for mass multiplication of bioagents, neem cake followed by FYM was found to be suitable substrate. Besides, neem cake completely arrested the mycelial growth of *P.palmivora*, pathogen of bud rot.

Talc formulation of *P.fluorescens /Trichoderma spp*

Talc formulations of the isolated native bacterial bioagent *P.fluorescens* and *Trichoderma spp* i.e., *T.viride*, *T.harzianum* and *T.hamatum* were developed by multiplying the bioagent on broth and incubating at room temperature. The culture was then homogenized and the homogenate was mixed with talc powder at 1 : 2 ratio along with 0.5 % carboxy methyl cellulose. King's B broth and potato dextrose broth was used for *P.fluorescnes* and *Trichoderma spp* respectively. Talc formulations of *P.fluorescens* and *Trichoderma spp* viz., *Trichoderma viride*, *T.harzianum* and *T.hamatum* were developed under laboratory conditions and the formulation was subsequently used for field studies.

Effect of Botanical extracts on mycelial growth of *P.Palmivora* :

Among the 20 botanicals tested against the *P.palmivora* by poisoned food technique, the fresh leaf extract (10% conc.) from henna (*lawsonia inermis finn*) completely suppressed the mycelial growth of P.palmivora (100% inhibition) on carrot agar under in vitro conditions at 10% concentration. Whereas at 5% concentration, henna leaf extract inhibited the mycelial growth of *P.palmivora* only up to 31%. Henna leaf extract at 10% and 5% level found to be inhibitory to all the tested fungal bioagents. While all other leaf extracts found ineffective. However, the mycelial growth as well as sporangia produced by P.palmivora not affected by other leaf extracts used in the present study.

Effect of fresh leaf, water extract and methanol extract of henna on *P.palmivora*

Among various extracts of henna were screened against mycelial growth of *P.palmivora* fresh leaf extract of henna at 10 % concentration was found to be effective and water extract of henna was also found to inhibited the mycelial growth at 70% and 100% concentration.

Screening of Fungicides, Fertilizers & Chemicals on mycelial growth of P.palmivora:

Seven fungicides namely copper oxychloride (Blitox) (0.3%), Bitertanol (Baycor) (0.1 %), Tridemorph (Calixin) (0.1 %), Bordeaux mixture (1 %), Hexaconazole (Cantof) (0.1%), Neem oil (2%). Fertilizers namely Urea (1%), Potash (2%), Super phosphate (2%), and Chemicals namely Zinc sulphate (2%), and Copper sulphate (0.3%) were screened against *P.palmivora* on PDA by poisoned food technique at room temperature. Bitertanol (0.1 %), Tridemorph (0.1 %), Triademiphon (0.1 %) and Hexaconazole (0.1%) were completely inhibited the mycelial growth of *P.palmivora* on PDA.

Prophylactic methods against bud rot disease in coconut:

Evaluation of developed formulations of bioagents on *P.palmivora* under in vitro conditions:

Effective bioagents viz., *Trichoderma viride* and *Pseudomonas fluorescens* were converted to talc based formulations and tested against *Phytophthora palmivora* infection by inoculation by *P.palmivora* inoculated coconut leaf petiole bits. All the treatments were imposed 7 days before inoculation with *P.palmivora*. No growth [-] of *P.palmivora* was recorded with talc formulation of *T.viride* followed by minimum growth (++) of test pathogen treatments viz., talc formulation of *T.viride* grown on henna extract, talc formulation of *T.viride* grown on henna extract + talc formulation of *P.fluorescens* grown on henna extract + neem cake. Maximum growth (++++) of test pathogen was recorded in untreated control.

Evaluation of developed formulations of antagonists on bud rot disease under field conditions (prophylactic method).

Field experiment on evaluation of various formulations of *T.viride* and *P.fluorescens* against bud rot disease of coconut was carried out (Crown rot infection). All the treatments [Table-11] were given 24 hours before inoculation with test pathogen, *P.palmivora*, to evaluate the efficiency of bioagents as prophylactic approach. Formulations (treatment) are applied @ 5 gin crown region of coconut seedlings. After 24 hours, the seedlings were inoculated with *P.palmivora*. None of seedlings in treatments expressed the bud rot symptoms, while in control, all the seedlings showed crown rot symptoms.

Evaluation of developed formulations of bioagents on bud rot disease under field conditions (curative methods):

Field experiment on evaluation of various formulations of *T.viride* and *P.fluorescens* against bud rot disease of coconut was carried out. Coconut seedlings were inoculated with *P.palmivora* and the seedlings with crown rot symptoms were selected to evaluate the bud rot curative efficiency of bioagents, as curative approach. Formulations (treatment) are applied @ 5 gin crown region (rotted portion) of coconut seedlings. Data on recovery of seedlings in each treatment are recorded. Application of talc formulation of *Pseudomonas fluorescens* or *Trichoderma viride* in the crown region (rotted portion) of the coconut seedlings completely controlled the bud rot disease in coconut. The bud rot infected coconut seedlings recovered with in a period of 9 and 15 days after the application of talc formulation of *P.fluorescens* and *T.viride* respectively. The required quantity of talc formulation of *P.fluorescens* or *T.viride* to

be applied in crown region of coconut seedlings against bud rot disease was found to be 5 g, 10 g, 75 g, 100 g, 150 g and 200 g for six months, 1 year, 2 years, 3 years, 4 years & 5 years and above age of coconut seedlings respectively.

Studies conducted on field evaluation of spray formulations of *Pseudomonas fluorescens* indicated that crown rot and nut rot infection can be checked by spraying 10 to 15 day old culture filtrates (100% concentration) of *P.fluorescens*.

Table -11 : Field evaluation of various formulations of bioagents against bud rot disease in coconut

	Treatments (5 g / each crown)	No. of seedlings recovered from crown rotting	Recovery period (No. of days)
T l	Talc formulation of *P.fluorescens*	5/5	9 - 12
T 2	Talc form ulation of *T.viride*	5/5	12 - 15
T 3	Neem cake formulation of *P.fluorescens*	3/5	15 - 20
T 4	Neem cake form ulation of *T.viride*	3/5	30 - 35
T 5	Talc formulation of *T.viride* grown on henna extract	2/5	14 - 16
T 6	Talc form ulation of *P.fluorescens* grown on henna extract	2/5	15 - 20
T 7	Neem cake	3/5	15 - 20
T 8	Dry henna powder	0/5	No recovery
T 9	Control	0/5	No recovery

Developed biocontrol based IDM package against bud rot in coconut:

- The garden should be kept clean.
- The trees died due to bud rot should be removed and burnt to avoid further spread of the disease.
- In the initial stages of bud rot, the rotten parts should be removed and destroyed.
- Application of talc powder formulation of *Pseudomonas fluorescence* or *Trichoderma viride* in crown region of coconut seedlings is recommended.
- The required quantity of talc formulation of *Pseudomonas fluorescens* or *Trichoderma viride* to be applied in the crown region of coconut seedlings is 5 g, 10 g, 75 g, 100 g, 150 g and 200 g for 6 months, 1 year, 2 years, 3 years, 4 years and 5 years and above age of coconut seedlings respectively.
- Spraying of 10 to 15 day old culture filtrate of *Pseudomonas fluorescens* at 100% concentration twice at 30 days interval on crown region and on nuts of coconut.

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BUD ROT SYMPTOMS IN COCONUT

Withering of spear leaf

Rotted leaf separates from crown

Rotted crown

Tender nut rot

Matured nut rot

Wilting of heart leaf

Bud rot affected plam

Immature nut fall

Copra rot

Bud rot affected bearing palm

BIOINTENSIVE IDM FOR BUD ROT IN COCONUT

Talc formulation of *P.fluorescens*

Application of talc formulation of *P.fluorescens*

Crown rot infection

P.fluorescens treatment

Recovery from crown rot

Spraying of liquid formulation of *P.fluorescens* on crown region and nuts of coconut