



# Biocontrol agents for Sustainable Management of Bud Rot Disease in Coconut Nursery

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## Abstract

*Phytophthora palmivora*, an oomycete pathogen responsible for coconut bud rot, poses a significant threat to palms of all ages, with substantial economic implications. This research aimed to address this challenge by implementing eco-sustainable management strategies through the utilization of a synergistic combination of biocontrol agents. The study focused on combating coconut bud rot in both nursery and main field settings. In the nursery, the study employed a soil application of *Trichoderma asperellum* at a rate of 25g per cent during sowing, combined with crown application of *Bacillus subtilis* at a concentration of 10g per liter of water, administered twice at three-month intervals and six months after sowing. Additionally, an application of Arbuscular Mycorrhizal (AM) fungi at 50g per cent three months after sowing was implemented. This multi-pronged approach proved 77.12 per cent effective in suppressing bud rot disease within the nursery environment. For young coconut gardens, a randomized design with three replicates was used to evaluate the treatments. The results indicated that a soil and crown application of *B. subtilis* at 10g per liter of water, performed twice - first during the pre-monsoon period (last week of May) and secondly in the post-monsoon period (September) - significantly reduced bud rot incidence. Additionally, the application of *T. asperellum* at a rate of 50g per palm and AM fungi at 50g per palm annually in January demonstrated 71.73 per cent effectiveness in mitigating the occurrence of bud rot in young coconut gardens. This research underscores the practicality and efficacy of employing a carefully curated combination of biocontrol agents within eco-sustainable management practices to combat the economically detrimental coconut bud rot disease in various coconut palm cultivation settings.

**Keywords:** Coconut, Bud rot, Nursery Diseases, Biocontrol agents, Eco-sustainable Management

## Introduction

The coconut tree (*Cocos nucifera* Linn.), a unique species within the genus *Cocos* and a member of the palm tree family (Arecaceae), holds paramount importance globally. It is cultivated in over 93 countries, spanning a vast land area of 12.78 million hectares and yielding a staggering 54 billion nuts annually (Report of the Coconut Development Board, 2022). However, this invaluable crop faces a significant threat from a range of diseases, with bud rot caused by the fungus *Phytophthora palmivora* emerging as a primary concern.

In India, the first documented report of bud rot dates back to 1906 when Butler identified this devastating disease. Subsequent research has

revealed its devastating impact, leading to yield losses of up to 10% and 13% (Ramesh *et al.*, 2013). Although bud rot is sporadic in nature, it can escalate to epidemic proportions, as highlighted by Gangaraj *et al.* (2021). The complex lifecycle of *P. palmivora* in tropical regions adds to the challenge, given its broad host range and the ability to infect various plant tissues, including roots, stems, leaves, flowers, and fruits. The production of diverse spores, such as zoospores, sporangiospores, chlamydospores, and oospores, allows for versatile modes of transmission, including airborne, soilborne, water-borne, and vector-borne dispersal of propagules (Drenth and Guest, 2013; Konam and Guest, 2004).

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The first visible symptom of bud rot manifests as the wilting of the spindle leaf, marked by a distinct pale coloration upon careful observation. This initial symptom progresses to the browning, drying, and bending of the spindle. Subsequently, the surrounding tissues of the terminal bud deteriorate, emitting a foul odor. Ultimately, the palm succumbs to the disease, with the heart leaf displaying chlorosis, wilting, and collapse. The disease can also extend to older, adjacent leaves and spathes, leading to a dead center with a fringe of surviving leaves. Light brown to yellow, sunken lesions may appear on leaf bases, stipules, or pinnae. Internally, the tissues beneath the bud rot change from pink to purple, eventually turning dark brown. Infected nuts exhibit brown to black necrotic areas with a yellow border on the surface, presenting a mottled appearance. Young nuts are particularly susceptible, failing to mature and subsequently falling from the tree. Older nuts, while infected, continue to ripen normally. The fungus can also affect nuts, causing the decay of immature nuts, leading to their premature fall during the rainy season. A water-soaked grayish-green region develops at the stalk end of the nuts, surrounded by the healthy, dark green area. These lesions later turn brown and become sunken due to underlying tissue decay. The rot can extend into the husk and, in some cases, deep into the endosperm cavity. Sharadraj and Chandramohan (2014) reported that the bud rot incidence has been recorded to range from 5.8% to 23.3% in coconut gardens.

The economic repercussions of bud rot outbreaks can be staggering, as demonstrated by Mosquera Montoya *et al.* (2014), who estimated losses of 250 million U.S. dollars resulting from bud rot in oil palm plantations in Colombia. Recognizing the importance of early detection, several research scholars, including Martínez (2009), Martínez *et al.* (2013), and Torres *et al.* (2010), have emphasized its pivotal role in bud rot management. Timely intervention is especially critical in young palms, as the removal of healthy tissue above the meristem is limited compared to older palms. Ariza *et al.* (2008) also stressed the

need to inspect and treat surrounding palms at the first signs of disease symptoms to prevent further spread.

As we contemplate disease management in agriculture, the extensive use of chemical fertilizers and pesticides has become common place in modern agriculture, with over two billion metric tons of pesticides being utilized annually worldwide. These chemicals are employed to combat infections caused by fungi, bacteria, viruses, nematodes, as well as control pests and weeds. However, the toxicity, persistence, and carcinogenic potential of many of these substances have raised concerns about their impact on soil quality, the hydrogeological environment, and both animal and human health. Consequently, there is a growing recognition of the need for sustainable alternatives, with biocontrol emerging as a viable and environmentally friendly option. Across the globe, antagonistic fungi and bacteria are harnessed as biocontrol agents (BCAs) to protect plants from diseases, enhance plant development, and boost productivity (Rajneesh *et al.* or Thakur *et al.*, 2022).

Biological control, characterized by the use of one organism to control another, is an environmentally sound and cost-effective approach for addressing pest issues in various ecosystems, including agriculture, forestry, natural environments, and urban settings (Hoddle *et al.*, 2021; Wittenberg and Cock, 2001). Numerous biocontrol agents are currently in commercial use, including *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum*, *T. viride*, and mycorrhizal fungi, specifically targeting soil-borne pathogens (Harrier and Watson, 2004). Furthermore, studies have shown that ectomycorrhizal and arbuscular mycorrhizal fungi can elicit similar chitin elicitors, inducing a defensive response (Salzer and Boller, 2000). This approach has been adopted as an effective means of plant protection (Oyewole *et al.*, 2017).

In light of the environmental and health concerns associated with chemical pesticides, this study was undertaken to investigate the effectiveness of various biocontrol agents with diverse modes of action. Our goal is to develop an affordable and user-friendly strategy for coconut

nursery garden management while reducing the harmful effects of pesticides. Specifically, we evaluated the efficacy of biocontrol agents and arbuscular mycorrhizal fungi (VAM) in mitigating bud rot disease in coconut nursery gardens.

## Materials and methods

### Coconut nursery experiments

The experiments were conducted at the Coconut Research Station, Aliyarnagar, Tamil Nadu, India, utilizing West Coast Tall (WCT) and Kenthali Dwarf (KTD) coconut seed nuts. A randomized block design (RBD) with four treatments and five replications was employed. Each replication consisted of 20 seed nuts.

1. Treatment 1 ( $T_1$ ): This treatment involved the soil application of talc based formulation of *Trichoderma asperellum* at a rate of 25 g/cent during sowing, along with spindle leaf stage application of *Bacillus subtilis* endophyte at 10 g/l of water, (250ml/seedlings) administered twice at three months and six months post-sowing. Additionally, arbuscular mycorrhizal (AM) fungi were applied at 50 g/cent three months after sowing.
2. Treatment 2 ( $T_2$ ): Soil drenching was performed with metalaxyl at 2 g/l of water during sowing, and spindle leaf stage application of metalaxyl (250ml/seedlings) at the same concentration was repeated twice at three months and six months post-sowing.
3. Treatment 3 ( $T_3$ ): Soil drenching was carried out with copper oxy chloride at 2.5 g/l of water during sowing, and the same treatment was repeated twice at three months and six months post-sowing (250ml/seedlings).
4. Treatment 4 ( $T_4$ ): An untreated control group.

Data was collected on seed nut germination for both WCT and KTD varieties at three months after sowing, including the calculation of germination percentages. Bud rot incidence was recorded at 15-day intervals, commencing from

three months post-sowing and continuing up to eight months for both WCT and KTD seedlings.

### Experiment in young coconut garden

An experiment was conducted in a young coconut garden featuring the ALR-1 variety. Observations were made on bud rot incidence and growth attributes, such as palm height, collar girth, and leaf count. The following treatments were applied:

1. Treatment 1 ( $T_1$ ): Crown application of *Bacillus subtilis* endophyte at 10 g/l of water (1 litre/tree) was conducted twice, first during the pre-monsoon period (last week of May) and then during the post-monsoon period (September). Soil application of *Trichoderma asperellum* was carried out at a rate of 50 g per palm, and arbuscular mycorrhizal (AM) fungi were applied at 50 g per palm annually during January.
2. Treatment 2 ( $T_2$ ): Crown application of metalaxyl at 2 g/l of water (1 litre/tree) was performed three times: during the pre-monsoon period (May), post-monsoon period (September), and January.
3. Treatment 3 ( $T_3$ ): Crown application of copper oxy chloride at 2.5 g/l of water (1 litre/tree) was also applied three times: during the pre-monsoon period (May), post-monsoon period (September), and January.
4. Treatment 4 ( $T_4$ ): An untreated control group.

In this experiment, a randomized block design (RBD) was used with four treatments and five replications, and it was conducted at Aliyarnagar, Anaimalai block, Coimbatore district, Tamil Nadu, India.

### Statistical analysis

Data from the *in vitro* fungicidal trial underwent statistical analysis to assess mean differences using Duncan's multiple range test. Before conducting analysis of variance (ANOVA), the percentage values of disease indices were transformed using the arcsine transformation. Mean values  $\pm$  standard errors were reported. Statistical

significance was determined at a level of  $P < 0.05$ , and means were compared using Duncan's multiple range test (DMRT).

## Results

In our study, we observed significant reductions in bud rot incidence and notable improvements in seedling germination and growth attributes across different coconut varieties and stages of cultivation.

In the case of the West Coast Tall (WCT) and Kenthali Dwarf (KTD) varieties, we found that Treatment  $T_1$ , which involved soil application of *Trichoderma asperellum* at 25 g/cent during sowing, spindle leaf stage application of *Bacillus subtilis* endophyte at 10 g/l of water twice at three and six months post-sowing, and the application of arbuscular mycorrhizal (AM) fungi at 50 g/cent three months after sowing, led to a substantial reduction in bud rot incidence. Specifically,  $T_1$  reduced bud rot by 5.15% in WCT and 5.27% in KTD varieties. Additionally, Treatment  $T_3$ , which included soil drenching with copper oxy chloride at 2.5 g/l of water at sowing and twice at three and six months post-sowing, exhibited effective control of bud rot, resulting in reductions of 6.5% and 6.35% in WCT and KTD, respectively (Table 1).

Further analysis of germination data in the WCT nursery demonstrated the superiority of Treatment  $T_1$ , which achieved the highest germination rate of 91.0% compared to the untreated control at 76.3%. Moreover,  $T_1$  excelled in promoting various growth attributes, including shoot length (98.8 cm), root length (35.8 cm), collar girth (11.3 cm), number of leaves (6.5/plant), root volume (12.8 mm<sup>3</sup>/plant), and root dry weight (4.42 g/plant), outperforming all other treatments (Table 2).

In the KTD nursery, Treatment  $T_1$  also stood out as the most effective in enhancing germination and growth attributes. It achieved a germination rate of 89.1%, with notable root and shoot lengths of 94.7 cm and 26.28 cm, a collar girth of 8.9 cm, 6.0 leaves per plant, root volume of 9.8 mm<sup>3</sup>/plant, and a root dry weight

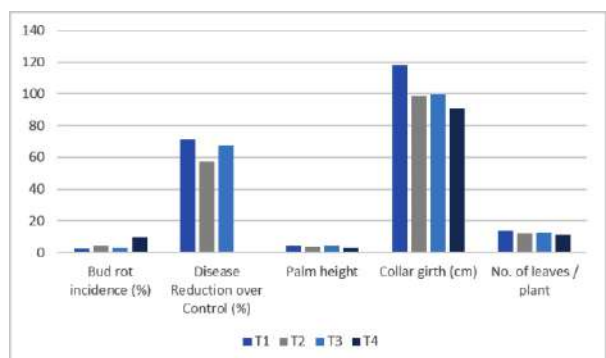
of 4.18 g/plant (Table 3).

Moving to the young coconut garden experiment, Treatment  $T_1$ , involving crown application of *Bacillus subtilis* endophyte at 10 g/l of water twice during the pre-monsoon (last week of May) and post-monsoon (September) periods, soil application of *Trichoderma asperellum* at 50 g/palm, and AM fungi at 50 g/palm/year during January, was exceptionally effective. It reduced bud rot incidence by a remarkable 71.73%, resulting in an incidence rate of only 2.6% compared to 9.4% in the untreated control.

Additionally, Treatment  $T_3$ , which included crown application of copper oxy chloride at 2.5 g/l of water thrice during the pre-monsoon, post-monsoon, and January periods, successfully controlled bud rot with a 3.0% incidence rate. Notably, Treatment  $T_1$  also exhibited promising results in enhancing palm growth, with the highest recorded palm height and collar girth of 118.2 cm and 13.5 leaves per palm (Table 4; Fig 1).

These findings underscore the effectiveness of the tested treatments in reducing bud rot incidence and promoting coconut seedling germination and growth attributes. It is essential to consider these integrated approaches as alternatives to relying solely on chemical fungicides, which can have economic and environmental drawbacks. Our study opens avenues for future research to identify the specific compounds responsible for disease reduction and to further investigate the application of these strategies in various coconut growth stages.

**Fig. 1. Efficacy of biocontrol agents and AM against bud rot disease in young coconut garden and their effect on growth parameters of coconut**



**Table 1. Efficacy of biocontrol agents and AM in management of bud rot disease of coconut seedlings**

Treatments	West Coast Tall (WCT)		Kenthali Dwarf KTD)	
	Bud rot incidence (%) (after 120 days)	Per cent Disease Reduction over control	Bud rot incidence (%) (after 120 days)	Per cent Disease Reduction over Control
T <sub>1</sub> : (Soil application of talc based formulation of <i>Trichoderma asperellum</i> at a rate of 25 g/cent during sowing, along with spindle leaf stage application of <i>Bacillus subtilis</i> endophyte at 10 g/l of water, (250ml/seedlings) administered twice at three months and six months post-sowing. Additionally, arbuscular mycorrhizal (AM) fungi were applied at 50 g/cent three months after sowing)	5.15 <sup>a</sup> (13.52)	77.12 <sup>a</sup> (61.81)	5.27 <sup>a</sup>	77.19 <sup>a</sup> (61.50)
T <sub>2</sub> : (Soil drenching was performed with metalaxyl at 2 g/l of water during sowing, and spindle leaf stage application of metalaxyl (250ml/seedlings) at the same concentration was repeated twice at three months and six months post-sowing)	7.19 <sup>a</sup> (15.35)	65.26 <sup>c</sup> (54.37)	7.54 <sup>c</sup>	66.92 <sup>c</sup> (54.65)
T <sub>3</sub> : (Soil drenching was carried out with copper oxy chloride at 2.5 g/l of water during sowing, and the same treatment was repeated twice at three months and six months post-sowing (250ml/seedlings))	6.50 <sup>b</sup> (14.62)	68.59 <sup>b</sup> (56.31)	6.35 <sup>b</sup>	72.15 <sup>b</sup> (58.28)
T <sub>4</sub> : (Control)	20.70 <sup>d</sup> (19.28)	0(2.86)	22.80 <sup>d</sup>	0.0(2.86)
SEd	0.22	0.74	0.27	0.71
CD (P=0.05)	0.48	1.60	0.50	1.55
CV (%)	2.22	2.65	2.71	2.58

Values are mean of five replications. Figures in the parentheses represent arcsine transformed values. In each treatment numbers followed by the same letter are not significantly different to LSD test at P<0.05%

**Table 2. Effect of biocontrol agents and AM on growth parameters of coconut seedlings West Coast Tall (WCT)**

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	No. of roots	Collar girth (cm)	No. of leaves/plant	Root Volume (mm <sup>3</sup> /plant)	Root dry weight (g/plant)
T <sub>1</sub>	91.0 <sup>a</sup> (73.58)	98.80 <sup>a</sup> (77.94)	35.80 <sup>a</sup> (36.36)	12.80 <sup>a</sup> (20.87)	11.30 <sup>a</sup> (19.53)	6.50 <sup>a</sup> (14.76)	12.80 <sup>a</sup> (20.49)	4.42 <sup>a</sup> (12.15)
T <sub>2</sub>	85.40 <sup>a</sup> (67.13)	93.10 <sup>a</sup> (74.85)	28.10 <sup>a</sup> (31.68)	7.00 <sup>a</sup> (15.97)	8.34 <sup>a</sup> (16.78)	5.50 <sup>a</sup> (13.52)	9.96 <sup>a</sup> (18.37)	3.80 <sup>a</sup> (10.87)
T <sub>3</sub>	88.90 <sup>b</sup> (71.45)	95.2 <sup>b</sup> (76.72)	32.10 <sup>b</sup> (34.95)	9.20 <sup>b</sup> (17.64)	9.36 <sup>b</sup> (17.81)	5.90 <sup>b</sup> (13.97)	11.50 <sup>b</sup> (19.17)	4.00 <sup>b</sup> (11.59)
T <sub>4</sub>	76.30 <sup>d</sup> (49.57)	89.50 <sup>d</sup> (69.65)	27.0 <sup>d</sup> (30.32)	6.10 <sup>d</sup> (13.90)	7.50 <sup>d</sup> (15.88)	4.75 <sup>d</sup> (12.57)	9.52 <sup>d</sup> (17.96)	3.34 <sup>d</sup> (10.55)
SEd	0.34	1.47	0.48	0.63	0.34	0.2	0.371	0.29
CD (P=0.05)	0.74	3.2	1.05	1.389	0.75	0.62	0.81	0.64
CV (%)	0.81	3.10	2.29	5.95	3.11	3.28	3.07	4.09

Values are mean of five replications. Figures in the parentheses represent arcsine transformed values. In each treatment numbers followed by the same letter are not significantly different to LSD test at P<0.05%

**Table 3. Effect of biocontrol agents and AM on growth parameters of coconut seedlings Kenthali dwarf (KTD) seedlings**

Treatments	Germination (%)	Shoot length (cm)	Root length (cm)	No. of roots	Collar girth (cm)	Number of leaves/plant	Root Volume (mm <sup>3</sup> /plant)	Root dry weight (g/plant)
T <sub>1</sub>	89.1 <sup>a</sup> (70.66)	94.7 <sup>a</sup> (76.83)	26.28 <sup>a</sup> (30.83)	10.7 <sup>a</sup> (19.07)	8.90 <sup>a</sup> (17.23)	6.0 <sup>a</sup> (14.16)	9.8 <sup>a</sup> (18.28)	4.18 <sup>a</sup> (11.79)
T <sub>2</sub>	83.7 <sup>a</sup> (66.19)	84.36 <sup>a</sup> (66.74)	16.9 <sup>a</sup> (24.29)	6.90 <sup>a</sup> (15.63)	7.90 <sup>a</sup> (16.30)	4.2 <sup>a</sup> (11.81)	8.3 <sup>a</sup> (16.80)	2.60 <sup>a</sup> (9.28)
T <sub>3</sub>	87.0 <sup>b</sup> (68.87)	90.6 <sup>b</sup> (71.84)	20.0 <sup>b</sup> (26.54)	8.36 <sup>b</sup> (16.79)	8.80 <sup>b</sup> (17.19)	5.6 <sup>b</sup> (13.67)	8.9 <sup>b</sup> (17.39)	3.24 <sup>b</sup> (10.36)
T <sub>4</sub>	73.0 <sup>d</sup> (58.45)	78.8 <sup>d</sup> (62.58)	16.6 <sup>d</sup> (24.00)	6.40 <sup>d</sup> (14.40)	7.00 <sup>d</sup> (15.78)	4.0 <sup>d</sup> (11.16)	6.9 <sup>d</sup> (15.22)	2.35 <sup>d</sup> (4.33)
Sed	0.68	0.61	0.75	0.71	0.12	0.39	0.06	0.28
CD (P=0.05)	1.49	1.33	1.63	1.53	0.26	0.87	0.13	0.60
CV (%)	1.64	1.39	4.49	6.77	1.13	4.97	0.60	4.36

Values are mean of five replications. Figures in the parentheses represent arcsine transformed values. In each treatment numbers followed by the same letter are not significantly different to LSD test at P<0.05%

**Table 4. Efficacy of biocontrol agents and AM in management of bud rot disease in young coconut garden and their effect on growth parameters of coconut**

Treatments	Bud rot incidence (%) (after 30 days)	Disease Reduction over Control (%)	Palm height	Collar girth (cm)	No. of leaves/plant
T <sub>1</sub> : (Crown application of <i>Bacillus subtilis</i> endophyte at 10 g/l of water (1 litre/tree) was conducted twice, first during the pre-monsoon period (last week of May) and then during the post-monsoon period (September). Soil application of <i>Trichoderma asperellum</i> was carried out at a rate of 50 g per palm, and arbuscular mycorrhizal (AM) fungi were applied at 50 g per palm annually during January)	2.6 <sup>a</sup> (9.93)	71.73 <sup>c</sup> (59.99)	4.2 <sup>c</sup> (11.50)	118.2 <sup>a</sup>	13.5 <sup>c</sup> (21.78)
T <sub>2</sub> : (Crown application of metalaxyl at 2 g/l of water (1 litre/tree) was performed three times: during the pre-monsoon period (May), post-monsoon period (September), and January)	3.9 <sup>c</sup> (11.49)	57.60 <sup>c</sup> (49.79)	3.7 <sup>c</sup> (10.07)	98.5 <sup>c</sup> (80.84)	12.0 <sup>b</sup> (20.51)
T <sub>3</sub> : (Crown application of copper oxy chloride at 2.5 g/l of water (1 litre/tree) was also applied three times: during the pre-monsoon period (May), post-monsoon period (September), and January)	3.0 <sup>b</sup> (10.72)	67.39 <sup>b</sup> (58.35)	3.9 <sup>b</sup> (11.28)	99.7 <sup>b</sup> (82.29)	12.8 <sup>b</sup> (20.04)
T <sub>4</sub> : Control	9.4 <sup>d</sup> (17.61)	0 (2.86)	3.0 <sup>d</sup> (10.25)	91.0 <sup>d</sup> (72.88)	11.3 <sup>c</sup> (19.96)
SEd	0.357	3.74	0.404	1.33	0.416
CD (P=0.05)	0.777	0.169	0.88	3.077	0.905
CV (%)	4.53	2.26	5.85	2.68	3.19

Values are mean of five replications. Figures in the parentheses represent arcsine transformed values. In each treatment numbers followed by the same letter are not significantly different to LSD test at P<0.05%

## Discussion

Our study aligns with existing research that highlights the effectiveness of employing multiple biocontrol agents as a reliable approach to enhance biological control against plant diseases, particularly bud rot in coconut palms. These findings underscore the potential of integrated disease management strategies for sustainable agriculture.

In previous studies, seed nut dipping, soil drenching, and foliar sprays of the biocontrol agent *Pseudomonas fluorescens*-talc (1% solution) and the chemical fungicide Copper oxy chloride (0.3%) demonstrated promising results for managing bud rot in coconut nurseries (Surilirajan, 2014). These treatments have shown potential for integration into comprehensive disease management strategies.

However, our study deviates from this conventional approach by emphasizing the combined use of multiple strains of biocontrol agents, which proved to be more effective in reducing bud rot incidence compared to combinations of biocontrol agents and chemical fungicides. This approach is in line with the observations made by various researchers who have reported that mixtures of biocontrol agents can exert a broader spectrum of activity, enhance

overall biological control efficacy, and induce greater production of defense enzymes compared to individual strains (Latha *et al.*, 2009; Senthilraja *et al.*, 2010).

The consistent success of our study, where *Bacillus subtilis* and *Trichoderma asperellum* were employed in both nursery and young coconut seedlings, can be attributed to several mechanisms. While we did not document the production of antibiotics, toxins, and siderophores in our research, previous studies have substantiated the role of these metabolites in disease inhibition (Daniel Jebaraj *et al.*, 2012). *Trichoderma asperellum*, in particular, is known to produce antibiotics and engage in mycoparasitism, which mechanically and chemically eliminates pathogens. Additionally, it competes for space, nutrients, and oxygen, contributing to disease suppression (Jayaratne *et al.*, 2015).

The role of *Bacillus* species in disease suppression can be linked to their production of various peptide antibiotics, low molecular weight volatile compounds, and lipopeptides with specific antifungal properties. These compounds, such as fengycin and iturin, are known for their antifungal activities and may have contributed to bud rot reduction in our study (Zhang *et al.*, 2013). Additionally, certain rhizosphere bacteria, including *Bacillus* species, can produce plant

growth-promoting substances like gibberellins and cytokinins, further enhancing crop health and yield (Pliego *et al.*, 2011; Kang *et al.*, 2009).

The concept of Induced Systemic Resistance (ISR) is significant in understanding how various microbes, including *Bacillus* and *Trichoderma* species, contribute to disease suppression. ISR can be induced by live or dead microbes, as well as specific bacterial molecules and organelles. These mechanisms have been reported to trigger the defense responses of plants against pathogens (Kloepper and Ryu, 2006). The mode of action of *Bacillus* spp. in our study may involve these mechanisms, although further research is needed to elucidate their specific contributions.

Our findings align with previous research, suggesting that *Bacillus* spp. possess significant antifungal potential. They have been demonstrated to suppress various plant pathogens, including *Fusarium oxysporum* and *Phytophthora capsici* (Shahzad *et al.*, 2017; Melnick *et al.*, 2008). Additionally, endophytic bacterial strains like *Bacillus subtilis* have shown efficacy against phytopathogenic fungi, such as *Rhizoctonia solani*, in cotton seedlings (Selim *et al.*, 2017).

Furthermore, the production of phytoalexins and other defense-related compounds by the host plant in response to microbial colonization, such as with Arbuscular Mycorrhizal Fungi (AMF), can contribute to disease suppression and promote plant growth (Jeandet *et al.*, 2013; Harrier and Watson, 2004). The enhanced nutrient uptake, particularly phosphorus, associated with mycorrhizal plants may play a role in disease reduction (Olowe *et al.*, 2018).

In conclusion, our study provides valuable insights into the effectiveness of integrated disease management strategies for combating bud rot in coconut palms. The combined use of multiple biocontrol agents, such as *Bacillus subtilis* and *Trichoderma asperellum*, holds promise for sustainable disease control. While the specific mechanisms involved in disease suppression were not fully explored in our research, the findings

corroborate previous studies and open avenues for future investigations into the modes of action of these biocontrol agents at different stages of coconut cultivation. An integrated approach that combines various factors, including cultivar selection, drainage, fertilization, monitoring, and infected tissue removal, should be considered for comprehensive disease management in coconut farming (Torres *et al.*, 2016).

## Conclusion

The comprehensive investigation into bud rot management in coconut cultivation has yielded valuable insights that can significantly contribute to the sustainable growth of this vital crop. Our findings underscore the efficacy of treatment, comprising the soil application of *T. asperellum* at 25 g/cent during sowing, crown application of *B. subtilis* endophyte at 10 g/liter of water at three and six months post-sowing, and the application of AM fungi at 50 g/cent at three months post-sowing. This treatment not only reduced bud rot incidence but also exhibited a positive impact on germination and growth attributes in coconut seedlings, outperforming alternative treatments.

Furthermore, the study extended its investigation to young coconut gardens, revealing that treatment, which included crown application of *B. subtilis* endophyte, soil application of *T. asperellum*, and AM fungi during different stages of growth, effectively mitigated bud rot incidence. These findings highlight the potential of integrated disease management strategies for the long-term health and productivity of coconut palms. While foliar application of fungicides showed efficacy in reducing fungal diseases' incidence, our study emphasizes the importance of avoiding sole reliance on chemical control measures. Such approaches not only prove uneconomical but also pose potential hazards to ecological and environmental health.

This research has not only addressed a significant challenge in coconut cultivation but has also paved the way for future investigations. Subsequent researchers can focus on identifying the specific antibiotics, enzymes, and other

components responsible for the observed reduction in coconut bud rot incidence during both nursery and young seedling stages. A deeper understanding of the underlying mechanisms will further enhance our ability to develop sustainable disease management practices and ensure the prosperity of coconut farming. In conclusion, this study contributes to the body of knowledge in coconut agriculture and provides valuable insights for future research and practical applications in the field.

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## References

- Ariza, J. G., Torres, G. A., Sarria, G. A., Varón, F., and Martínez, G. 1977. Relación entre los síntomas externos y el avance interno de la lesión causada por la Pudrición del cogollo (PC) en palmas de vivero en Tumaco. *Nuestra Portada*, 36.
- CDB. 2022. Coconut Development Board, India. <http://www.coconutboard.gov.in>.
- Daniel Jebaraj, M., Manjunath Hubballi, Selvaraj, M., Jagannathan, R. and Srinivasan, V.M. 2012. Combining and strains with organic amendments to enhance suppression of root rot disease incidence in coleus. *Inter J Cur Res*. 4:116-126.
- Drenth, A., and Guest, D. I. 2013. *Phytophthora palmivora* in tropical tree crops. Page 244 in: *Phytophthora: A Global Perspective*. K. H. Lamour, ed. CABI, Wallingford, U.K.
- Gangaraj Karyath Palliyath, Muralikrishna Kilingar Subrahmanya, Ginny Antony, Sahu Binod Bihari, Vinayaka Hegde and Rajesh Muliya Krishna. 2021. A rapid in vitro leaf inoculation assay to investigate *Phytophthora palmivora*–coconut interactions. 169:5316-328.
- Harrier, L. A., Watson, C. A. 2004. The potential role of Arbuscular Mycorrhizal (AM) fungi in the bioprotection of plants against soil-borne pathogens in organic and/or other sustainable farming systems. *Pest management Science* 60: 149-157.
- Hoddle, M.S., Lake, E.C., Minter, C.R. and Daane, K.M. 2021. Importation biological control. CSIRO Publishing, Victoria, pp 67–89.
- Jayarathne, D. L. and Dayaratne, M. T. A. 2015. Phenotypic variability of *Ceratocystis paradoxa* isolated from North Western and Western Provinces of Sri Lanka and its Bio control by potential bio-control agent; *Trichoderma viridae*. *International Journal of Coconut R & D*, 31(20): 42-51.
- Jeandet, P., Clément, C., Courot, E. and Cordelier, S. 2013. Modulation of phytoalexin biosynthesis in engineered plants for disease resistance. *International Journal of Molecular Sciences*, 14(7): 14136-14170.
- Kang, S. M., Joo, G. J., Hamayun, M., Na, C. I., Shin, D. H., Kim, H. Y. and Lee, I. J. 2009. Gibberellin production and phosphate solubilization by newly isolated strain of *Acinetobacter calcoaceticus* and its effect on plant growth. *Biotechnology letters* 31:277-281.
- Kloepper, J.W. and Ryu, C.M., 2006. Bacterial endophytes as elicitors of induced systemic resistance. In: *Microbial root endophytes*; Schulz B, Boyle C, Sieber T, Eds. *Springer-Verlag*: Berlin Heidelberg .33-52.
- Konam, J. K. and Guest, D. I. 2004. Role of beetles (Coleoptera: Scolytidae and Nitidulidae) in the spread of *Phytophthora palmivora* pod rot of cocoa in Papua New Guinea. *Australas. Plant Pathol.* 33:55-59.
- Latha, P., Anand, T., Ragupathi, N., Prakasam, V. and Samiyappan, R. 2009. Antimicrobial activity of plant extracts and induction of systemic resistance in tomato plants by mixtures of PGPR strains and Zimmu leaf extract against *Alternaria solani*. *Biol. Cont.* 50:85–93.
- Martínez, G., Arango, M., Torres, G., Sarria, G., Vélez, D., Rodríguez, J. and Guest, D. 2013. Avances en la investigación sobre las dos enfermedades más importantes en la palma de aceite en Colombia: la Pudrición del cogollo y la Marchitez letal. *Palmas*, 34(1):39-47.
- Martinez, G. 2009. Identificación temprana y manejo integrado de la pudrición del cogollo. *Palmas* 30:63-77.
- Melnick, R.L., Zidack, N.K., Bailey, B.A., Maximova, S.N., Gultinan, M. and Backman, P.A. 2008. Bacterial endophytes: *Bacillus* spp. from annual crops as potential biological control agents of black pod rot of cacao. *Biological control* 46(1): 46-56.
- Montoya, M. M., Evans, E., Grogan, K., and Díaz, C. A. F. 2014. Un modelo de simulación discreto para determinar la edad óptima de replantación en



- presencia de la Pudrición del cogollo (PC). *Palmas*, 35(1): 19-32.
- Olowe, O. M., Olawuyi, O. J., Sobowale, A. A., and Odebode, A. C. 2018. Role of arbuscular mycorrhizal fungi as biocontrol agents against *Fusarium verticillioides* causing ear rot of *Zea mays* L.(Maize). *Current Plant Biology* 15: 30-37.
- Oyewole, O. B., Olawuyi, J. O., Odebode, C. A. and Abiala, A. M. 2017. Influence of Arbuscular mycorrhiza fungi (AMF) on drought tolerance and charcoal rot disease of cowpea. *Biotechnology Reports*, 14: 8-15.
- Pliego, C., Kamilova, F. and Lugtenberg, B. 2011. Plant Growth-promoting Bacteria: Fundamentals and Exploitation. In: *Bacteria in Agrobiolgy: Crop Ecosystems*. Maheshwari DK, ed. *Springer, Germany*. 295-343.
- Ramesh, R., Maruthadurai, R., and Singh, N. P. 2013. Management of bud rot disease in the coconut plantations of Goa. <https://www.researchgate.net/publication/267029217>.
- Salzer, P. and Boller, T. 2000. Elicitor-induced reactions in mycorrhizae and their suppression. In: Podila GK, Douds DD, eds. *Current advances in mycorrhizae research*. Minnesota, USA: *The American Phytopathological Society*, 1–10.
- Selim, H. M., Gomaa, N. M. and Essa, A. M. 2017. Application of endophytic bacteria for the biocontrol of *Rhizoctonia solani* (Cantharellales: ceratobasidiaceae) damping-off disease in cotton seedlings. *Biocontrol Science and Technology*, 27:81-95.
- Senthilraja, G., Anand, T., Durairaj, C., Kennedy, J.S., Suresh, S., Raguchander, T. and Samiyappana, R. 2010. A new microbial consortia containing entomopathogenic fungus, and plant growth promoting rhizobacteria *Beauveria bassiana*, *Pseudomonas fluorescens* for simultaneous management of leaf miners and collar rot disease in groundnut. *Biocont Sci Technol*. 20:449–464.
- Shahzad, R., Abdul Latif, Khan., Saqib, Bilal., Sajjad, Asaf. and In-Jung, Lee . 2017. Plant growth-promoting endophytic bacteria versus pathogenic infections: an example of *Bacillus amyloliquefaciens* RWL-1 and *Fusarium oxysporum* f. sp. *lycopersici* in tomato. *PeerJ* 5:p3107.
- Sharadraj, K.M. and Chandramohanam, R. 2014. A new detached coconut leaf let technique for bioassay of Fungicides against *Phytophthora palmivora* – the incitant of coconut bud rot. *International journal of plant protection*. volume 7: 161-165.
- Surulirajan, M., Rajappan, K., Sathesh Kumar, N., Annadurai, K., Jeevan Kumar, K. and Asokhan, M. 2014. Management of bud rot disease of coconut in nursery. *International Journal of Tropical Agriculture* 32:415-418.
- Thakur, R., Gupta, D., and Jandaik, S. 2022. Bio-control by using Antagonistic (Filamentous Fungi and VAM) and Bacteria against *Macrophomina phaseolina*. *international journal of plant and environment*. 8(02):106-110.
- Torres, G. A., Sarria, G. A., Martinez, G., Varon, F., Drenth, A., and Guest, D. I. (2016). Bud rot caused by *Phytophthora palmivora*: a destructive emerging disease of oil palm. *Phytopathology* 106(4): 320-329.
- Wittenberg, R. and Cock, M.J.W. (eds.) 2001. *Invasive Alien Species: A Toolkit of Best Prevention and Management Practices*. CAB International, Wallingford, Oxon, UK.
- Zhang, X., Li, B., Wang, Y., Guo, Q., Lu, X., Li, S. and Ma, P. 2013. Lipopeptides, a novel protein, and volatile compounds contribute to the antifungal activity of the biocontrol agent *Bacillus atrophaeus* CAB-1. *Appl. Microbiol. Biotechnol.* 97: 9525–9534.