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Research Article

Field evaluation of *Trichoderma reesei* (Drysrhu), an effective biocontrol agent against *Phytophthora* pod rot in cocoa

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ABSTRACT: Cocoa (*Theobroma cacao* L.) is a commercially valuable plantation crop grown in tropical regions, including India, where it is commonly cultivated as an intercrop in coconut, oil palm, and arecanut-based farming systems. Although cocoa is a cash crop for farmers, in recent years, black pod rot caused by *Phytophthora palmivora* (E.J. Butler), a hemibiotrophic oomycete with a broad host range, has emerged as a major constraint in cocoa cultivation. This pathogen primarily affects cocoa pods, resulting in severe yield losses. In the present study, the efficacy of *Trichoderma reesei* (Drysrhu) was assessed under field conditions from 2020-2022 for two years through foliar and soil applications. Foliar application at a concentration of 2×10^6 cfu/ml led to a significant reduction in disease incidence (25.04%), whereas soil application of 50 g *T. reesei* combined with 5 kg neem cake further reduced the incidence to 17.03%. These results suggest that *T. reesei*, particularly when applied as a foliar spray, holds promise as an effective biocontrol agent for the integrated management of black pod rot disease in cocoa.

KEYWORDS: Andhra Pradesh, biocontrol, cocoa, pod rot, *T. reesei*

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INTRODUCTION

The Cocoa tree (*Theobroma cacao* L., Malvaceae) is an understory plant native to the Amazon region of South America. This tropical tree produces fruits, known as pods, which contain seeds, commonly referred to as cocoa beans. These beans are the primary raw material in the confectionery industry, especially for chocolate production.

Nationwide, cocoa occupies an area of approximately 105,975 ha with an estimated annual production of 28,426 metric tonnes and a productivity of 350-400 kg/ha. Andhra Pradesh stands as the leading cocoa-producing state, contributing about 11,448 MT from 41,874 ha (DCCD, 2021-2022). Cocoa cultivation in the state has significantly enhanced farmers' incomes, especially when grown as an intercrop with coconut, oil palm and arecanut, making it a profitable component of integrated farming systems.

Cocoa cultivation in India has seen significant growth, particularly in Andhra Pradesh (AP), which has emerged as the country's leading producer. Cocoa was introduced in Andhra Pradesh during the 1990s, primarily as an intercrop in coconut groves in the Erstwhile East Godavari district. Over time, its cultivation expanded to other coastal districts, including West Godavari, Eluru, Srikakulam, Konaseema, Kakinada, Krishna, and Parvathipuram Manyam. The state's favourable agro-climatic conditions, combined with the practice of intercropping, have made cocoa cultivation both viable and profitable for farmers.

Cocoa farming is a lifeline for approximately 5.5 million smallholder cocoa farmers and employs 14 million rural workers worldwide (Fountain & Hutz-Adams, 2015). The nutritional and health benefits of cocoa, owing to its high polyphenol content, have further amplified its demand.

The potential cocoa farming faces a significant threat from the various fungal diseases, among which *Phytophthora palmivora* has the ability to attack pods at any stage of development. Sporangia or zoospores that germinate and penetrate the pod surface may cause infection. Disease symptoms may appear 3-4 days after infection, and sporangia production may begin on the night following the second day of

visible symptoms (Spence, 1961). Adegbola (1993) described a typical black pod in addition to 'the seaweed-like' odour that characterises pod rot caused by *P. palmivora* (Sreenivasan & Quesnel, 1977). The disease's most common symptom is fruit rot, also known as black pod or *Phytophthora* pod rot. However, stems and trunks can also be affected, with cankers appearing on the bark and flower cushions disappearing. Infections have been noticed on leaves and roots as well, although less frequently and with a much lower frequency.

When *P. palmivora* infects cocoa, the pathogen penetrates the waxy cuticle and attacks the epidermis. At the site of infection, a small brownish spot appears. Such infections can start at the stem or blossom ends of fruits and spread to the fruit. Infection spreads quickly across the outer surface, eventually covering the entire pod in a matter of days. Infected areas turn brown to black, and if conditions are favourable, clusters of white sporangia appear on the pod's outer surfaces. As visible symptoms progress, the pathogen moves deeper into the pod, infecting and destroying the beans. Infected beans quickly deteriorate and rot, rendering the pod useless. The pods then dehydrate and mummify.

Current management strategies for black pod disease rely on a combination of chemical and cultural methods. Fungicides, such as copper-based formulations, are commonly used to prevent and control fungal infections on pods (Akrofi *et al*., 2012). However, frequent fungicide application can be expensive, environmentally harmful, and lead to the development of fungal resistance (N'Guessan *et al*., 2005). Hence,biocontrol-based management of pod rot disease was conducted for the benefit of the farming community.

MATERIALS AND METHODS

Field trials were conducted during 2020-2021 and 2021-2022 at Avidi village (Latitude 16.674415, Longitude 81.904060) of Kothapeta Mandal of Dr. B. R. Ambedkar Konaseema district, Andhra Pradesh, India, in the Coconut-cocoa cropping system of Konaseema district. These areas had a previous history of a high incidence of *Phytophthora* diseases in cocoa. These gardens have approximately 140-150 plants per acre, and they were 15-20-year-old plantations. The causal organism of black pod disease in the experimental locality was identified as *P. palmivora* based on the laboratory examination of morphological characters and molecular conformity with ITS1 and ITS4 primers (Neeraja *et al*., 2025) collected randomly from the cocoa garden during the south-west monsoon period of 2019 and 2020.

The experiment was laid out in a Randomised block design with four treatments and seven replications. The treatment details are as follows.

T_1- Spraying of *Trichoderma reesei* (Drysrhu- *T. reesei* 1) spore suspension (2×10^6 cfu/ml)

T_2 - Soil application of 50 g of *T. reesei* along with 5kg Neem cake

T_3 - Spraying of copper oxychloride (3 g/l of water)

T_4 - Control

The first spray was given soon after disease appearance and subsequently, at a 45-day interval. The fresh microbial spore suspension of *T. reesei* (talc-based) containing 2x106 cfu/g was obtained from Horticulture Research Station, Ambajipeta and sprayed by 10 g in one litre of water (1%), soil application of 50 g talc-based formulation of *T. reesei* along with 5kg Neem cake was applied at the basins of the cocoa tree and spaying of Copper oxy chloride 3 g dissolves in one litre of water applied as foliar spray on cocoa pods. The per cent disease incidence was calculated. The fungicides and biocontrol agents were sprayed on pods, main stem and branches. Cultural practices, including Integrated Nutrient Management (INM), plant and field hygiene, pruning and other recommended cultivation practices were adopted in all the treatments.

The disease incidence in the fields was assessed with the following formula (Jayalakshmi *et al*., 2017):

Per cent Disease Incidence = (Number of infected Plants/Total number of plants examined) * 100

The per cent reduction over the untreated control was computed using the following formula:

Per cent reduction over control = [(Per cent disease incidence in control – Per cent disease Incidence in treatment)/ Per cent disease Incidence in control] * 100

The values were arc sine transformed prior to statistical analysis to stabilise variances and meet ANOVA assumptions (Gomez & Gomez, 1984). The transformed data were subjected to Analysis of Variance (ANOVA) to determine the significance of differences among treatments using the RBD model.

The Critical Difference (CD) at 5% level of significance ($p \leq 0.05$) was used to compare treatment means. The Standard Error of Mean (SEM) was also calculated to assess the precision of the mean estimates. All statistical analyses were carried out using OPSTAT software (Sheoran *et al*., 1998). The results were interpreted using transformed values, while actual percentage values were presented in the tables. Graphs were prepared using MS Excel 2021 for visual interpretation of treatment effects.

RESULTS AND DISCUSSION

The efficacy of different treatments against the disease was evaluated based on disease incidence recorded at three intervals: Pre-treatment, 45 Days After Treatment (DAT), and 90 DAT (Table 1). All treatments significantly reduced disease incidence compared to the untreated control.

At 45 DAT, the lowest disease incidence (12.04%) was recorded with foliar spraying of copper oxychloride (3 g/l) (T3), showing a 52.00% reduction over the control. The high initial efficacy of copper oxychloride may be attributed to its protective action, forming a persistent fungitoxic barrier on plant surfaces, thus preventing spore germination and pathogen entry. These findings are consistent with earlier studies by Hebbar (2007) and Gowda *et al*. (2012), who demonstrated that copper fungicides are effective in reducing Phytophthora pod rot and leaf spot diseases in perennial crops like cocoa and coconut.

The second most effective treatment was foliar application of *T. reesei* (T1) at 2×10^6 cfu/ml, which resulted in a disease incidence of 14.10% and a 32.30% reduction over control. *T. reesei* is known to possess potent antagonistic properties, including competition, mycoparasitism, and production of hydrolytic enzymes like chitinases and glucanases (Harman *et al*., 2004). These enzymes degrade fungal cell walls, inhibiting pathogen development. Moreover, foliar application facilitates phyllosphere colonisation, triggering Induced Systemic Resistance (ISR) in host plants, as reported by Yedidia *et al*. (2003) and Woo *et al*. (2014).

Soil application of *T. reesei* (T2), supplemented with neem cake (50 g + 5 kg), showed 16.08% disease incidence and 31.25% reduction. The organic amendment not only served as a substrate for *Trichoderma* multiplication but also enriched the soil microflora, creating an unfavourable environment for soil-borne pathogens. Neem cake is known to release azadirachtin and related limonoids that have antifungal and nematicidal properties (Gopalakrishnan *et al.*, 2011). These results corroborate findings by Senthilkumar *et al*. (2020), who showed that integrating *Trichoderma* with neem cake reduced disease incidence in plantation crops under field conditions.

In contrast, the untreated control plots (T4) recorded the highest disease incidence of 24.96% at 45 DAT, emphasising the natural progression of the disease under favourable environmental conditions such as high humidity and intermittent rains during monsoon months, which enhance pathogen sporulation and infection. At 90 DAT, the trend was largely maintained. The lowest disease incidence was recorded with T1 (10.57%), achieving a 25.04% reduction over control, followed by T2 with 13.34% and 17.03% reduction, and T3 with 11.52% and 4.31% reduction. Though copper oxychloride showed high efficacy initially, its effectiveness waned over time, possibly due to leaching or degradation under field conditions. This reduction in residual activity over time has been previously reported by Anandaraj *et al*. (2009) and Roy *et al*. (2017), suggesting the need for repeated applications or integration with biocontrol agents for sustainable disease suppression.

Interestingly, biocontrol treatments (T1 and T2) maintained consistent disease suppression over the 90 days. This may be due to the ability of *Trichoderma* spp. to establish themselves in both the rhizosphere and phyllosphere, offering long-term protection (Monte, 2001). Moreover, *T. reesei* has been genetically characterised to possess strong mycoparasitic ability and metabolic versatility, enabling it to persist in diverse environmental niches (Kubicek *et al*., 2011). The combination of biocontrol agents and organic amendments appears to promote plant vigour and enhance

Table 1. Pooled mean pod rot disease incidence during 2020-2022

Treatment No.	Name of the treatment	Mean per cent disease incidence				
		Pretreatment data	45 days after treatment	Reduction over control	90 days after treatment	Reduction over control
T_1	Spraying of *Trichoderma reesei* – Drysrhu- *T. reesei* 1@ 2×10^6 cfu/ml)	20.83 (24.28)	14.10 (18.25)	32.30 (30.28)	10.57 (12.89)	25.04 (26.52)
T_2	Soil application of 50 g *T. reesei* along with 5 kg neemcake	23.39 (29.39)	16.08 (22.05)	31.25 (35.26)	13.34 (15.36)	17.03 (22.31)
T_3	Spraying of copper oxychloride (3 g/l of water)	25.09 (26.32)	12.04 (16.05)	52.0 (65.32)	11.52 (15.26)	4.31 (8.56)
T_4	Control (without any treatment)	23.95 (31.25)	24.96 (32.43)	4.21* (8.21)	26.50 (31.25)	6.16* (11.45)
SEM (±)		2.59	2.52	-	2.28	-
CD (0.05%)		6.06	8.05	-	7.46	-

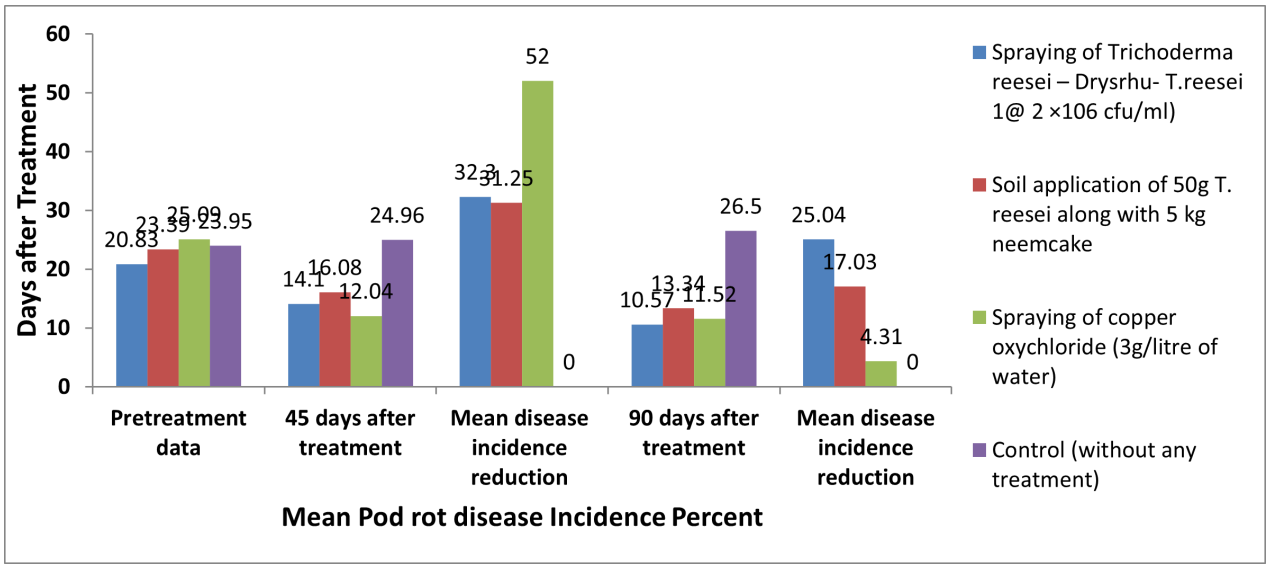


Figure 1. Effect of different treatments on pod rot disease incidence (%) in cocoa at pretreatment, 45 and 90 days after application. Treatments: T1- Foliar spray of *T. reesei* @ **2 ×** 10^6 cfu/ml, T2 - Soil application of 50 g *T. reesei* + 5 kg neem cake, T3 - Spraying of copper oxychloride (3 g/l), T4 - Untreated control.

the plant's innate defence mechanisms, which aligns with the findings of Verma *et al*. (2017), who emphasised the synergistic effects of PGPR and organic inputs in disease suppression.

The results of the field experiment conducted to evaluate the efficacy of different treatments on the management of *Phytophthora* pod rot disease in cocoa are presented in Figure 1. At pretreatment, disease incidence ranged between 20.83% and 29.95% across all treatments and controls, indicating uniform initial infection levels and validating the comparability of treatments. A significant reduction in pod rot incidence was observed 45 days after treatment. Among the treatments, foliar spraying of copper oxychloride (3 g/l) recorded the lowest disease incidence (12.04%), followed by soil application of *T. reesei* along with neem cake (14.1%) and foliar spray of *T. reesei* (16.08%). The untreated control plot recorded the highest disease incidence (24.96%) at 45 days after treatment. The efficacy of copper oxychloride was further reflected in its superior mean disease incidence reduction, which was 52% and 4.31% at 45 and 90 days after treatment, respectively. However, the efficacy diminished slightly at 90 days, where disease incidence increased to 11.52%. In contrast, *T. reesei* foliar application maintained a consistent reduction (32.3% and 25.04% at 45 and 90 days, respectively), indicating sustained biocontrol activity.

The soil application of *T. reesei* with neem cake showed moderate effectiveness, with 31.25% and 17.03% disease reduction at 45 and 90 days, respectively. The untreated control consistently recorded significantly higher disease levels at all intervals, highlighting the importance of active disease management strategies. These findings align with earlier studies by Singh *et al.* (2019), who reported significant suppression of *Phytophthora* pod rot in cocoa following application of *Trichoderma* spp. The combination of *T. reesei* and neem cake likely enhanced soil microbial activity and induced systemic resistance, as also documented by Sharma and Goswami (2021). The rapid reduction observed with copper oxychloride corroborates the fungicidal efficiency reported in chemical management of cocoa diseases (Rahman *et al.*, 2020), though its long-term sustainability and environmental concerns highlight the relevance of biological alternatives. The long-term sustainability of the bioagent is closely linked to its ability to colonise and persist at the site of application. Although this study did not directly assess colonisation, future work should include quantification of establishment, niche-occupation and temporal persistence (*e.g.*, by re-isolation, marker-based tracking or molecular quantitation) so as to confirm ecological stability of the treatment. Recent studies highlight the importance of colonisation capacity for biocontrol and plant-beneficial microbes (Riseh *et al*. 2025; Knights& Poole, 2024).

COCLUSION

Thus, the current study demonstrates that both biological control using *T. reesei* and chemical control with copper oxychloride are effective strategies, though biocontrol offers more sustainable and persistent protection. These findings highlight the potential of integrating eco-friendly biocontrol options into crop disease management frameworks, especially in plantation crops like cocoa or coconut, where long-term sustainability is crucial.

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