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The Efficacy of Bordeaux Mixture and Trichoderma Powder in Preventing Fungal Diseases on Cashew Nut Wounds after Pruning

In Sokra[2,3,*] and Srun Somaly[1]
[1]Department of Soil and Crop Production, Faculty of Agronomy, University of Kratie, Camodia
[2]Department of Food Engineering, Faculty of Agro-Industry, University of Kratie, Cambodia
[3]Metabolic Engineering Research Unit, School of Biotechnology, Suranaree University of Technology, 111 University Avenue, Suranaree, Muang, Nakhon Ratchasima, 30000, Thailand

Abstract:- Cashew cultivation is an important agricultural industry in Cambodia, providing high income for farmers. Pruning cashew trees helps maintain their health and ensures adequate light exposure, but it also makes them susceptible to fungal infections, which can lead to reduced yields and cause some farmers to abandon cultivation. This study aimed to identify effective biological products for protecting cashew wounds from fungal infections. It was conducted in two phases: the first phase determined the inhibitory effects of *Trichoderma asperellum* on pathogenic fungi grown on potato dextrose agar plates, after which *T. asperellum* was produced in powder form. The second phase applied Bordeaux mixture and Trichoderma powder to cashew wounds, using a completely randomized design with three treatments: T1: 10% Bordeaux mixture, T2: 40% Trichoderma powder, and T3: 50% Trichoderma powder. Results showed that *T. asperellum* completely inhibited *Rhizopus* fungus within 6 days. One-way ANOVA analysis of the percentage of healing showed a significant difference ($p < 0.01$). T3 (50% Trichoderma powder) was the most effective, preventing fungal infection for up to 60 days and reducing the healing period from 90 to 3.83 ± 4.4%. The use of Trichoderma powder also protected against fungal diseases like gummosis, *Penicillium*, and *Rhizopus*, with 50% Trichoderma powder proving most effective in preventing fungal infections and speeding up wound healing after pruning.

Keywords:- *Bordeaux Mixture; Trichoderma Powder; Fungal Disease; Cashew Wound; Pruning.*

I. INTRODUCTION

The cashew tree is a versatile crop that serves multiple purposes and contributes significantly to the global economy (Oliveira et al., 2020). Currently, cashew farming is a high-profit venture for farmers in Cambodia as well (Koh et al., 2021). Cambodia has strong potential in both cashew cultivation and nut processing. Cashew nuts are processed domestically and sold to Cambodian industries before being exported internationally (Prak & Rot, 2019). A decline in cashew production would significantly affect Cambodia's economy (Prak & Rot, 2019). Factors contributing to the decline in cashew cultivation and production include land size, fertilizer usage, pesticides, insect control, technical training in cultivation, and market access (Wongnaa, 2013). To achieve high-yield cashew crops, farmers need to focus on pruning and orchard sanitation for effective orchard management (Kumar et al., 2015). Pruning is crucial for cashew trees as it reduces various risk factors on the entire tree and ensures sufficient light for all parts (Kumar et al., 2015). However, pruning can also cause wounds that may lead to fungal diseases and affect crop health. In Cambodia, three major diseases commonly affect cashew trees: Gummosis, Anthracnose, and Powdery mildew (Faust, 2023). Gummosis, often marked by sap oozing from wounds, weakens the tree and is caused by a particular fungus (Alves et al., 2015; Peralta-Ruiz et al., 2023). Generally, Gummosis affects wounds on plants and is treated with Bordeaux mixture (Gade & Koche, 2012). However, many farmers lack control measures, while others receive guidance from the Climate Resilience of Agricultural Systems (CRAS) project (Faust, 2023). A 10% Bordeaux mixture is applied to cashew wounds, and a 1% solution is used for general crop spraying (Faust, 2023). Despite its effectiveness, Bordeaux mixture is expensive and complicated to produce, making it less accessible to some farmers. In addition to Bordeaux mixture, biological control methods are used to manage fungal diseases on cashews and other crops. Among these, *Trichoderma* spp. is a popular biofungus in agriculture due to its efficiency in promoting growth and protecting against various fungal diseases (Zin & Badaluddin, 2020). Ramirez-Olier et al. (2019) found that Anthracnose, a disease caused by fungi, weakens cashew trees and can be managed with Trichoderma spp., which works either directly or indirectly against pathogens by producing antifungal compounds, degrading pathogen cell walls, or competing for nutrients (Asad, 2022). Ruangwong et al. (2021) found that *Trichoderma* spp. produces organic compounds that inhibit fungal growth, causing a decrease in fungal viability. Trichoderma powder, which is easier and cheaper to use, has also shown high effectiveness (Yao et al., 2023). New research highlights the need for effective methods to protect cashew trees from fungal infections post-pruning, especially in the context of Cambodia where Bordeaux mixture, despite its effectiveness, has limitations and potential negative effects if overused

(Gade & Koche, 2012). Trichoderma powder has shown high efficacy in agriculture, but specific studies evaluating its impact on cashew wounds post-pruning remain limited. Therefore, this study aims to address this gap by investigating cashew wound treatment using Bordeaux mixture, 40% Trichoderma powder, and 50% Trichoderma powder to provide better options for Cambodian farmers.

II. MATERIALS AND METHODS

A. Location and Experimental Period

The experiment on the production of Trichoderma powder was conducted at the Microbiology Laboratory of University of Kratie, located in Srae Sdao Village, Ou Russei Commune, Kratie City, Kratie Province, Cambodia. The field trial on cashew wound treatment was conducted in Saob Krom Village, Saob Commune, Prek Brasop District, Kratie Province. The study was carried out from June 1 to September 30, 2023, with 30 days allocated for laboratory work and 90 days for the field experiment. The experiment was conducted during the rainy season.

B. Raw Material

The raw materials used in this experiment included *Trichoderma asperellum*, rice, potato dextrose agar (PDA) powder, agricultural lime, and water. In the field experiment, 45 cashew trees, all of the same age (5 years), with similar trunk diameters and comparable wound sizes, were selected for the study.

C. Fungus Culture Media, Bordeaux Mixture, and Trichoderma Powder Preparation

A 4% PDA powder mixture was combined with water and subjected to sterilization at 121°C and 106 kPa (1 atm) pressure for 20 minutes. After sterilization, it was allowed to cool to 60°C before being poured into culture dishes for use in cultivating the fungal pathogens and T. asperellum. A 10% Bordeaux mixture was prepared by mixing 1 kg of copper sulfate with 10 liters of water, ensuring the solution was thoroughly dissolved and adjusting the pH to 7. For the Trichoderma powder, 400 grams were mixed with 1 liter of water to create a 40% Trichoderma powder suspension, and 500 grams were mixed with 1 liter of water to create a 50% Trichoderma powder suspension.

D. Field Experiment Design and Treatments

This experiment was conducted using two levels of Trichoderma powder (40% and 50%), which are easy-to-apply liquid suspension concentrations suitable for treating wounds on cashew trees, along with a 10% Bordeaux mixture, which is the concentration commonly used by farmers in the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) project. The experiment involved three factors, each repeated three times, with each replicate consisting of five cashew trees, for a total of 45 trees. The selected cashew trees were of the same age, had similar trunk diameters, and comparable wound sizes. The experimental design followed a completely randomized design (CRD).

$$I = \frac{C - T}{C} \times 100$$

Treatment 1 (T1): 10% Bordeaux mixture (Faust, 2023)
Treatment 2 (T2): 40% Trichoderma powder
Treatment 3 (T3): 50% Trichoderma powder

E. In Vitro Antagonism By Fresh Culture Technique

The antagonistic activity of *T. asperellum* against Rhizopus spp., a pathogenic fungus, was studied using the fresh culture technique (Nair, 2020) on PDA plates. Freshly prepared PDA plates were inoculated with 3-day-old cultures of *T. asperellum* and Rhizopus. Colonies of *T. asperellum* and *Rhizopus* were picked and inoculated on opposite edges of new PDA plates, while control plates were prepared by inoculating *Rhizopus* alone on one side. The plates were incubated at 28°C for 6 days to observe antagonistic activity.

The percentage inhibition of the radial growth of Rhizopus was calculated following the method described by Rahman et al. (2009).

$$I = \frac{C - T}{C} \times 100$$

Where:
I = Percentage inhibition of radial growth (%)
C = Radial growth of *Rhizopus* in the control plate (cm)
T = Radial growth of *Rhizopus* in the presence of *T. asperellum* (cm)

F. Measurement of the Percentage of Injury Area on Cashew Wounds

The percentage of injury on cashew wounds was determined by measuring the wound surface area of the cut cashew (circular shape, $A = \pi r^2$) in millimeters. The injury percentage was calculated at each time point (0, 15, 30, 45, 60, 75, and 90 days) to monitor the healing activity.

The percentage of wound area was calculated using the formula:

$$\text{Percentage of Wound Area} = \frac{\text{Wound Area on Day n}}{\text{Initial Wound Area}} \times 100$$

(Thakur et al., 2011).

G. Data Collection

In this study, the primary data were measured and calculated at each designated time point, recorded as numerical values and quantitative data. The growth of the fungal culture *T. asperellum* was measured daily over a 7-day period using a scale graduated in centimeters. The percentage of wound area was measured and calculated every 15 days over a 90-day period using the formula *F*.

H. Data Analysis

All experimental data were analyzed by calculating the mean values. After data collection, one-way ANOVA was performed using GraphPad Prism 9 version 9.5.1 (733), followed by Tukey's multiple comparisons test. Data points in Figures 4 and 7, and Table 1, are presented as mean values (M) with standard deviation (SD), and p-values < 0.01 were considered statistically significant for the analysis.

III. RESULT AND DISCUSSION

A. Antagonistic Activity

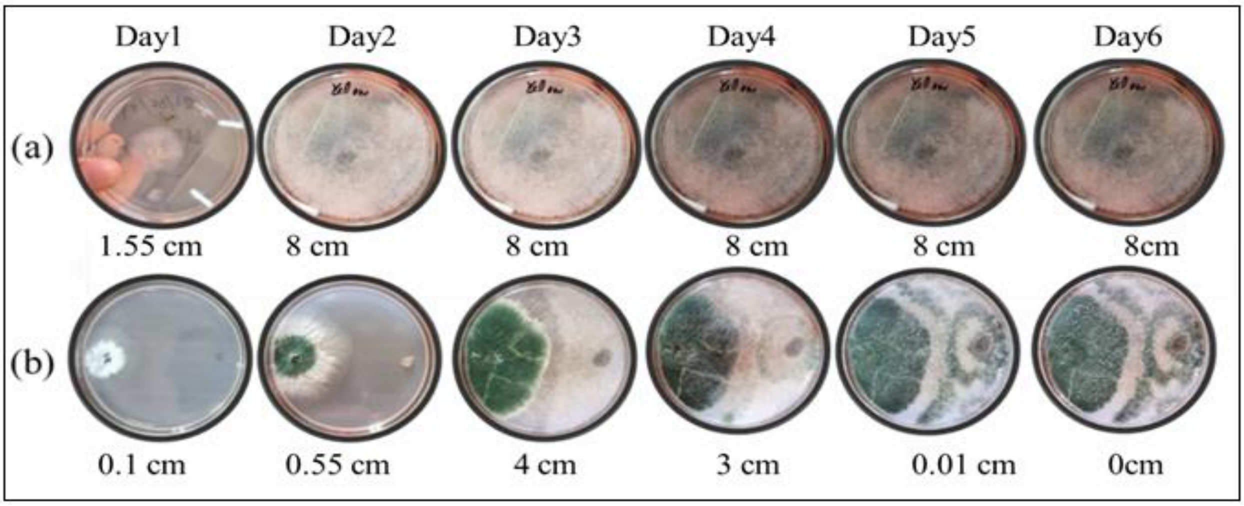

Fig 1. Cultivation of the biocontrol fungus T. asperellum and the pathogenic fungus (Rhizopus) on plant culture media. (a) Cultivation of the pathogenic fungus on PDA culture plates and (b) Cultivation of both the pathogenic fungus and the biocontrol fungus T. asperellum on a single PDA culture plate. Cm = centimeters.

Fig 1a Observation of the growth of the pathogenic fungus on cashew wounds showed rapid and vigorous growth in the absence of biocontrol fungi. Within just 2 days, the fungus had spread across the PDA plate, reaching a diameter of 8 cm. In contrast, the pathogenic fungus grown on the same PDA plate with the biocontrol fungus *T. asperellum* showed that the growth of the pathogen was inhibited (Figure 1b). On day 2, the pathogen's growth was limited to 0.55 cm, and by day 6, the pathogen was completely destroyed by *T. asperellum*. These results are consistent with the study by Go et al. (2019), which found that *T. asperellum* can inhibit pathogen growth within about a week. Similarly, Elshahawy & Marrez (2023) showed that the pathogen grows rapidly on PDA plates without *T. asperellum*, but *T. asperellum* effectively controls fungal growth with high efficacy. This study also aligns with the findings of Cuervo-Parra et al. (2022), which demonstrated that *T. asperellum* is effective in destroying pathogenic fungal hyphae. Within 2 days, the use of this biocontrol fungus resulted in a 47% reduction in pathogen growth.

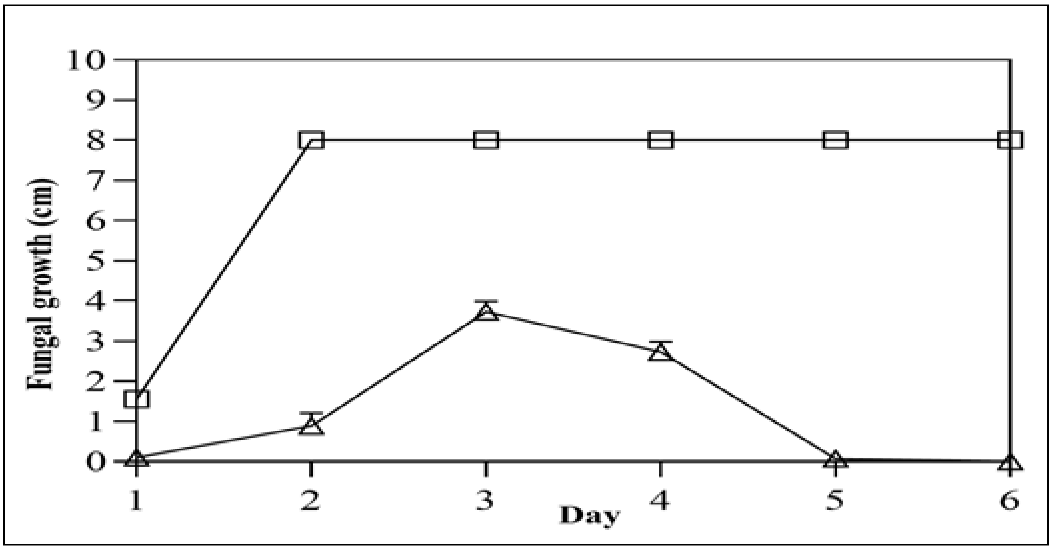

Fig 2. Growth length of the pathogenic fungus. Square = fungal hyphae of the pathogenic fungus on the culture plate, Triangle = fungal hyphae of the pathogenic fungus on the culture plate with the presence of the biocontrol fungus T. asperellum, cm = centimeters.

As shown in ***Fig 2***, the control factor (C) exhibited continuous growth, with the fungus completely covering the PDA plate within 2 days. In contrast, for the treatment (T), where the biocontrol agent *T. asperellum* was applied, the growth of the pathogen did not exceed 3.7 cm. The inhibitory effect continued to increase until the 6th day, when the pathogen was completely eradicated by *T. asperellum*, resulting in 100% biocontrol activity (Table 1). These results demonstrate that *T. asperellum* effectively suppressed the growth of the pathogen by overgrowing the fungal hyphae and halting their growth, consistent with the findings of Guzmán-Guzmán et al. (2023), who used *T. asperellum* and other Trichoderma species for biocontrol. Moreover, Yao et al. (2023) highlighted that *T. asperellum* produces antifungal and antibacterial compounds, enabling it to inhibit the growth of pathogens on various crops. It also collaborates with enzymes to degrade the cell walls of fungal pathogens, leading to enhanced crop growth. This aligns with our study, where *T. asperellum* demonstrated its antifungal activity, effectively eliminating the pathogens. Furthermore, when the pathogen was grown on PDA plates without any biocontrol agent, it exhibited rapid and healthy growth. However, when *T. asperellum* was introduced, the pathogen's growth was slowed significantly, and it was eventually eradicated. These findings are consistent with studies by Tamizi et al. (2022) and Martínez-Salgado et al. (2021), confirming the efficacy of *T. asperellum* as a biocontrol agent.

B. Fungal Presence on Cashew Wounds During Healing Process

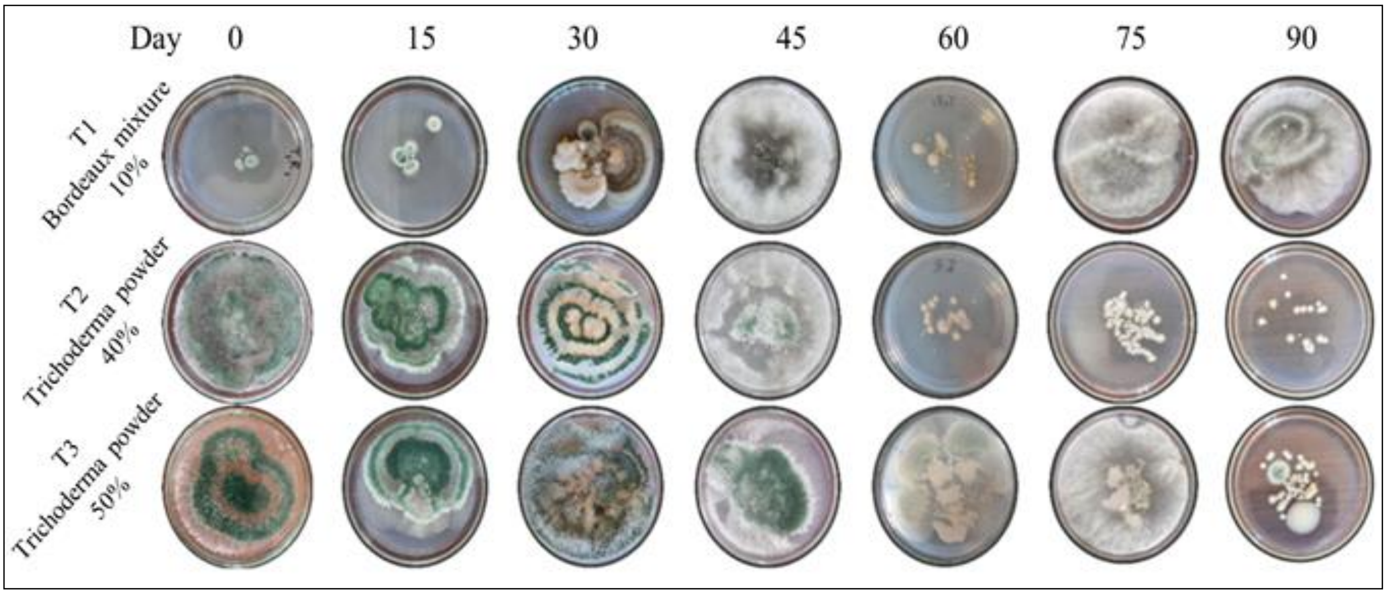

Fig 3. Fungi on the cashew wound of each treatment grown on PDA plates.

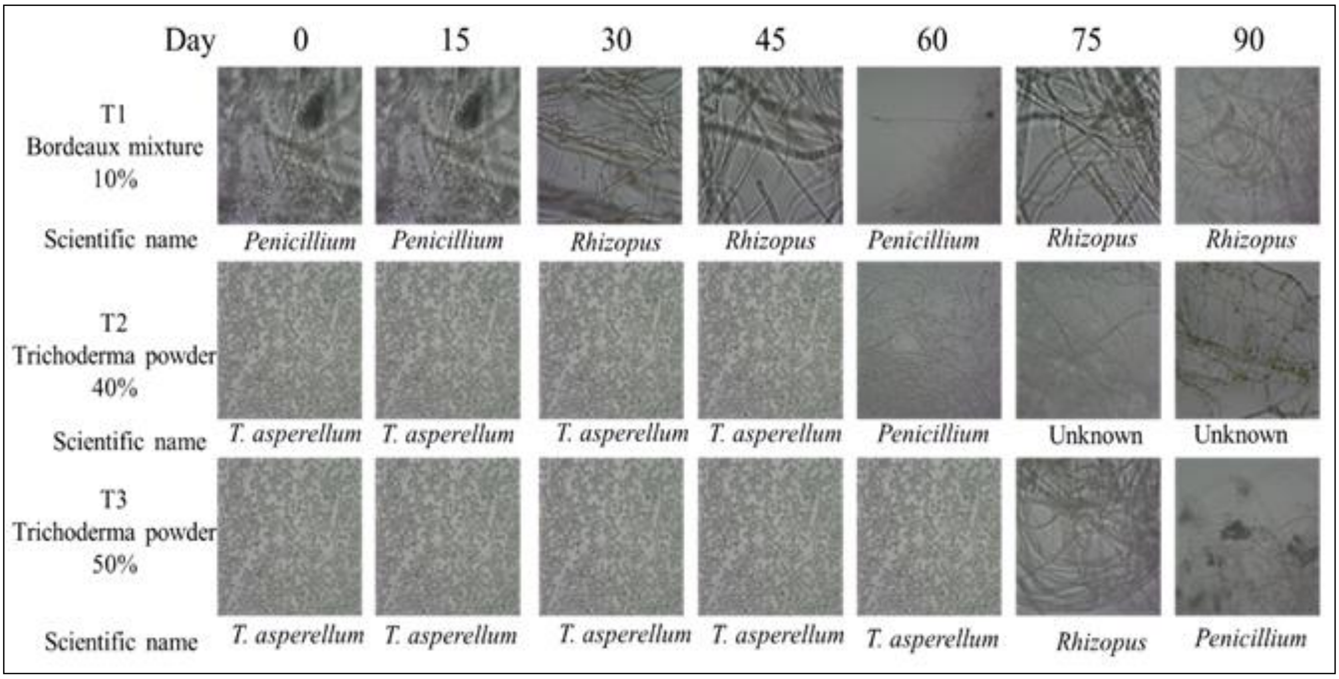

Fig 4. Structure of fungi on cashew wounds observed using an LB-1270 compound microscope.

T. asperellum was identified using the method described by Rhaman et al. (2009).
Penicillium was identified using the method described by Pitt et al. (2014).
Rhizopus was identified using the method described by Lennartsson et al. (2014).
Unknown = Unable to identify.

According to ***Fig 3*** and ***4***, the application of 10% Bordeaux mixture (T1) from Day 0 to Day 90 led to the growth of various fungi on cashew wounds, but not the biocontrol fungus *T. asperellum*. The fungi that developed on the culture plates of T1 included Penicillium and Rhizopus (Figure 4). In contrast, the use of 40% Trichoderma powder (T2) effectively prevented the growth of other fungi until Day 45, during which only *T. asperellum* was observed. From Day 45 to Day 90, however, Penicillium and other unidentified fungi began to appear. For 50% Trichoderma powder (T3), other fungi were effectively inhibited for up to 60 days, during which only the biocontrol fungus *T. asperellum* was observed. From Day 60 to Day 90, *Penicillium* and *Rhizopus* reappeared (***Fig 4***).

The findings of this study align with those of Freire et al. (2002) and Adebajo and Diyaolu (2003), who reported that cashew crops face multiple unprotected diseases, including anthracnose, gummosis, and leaf blight, caused by fungi such as Colletotrichum spp., Fusarium spp., Rhizopus, Aspergillus, Penicillium, and other pathogens. This study also supports the findings of Dominic et al. (2014) and Khatoom et al. (2017), who noted that cashew, despite its high market value, is vulnerable to pathogenic fungi such as *Pestalotiopsis palmarum*, *Phyllosticta* spp., *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Chaetomium brasiliense*, and *Cryptosporiopsis* spp.

Furthermore, this study is consistent with the research of Parlindo et al. (2023), which found that cashew crops are affected by fungal diseases on the roots, stems, and leaves. Parlindo et al. (2023) highlighted that the use of Trichoderma powder is one of the most effective methods for preventing and treating fungal diseases, a result corroborated by this study.

C. Reduction of Cashew Wound Area During Healing Process

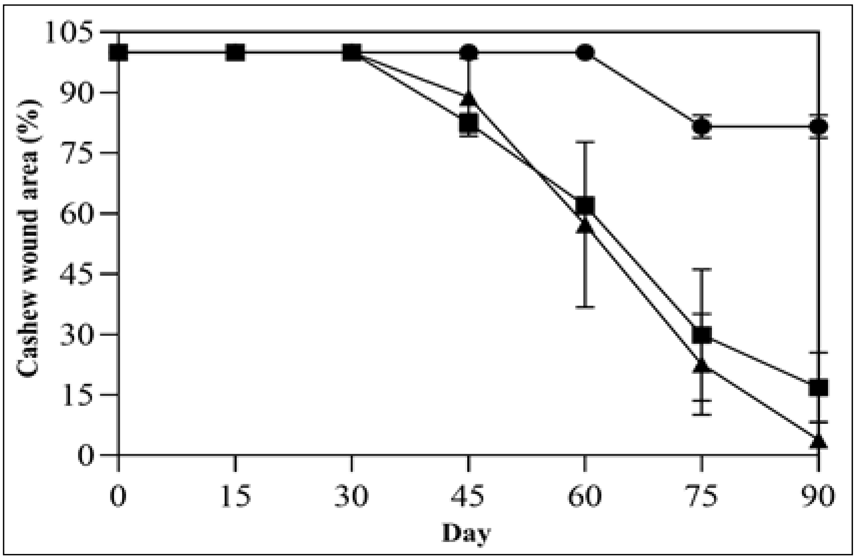

Fig 5. Changes in the surface area of cashew wounds expressed as a percentage for each treatment. Circle = percentages area of wounds using Bordeaux mixture (T1), Square = percentages area of wounds using Trichoderma powder 40% (T2), Triangle = percentages area of wounds using Trichoderma powder 50% (T3).

Fig 5 of this study showed that the percentage change in the surface area of cashew wounds treated and protected for 90 days differed based on the type of treatment and the concentration used. Cashew wounds treated with 10% Bordeaux mixture (T1) did not show significant healing or increased wound closure rates over 90 days, with the wound area remaining at 82 ± 2.8%. In contrast, treatment with 40% Trichoderma powder (T2) effectively healed the wounds, with the wound area decreasing to 17 ± 8% by Day 90, on average. The 50% Trichoderma powder treatment (T3) showed the highest healing capacity, with the wound area reduced to an average of 3 ± 3.85% by Day 90. One-way ANOVA analysis revealed that the percentage of wound area in each treatment group was significantly different ($p < 0.01$).

Table 2 shows that the mean variation in the cashew wound area across the different treatments was significantly different. There were significant differences between T1 and T2, and between T1 and T3 ($p < 0.01$), while no significant difference was observed between T2 and T3 ($p > 0.05$).

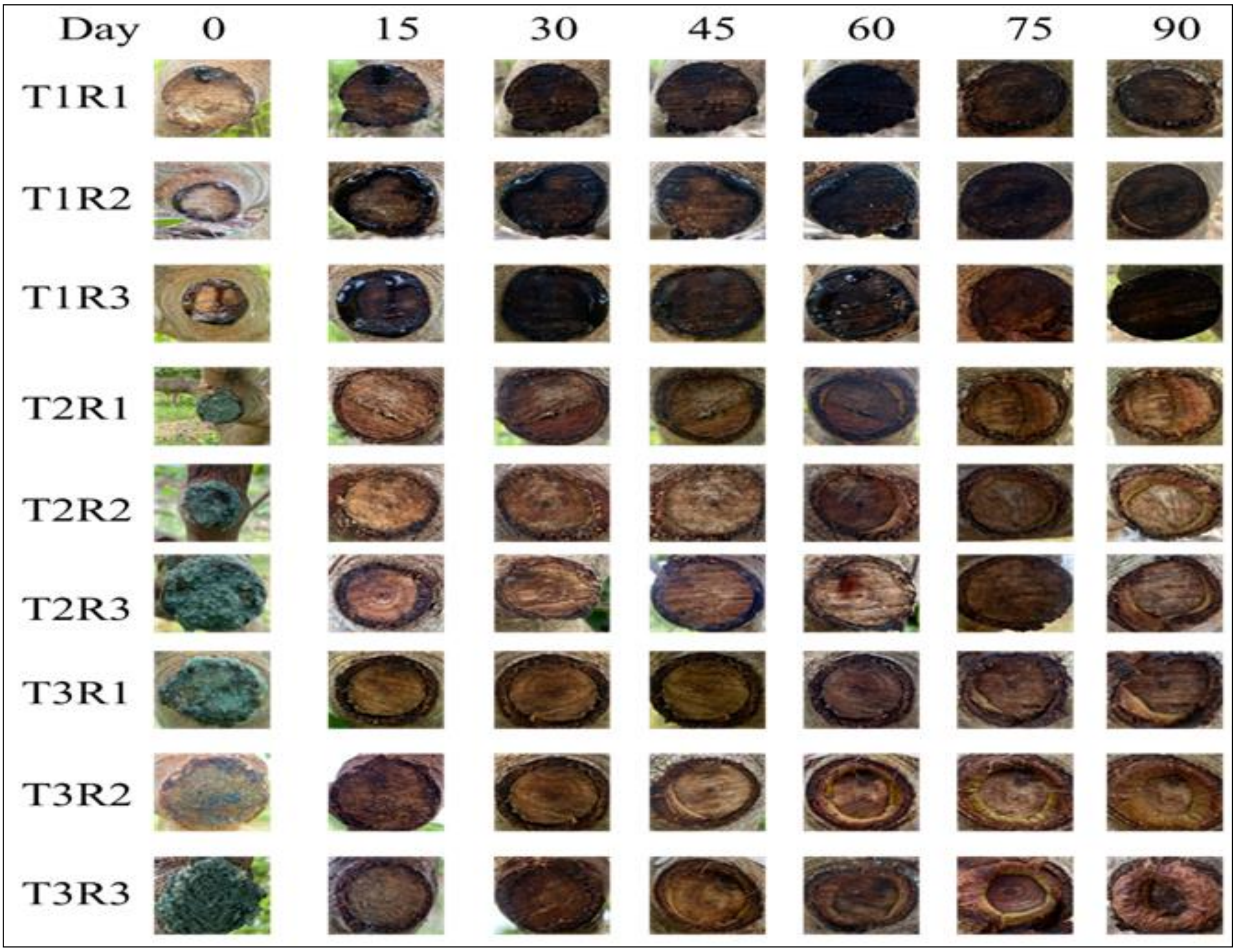

Fig 6. Condition of cashew wounds in each treatment from pruning day to day 90. T1R1 = Treatment 1 and Replicate 1, T1R2 = Treatment 1 and Replicate 2, T1R3 = Treatment 1 and Replicate 3, T2R1 = Treatment 2 and Replicate 1, T2R2 = Treatment 2 and Replicate 2, T2R3 = Treatment 2 and Replicate 3, T3R1 = Treatment 3 and Replicate 1, T3R2 = Treatment 3 and Replicate 2, T3R3 = Treatment 3 and Replicate 3.

The results indicate that the use of Bordeaux mixture caused wounds on cashew trees to turn black and exude gum, symptoms characteristic of gummosis. However, the application of Bordeaux mixture did not significantly accelerate wound healing. In contrast, the use of Trichoderma powder notably enhanced wound healing, with the 50% concentration proving most effective (***Fig 6***). The inhibitory effects of Trichoderma powder on pathogenic fungi such as *Rhizopus*, *Aspergillus*, and *Penicillium* were evident, preventing fungal colonization on cashew wounds. This aligns with previous findings by Lennartsson et al. (2014) and Mousavi et al. (2016), which demonstrated the effectiveness of Trichoderma in suppressing fungi responsible for fruit rot. In comparison, the Bordeaux mixture was effective in preventing fungal infections, consistent with findings by Broome et al. (2010) and Faust (2023). However, it did not promote wound healing due to oxidative reactions leading to tissue darkening. The antifungal activity of lime sulfur and calcium oxide was observed but resulted in tissue blackening, likely due to chemical absorption by the wound. Our results reaffirm the biocontrol potential of *T. asperellum*, as also reported by Ramsés et al. (2018) and Tyśkiewicz et al. (2022), highlighting its ability to enhance plant health without adverse effects. Furthermore, this study underscores that the 50% Trichoderma powder concentration optimally accelerates wound healing in cashew trees while effectively suppressing pathogenic fungal growth.

IV. CONCLUSION

This study demonstrated that Trichoderma powder is significantly more effective than Bordeaux mixture in preventing fungal growth and accelerating wound healing in cashew trees after pruning. The 50% Trichoderma powder concentration provided superior fungal protection, reducing the wound area to just 3 ± 4% within 90 days, compared to Bordeaux mixture, which did not promote wound healing. The findings highlight the critical role of the biological fungus *T. asperellum* in protecting cashew trees from pathogenic fungi and facilitating wound recovery. Among the tested concentrations, 50% Trichoderma powder was found to be the optimal treatment, offering effective protection and healing while minimizing fungal infections.

However, this research was conducted during the rainy season, which posed challenges due to the washing off of the wound dressing after application. Additionally, disease conditions and environmental factors in cashew orchards vary between regions, representing a limitation of this study. Future research should include experiments conducted during the dry season and in different regions to evaluate the effectiveness of Trichoderma powder under varying seasonal and environmental conditions.

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