Evaluating the Efficacy of Various Fungicidal Agents and Phytohormone to Manage Rough Bark Disease of Cinnamon (*Cinnamomum zeylanicum* B.)

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Abstract

Cinnamon, a vital spice crop exported from Sri Lanka, faces the economic threat of Rough Bark Disease (RBD), a major biotic factor affecting cinnamon production. In response to the environmental and health concerns associated with chemical pesticides, environmentally friendly alternatives to control RBD are becoming increasingly popular. This study compares the effectiveness of multiple fungicide treatments and a phytohormone in controlling RBD under field conditions. The field experiment was based on a factorial design and included six treatments with ten replicates each. Both in vitro and in vivo evaluations of the treatments were carried out, which included tebuconazole at a concentration of 0.05% (T1), tebuconazole at a concentration of 0.1% (T2), aqueous neem extract (T3), 5 ml salicylic acid (T4) and 1% Bordeaux mixture included (T5) and a control group (T6). The results showed that the most effective inhibition of mycelial growth (100%) occurred with 1% Bordeaux mixture treatments in in vitro evaluations. Tebuconazole also significantly inhibited mycelial growth at concentrations of 0.05% and 0.1%. In the field trial, which included two application periods, no differences were evident between the treatments with 1% Bordeaux mixture and 0.05% or 0.1% tebuconazole in the occurrence and severity of RBD. However, combined in vitro and in vivo studies highlight the superiority of the 1% Bordeaux mixture as the most effective antifungal agent for controlling RBD in cinnamon compared to other treatments.

Keywords: Cinnamomum zeylanicum B., Rough bark disease, Fugicidal agents, Phytohormones

I. INTRODUCTION

Cinnamon (*Cinnamomum zeylanicum* B.) is the most valuable spice crop exported from Sri Lanka (Suriyagoda et al., 2021). In Sri Lanka, cinnamon is grown in the Sinharaja and Knuckles forest reserves and the central highland regions. According to EDB (2020), there will be 33,000 ha of cinnamon cultivation in Sri Lanka in 2021, and according to Jayasinghe et al. (2016), 60,000 farming families, or around 300,000 people, are involved in the industry. Cinnamon exports totaled 18,722.8 metric tonnes, in 2020resulting a foreign exchange earnings of Rs. 38.2 billion.

The presence of pests and diseases, some of which have attracted significant attention in Southeast Asian countries, poses a significant threat to cinnamon production (Khan et al., 2020). Among these challenges, Rough Bark Disease (RBD), caused by *Phomopsis* spp., stands out as the most widespread, damaging and economically important fungal disease in Sri Lanka's cinnamon (Ashan et al., 2020; Jayasinghe et al., 2017). Treatment for this condition typically involves a combination of cultural practices and chemical treatments.

Although chemical fungicides are known for their effectiveness, there is growing recognition that organic fungicides offer a more cost-effective and environmentally friendly approach to crop protection, providing effective disease control without negative environmental impacts. In addition, the application of plant hormones has been shown to significantly increase a plant's resistance to disease and infection (Burketova et al., 2015). Despite their potential benefits, there is limited research exploring alternative fungicidal agents to replace traditional commercial fungicides for the treatment of plant diseases. This study aims to fill this knowledge gap by

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comparing different fungicidal treatments and phytohormones related to the treatment of bark disease in cinnamon and assessing their overall effectiveness. In this way, it aims to provide valuable insights into sustainable and environmentally friendly approaches to disease control in cinnamon cultivation.

II. MATERIALS AND METHODOLOGY

A. *Study Area*

The study was carried out from February to August 2022 at the National Cinnamon Research and Training Centre (NCR & TC), Palolpitiya, Matara (latitude 6°01'42.1"N and longitude 80°33'34.2"E; 38 m amsl). The main type of soil in the field study area is red-yellow podsolic, and it is located in agro ecological zone IL10. The annual precipitation is 2261 mm.

B. *In vitro Experiment*

First, samples of the rough bark disease were collected, and then, using the hyphal tip transfer technique, the isolated fungus was cultivated in petri plates (Heath, 2011). Tebuconazole (EW 250 g/l), a chemical fungicide, was employed in two quantities to prepare the testing reagent. 1 ml and 0.5 ml per 1 liter of water. 5 ml of a salicylic acid solution were used as a plant hormone. Freshly made Neem extraction (a strained solution generated from 100g of Neem leaves cooked in 1 l of water) and a 1% Bordeaux mixture were used as homemade reagents. 10g of $CuSO_4$ was dissolved in 100 ml of hot water to create a 1% Bordeaux mixture. 900 ml of tap water was used to dissolve 10 g of lime. Then, two solutions were properly combined.

The poison food technique was used to assess the fungicidal impact of testing regents. Using a micropipette, precisely 1 ml of filter-sterilized testing solution (Whatman Slinger filters, 0.45 cm diameter) was applied to a 9 cm diameter sterilised petri plate. Next, 15 ml of potato dextrose agar (PDA) was added to the plate. With 15 ml of PDA and 1 ml of sterilised distilled water, control plates were run. The centre of each PDA plate was then implanted with a 0.5 cm diameter mycelial plug that was taken from the pathogen culture that had been growing for 7 days. Each testing regent underwent five duplicates of these steps. The PDA plates were appropriately identified and parafilm-sealed. At 25˚C, plates were kept in an incubator. Following a 48-hour observation period, the colony diameters (measured in cm) of each plate were assessed, and the percentage inhibition of diameter growth (PIDG) was determined using the equation shown below (Khan et al., 2021).

$$PIDG = \frac{(Diameter\ of\ control - Diameter\ of\ sample)}{Diameter\ of\ control} * 100\%$$

Field Experiment Two months following the harvesting, a field trial was established on the grounds of NCR and TC. After 3–4 weeks following harvest, the beginning of new shoots can be observed. Then, for the experiment, bushes with at least two new shoots were chosen. The two-factor factorial design was used for the study (Figure 01). The application of the treatment was done once in every two weeks and once a month.

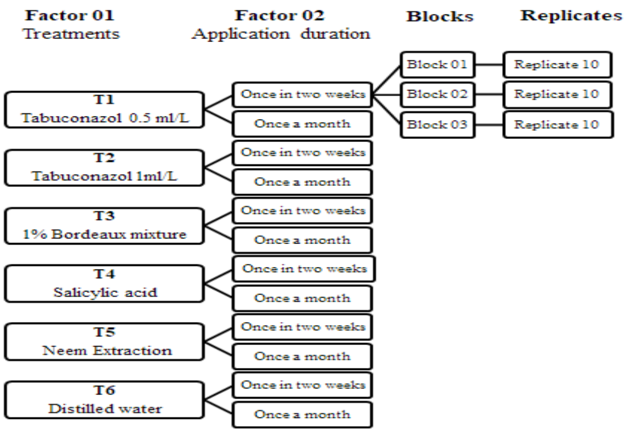

Figure 01: Design of the study

The number of typical Rough Bark Disease spots that appeared on new shoots was recorded once a month separately, and disease incidences (number of initial spots) and severity (area of initial spots) were calculated.

III. RESULTS AND DISCUSSION

The upper and lower surfaces of the colony that was isolated on the diseased cinnamon plants are shown in Figure 02. After seven days, fast-growing off-white, pale yellow-coloured mycelia were visible in the PDA medium. Table 01 shows the percentage inhibition of *Phomopsis* spp by different treatments. Diameter growth inhibited by the poison food technique. The results showed that the 1% Bordeaux mixture performed at its highest level by completely preventing mycelial growth over the 6 day observation period. The

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media supplemented with 5 mL of salicylic acid showed the lowest PIDG values, indicating no fungicidal activity. The three treatments, for example 0.05% tebuconazole, 0.1% tebuconazole and 1% Bordeaux combination, do not differ significantly when considering the PIDG values obtained for the fourth, fifth and sixth days. However, considering the aqueous neem extract over the entire observation period, the concentration tested was not sufficient to inhibit the RBD-causing active ingredient by 50%, nor was there any noticeable difference between the two treatments (5 ml salicylic acid and aqueous neem extraction) during the entire observation period.

Table 02 shows the effects of application to six different treatments to reduce RBD under field conditions over a five-month period. According to the table, after 5 months of use, the treatment effect for the two observed parameters (number of spots and area of spots) became significant (p 0.05), while the duration of use and the interaction of treatment and duration remained non-significant. This is further supported by Figure 03, which shows the average number RBD spots appearing in fresh cinnamon shoots at the end of the five-month treatment period. Over a period of five months, T1, T2 and T5 caused the least RBD spot development, while T3 and T4 also prevented RBD spot development compared to the control, once applied every two weeks, but no significant difference between T3 when used once monthly, T4 and control. Regarding the number of RBD spots that have developed, there is no discernible difference between T3 and T4, similar to how there is no discernible difference between T1, T2, and T5. (Figure 04).

Figure 04 shows the average number of spots in new shoots in six different treatments over the period of two applications. The figure shows that there is no difference between the two application periods T1, T2, T5 and T6 ($p > 0.05$). The results showed that using treatments T1, T2 and T5 once a month rather than every two weeks is sufficient. Compared to once-monthly dosing, T3 and T4 applications once every two weeks demonstrated significant disease suppression. The control group discovered the most stains for both durations of use. The average spot area on new shoots treated with six different substances in two applications also shows the same treatment.

Figure 02: Upper and lower surface of *phomopsis spp*

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Table 01: Percentage Inhibition of Diameter Growth of Isolated *Phomopsis spp*. under the Five Treatments in the Culture

Treatment	PIDG* value				
	Day 02	Day 03	Day 04	Day 05	Day 06
Tebuconazole 0.05% (T1)	36.11^{b} ± 5.01	60.83^{b} ± 2.20	75.00^{a} ± 1.80	80.00^{a} ± 2.22	79.29^{a} ± 1.71
Tebuconazole 0.1% (T2)	100.00^{a} ± 0.00	90.00^{ab} ± 10.00	88.02^{a} ± 6.14	91.11^{a} ± 4.63	91.91^{a} ± 4.13
Neem aqueous extraction (T3)	18.06^{b} ± 5.01	31.67^{d} ± 8.33	39.06^{b} ± 10.97	41.85^{b} ± 8.07	44.01^{b} ± 7.85
5 ml Salicylic acid (T4)	18.06^{b} ± 12.34	21.67^{d} ± 11.67	10.52^{b} ± 9.00	12.22^{b} ± 3.57	17.15^{b}±13.32
1% Bordeaux mixture (T5)	100.00^{a} ± 0.00	100.00^{a} ± 0.00	100.00^{a} ± 0.00	100.00^{a} ± 0.00	100.00^{a}±0.00

Table 02: Effect of Application on Six Different Treatments for Controlling RBD under Field Conditions in 5 Months Period

Data set	Factor	P - values generated in ANOVA	
		For no. of spots	For area of spots
After 2 months of application	Treatments (A)	0.520	–
	Application duration (B)	0.269	–
	(A) X (B)	0.246	–
After 3 months of application	Treatments (A)	0.270	0.183
	Application duration (B)	0.086	0.215
	(A) X (B)	0.587	0.702
After 4 months of application	Treatments (A)	0.470	0.164
	Application duration (B)	0.133	0.210
	(A) X (B)	0.906	0.837
After 5 months of application	Treatments (A)	0.000	0.004
	Application duration (B)	0.374	0.332
	(A) X (B)	0.155	0.472

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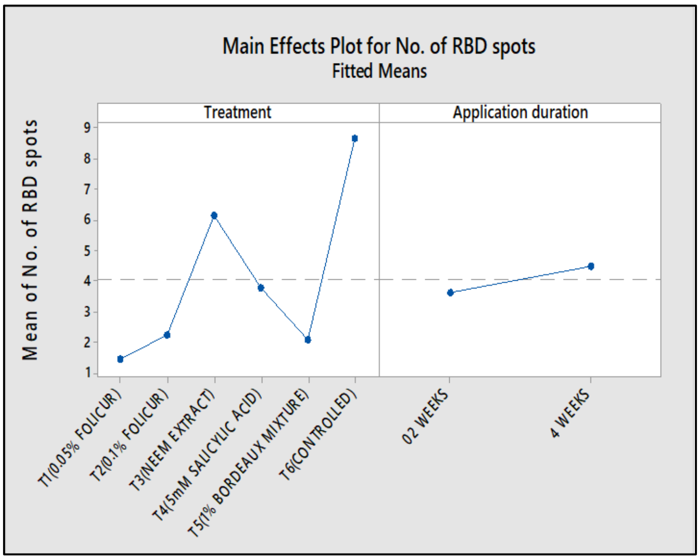

Figure 03: Mean Number of RBD Spots Appeared in New Shoots of Cinnamon after the Treatment Application at the End of the Five Months

In vitro studies have shown that various fungicidal solutions have a suppressive effect on the growth of *Phomopsis* sp. The phytohormone and other fungicidal compounds have varying degrees of inhibitory effect. In this study, a 1% Bordeaux mixture was found to be most effective in suppressing mycelial growth, consistent with the findings of Park et al. matches. (2016) that 1% Bordeaux mixture was the best treatment for both *in vivo* and *in vitro* assays, Bordeaux mixture was the best treatment for reducing the incidence of *Phomopsis* sp. caused gummosis diseases (Farias et al., 2021). With increasing concentrations of tabuconazole at two concentrations of 0.05% and 0.1%, respectively, there was a progressive increase in PIDG for *Phomopsis* sp. was seen. The PIDG values on the sixth day for these two concentrations were 79.29% and 91.91% respectively. This is consistent with the findings of Najoi et al. (2018) to control a wide range of pathogenic fungi, tabuconazole has been widely used on a variety of crops in several countries. In the current study, aqueous neem extraction achieved a PIDG value of 44.01% on the sixth day. In the in vitro study there was only minimal inhibition of diameter growth. The absence of *Phomopsis* spp. the inhibition in cinnamon RBD may be due to the low concentration. Aqueous neem extraction reduces mycelial growth of both pathogenic fungi and the degree of suppression progressively increases with concentration (Kumar et al., 2016; Nandini et al., 2018). Furthermore, it was observed that all symptom parameters were recorded less in other treatments than in the control treatment as plant hormones have immense value in disease control by managing plant biotic stress (Ali and Baloch, 2020).

IV. CONCLUSION

In comparison to the two positive controls examined, 0.05% and 0.1% Tebuconazole, the 1% Bordeaux combination emerged as the most effective fungicidal treatment for managing Rough Bark Disease (RBD) in cinnamon. However, our findings suggest that applying 0.05% Tebuconazole once a month is sufficient for successful RBD management. Interestingly, the phytohormone salicylic acid did not exhibit any significant suppression of diameter growth in the in vitro study. Nevertheless, it positively impacted the outcomes of the field trial by reducing the frequency and severity of the disease. These results indicate that salicylic acid may possess biotic stress-supporting capabilities rather than fungicidal ones, enabling the plant to better withstand against biotic stress caused by pathogen.

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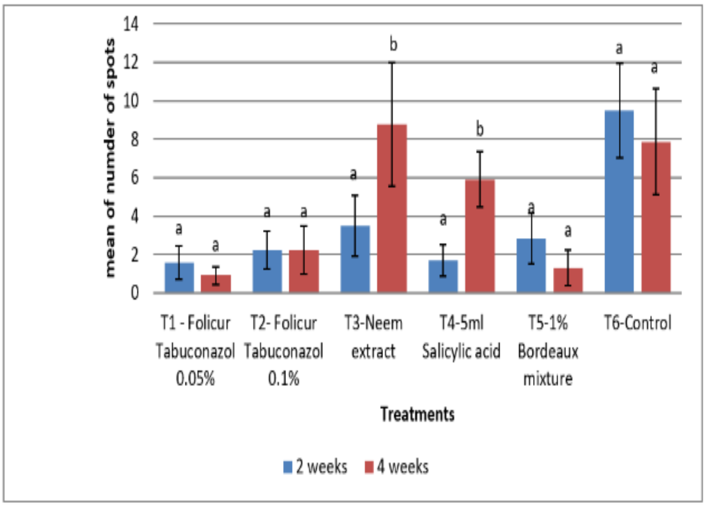

Figure 04: Mean Number of Spots in New Shoots with Six Different Treatments with Two Application
Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test.

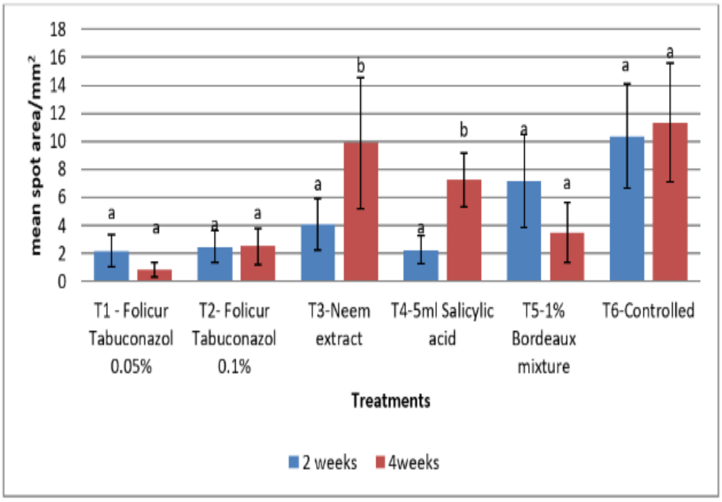

Figure 05: Mean of Spot are in New Shoots with Six Different Treatments with Two Application Duration at the End of Five Month.
Means sharing a common letter(s) are not significantly different by Tukey's multiple comparison test.

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