Evaluating the effectiveness of botanical extracts on cotton sclerotial fungi

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Abstract. *Sclerotium rolfsii*, *Rhizoctonia solani*, and *R. bataticola* caused damping-off, root rot, and charcoal rot on cotton plant. The fungi produced sclerotia, and hence they are difficult to control. Synthetic fungicides had negative effects on the environment, but fungicidal compounds of botanical extracts were environmentally friendly. This research aimed to test several botanical extracts on the three fungi, i.e., extract from neem seed, the mixture of neem seed with clove oil, and citronella oil with ratio 7:1; 3:1; 1:1. The concentration used for the test were: 500, 1250, 2500, and 3750 ppm. Each of the tested fungi was placed in the middle of PDA medium with the botanical extract. Each treatment was replicated three times. The growth of the tested fungi was measured every two days. Neem extract inhibited the mycelial growth of the three tested fungi 2-40%. When the neem was mixed with clove oil, its effectiveness escalated to 100%, especially at ratios 3:1 and 1:1. The mixture of neem extract with citronella oil was less effective than that of neem extract with clove oil. Of the three tested fungi, *S. rolfsii* was the most vulnerable fungus to the tested botanical extract.

Keywords: *Sclerotium rolfsii*, *Rhizoctonia solani*, *Rhizoctonia bataticola*, neem, clove, citronella

1. Introduction

Besides for textile industries, cotton is also used for banknote paper. Indonesian cotton demand for textile industries was 454-762 metric tons, while the national cotton production was only 1600-2500 tons. It means that the national cotton production only fulfills 0.5% of the national demand. Therefore, the national cotton development programs should be encouraged to increase domestic production by providing productive areas and cultivation technology.

One of the problems of cotton production was the infection of sclerotial fungi such as *Sclerotium rolfsii*, *Rhizoctonia solani*, and *R. bataticola*, which causes damping-off, neck rot root, and charcoal rot [1]. The three fungi are saprophytes and survive in unfavorable conditions by forming sclerotia. They also have many host plants, especially in tropical climates. Therefore, these fungi are difficult to control and become one of the problems in tropical agriculture.

Farmers usually relied on synthetic fungicides to control pathogenic fungi. However, using synthetic fungicides for a long period created environmental problems and human health [2]. These negative effects can be overcome by using more environmentally friendly pesticides [3], such as plant extracts that contain fungicidal effects [4]. Plant-based fungicides could break down faster than synthetic chemical pesticides, so they do not accumulate for a long time in the environment [5].
Indonesia has a very high biological diversity plant, including botanical fungicide plants such as neem (*Azadirachta indica*), cloves (*Syzygium aromaticum*), and citronella (*Cymbopogon nardus*). Neem seed is widely known as the best source of botanical pesticide because of its bioactive compounds such as azadirachtin, salanin, nimbin, nimbidin, and meliantriol. Azadirachtin acts as an insecticide, nematicide, mitiside, and fungicide and works as an antifeedant [6, 7]. Neem seeds contain 0.2-0.6 % azadirachtin [8]. Cloves are also widely used as medicines and antibiotics (bacteria and fungi). Clove seeds contain eugenol, and citronella contains citronella [9], which has antifungal and antibacterial properties [10].

In this study, extracts of neem seed, clove, and citronella were mixed in several combinations to see their effectiveness in inhibiting the growth of the fungi mentioned above.

2. Materials and methods
This research was conducted in the Phytopathology Laboratory of the Sweetener and Fiber Crops Research Institute. The tested botanical fungicides were from extracts of neem seeds (M) only (1), neem seed extracts (M) mixed with clove flower oil (C), or with citronella oil (S) with the following ratio: (2): M: C: 175: 25 mL (7: 1); (3) M: C: 150: 50 mL (3: 1); (4) M: C 100: 100 mL (1: 1); (5) M: S: 175: 25 mL (7: 1); (6) M: S: 150: 50 mL (3: 1); and (7) M: S 100: 100 mL (1: 1); and as a control, the tested fungus was cultured in PDA without extracts. The tested pathogenic fungi were *S. rolfsii*, *R. solani*, and *R. bataticola*, isolated from diseased cotton plants with specific symptoms associated with the fungi. The medium used to culture the fungus was potato dextrose agar (PDA). The extracts were diluted to four series of concentrations: 500 ppm; 1250 ppm; 2500 ppm; and 3750 ppm. The 10-day-old fungal culture (3 mm diameter) was placed in the center of a petri dish (9 mm diameter) containing PDA with tested botanical extracts. The fungus was then incubated at 26°C, and the growth was observed every two days until the fungus reached the edge of the Petri dish. Each treatment consisted of 1 Petri dish and was repeated 3 times. The experimental design used a completely randomized design. Data were analyzed using ANOVA, and differences in each treatment will be further tested using Duncan's multiple range test at a 5% level. Calculation of the percentage of fungicide inhibition based on the difference between control and treatment [10] using the formula:

\[ H = (((K - P) / K) \times 100 \]

H: Percentage of inhibition
K: mycelial growth on control dish
P: mycelial growth on treatment dish

We also calculated the value of median lethal dose (LD₅₀) to determine the botanical extracts’ toxicity. The LD₅₀ was estimated the dose of the active ingredient of the botanical extracts required to kill half of the population of the tested target after a specified test duration.

3. Result
3.1. *Sclerotium rolfsii*

The normal growth of *S. rolfsii* on PDA reached the edge of the petri dishes on day 5-6. When the medium was added with the neem seed extracts, the growth of the fungus slowed down (Figure 1a). However, the efficacy of neem seed extract was only able to inhibit the mycelial growth of *S. rolfsii* 5-40 % (Table 1), but when neem was mixed with clove oil, its effectiveness increased. When the concentration of clove oil was raised, the inhibitory effect on *S. rolfsii* was increased too. The growth of *S. rolfsii* decreased sharply, particularly at higher concentrations. *S. rolfsii* did not grow in medium added with the mixture of neem seed extract with clove oil at ratios of 3:1 and 1:1 with concentration at a range of 1250-3750 ppm (Figure 1c-d). The mixture of neem extract and clove oil also reduced mycelial density. The inhibitory effect of neem extract mixed with citronella oil was less effective, compared to the mixture of neem seed extract with clove oil. The fungicidal effect on *S. rolfsii* was shown when the neem extract was mixed with citronella oil at ratios of 3: 1 and 1: 1 (Figure 1 e-g).
From table 1, it can be seen that the mixture of neem seed extracts with clove oil in a ratio of 1:1 had a fungicidal effect on *S. rolfsii*, followed by the mixture of neem seed extract with clove oil in a ratio of 3:1. The mixture of neem seed extract mixed with citronella oil with a ratio of 1:1 gave the highest inhibition, but only had a fungicidal effect when applied at concentrations of 2500-3750 ppm.

**Table 1.** The inhibitory effect of botanical extracts on *Sclerotium rolfsii*

| Concentration | Inhibition of botanical extracts (%) |
|---------------|--------------------------------------|
|               | M | M:C(7:1) | M:C(3:1) | M:C(1:1) | M:S(7:1) | M:S(3:1) | M:S(1:1) |
| 500 ppm       |   | 22.4 d   | 22.2 d   | 1.9 a    | 100.0 j  | 5.1 ab   | 8.0 b    | 8.6 b    |
| 1250 ppm      |   | 5.1 ab   | 24.4 d   | 100.0 j  | 100.0 j  | 10.4 b   | 16.8 c   | 33.5 e   |
| 2500 ppm      |   | 40.3 f   | 83.1 i   | 100.0 j  | 100.0 j  | 22.4 d   | 72.5 h   | 100.0 j  |
| 3750 ppm      |   | 7.9 b    | 94.8 j   | 100.0 j  | 100.0 j  | 57.9 g   | 100.0 j  | 100.0 j  |

Note: M: Neem seed extract; M: C(7:1): Neem seed extract with clove oil in a ratio of 7:1; M: C(3:1): Neem seed extract with clove oil in a ratio of 3:1; M: C(1:1): Neem seed extract with clove oil in a ratio of 1:1; M:S(7:1): Neem seed extract with citronella oil in a ratio of 7:1; M:S(3:1): Neem seed extract with citronella oil in a ratio of 3:1; M:S(1:1): Neem seed extract with citronella oil in a ratio of 1:1.
3.2. *Rhizoctonia solani*

The use of neem seed extract alone was less effective in inhibiting the mycelial growth of *R. solani*. The growth of the fungus on neem seed extract added PDA in a range of concentration 500-3750 ppm was almost the same (Figure 2a). The inhibitory effect of neem extract was not significantly different and was only 2.2-7.8% (Table 2). Its effectiveness increased when neem seed extract was mixed with clove oil. The inhibitory effect on the growth of mycelia was better when the clove concentration increased. *R. solani* did not grow at all on PDA contained the mixture of neem seed extract and clove oil in a ratio of 7:1 at concentrations of 2500 up to 3750 ppm. A mixture of neem seed and clove oil in a ratio of 1:1 at a concentration of 500-3750 ppm was lethal to *R. solani* (Figure 2). The effect of the addition of citronella oil on *R. solani* was almost similar to *S. rolfsii*. The citronella oil was less effective compared to clove oil (Table 2).

**Table 2.** The inhibitory effect of botanical extracts on *Rhizoctonia solani*.

| Concentration | M  | M:C(7:1) | M:C(3:1) | M:C(1:1) | M:S(7:1) | M:S(3:1) | M:S(1:1) |
|---------------|----|----------|----------|----------|----------|----------|----------|
| 500 ppm       | 7.6 a | 55.2 e   | 34.6 c   | 100.0 h  | 4.8 a    | 1.7 a    | 23.7 b   |
| 1250 ppm      | 7.4 a | 69.8 f   | 100.0 h  | 100.0 h  | 19.3 b   | 37.8 c   | 92.2 g   |
| 2500 ppm      | 2.2 a | 100.0 h  | 100.0 h  | 100.0 h  | 58.5 e   | 48.7 d   | 100.0 h  |
| 3750 ppm      | 7.8 a | 100.0 h  | 100.0 h  | 100.0 h  | 65.6 ef  | 88.5 g   | 100.0 h  |

Note: **M**: Neem seed extract; **M**: C(7:1): Neem seed extract with clove oil in a ratio of 7:1; **M**: C(3:1): Neem seed extract with clove oil in a ratio of 3:1; **M**: C(1:1): Neem seed extract with clove oil in a ratio of 1:1; **M**: S(7:1): Neem seed extract with citronella oil in a ratio of 7:1; **M**: S(3:1): Neem seed extract with citronella oil in a ratio of 3:1; **M**: S(1:1): Neem seed extract with citronella oil in a ratio of 1:1.
Figure 2. Mycelial growth of *R. solani* on PDA added with concentration series of botanical extracts.
3.3. *Rhizoctonia bataticola*

The growth of *R. bataticola* decreased when the PDA medium was added with neem seed extract (Figure 3a), but the growth was not affected when the neem seed extract was added with clove oil in a ratio of 7:1, except at a concentration of 3750 ppm (Table 3). The mixture of neem seed extract and clove oil in a ratio of 3:1 inhibited the growth of *R. bataticola* by 100% at concentrations of 2500-3750 ppm. The growth inhibition capability of the mixture increased when the concentration of clove increased, i.e., in a ratio of 1:1 (Table 3).

**Table 3.** The inhibitory effect of botanical extracts on *Rhizoctonia bataticola*.

| Concentration | M: C(7:1) | M: C(3:1) | M: C(1:1) | M: S(7:1) | M: S(3:1) | M: S(1:1) |
|---------------|-----------|-----------|-----------|-----------|-----------|-----------|
| 500 ppm       | 25.9 f    | 0.0 a     | 19.1 e    | 18.1 e    | 17.4 de   | 0.0 a     | 14.6 |
| 1250 ppm      | 9.7 c     | 2.0 a     | 85.2 k    | 100.0 m   | 30.0 f    | 2.4 a     | 16.9 |
| 2500 ppm      | 13.0 cd   | 2.0 a     | 100.0 m   | 100.0 m   | 40.0 g    | 6.5 b     | 80.2 |
| 3750 ppm      | 15.0 de   | 47.6 hi   | 100.0 m   | 100.0 m   | 50.6 i    | 45.4 h    | 92.2 |

Note: M: Neem seed extract; M: C(7:1): Neem seed extract with clove oil in a ratio of 7:1; M: C(3:1): Neem seed extract with clove oil in a ratio of 3:1; M: C(1:1): Neem seed extract with clove oil in a ratio of 1:1; M: S(7:1): Neem seed extract with citronella oil in a ratio of 7:1; M: S(3:1) Neem seed extract with citronella oil in a ratio of 3:1; M: S(1:1) Neem seed extract with citronella oil in a ratio of 1:1.
Based on LD$_{50}$, S. rolfsii was the most vulnerable fungus compared to other tested fungi against the botanical extracts used in this study (Table 4). Lower LD$_{50}$ is indicative of increased toxicity of the extracts.

**Table 4.** The value of LD50 of the tested botanical extracts for the three fungi.

| Treatment                              | S. rolfsii | R. solani | R. bataticola |
|----------------------------------------|------------|-----------|---------------|
| Neem                                   | 1.55       | 13.15     | 10.35         |
| Neem seed extract: clove oil (7:1)     | 1.75       | 1.3       | 4.05          |
| Neem seed extract: clove oil (3:1)     | 0.6        | 0.6       | 1             |
| Neem seed extract: clove oil (1:1)     | 0.45       | 0.25      | 0.65          |
| Neem seed extract: citronella oil (7:1)| 3.6        | 2.55      | 3.1           |
| Neem seed extract: citronella oil (3:1)| 1.85       | 2.15      | 4.45          |
| Neem seed extract: citronella oil (1:1)| 1.3        | 0.95      | 1.85          |

4. Discussion

The effectiveness of neem seed extracts against R. solani and R. bataticola was lower (LC$_{50}$: 13.15 and 10.35), compared to S. rolfsii (LC$_{50}$: 1.55). Three types of azadirachtin (A, B, and H) produced from neem extraction had the ability to inhibit S. rolfsii with LC$_{50}$ 43.9 ppm and LC$_{50}$ for R. solani was 63.7
Niaz also reported that the growth of *R. solani* and *R. bataticola* (*Macrophomina phaseolina*) was inhibited by Azadirachtin at a concentration of 1000 ppm [12]. Neem extract was also inhibited *in vitro* growth of *Penicillium digitatum* up to 100% at a concentration of 3000 ppm [5]. In general, Locke 1995 stated that neem was used to control plant pathogenic fungi such as *R. solani*, *S. rolfsii*, *Sclerotinia sclerotiorum*, *B. cinerea*, *P. expansum*, *Glomerella cingulata*, *Alternaria alternata*, *Aspergillus niger*, *Curvularia lunata*, *Sphaerotheca fuliginea*, *Plasmovara viticola*, *Diplocarpon rosae*, and some rust fungi ranged from 2-10% [13].

The effectiveness of neem seed extract increased when mixed with clove or citronella. However, the mixture of neem with cloves was better than neem mixed with fragrant citronella. Clove flower extract contained eugenol. The mycelial growth of *S. rolfsii* inhibited on media containing eugenol 200 ppm or clove powder containing eugenol 0.2% and prevented sclerotial germination when grown on media containing eugenol 300 ppm or clove powder containing eugenol 0.3% [14].

Some bioactive compounds produced by certain plants have fungicidal or fungi toxic activities because of their ability to inhibit the growth of fungi and their spores [5] and also have the ability to block the biosynthetic process of pathogenic metabolite [3]. Citronellal, linalool, and α and β pinenes produced by *Cymbopogon* inhibited fungal growth effectively [15]. This is because they damaged cell structures, macromolecule cells, organelles and disrupted the process of metabolism [16]. The toxicity of citronella to fungi persisted for 210 days at various temperatures [17]. In another study, citronella extract with a concentration of 0.02-5% was able to inhibit mycelial growth of *Pestalotia* sp., the causal agent of leaf spot disease on angasana tree (*Pterocarpus indicus*) [10], but in our results, the citronella oil was less effective compared to clove oil.

5. Conclusion
Neem extract inhibited the mycelial growth of *S. rolfsii*, *R. solani*, and *R. bataticola* ranged from 2-40%. When the neem was mixed with clove oil, the effectiveness of the neem increased up to 100%, especially at ratios of 3:1 and 1:1. The mixture of neem extract with citronella oil was less effective compared to the mixture of neem extract with clove oil. Of the three tested fungi, *S. rolfsii* was the most vulnerable fungus to the tested botanical extracts.

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