

Botanical pesticide ability to control black pod rot of cacao *Phytophthora palmivora* (Butl.) Butl in the field

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Abstract

The use of synthetic chemical pesticides for cacao black pod rot disease control to remove heavy metal residues on cacao beans needs to be reduced. This research aimed to find out the ability of botanical pesticides to lower BPR incidence, BPR infection rate, and botanical pesticides' efficacy in controlling BPR. It was conducted at the Cacao Research Substation Garden of the Plantation and Horticulture Office of Southeast Sulawesi. The treatments experimented included control (no pesticide), CNSE, CNSE + CSLS, CNSE + whiteflower albizia bark extract, CNSE + siam weed leaf extract, CNSE + candle bush leaf extract, CNSE + whiteflower albizia bark extract + siam weed leaf extract + candle bush leaf extract, and synthetic pesticide with active ingredient copper oxysulfate at 345 g/L. Group-randomized trial was used as design in triplicate. The research results showed that the botanical pesticides of CNSE, and the was mixture with of CSLS, siam weed leaf extract, candle bush leaf extract, and whiteflower albizia bark extract were able to lower the disease incidence and slow down the infection rate of BPR. The lowest BPR incidence and infection rates were discovered in the CNSE + Siam weed leaf extract treatment, each at 38.90% and 0.087 per pod per week. The CNSE botanical pesticide mixed with Siam weed leaf extract, whiteflower albizia bark extract, and candle bush leaf extract demonstrated good efficacy above 50%, hence feasible to use as an alternative to chemical pesticides in BPR control.

Keywords: Botanical pesticides; Black pod rot; Cacao; CNSL; *Chromolaena odorata*; Efficacy

1. Introduction

Cacao is one of Indonesia's flagship commodities with the largest production center established on Sulawesi Island. The cacao production in Indonesia is predominantly intended to meet export needs, requiring that attention be drawn toward cacao beans quality improvement. It is pests and diseases in cacao which is responsible for the decline in the production quality and even for harvest failures. It is known that cacao is susceptible to a wide array of diseases; hence, an effective disease control strategy is needed in order to achieve sound production.

The primary disease attaching to cacao crops all around the world is the black pod rot (BPR) disease which causes a decline in cacao production. This disease in Indonesia is initiated by *Phytophthora palmivora* (Butl.) Butl. [1] which can also cause stem cancer in cacao. The incidence rate of the disease in susceptible clones may reach up to 85.4% [2], while the loss of cacao bean production caused amounts to 74.96% [3] or may even touch as high as 90% [4].

Many have primarily utilized synthetic chemical pesticides for disease control, but their repeated, continuous use has brought about various problems such as environmental pollution, pesticide residue, and pathogenic resistance.

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Synthetic chemical pesticide residue has been encountered in cacao beans, hence reducing the competitiveness of Indonesia's cacao products on the international market [5]. On the cacao beans from South Sulawesi and Southeast Sulawesi Provinces, the Cu (copper) heavy metal residue has even been discovered [6]. Pesticides with copper as active ingredient are generally used for controlling the black pod rot disease of cacao. Humans' long-term exposure to synthetic chemical pesticides which contain heavy metals can lead to kidney dysfunction [6].

Environmentally friendly techniques for controlling the black pod rot disease of cacao have been developed by using pesticides from plants (botanical pesticides). It is expected that botanical pesticide use can reduce the deleterious effects of synthetic chemical pesticides in order to realize healthy plant cultivation [7]. Reports on botanical pesticide use for controlling plant diseases have grown on a yearly basis [7; 8]. The advantages of using botanical pesticides are that they are quickly degradable in nature, hence leaving no residue in soil or agricultural products [9], and that they do not influence the lives of botanical agents like *Trichoderma* sp. and *Gliocladium* sp. [10].

The most abundant source of botanical pesticides in Indonesia is the cashew nut plant (*Anacardium occidentale*). The shell of cashew nut contains cashew nut shell liquid (CNSL) with its ability to control cacao diseases [11] and cacao pod borers [12]. The botanical pesticide ability of cashew nut shell to control black pod rot of cacao is currently under continuous improvements through mixing with other botanical pesticide extracts. Results of a laboratory investigation showed that the mixtures of cashew nut shell extract (CNSE) and coconut shell liquid smoke (CSLS), whiteflower albizia (*Albizia saponari*) bark extract, Siam weed (*Chromolaena odorata*) leaf extract, and candle bush (*Senna alata*) leaf extract were able to inhibit the growth of *P. palmivora* biomass and the development of cacao black pod rot symptoms [13].

Further research on the field scale of the extent of ability of botanical pesticides from cashew nut shell and other ingredients in cacao black pod rot [BPR] disease is required. This research aims to figure out the ability of botanical pesticides against BPR incidence, BPR progression, and botanical pesticides' efficacy in controlling BPR.

2. Material and methods

2.1. Study area

This study was conducted at the Cacao Research Substation garden of the Plantation and Horticulture Office of Southeast Sulawesi Province, South Konawe Regency, Southeast Sulawesi Province from October 2018 to May 2019.

2.2. Material and research design

The materials used in this research included cacao tree, cashew nut shell extract (CNSE), coconut shell liquid smoke (CSLS), whiteflower albizia bark extract, Siam weed leaf extract, candle bush leaf extract, synthetic pesticide with the active ingredient copper oxysulfate (345 g/L), water, and detergent. The tools used included a machete for cutting down the botanical pesticide ingredients, blender for pulverizing the botanical pesticide ingredients, gauze for filtering, jerry cans for storing the botanical pesticide solutions, and knapsack sprayers for spraying the pesticides.

The research design employed was the group-randomized trial (GRT) design. There were eight treatments administered: P₀ = control (no pesticide); P₁ = CNSE; P₂ = CNSE + CSLS; P₃ = CNSE + whiteflower albizia bark extract; P₄ = CNSE + Siam weed leaf extract; P₅ = CNSE + candle bush leaf extract; P₆ = CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract; and P₇ = synthetic pesticide with 345 g/L of the active ingredient copper oxysulfate. Each treatment was conducted in triplicate, and each treatment unit was assigned to two cacao plants.

2.3. Botanical Pesticide Fabrication and Application

Botanical pesticide extracts were made per Bande et al.'s method [13]. The botanical pesticide ingredients (Siam weed leaf, candle bush leaf, and whiteflower albizia bark) were dried, and upon drying, they were cut to a 0.5 cm size and pulverized. Maceration was performed by mixing the ingredients each at 250 grams and 5000 ml of water with an addition of 1 g of detergent, then storing the mixtures inside tightly closed buckets for 3 days with stirring for the botanical pesticides to be well-mixed with the solvent. After the 3 days' submersion, filtering was performed by gauze, leaving suspensions which were to be stored in jerry cans, ready for use. CSLS and CNSE were obtained from Ir. Mariadi, M.S. of the Faculty of Agriculture, Universitas Halu Oleo.

Spraying of pesticides was performed weekly for nine weeks. The CNSE pesticide was sprayed at a concentration of 2.5 ml L⁻¹, the CNSE pesticide mixed with other ingredients at 250 ml L⁻¹, and chemical pesticide at 2 ml L⁻¹. The time of

spraying was morning. Every treatment was sprayed on pods 5–8 cm in size and other pods emerging during the research. The pods sprayed were labeled on the stalks by treatment.

2.4. Observed variabel

The variables observed were the disease incidence, disease progression, and botanical pesticide efficacy. Observation of the incidence rate of cacao black pod rot was conducted in the third week after the first treatment application. It was conducted weekly on the labeled pods; seven days was the observation interval. Disease incidence was given by the formula below:

$$DI = \frac{a}{b} \times 100\%$$

DI = Diseases incidence
 a = Number of pods infected by black pod rot
 b = Number of all pods observed

Analysis of cacao black pod rot disease progression was conducted based on the calculation of the infection rate and the area under disease progress curve (AUDPC). The formula for calculating the cacao black pod rot infection rate was established based on the goodness of fit of three models: monomolecular, logistic, and Gompertz. The best model selected was one which had the greatest coefficient of determination (R^2), and if two models had the same coefficients of determination, selection was to be made on the model with the smallest mean squared error [14]. The infection rate was calculated by the formula proposed by Neher and Campbell [15]. If the disease progression was to be measured under the monomolecular model, then the formula was as follows:

$$r_i = \frac{1}{t} \left(\ln \frac{1}{1 - X_t} - \ln \frac{1}{1 - X_0} \right)$$

r_i = Infection rate
 X_t = Disease proportion at time t_i
 X_0 = Disease proportion at the onset of the observation ($t = 0$)
 t = Time

The disease progression as shown in the AUDPC was calculated by the following formula Campbell and Madden [14]:

$$AUDPC = \sum_{i=1}^{n-1} \left(\frac{X_i + X_{i+1}}{2} \right) (t_{i+1} - t_i)$$

AUDPC = Area under disease progress curve
 y = Disease incidence
 t = Time

The botanical pesticide's efficacy in controlling BPR was measured after a fifth time of spraying using the formula below Department of Agriculture [16]:

$$EP = \frac{DIc - DI_t}{DIc} \times 100\%$$

EP = Efficacy of tested botanical pesticide (%)
 DIc = Disease incidence in the control
 DI_t = Disease incidence in the treatment plot after application of botanical pesticide

2.5. Data Analysis

The data from the disease incidence observation were analyzed by ANOVA with the SPSS version 20 program. If the ANOVA result indicated that the treatments were of significance, Duncan's multiple range test at a 5% significance level was then conducted. AUDPC, infection rate, and efficacy level were calculated by the Microsoft Excel 2010 program.

3. Results

3.1. Black pod rot [BPR] disease incidence

Cacao black pod rot symptoms were captured in all treatments, with and without application of botanical or chemical pesticide treatment. The rate of the disease incidence varied for each treatment. The results of the BPR incidence rate observation are presented in Figure 1. Based on Figure 1, the pods left unsprayed with pesticide demonstrated the highest incidence rate of 75.6%, which indicates that only 24.4% of the pods were in a healthy state. The incidence rate in the application of synthetic pesticide was not different in a statistically significant way from those in the botanical pesticide applications. This reflects that the botanical pesticide of CNSE and its mixtures with other ingredients possessed the potential as alternatives to synthetic chemical pesticides.

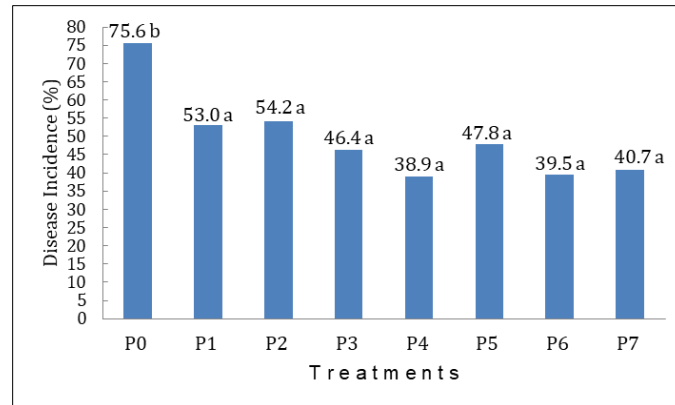


Figure 1 Mean cacao black pod rot incidence rates of various treatments with CSNE as the main ingredient. P₀: control (no pesticide); P₁: CNSE; P₂: CNSE + CSLS; P₃: CNSE + whiteflower albizia bark extract; P₄: CNSE + Siam weed leaf extract; P₅: CNSE + candle bush leaf extract; P₆: CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract; and P₇: synthetic pesticide with 345 g/L of the active ingredient copper oxysulfate. Figures followed by the same letters are not different significantly in the Duncan's multiple range test with $\alpha = 5\%$

The CNSE and the CNSE + CSLS treatments yielded disease incidence rates above 50%, but when CNSE was mixed with whiteflower albizia bark extract, Siam weed leaf extract, and candle bush leaf extract, the rates went down below 50%. The lowest disease incidence was discovered in the CNSE + Siam weed leaf extract treatment (39.52%), whose rate was almost identical to that of the synthetic pesticide application (40.72%).

3.2. Black pod rot infection rate

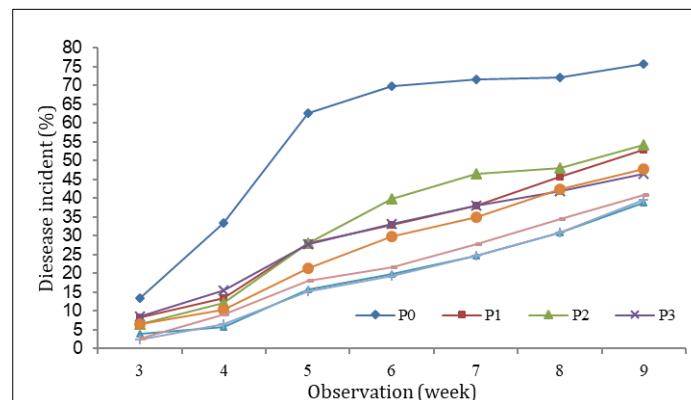


Figure 2 Cacao black pod rot progression in each treatment. P₀: control (no pesticide); P₁: CNSE; P₂: CNSE + CSLS; P₃: CNSE + whiteflower albizia bark extract; P₄: CNSE+ Siam weed leaf extract; P₅: CNSE + candle bush leaf extract; P₆: CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract; and P₇: synthetic pesticide with 345 g/L of the active ingredient copper oxysulfate

The progression of the black pod rot continuously increased from week to week (Figure 2). The results of the goodness of fit of the BPR progression model are presented in Table 1. Based on Table 1, the highest coefficient of determination (R^2) of the linear regression analysis obtained was yielded by the monomolecular model. Hence, this model was applied in all treatments.

Table 1 Results of goodness of fit test of the cacao black pod rot disease progression

Treatments	Coefficient of Determination (R^2)			Model's Fit
	M	L	G	
P ₀ : control (no pesticide)	0.8506	0.8013	0.8336	M
P ₁ : CNSE	0.9866	0.9332	0.9614	M
P ₂ : CNSE + CSLS	0.9689	0.8894	0.9317	M
P ₃ : CNSE + whitlower albizia bark extract	0.9818	0.8971	0.9398	M
P ₄ : CNSE + Siam weed leaf extract	0.9751	0.9399	0.9735	M
P ₅ : CNSE + candle bush leaf extract	0.9926	0.9470	0.9784	M
P ₆ : CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract	0.9785	0.9244	0.9754	M
P ₇ : synthetic pesticide with 345 g/L of copper oxysulfate	0.9924	0.8937	0.607	M

Description: M = Monomolecular, L = Logistic, G = Gompertz

The results of the BPR infection rate and AUDPC calculation are presented in Table 2; the higher the r , the faster the disease's progression. The AUDPC and r values of a plant can be used to identify the disease progression rate. The BPR infection rate was the highest in the control (with no pesticide application), which was 2–3 times higher than the rates in other treatments (0.201 pods per week). Low infection rates were encountered in the CNSE + Siam weed leaf extract treatment (P₃), CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf treatment extract (P₅), and synthetic pesticide with 345 g/L of copper oxysulfate treatment (P₆). These low infection rates pushed the disease's incidence rates in the same treatments lower than the incidence rate in the treatment without any application of pesticide, botanical or synthetic chemical. The lower the infection rate, the lower the BPR incidence. The cacao black pod rot progression by AUDPC value was the highest in the treatment without pesticide application. This was presumed to be because the colonization of the pathogen *Phytophthora palmivora* on the cacao pods took place in a massive, uninhabited way. The pesticide application triggered the plant's defensive response to the pathogenic infection and caused the disease progression to slow down.

Table 2 Infection rates (r) and AUDPC of cacao black pod rot disease in various treatments with the mix of CSNE as the main ingredient and various other botanical pesticide ingredients

Treatments	Infection rate (pods/week)	AUDPC (%*day)
P ₀ : control (no pesticide)	0.211	2477.30
P ₁ : CNSE	0.111	1321.67
P ₂ : CNSE + CSLS	0.119	1434.02
P ₃ : CNSE + whiteflower albizia bark extract	0.089	1286.85
P ₄ : CNSE + Siam weed leaf extract	0.075	826.60
P ₅ : CNSE + candle bush leaf extract	0.097	1162.00
P ₆ : CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract	0.080	823.45
P ₇ : synthetic pesticide with 345 g/L of copper oxysulfate	0.082	928.03

3.3. Black pod rot of cacao

Cacao black pod rot symptoms were captured in all treatments, with and without application of botanical or chemical pesticide treatment. The rate of the disease incidence varied for each treatment. The results of the BPR incidence rate observation are presented in Figure 1. Based on Figure 1, the pods left unsprayed with pesticide demonstrated the high

3.4. The efficacy of botanical pesticides

The treatments experimented yielded varied rates of efficacy (Figure 3), which suggests that each of the treatments had a unique efficacy in inhibiting BPR progression. According to Figure 3, the botanical pesticides applied were effective in controlling the BPR disease as proven by efficacy values greater than 50%, except for the CNSE + CSLS treatment, whose efficacy rate was lower than 50%. The highest efficacy was exhibited by the CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract treatment (72.4%), followed by the CNSE + Siam weed leaf extract (71.6%). The efficacy levels of the two types of pesticides above exceeded that of the chemical pesticide (69.0%), hence applicable as alternatives to chemical pesticides. Such high efficacy levels slowed the cacao black pod rot disease infection rate down and lowered the disease incidence.

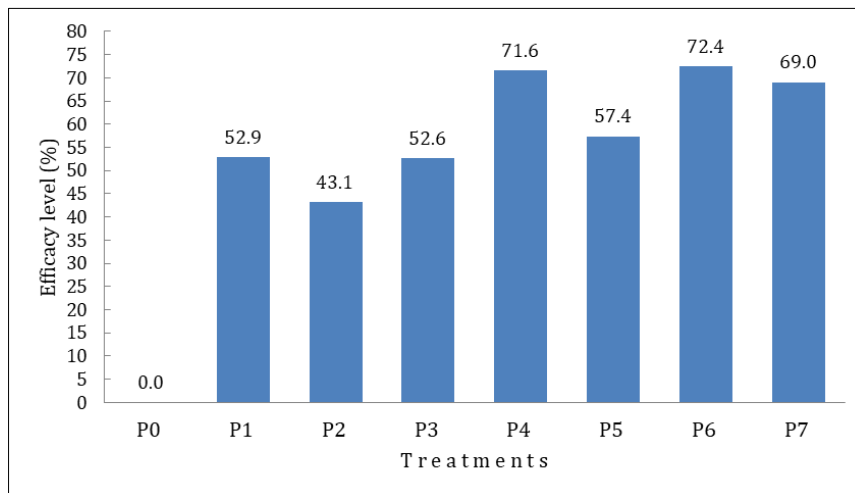


Figure 3 Botanical pesticides' efficacy levels (%) against BPR. P₀: control (no pesticide); P₁: CNSE; P₂: CNSE + CSLS; P₃: CNSE + whiteflower albizia bark extract; P₄: CNSE + Siam weed leaf extract; P₅: CNSE + candle bush leaf extract; P₆: CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract; and P₇: synthetic pesticide with 345 g/L of the active ingredient copper oxysulfate

4. Discussion

The application of botanical pesticides in this research was able to reduce the disease incidence and decelerate the disease progression better than the non-application. The botanical pesticides' ability to control the disease had no significant difference statistically from synthetic chemical pesticides, thus feasible to use as alternatives to synthetic chemical pesticides. The effectiveness of the botanical pesticides in controlling cacao black pod rot was good enough as can be seen in the yielded efficacy rates upward of 50%, particularly those of the CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract treatment and of the CNSE + Siam weed leaf extract treatment.

The low disease incidence in the botanical pesticide applications was attributed to the antifungal phytochemical contents. The shell of cashew nut contains cashew nut shell liquid (CNSL) which can reduce the incidence of cacao black pod rot [11]. Coconut shell liquid smoke contains phenol which is inhibitive of *Phytophthora* sp. growth [17] and antifungal to *Polyporus sanguineus* and *Tyromyces palustris* growths at 81.5% and 90%, respectively [18]. Siam weed leaf extract contains saponins, phenols, glucosinolates, and flavonoids, which exert an antifungal effect on *Phytophthora megakarya* [19]. Similarly, candle bush leaf extract is able to inhibit fungal growth [20].

The botanical pesticide of CNSE + Siam weed leaf extract was able to reduce the BPR infection rate to the lowest among the treatments (38.90%). Such a decrease in the disease intensity was because the mixture of CNSE and Siam weed leaf extract has the ability to inhibit the growth of *Phytophthora palmivora* mycelia and BPR symptoms [13]. Such an ability shows that the active substances in the two ingredients were antifungal and in synergy to control BPR disease. A botanical pesticide mixture may exhibit synergism and hence improve its utilization effectiveness [21]. The CNSL in cashew nut shell is rich in such secondary metabolites as alkaloids, saponins, tannins, flavonoids, amino acids,

diterpenes, volatile oils, and terpenoids, all of which are antifungal [22]. Meanwhile, the extract of Siam weed leaf, as shown by gas chromatography analysis, contains antifungal α -pinenes, β -pinenes, and germacrene D [23].

The disease incidence in the application of the chemical pesticide with 345 g/L of copper sulfate was 40.73%, which statistically had no significant difference from the disease incidence in the botanical pesticide treatments. This rate was especially akin to the incidence rate in the CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract treatment (39.52%). The mixture of a number of botanical pesticide ingredients allows for the intersection of a number of active substances which are able to inhibit pathogen growth. Botanical pesticide active substances such as terpenes, phenols, alkaloids, tannins, and other secondary metabolites are able to induce the toxicity in fungi cell wall, membrane, and other organelles [7], influence spore germination, and delay sporulation [9].

The results of the model goodness of fit test suggested that the investigation into BPR progression in all treatments in this research be conducted under the monomolecular model. The treatments applied were shown non-causative of any change to the BPR progression model. This monomolecular disease progression model generally occurs in soil-borne pathogens. Data showed that the pathogenic inoculum of the BPR disease predominantly was sourced from the soil around the cacao tree canopy. This finding was supported by the fact that the soil surface beneath the cacao tree canopy was in an open state, hence allowing the splash of raindrops from the soil to reach healthy cacao pods. Soil is the main, fixed inoculum source of *Phytophthora palmivora* in cacao plantation, whose spread can be mediated by rain water splash [24]. The disease progression model was highly influential to the infection rate of the BPR-causing *Phytophthora palmivora*. The infection rates (r) of the cacao BPR over the observation period fluctuated and differed from one treatment to another. Calculation of infection rate demonstrated that the various botanical pesticide mixes influenced the progression of the cacao BPR. The infection rate of the synthetic chemical pesticide treatment was not far different from those of the CNSE + Siam weed leaf extract treatment and the CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract treatment. The three treatments abovementioned were able to reduce the progression of the cacao black pod rot disease. Although they are able to control plant diseases effectively and efficiently, synthetic chemical pesticides must be used in a limited way as they are toxic [25].

An AUDPC value illustrates to which extent a disease disturbs a plant in the long run, while disease is disturbance which occurs during the observation [26]. The lower the AUDPC value, the higher the inhibitory ability of a treatment is against a disease's progression. In this research, the lowest AUDPC values were found in the CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract treatment, the CNSE + Siam weed leaf extract treatment, and the synthetic pesticide with 345 g/L of copper oxysulfate treatment, showing that the three were able to protect cacao pods against BPR-causing *Phytophthora palmivora* colonization. A high AUDPC value in the non-pesticide treatment indicates that the pathogen was free to infect cacao pods until the end of the observation variable without any inhibiting factors. AUDPC illustrates a disease's cumulative amount within a single season [27]; the higher the AUDPC value, the more cacao pods are infected by BPR.

The efficacy value of the CNSE pesticide mixed with coconut shell liquid smoke was very low (43.1%), indicating a weak ability to control cacao BPR. The efficacy of CNSE pesticide would increase if mixed with whiteflower albizia bark extract, Siam weed leaf extract, and candle bush leaf extract. A pesticide was to be said effective if the efficacy rate was at least 50%, provided that the disease incidence a week after spraying was significantly different from the control (without fungicide application) [16]. The high rates of botanical pesticide efficacy in the CNSE + whiteflower albizia bark extract + Siam weed leaf extract + candle bush leaf extract and the CNSE + Siam weed leaf extract treatments show that the phytochemistry in the ingredients were in synergy to inhibit pathogenic colonization on cacao pods. From *in vitro* analysis, it was discovered that the botanical pesticide of CNSE + Siam weed leaf extract was able to suppress *Phytophthora palmivora* growth at 63.59% [13].

5. Conclusion

The botanical pesticides of CNSE, and the was mixture with of coconut shell liquid smoke, siam weed leaf extract, candle bush leaf extract, and whiteflower albizia bark extract were able to lower the disease incidence and slow down the infection rate of BPR. The lowest BPR incidence and infection rates were found in the CNSE + siam weed leaf extract treatment, each at 38.90% and 0.087 per pod per week. The CNSE pesticide mixed with siam weed leaf extract, and CNSE mixed with whiteflower albizia bark extract, siam weed leaf extract, and candle bush leaf extract had good efficacy rates of 71.6% and 72.4%, hence potential to use as alternatives to chemical pesticides in cacao black pod rot control.

Compliance with ethical standards

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Disclosure of conflict of interest

La Ode Santiaji Bande, Gusnawaty HS, and Mariadi were research design, collected, analyzed and interpreted the data, and wrote manuscript. Muhidin and Abdul Rahman were reviewed the manuscript. All authors read and approved the final version. The authors declared that present study was performed in absence of any conflict of interest.

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