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Research Article
Effectiveness of Botanical Pesticides Composite to Decrease of *Phytophthora palmivora* Caused Black Pod Rot on Cocoa

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Abstract

Background and Objective: Pod rot disease caused by *Phytophthora palmivora* is one of the causes of low productivity in cocoa plants. The objective of the study was to determine the effectiveness of botanical pesticides composite inhibiting the growth of *Phytophthora palmivora* biomass and to know the type of botanical pesticides composite that able to inhibit the black pod rot disease in cocoa. **Materials and Methods:** The research was conducted in Plant Protection Laboratory, Faculty of Agriculture, Halu Oleo University. The research designed using a completely randomized design, consisting of six treatments as follows: C_0 = Without pesticide, C_1 = Composite of cashew nut shell extract+liquid smoke, C_2 = Composite of cashew nut shell extract+tree bark of *Albizia saponaria*, C_3 = Composite of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.), C_4 = Composite of cashew nut shell extract+leaf of *Cassia alata*, C_5 = Composite of cashew nut shell extract+tree bark of *Albizia saponaria*+leaf of siam weed (*Chromolaena odorata* L.)+Leaf of *Cassia alata* and C_6 = Chemical pesticide as a control treatment with active ingredients *copper oxysulfate* (345 g L^{-1}). The parameters observed were dry weight of *Phytophthora palmivora* biomass and extensive spotting of cacao pod rot. Botanical pesticides were applications in two phase's inhibitory test. Firstly, is in petri dish using *vegetable juice* (V4) media. Secondly, is in the laboratory using healthy cocoa fruit. **Result:** The results showed the effectiveness of botanical pesticides from composite of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.) to inhibit the growth of *Phytophthora palmivora* biomass is 60.92%. **Conclusion:** The composite of botanical pesticides is able to inhibit the development of symptoms of black pod rot disease by 63.59%.

Key words: Botanical pesticides, black pod rot, cocoa, *Phytophthora palmivora*, *Chromolaena odorata*, Siam weed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cacao is an important cash crop in Indonesia, cultivated by an estimated one million smallholders. Indonesia currently is the world's third-largest cocoa producer[1]. In recent years, however the country's cocoa production growth has been limited and declined. Smallholders produce almost 90% of Indonesia's cocoa output. Cacao smallholders are suffering from declines in production negatively affecting household income[2]. The most significant reasons for low production were aging trees and infestations from pest and disease.

Many factors contribute to a decline in production of cocoa beans worldwide. Plant diseases are major components of the decline in production. One of the causes of low productivity of cocoa due to black pod rots (BPR), which can reduce production to 54%[3]. Black pod rot caused by various species of *Phytophthora*[4,5], is the most important disease of cacao on a global scale[6]. *Phytophthora palmivora* is a pathogen of many plant species including citrus (*Citrus* sp.), rubber (*Hevea brasiliensis*) and several palm species such as coconut (*Cocos nucifera*) and the African oil palm (*Elaeis guineensis*)[7]. BPR disease of cacao is an economically serious problem in all areas of the world where cacao is grown[8].

In Indonesia, Southeast Sulawesi is major cocoa production. Contrary, the productivity of cocoa in Southeast Sulawesi is very low at only 829 kg ha^{-1} every year[9], when compared to the normal conditions that can reach 2 t ha^{-1} yearly[10]. The extent damage on cocoa crops due to BPR in Southeast Sulawesi was about 526.71 ha from total cocoa plantation of 249,234 ha and *P. palmivora* pathogen has been proven to infect and cause disease in almost all cocoa plantation centers in Southeast Sulawesi[11].

The method to control BPR commonly still rely on synthetic chemical pesticides, which is often use inappropriate dose recommended and time application. Chemical control remains expensive and poses an enormous risk of poisoning for the users and the environment[12]. The application of chemical pesticides can lead to poisoning in humans, environmental pollution, the emergence of resistance and pest resurgence and the presence of pesticide residues in agricultural products[13]. It is necessary to develop and use environmental friendly pesticides such as the botanical pesticides and become an alternative to the integrated control strategy against BPR[12].

Much plant can be use as source active ingredient for botanical pesticides and have ability to control *P. palmivora* agent of BPR. Plants that can be used as a source of botanical pesticides such as cashew nuts (*Anacardium occidentale*), leaf of siam weed (*Chromolaena odorata*), leaf of *Cassia alata* and bark of *Albizia saponaria*. Extracts of cashew nut shells is effective as molluscicidae, fungicide and bactericide[14]. Siam weeds leaf extract (*Chromolaena odorata*) that can inhibits *Aspergillus niger*[15]. Leaf of *Cassia alata* is used to suppress the *Cercospora personatum* fungus of pea[16] and the bark of the tree *Albizia saponaria* containing antimicrobial[17]. These extract contain the main different ingredients that can reduce various plant diseases.

Synergism effect of various active ingredients mixture from botanical pesticide is expected to increase the effectiveness in controlling BPR. It could be used as an alternative of chemical pesticide so that the environmental pollution and chemical pesticide residues in agricultural products can be reduced. The study aim was to determine the effectiveness of botanical pesticides composite inhibiting the growth of *Phytophthora palmivora* biomass and to know the type of botanical pesticides composite that able to inhibit the black pod rot disease in cocoa.

MATERIALS AND METHODS

The research was conducted in Plant Protection Laboratory, Faculty of Agriculture, Halu Oleo University from January to May 2017. The research was design using a completely randomized design (CRD), consisting of six treatments as follows: C_0 = Without pesticides treatment, C_1 = Botanical pesticide composite of cashew nut shell extract+liquid smoke, C_2 = Botanical pesticide composite of cashew nut shell extract+tree bark of *Albizia saponaria*, C_3 = Botanical pesticide composite of cashew nut shell extract+leaf of Siam weed (*Chromolaena odorata* L.), C_4 = Botanical pesticide composite of cashew nut shell extract+leaf of *Cassia alata*, C_5 = Botanical pesticide composite of cashew nut shell extract+tree bark of *Albizia saponaria*+leaf of *Chromolaena odorata* L.+leaf of *Cassia alata* and C_6 = Chemical pesticides as a control treatment with active ingredients *copper oxysulfate* (345 g L^{-1}).

The application of pesticide consists of two phases of inhibitory test. In petri dish, use *vegetable juice* (V4) as media and in the laboratory, using healthy cocoa fruit. Isolation of pathogenic *P. palmivora* is done by taking the cocoa bean in the field that has a symptoms of rotting fruit. The test on the petri dish is done by mixing V4 juice medium with pesticide extract and laying the pure culture pieces of *P. palmivora,* then in tape after that stored in the rack for further observation. Test on cocoa fruit was done by taking the fruit of cocoa with a large uniform fruit and then washed until clean after that fruit surface sterilize using disinfectant.

The observational variables in the observed petri dish test were biomass mycelium weight (mycelium), observed by taking each mycelium from a petri dish tested aged 8 days after the incubation mass with indicator of one mycelium cup of test (control) was full and then dried in oven with temperature 40°C for 6 h using tissue. Drying is continued when the weight is not stable, then weighed using an analytic scale. The effectiveness of the mixture of botanical pesticides was calculated by the percentage inhibition of botanical pesticides (IBP), by the formula:

$$IBP = \frac{Dc - Dp}{Dc} \times 100\ (\%)$$

IBP = Inhibit of botanical pesticides (%)
Dc = Dry weight of biomass *P. palmivora* to control
Dp = Dry weight of biomass *P. palmivora* with botanical pesticides

The observed test on cocoa fruit included spotted diameter measured 4 days after the test by observing the appearance of black spots on the fruit for all treated treatments. The effectiveness of the mixture of vegetable pesticides was calculated by the Disease Suppression Index (DSI)[18] with the formula:

$$DSI = \frac{Sc - St}{Sc} \times 100(\%)$$

DSI = Disease suppression index
Sc = Spacious spots on the controls
St = Extensive spots on treatment

Data analysis: Data analyses using two-ways analysis of variances (ANOVA) using the Statistical Package of Social Sciences (SPSS) program version 20 for Windows (Chicago, IL, USA). If the test result showed a significant difference, then tests of treatment differences were performed using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Result: The results showed that the botanical pesticide has effective inhibiting growth of *P. palmivora*. The dry weight biomass (DWB) of *P. palmivora* was lower when treatment with botanical pesticides, compared than that without botanical pesticides treatment. The different composition of botanic al pesticides has a given different effect on DWB of *P. palmivora* (Table 1). The lower DWB *P. palmivora* in each botanical pesticide treatment has indicated that the higher botanical pesticide capability to inhibit the growth of the *P. palmivora* mycelium. Botanical pesticide treatment was able to reduce the DWB of pathogenic *P. palmivora*, with relative resistance level between 31.41-60.92% compare to control treatment using chemical pesticide. The higher the relative resistance value indicated the higher botanical pesticides effective in reducing *P. palmivora* growth. The highest relative resistance still in chemical pesticide (92.14%) and DWP of *P. palmivora* was only reached 12 mg.

The highest effectiveness of botanical pesticide compound was inhibiting the DWB of *P. palmivora* found in the mixture of cashew nut leaf extract and Siam weed leaf that reached 60.92%. Other botanical pesticide mixtures that also have potential to be developed as a substitute for chemical pesticides are a mixture of cashew nut shell+tree bark of *Albizia saponaria*, that has inhibition level reached 50.41%, compared that treatment without application of botanical pesticides.

The result showed that application of various botanical pesticides has significant effect on *P. palmivora* spots on cocoa pod a few day after inoculation (DAI). The mixture of botanical pesticide was able to reduce the symptoms of BPR compared to the control treatment. The smaller value indicated the larger capability to reduce the extent of BPR (Table 2).

The botanical pesticides compound can reduce the extent of BPR spotting on cocoa pod. The lowest spotted area was found in the botanical pesticide treatment mixture of cashew nut shell extract+Siam weed leaves. The synergism effect of botanical pesticides even lower than that of chemical pesticides but all kind mixtures of botanical pesticide have the ability to reduce the extent of the spots.

The botanical pesticides can inhibit the symptoms of black pod rot disease, with varying disease suppression index value. The higher the index suppression value on each treatment is the more effective in reducing the occurrence of BPR on cocoa a pod (Table 3).

The highest suppression index of botanical pesticide mixture to the extent of late blight of cocoa in 7 DAI (day after inoculation) found in mixed treatment between cashew nut leaf extract and siam weed leaf. The disease suppression index above 50% in the seventh day after inoculation, found in the mixed treatment of cashew nut leaf extract+siam weed (63.59%), mixture of cashew nut shell+tree bark of *Albizia saponaria* (60.43%) and mixture of cashew nut shell+tree bark of *Albizia saponaria*+siam weed+*Casia alata* (54.70%).

Table 1: Average dry weight biomass of *P. palmivora* and the relative inhibition level of botanical pesticides compound

	Botanical pesticides treatment	Dry weight biomass (mg)	Inhibition level (%)
C_1	Composite of cashew nut shell extract+liquid smoke)	87.33^{b}	42.12^{bc}
C_2	Composite of cashew nut shell extract+tree bark of *Albizia saponaria*	81.00^{b}	50.41^{bc}
C_3	Composite of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.)	68.67^{bc}	60.92^{b}
C_4	Composite of cashew nut shell extract+leaf of *Cassia alata*	105.30^{ab}	31.71^{c}
C_5	Composite of cashew nut shell extract+tree barks of *Albizia saponaria*+leaf of siam weed *Chromolaena odorata* L.)+leaf of *Cassia alata*	102.73^{ab}	31.41^{c}
C_6	Chemical pesticide as a control treatment with active ingredient *copper oxysulfate* (345 g L^{-1})	12.00^{c}	92.14^{a}

Numbers followed by the same letter in the same column are not significantly different at the 95% confidence level

Table 2: Average broad spotted symptoms on black pod rot disease in various mixtures of botanical pesticides

		Spotting area (cm^2)			
		Day after inoculation (DAI)			
	Botanical pesticides treatment	4	5	6	7
C_0	Without botanical pesticide	13.55^{a}	20.62^{a}	26.77^{a}	41.74^{a}
C_1	Composite of cashew nut shell extract+liquid smoke	7.12^{b}	10.31^{b}	14.03^{bc}	20.99^{bc}
C_2	Composite of cashew nut shell extract+tree bark of *Albizia saponaria*	7.43^{b}	9.11^{b}	12.04^{bc}	16.56^{bc}
C_3	Composite of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.)	6.33^{b}	8.87^{b}	11.30^{c}	15.10^{c}
C_4	Composite of cashew nut shell extract+leaf of *Cassia alata*	10.41^{ab}	13.48^{b}	17.64^{b}	25.72^{b}
C_5	Composite of cashew nut shell extract+tree bark of *Albizia saponaria*+leaf of siam weed (*Chromolaena odorata* L.)+leaf of *Cassia alata*	6.54^{b}	10.05^{b}	13.79^{bc}	18.26^{bc}
C_6	Chemical pesticide as a control treatment with active ingredients *copper oxysulfate* (345 g L^{-1})	6.25^{b}	8.48^{b}	10.91^{c}	14.44^{c}

Numbers followed by the same letter in the same column are not significantly different at the 95% confidence level

Table 3: Suppression index spotted the symptoms of black pod rot disease in various mixtures of botanical pesticide

		Suppression index (%)			
		Day after inoculation (DAI)			
	Botanical pesticides treatment	4	5	6	7
C_0	Without botanical pesticide	00.00^{b}	00.00^{b}	00.00^{b}	00.00^{b}
C_1	Composite of cashew nut shell extract+liquid smoke	44.41^{a}	47.79^{a}	47.94^{a}	47.50^{a}
C_2	Composite of cashew nut shell extract+tree bark of *Albizia saponaria*	43.17^{a}	55.30^{a}	55.15^{a}	60.43^{a}
C_3	Composite of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.	49.00^{a}	55.41^{a}	57.61^{a}	63.59^{a}
C_4	Composite of cashew nut shell extract+leaf of *Cassia alata*	20.84^{ab}	33.83^{a}	33.95^{a}	38.16^{a}
C_5	Composite of cashew nut shell extract+tree bark of *Albizia saponaria*+leaf of siam weed (*Chromolaena odorata* L.)+leaf of *Cassia alata*	49.23^{a}	47.79^{a}	47.82^{a}	54.70^{a}
C_6	Chemical pesticides as a control treatment with active ingredients *copper oxysulfate* (345 g L^{-1})	49.88^{a}	59.05^{a}	59.11^{a}	64.79^{a}

Numbers followed by the same letter in the same column are not significantly different at the 95% confidence level

DISCUSSION

Botanical pesticides from cashew nuts produce CNSL oil which has active ingredients of *anacardic acid*[19,20], *cardol* and *cardanol*[21,22]. The CNSL liquid is a natural source of phenolic compounds that contribute to its antioxidant[23,24], antifungal[25,26], antibacterial[27,28] and larvacidal[29,30]. CNSL have been used to control of *S. aureus*[27], *Enterococcus faecalis* and *Salmonella typhi*[26,31] and also antifungal activity to fungi namely *Epidermophyton floccosum, A. niger* and *A. flavus*.

The active ingredients in siam weed (*Chromolaena odorata* L.) leaves were contained alkaloids, phenols, flavonoids, saponins, cardenolides, anthraquinones and tannins[19,32-34]. The presence of the phytochemical alters several biochemical functions[35]. The siam weed has a potential as antibacterial, antifungal[36] and nematicidal[37]. Extracts of *Chromolaena odorata* used to control *Plutella xylosteila*[38], *Sitophilus oryza*[39], *Aedes aegypti*[40,41], *Musca domestica*[42], *Periplaneta americana*[43] and *Vibrio harveyi*[44].

The synergistic effect of the *anacardic acid* from cashew nut shells and active ingredient from siam weed leaves caused the botanical pesticide mixture to be superior, compared that of other pesticides in inhibiting the growth of *P. palmivora*. The higher CNSL concentration applied to cocoa fruit causes the lower diameter of pod rot symptoms due to *P. palmivora* infection[45]. The bark *Albizia saponaria* contains triterpenescent, saponins, alkaloids, steroids and flavonoids and contains potentially pesticides compounds[46]. Saponins are

bioactive compounds generally considered to be produced by plants[47] to counteract pathogens and herbivores[48-50]. Extract of *Albizia saponaria* has antifungal and antimicrobial activity against human cancer[51,52], also as medicinal plant in veterinary practice[53]. Saponin powder could utilized as natural insecticides[54] as part of Integrated Pest Management (IPM) programs[55].

The botanical pesticides can reduce the occurrence of late fruit. The botanical pesticide used was all mixed with cashew nut shell extract to increase the ability in reducing the growth of *P. palmiviro* caused of BPR. The extract of cashew nut shell has anti microbial effect[56]. Liquid coconut shell (liquid smoke) is capable to control growth of *P. palmivora* fungus that inhibits the growth of mycelium and the formation of its generative structure[57]. Siam weed extract has anti-fungal activity[15]. The siam weed leaf extract provides the best inhibitory ability against growth of *P. palmivora* in vitro at a concentration level of 40%[58].

CONCLUSION

Base on research it concluded that botanical pesticide has effective to inhibit the growth of *Phytophthora palmivora* caused BPR on cocoa. The botanical pesticides mixture of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.) inhibit the growth of biomass *Phytophthora palmivora* reached 60.92% and inhibit the development symptoms of BPR 63.59%.

SIGNIFICANCE STATEMENT

This study discovers the possible develop botanical to control of *P. palmivora* agent of black pod rot on cocoa. That can be beneficial for improving the alternative pesticide that environmental friendly to reduce the negative impact of chemical pesticides. This study will help the researcher to uncover the critical area of the potential of botanical pesticides to control of black pod rot cocoa that many researchers were not able to explore. The finding revealed that the botanical pesticides mixture of cashew nut shell extract+leaf of siam weed (*Chromolaena odorata* L.) effective in the inhibiting growth of *P. palmivora* and reduce the development symptoms of BPR.

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