Botanical pesticides effect from shells of bean's cashew nut on biological agents of *trichoderma* sp. and *gliocladium* sp.

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Abstract. A shell of cashew nut (*Anacardium occidentale*) has contained Cashew Nut Shell Liquid (CNSL) that is used as botanical pesticides. CNSL oil consists of active substance such as anacardat acid, cardol and cardanol. Utilization of the pesticides from shells of cashew nut to control pests and diseases of plants would be affected on biological agents. The objective of this research was to investigate pesticides inhibition on the increase of mycelium *Trichoderma* sp. and *Gliocladium* sp. by in vitro method. The tested concentration sample consisted of 0.0% (control), 2.5%, 7.5% and 10.0% in PDA media. The results of this research showed that 2.5% botanical pesticides concentration could minimize mycelium of *Trichoderma* sp. and *Gliocladium* sp. 22.73% and 21.04% respectively and also the increase shells of cashew extract could be affected the increase of mycelium inhibition. The extract with 2.5% concentration was the recommended concentration to control of fruit rot diseases and if concentration was 10.0% then its inhibition become 54.98% and 49.35%, respectively. The results proved that uncontrolled utilization of the pesticides could be affected on decrease of *Trichoderma* sp. and *Gliocladium* sp. growth.

1. Introduction

Current, utilization of the chemical pesticides is very massive on agriculture area, therefore it affects on the decrease of agricultural products. Utilization of pesticides in cocoa farming area has been used by farmers for cultivation, land preparation, plant maintenance and harvest. Excessive use of pesticides will cause health problems and environmental pollution [1], decreasing of soil quality [2] and pesticides residue on cocoa beans [3]. There were many methods to reduce the use of chemical pesticides, such as utilization of biological agents, botanical pesticides, resistant varieties, setting cropping pattern and habitat management (4,5).

In South east Sulawesi, extract of cashew nut shells is one of potential source of botanical pesticides. Cashew nut shells contain cashew nut shell liquid (CNSL) oil, which has fungicidal effect [8] and insecticidal effect [9]. Active ingredients on CNSL are anacardat acid, cardol and cardanol [10,11,12]. Laboratory testing showed that 3.5% botanical pesticide concentration could control *Phytophthora palmivora* growth, pod rot disease in cocoa plants by 87.67% [6] and 2.5% of botanical pesticide concentration could be decreasing pod rot diseases in cocoa plants by 64.16-71.02% [7].

*Trichoderma* sp. and *Gliocladium* sp. are biological control agents for controlling diseases that are widely used [13, 14]. Utilization of uncontrolled chemical pesticides can be affected on decreasing of pathogen control ability [14]. On the other hand, utilization of botanical pesticides can also be
decreasing the pathogen. Application of botanical pesticides from extract of cashew nut shells to control pest and disease will minimize of Trichoderma sp. and Gliocladium sp. on plant roots.

The recommendation concentration of botanical pesticides from cashew nut shells to control of fruit rot disease in cocoa plants is 2.5% [17]. Study of biological safety against using biological pesticides of cashew nut shells should be studied further, this is to avoid the negative effects of these vegetable pesticides. The objective of this research was to observe the inhibition of botanical pesticides from cashew nut shells extract against mycelium growth of Trichoderma sp. and Gliocladium sp. by in vitro method.

2. Materials and methods
This research was conducted in Plant Protection Laboratory, Agriculture Faculty, Halu Oleo University from March to May 2017. There were five different treatments that were arranged randomly with three replications. The treatments were $H_0$ (0.0%), $H_1$ (2.5%), $H_2$ (5.0%), $H_3$ (7.5%), and $H_4$ (10.0%). This research used a botanical agents of Trichoderma sp. and Gliocladium sp., which were the collection of Plant Protection Laboratory, Agriculture Faculty, Halu Oleo University. Trichoderma sp. and Gliocladium sp. are reproduced through Potato Dekstrosa Agar (PDA) medium. As for the botanical pesticides from extract of cashew nut shell is produced by Ir. Mariadi, M.S that contain active acid anakardat.

The experiment of botanical pesticides to inhibit Trichoderma sp. and Gliocladium sp. used toxic medium method, which was the mixture of botanical pesticides based on concentration on PDA medium [6]. After mixing process, the botanical pesticides was sterilized at 121 °C and 151 psi for 20 minutes. After that, the medium was cooled to temperature of 65 °C and 10 ml of the sterilized mixture pesticide medium was poured into a petridish and cooled. Inoculum of Trichoderma sp. and Gliocladium sp. was cultured on the center of petridish and incubated at room temperature separately.

The parameters were dry weight of mycelium and inhibitory of botanical pesticides against dry weight of mycelium. Observation of the mycelium was started ± 3-5 days after inoculation or if biological agents colony fulfilled a petridish. Meanwhile, the fungal mycelium was harvested by adding sterile aquadest on the medium sufficiently and then heated. The suspension was filtered, therefore only fungal mycelium was obtained. The fungal mycelium was dried in the oven at 50 °C until its weight is stable or the weight is not changing again. The inhibition of botanical pesticides was calculated by the equation as [6] as follows:

$$IB = \frac{DWC_1 - DWB_2}{DWC_1} \times 100\%$$

IB: The inhibit of botanical pesticides (%)
DWC_1: The dry weight of mycelium without botanical pesticides (control)
DWB_2: The dry weight of mycelium with botanical pesticides

Data was analyzed and if it has a significant affect then continue with Duncan's multiple-range test in 95% level.

3. Results and Discussion
The result of this study showed that the higher stimulation of botanical pesticides from cashew nut shells extract by in vitro method could be affected on decreasing of Trichoderma sp. and Gliocladium sp. biomass (Tables 1 and 2). Data analyzed of biomass weight showed that botanical pesticide concentration had a significant effect against biomass weight of Trichoderma sp. and Gliocladium sp. Clearly, this research implied that botanical pesticide was one of some alternatives to control pets and also to minimize the use of chemical pesticides. This result similar with some other research that mention about the advantages of botanical pesticides such as; environmentally friendly, easily degraded, without toxic, and according to the concept of sustainable agriculture [15,16].

The other result of the research (Table 1) show that the higher concentration could be decreased biomass weight Trichoderma sp. This is in line with the result of previous research which mentions that utilization dose or concentration of pesticides should be larger to get maximum results.
In the another hand, the Table 1 given information that utilization of botanical pesticides should be precise, so negative effect is neglected. Inhibition of 2.5% of botanical pesticides against Trichoderma sp. was 22.73% while the use of 5.0% botanical pesticides was not significantly different to 2.5% concentration (30.52%). If the concentration become 10.0%, it could be affected on increasing of inhibition of botanical pesticides by 54.98%. In this condition, Trichoderma sp. only growth 45.20% normally. The utilization of combination of Trichoderma sp. and botanical pesticides from cashew nut shells extract showed a good interaction if both of them will use to control pest simultaneously. Studied that Trichoderma sp. and botanical pesticides from clove extract had effect to control ginger rhizome rotten disease [18].

Inhibition of 2.5% botanical pesticides (%)

| Treatments   | Biomass weight (g) | Inhibition of botanical pesticides (%) |
|--------------|--------------------|---------------------------------------|
| Concentration 0.0% (H₀) | 0.13 d            | 0.00 a                               |
| Concentration 2.5% (H₁)   | 0.10 c            | 22.73 b                              |
| Concentration 5.0% (H₂)   | 0.09 bc           | 30.52 bc                             |
| Concentration 7.5% (H₃)   | 0.07 ab           | 44.81 cd                             |
| Concentration 10.0% (H₄)  | 0.06 a            | 54.98 d                              |

Notes: the number is followed by alphabet symbol is not different and the degree of trust is 95%

Biomass weight of Gliocladium sp. decreased by the increasing of botanical pesticides of cashew nut shells extract (Table 2). Higher biomass occurred on control treatment (without botanical pesticides) and it differed significantly to other treatments. Biomass weight of Gliocladium sp. on 2.5% as many as 0.12 g is not different with biomass weight on 5.0% as many as 0.10 g. This data showed that utilization of 5.0% biological pesticides from cashew nut shells extract had affect on the increase of Gliocladium sp., and this is almost same with the 2.5% concentration of biological concentration.

Inhibition of 2.5% botanical pesticides against Gliocladium sp. did not differ significantly with control treatment, which means that the ability of botanical pesticides still normal on this concentration. This study proved that botanical pesticides from cashew nut shells extract could be applied with Gliocladium sp. to control pest simultaneously. This data is also important as information to control pest of plants. Supriadi [19] said that one of efforts to minimize of chemical pesticides was optimization of botanical pesticides and biological control that are compatible simultaneously.
4. Conclusions

In summary utilization of botanical pesticides from shells of cashew nut by in vitro method could decrease biomass weight of *Trichoderma* sp. and *Gliocladium* sp. There are decreasing of weight’s *Trichoderma* sp. and *Gliocladium* sp. against inhibition of botanical pesticides using extract of cashew nut shells. The amount of inhibition using 2.5% extract of cashew nut shells against biomass weight of *Trichoderma* sp. and *Gliocladium* sp. were 22.73% and 21.04% respectively. Meanwhile, 10.0% concentration of botanical pesticides could inhibit by *Trichoderma* sp. and *Gliocladium* sp. by 54.98% and 49.35% respectively.

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