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Endophytic Fungi to Control of Cocoa Pod Borer (*Conopomorpha cramerella*) on Cocoa Plantation

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ABSTRACT

Endophytic fungi are quite common in nature and some of them had been shown to have adverse effects against insects. The previous study have documented the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore, parasitic nematodes and plant pathogens. Four isolates of fungal endophyte were isolated from cacao fruit and leaves from West Sulawesi, Indonesia. The isolates were identified such as *Trichoderma* sp., *Fusarium* sp., *Acremonium* sp., and *Aspergillus s*p. The isolates were examined for their control to cocoa pod borer (*C. cramerella*). The intensity damage by cocoa pod borer in all treatments of fungal endophyte is always lower than the control treatment.

Keyword: endophytic fungi, cocoa pod borer, *Conopomorpha cramerella*, cocoa plantation

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INTRODUCTION

Main obstacle of cocoa growing is pest and disease infestations such as cocoa pod borer (CPB), Vascular streak disease of cocoa (VSD) and black pod disease, resulting in reduction of yield producitivy about 40% (660 kg/ha/year) of 1100 kg/ha/year. Pest and disease infestation led to lose yield around 198,000 tonnes per year or it equaled to IDR 3.96 triliun per year. In addition, reduction of their infestations can cause poor bean quality so that cocoa bean export to United State of America faced potential loss around US$ 301.5 per ton.

Endophytic fungi are quite common in nature and some of them had been shown to have adverse effects against insects. The previous study have documented the presence of endophytic fungi provide a protection of the plant hosts against insect herbivore (12; 13; 5), parasitic nematodes (10; 14; 15; 17), and plant pathogens (11; 17 18). The present study aimed to isolates of endophytic fungi and to investigate it against cocoa pod borer *C. cramerella* in the field.

MATERIALS AND METHODS

Isolation of Endophytic Fungi:

Endophytic fungi were isolated according the protocols described by (20), which were slightly modified based on preliminary tests. The health cocoa pods taken from the field were washed twice in distilled water then surface sterilized by immersion for 1 minute in 70% (v/v) ethanol, 5 minutes in sodium hypochlorite (2.5 % (v/v) available chlorine) and 30 seconds in 70% (v/v) ethanol and then washed three times in sterilized distilled water for 1 minute each time. After surface sterilization, the samples were cut into 5-7 mm pieces and aseptically transferred to plates containing potato dextrose agar (PDA, pH 6.8, containing (g/l): potato 200; dextrose 20; agar 15.), which had been autoclaved for 15 minutes at 121ºC and then aseptically supplemented with 100 mg/ml of chloramphenicol (Pfizer) to suppress bacterial growth. Aliquots from the third wash were plated onto PDA to check that surface sterilization had been effective and they were then incubated at 28ºC. Any fungi present was isolated, purified and then maintained at 4ºC on PDA slopes for further identification. After 5 days of incubation the grown fungi were identified with reference to (1; 3).

Production of Endophytic Fungi in Powder Form:

The rice medium that has been soaked for 3 hours put into a flask 100 grams, and autoclaved at 121 ^{0}C for 30 minutes and after which by using a corkborer (diam. 0.5 mm), five pieces of endophytic fungi were inoculated in once the fungi started growing, the flask were shaken to assure an even fungal growth. The grown fungi then incubated at 30 ^{0}C for 48 hours. The rice medium along with the fungi then blended to produce a powder form for further study.

Production of Endophytic Fungi In Form of Pellet:

Isolate of endophytic fungi in a state of powder and pellets produced with using alginate. Isolates were added a solution of $CaCl_2$. The suspension is then mixed with 2% (w / v) Sodium-Alginate (Fluka, Biochemica) and by using ultra-Turrax stirring with a flat suspension. By using a plastic pipette tip which is made of Nalgene granules will be formed pellets. Pellets are stored for 45 minutes for further passed on a series of filters and washed with water. For the drying process performed on a tissue paper overlay that easily absorbs water at room temperature. Alginate granules of the fungal endophyte were stored in the refrigerator at 4 ^{0}C before being used in research.

Field Investigation of Endphytic Fungi against Cocoa Pod Borer :

The research were used of sample plot and about 1 (one) Ha and will be held in the village of Rappang, District Tappango, Polewali Mandar, West Sulawesi. Each test sample was taken as many as 10 pieces measuring between 3-5 cm (pod) and marked with rope on the ground for with other pod that are not included in the study. Endophytic fungi, which has been in the form of pellets first dissolved in water with a concentration of 1 gram of pellets versus 100 ml of water (1: 100 w / v). Sample pieces are then sprayed with a solution of endophytic fungi with the concentration of 10^8 colony forming units per ml by using hand sprayer.

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Spraying done in the morning 3 times within intervals of 10 days. Parameters as observed in this study is to examine the damage of pod at harvest time by separating the pod from the rind. The intensity of fruit damage is calculated based on the formula of Lee et al, (6), namely:

$$I = \frac{(Ri \times 0{,}093) + (Se \times 0{,}297) + Be}{TB} \times 100\ \%$$

Description :

I : Intensity of damage
Ri : Light Damage (1 – 15 % seed damage)
Se : Medium Damage (> 15 % - 40 %seed damage)
Be : Heavy Damage (> 40 % seed damage)
TB : Total of Pod

Data Analysis:

Anova was also performed to determine the effects of endophytic fungi to intensity damaged. The percent data were arcsine-transformed before being subjected to Anova. When significant differences were detected, means were separated using Tukey's test at 5% probability level.

RESULT AND DISCUSSION

Isolation of Endophytic Fungi

Isolation of Endophytic Fungi Isolates were obtained sevent isolates such as *Trichoderma* sp. (P1), *Aspergillus* sp. (P2), *Acremonium* sp. (P3), *Aspergillus* sp. (P4), *Fusarium* sp. (P5), *Fusarium* sp. (P6) and *Aspergillus* sp. (P7). (Table1).

Table 1: Isolation and Identification of Endophytic Fungi isolates on Pod, Larvae and Pupa of Cocoa Pod Borer

No	Endophytic Fungi Isolate	Source	Information
1.	*Trichoderma* sp.	Pod	P1
2.	*Aspergillus* sp.	Pod	P2
3.	*Acremonium* sp.	Pod	P3
4.	*Aspergillus* sp.	Larvae	P4
5.	*Fusarium* sp.	Larvae	P5
6.	*Fusarium* sp.	Pupa	P6
7.	*Aspergillus* sp.	Pupa	P7

Field Testing of Endophytic Fungi Against Cocoa pod borer Conopomorpha cramerella Intensity of Damage

The average intensity of damage to pod by *C. cramerella* in the treatment of fungal endophyte are always lower and statistically significantly different compared with the control (Figure 1).

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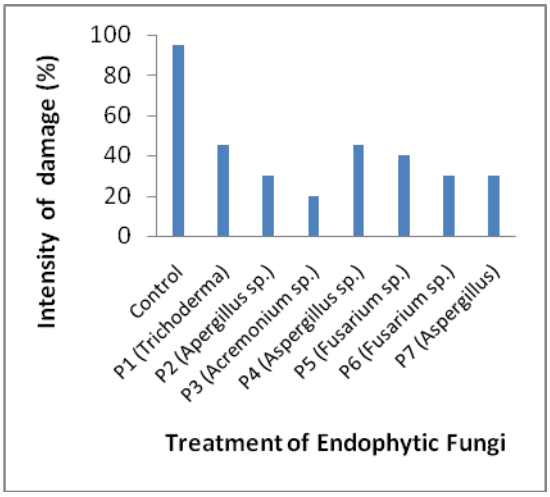

Figure 1: Average of Percentage of Intensity Damage by Cocoa Pod Borer *(C. Cramerella*)

Isolation of Endophytic Fungi

Isolation of Endophytic Fungi were obtained sevent isolates from four genus such as *Trichoderma* sp., *Aspergillus* sp., *Acremonium* sp and *Fusarium* sp. Some endophytic fungi have been reported to be isolated from various plants such as *Fusarium* sp. *Trichoderma* sp. (12). In other study in South Sulawesi in addition to *Fusarium* sp and *Aspergillus* sp, *Curvularia* sp., *Geotrichum* sp., *Gliocladium* sp., and *Colletotrichum* sp., has been isolated from the health leaves of cocoa plants which resistant to VSD disease (11). A number of researches also reported the presence of endophytic fungi in cocoa plants. Hanada (4) isolated a number of genera of fungal endophytes including *Trichoderma*, *Fusarium* from braches and twigs tissues of cocoa trees in Brasil. They found that *Trichoderma* and *Fusarium* showed a high level activity against *Phytophthora palmivora*, the causal agent of the black pod rod disease of cocoa. The presence of *Trichoderma* and *Fusarium* on the tissues of cocoa branches was also reported by (21; 19) reported the establishment of entomopathogen *Beauveria bassiana* as endophytes of cocoa plants if the fungus is inoculated in radicals of seedlings. Three fungal endophyte isolated from healthy Theobroma cocoa tissue and screened in vitro for antagonism against major pathogen of cocoa (9).

Field Testing of Endophytic Fungi Against Cocoa pod borer Conopomorpha cramerella

The average intensity of damage to pod by *C. cramerella* in the treatment of fungal endophyte are always lower and statistically significantly different compared with the control (Figure 1).

The field testing showed that the inoculation of mutualistic endophytic fungi into the soil reduced significantly the number of cocoa pod borer compared to the untreated control (Figure 1). The treatment with the highest biocontrol activity was endophytic fungi. The presence of this fungus decreased the number of cocoa pod borer *C. cramerella* on the treated plants over that of the other fungal treatments. However, at the end of the evaluation period the treatment of *Acremonium* sp. (P3) reduced significantly the number of cocoa pod borer *C. Cramerella* on the treated plants and significantly with the control. It is the same result to effect of *Acremonium* sp. Endophyte on four species of Bilbugs *Sphenophorus parvulus*, *S. Venatus*, *S. Inaequalis* and *S. Minimus* (8).

Webber (22) was probably the first researcher to report an example of plant protection giving by an endophytic fungus, in which the endophyte *Phomopsis oblonga* protected elm trees against the beetle *Physocnemum brevilineum*. It was suggested that the endophytic fungus *P. oblonga* was responsible for reducing the spread of the elm Dutch disease causal agent *Ceratocystis ulmi* by controlling its vector, the beetle *P. brevilineum*. The author associated the repellent effect observed towards the insect to toxic compounds produced by the fungi. This was confirmed four years later by Claydon (2), who showed that endophytic fungi belonging to the Xylariacea family synthesize secondary metabolites in hosts of the genus Fagus and that these substances affect the beetle larvae.

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CONCLUSION

It can be concluded that, Acremonium sp. is endophytic fungi which provide potential as biological control agent for cocoa pod borer *C. cramerella* of cocoa plant.

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