The Symptomp Rate of Cocoa Pod Borer (*Conopomorpha cramerella*) due to the Combination of Several Concentrations of Neem (*Azadiracta indica*) Solution Given the Biosurfactant of Diethanolamide Olein Palm and Different Fruit Sizes

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Abstract. One of the main pests on the cocoa plant is the cocoa pod borer. The use of natural pesticides can be an alternative solution in overcoming cocoa pod borer to replace synthetic pesticides. Neem is a plant that can be used as a base for natural pesticides. The main ingredients of neem are azadirachtin, meliantriol, salanin, nimbin, and nimbidine. Azadirachtin does not directly kill pests but can affect the life cycle of pests, as food repellents, and as a repellent. The purpose of this study was to determine the level of attack of the cocoa pod borer (*Conopomorpha cramerella*) due to the combination of several concentrations of neem (*Azadiracta indica*) solution. This research was conducted in a cocoa farm belonging to the residents of Lurang hamlet, Sukalaba Village, Gunung Sari District, Serang Regency, Banten Province. This research was conducted from November 2019 to April 2020. The experimental design used in this study was a one-factor combination of randomized block design repeated four times and the treatments were control, concentration of 15%, 25%, and 35% solution of young leaves and neem seeds plus palm olein biosurfactant diethanolamide (DEA) with fruit sizes 0.1-2.0 cm and 8.0-10.0 cm. The data were analyzed using analysis of variance (ANOVA) then tested using Duncan’s Multiple Range Test at the 5% level. The results showed that the combination treatment of concentration 15% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 0.1-2.0 cm and the combination treatment of concentration 35% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 8.0-10.0 cm performed the best effect on attack intensity.

Keywords: DEA biosurfactant olein palm, neem, Cocoa Pod Borer, natural pesticides, fruit size

1. Introduction

Indonesia is one of the third largest cocoa producing countries in the world, so that the potency of cocoa products are undoubtedly in the international trade arena. Moreover, Banten province is one of the largest cocoa producers in Indonesia, where in 2017 it produced 2,230 tons per ha and most of the cocoa was produced from smallholder plantations of farmers [1]. On the other hand, one of the obstacles in conducting a cocoa plantation business is the attack of pests. The main pest that can disrupt and even reduce cocoa production is the cocoa pod borer (CPB). According to Suparno [2], CPB attacks can reduce production by up to 80% and damage to beans by up to 82%, so that cocoa
farmers and entrepreneurs have a high concern to face this matter. This will have a big impact to decreased weight and quality of products and increased harvesting costs due to a long time to separate the good seeds and damaged seeds.

CPB pests are very detrimental and difficult to control so that most of farmers still use chemical insecticides as the first alternative to control the damage. Also chemical insecticides are easy to get, with relatively low cost and the effects can be seen quickly in the field. However, the continuous use of chemical insecticides will cause more serious problems, such as pest resistance, environmental pollution, death of natural enemies and rejection of products due to residues that exceed the tolerance threshold.

The compounds contained in the neem plant can inhibit pest attacks. However, the use of natural insecticides is generally less effective because the formulation is still very simple so that the additives formula are needed to combine, such as surfactants, which have functions as dispersing and leveling active ingredients as well as adhesives commonly used in synthetic pesticides [3]. In addition, surfactants are used as components of adhesives, coagulating agents, wetting agents, foaming agents, emulsifiers, penetration agents, and dispersants. One type of surfactant that can be applied to neem vegetable pesticides is diethanolamide (DEA) biosurfactant olein palm.

[4] stated that the prevention of CPB attack on cocoa fruit size 8-10 cm using the covering method is more effective than fruit size 11-15 cm. Covering the young fruit can reduce the CPB attack to below 20%. Whereas in the covering treatment combined with Heterorhabditis sp. can reduce CPB attack by up to 10%. The performance of Heterorhabditis sp. is synergistic with the covering of young fruit in controlling CPB larvae that have entered the cocoa fruits. According to that study, CPB control would be more effective if the fruit size was 8-10 cm or less.

The study of [5] showed that testing on the cocoa pod borer with the extract formulation of young leaves and neem seeds 100 g / 500 ml of water + 5% DEA biosurfactant olein palm at a concentration of 15 ppm was able to provide the fastest time of death (0-20 seconds) with mortality of 100%. Therefore, it is necessary to conduct a research to apply biopesticide of young leaves and neem seeds (Azadirachta indica) with the addition of DEA biosurfactant olein palm against CPB to determine its effectiveness in the field. The purpose of this study was to determine the level of attack of the cocoa pod borer (conopomorpha cramerella) due to the combination of several concentrations of neem (Azadiracta indica) solution and different fruit sizes.

2. Materials and Method
2.1. Place and Time
This study was an experimental research and was conducted in the Laboratory of Basic Science and Plant Protection and screen house, Department of Agroecotechnology, Faculty of Agriculture, University of Sultan Ageng Tirtayasa and in a cocoa farm belonging to the residents of Lurang hamlet, Sukalaba Village, Gunung Sari District, Serang Regency, Banten Province. This research was conducted from November 2019 to April 2020.

2.2. Materials
The materials used in this study were cocoa fruits (0.1-2.0 cm and 8.0-10.0 cm), neem seeds and leaves, DEA biosurfactant olein palm, and water. The tools used were blender, bottle, beaker, measuring cup, strainer, balance, scissors, funnel, hand sprayer, knife, camera, spoon, sieve, basin, stirrer, hot plate, stirrer, wood board, spatula, mortar and pestle, and stationery.

2.3. Research Design
The experimental design used in this study was a one-factor combination of randomized block design and the treatments were control, concentration of 15%, 25%, and 35% solution of young leaves and neem seeds plus palm olein biosurfactant diethanolamide (DEA) with fruit sizes 0.1-2.0 cm and 8.0-10.0 cm. So that this study had 9 treatments repeated four times and being 36 experimental units. The
data were analyzed using analysis of variance (ANOVA) then tested using Duncan’s Multiple Range Test at the 5% level. The detail treatments were as follow:

\[ P_0 = \text{Control} \]

\[ P_1 = \text{Combination treatment of concentration 25% solution of young leaves and neem seeds without 5% DEA biosurfactant olein palm and with a fruit size of 0.1-2.0 cm} \]

\[ P_2 = \text{Combination treatment of concentration 25% solution of young leaves and neem seeds without 5% DEA biosurfactant olein palm and with a fruit size of 8.0-10.0 cm} \]

\[ P_3 = \text{Combination treatment of concentration 15% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 0.1-2.0 cm} \]

\[ P_4 = \text{Combination treatment of concentration 15% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 8.0-10.0 cm} \]

\[ P_5 = \text{Combination treatment of concentration 25% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 0.1-2.0 cm} \]

\[ P_6 = \text{Combination treatment of concentration 25% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 8.0-10.0 cm} \]

\[ P_7 = \text{Combination treatment of concentration 35% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 0.1-2.0 cm} \]

\[ P_8 = \text{Combination treatment of concentration 35% solution of young leaves and neem seeds added with 5% DEA biosurfactant olein palm with a fruit size of 8.0-10.0 cm} \]

The parameters observed consisted of the percentage of CPB attack, intensity of CPB attack, and loss yield in the sample fruit harvested. The formula for calculating the percentage of fruit attacked by CPB is as follow:

\[ P = \frac{a}{a+b} \times 100\% \]

Notes:

- \( P \) = percentage of attacked cocoa fruit by CPB
- \( a \) = number of attacked cocoa fruit by CPB
- \( b \) = number of healthy cocoa fruit

The category of attacked cocoa fruit level is based on the percentage of sticky seeds which is stated in four categories, namely zero, mild, moderate, and heavy. The variations in the level of fruit damage are shown in Figure 1 and Table 1.

**Figure 1.** Categories of attacked fruit: (a) Zero/Healthy, (b) Mild, (c) Moderate, and (d) Heavy (Source: [6])
Table 1. Categories of attacked fruit by CPB

| Category       | Sticky seed criteria                                                                 | Value |
|----------------|-------------------------------------------------------------------------------------|-------|
| Zero/Healthy   | All cocoa seeds are easily removed from the skin of the pods, they are not sticky between the seeds | 0     |
| Mild           | All cocoa beans are easily removed from the pod skin, less sticky between beans (sticky seeds <10%) | 1     |
| Moderate       | The seeds are sticky to each other but can still be removed from the skin of the fruit (sticky seeds = 10-50%) | 3     |
| Heavy          | The seeds are sticky to each other and cannot be removed from the skin of the fruit (sticky seeds > 50%) | 9     |

The intensity of the attack of CPB (I) is calculated by using the formula:

\[ I = \left(\frac{(I \times R) + (3 \times S) + (9 \times B)}{9 \times A}\right) \times 100\% \]

Notes:
I = intensity of CPB attack
R = number of mild attacked fruits
S = number of moderate attacked fruits
B = number of heavy attacked fruits
A = number of fruits observed

The percentage of loss yield is calculated based on the equation cited in [6] as follow:

\[ Y = -0.0210 + 0.1005X \]

where X is obtained from:

\[ X = \left(\frac{(0 \times Sh) + (I \times R) + (3 \times S) + (9 \times B)}{A}\right) \]

Notes:
Y = loss yield
S = number of moderate attacked fruits
X = intensity score of CPB attack
B = number of heavy attacked fruits
Sh = number of healthy fruits
A = number of fruits observed
R = number of mild attacked fruits

The value obtained from the yield loss equation (Y) above is multiplied by 100% to show the percentage of yield loss due to CPB attacks.

3. Results and Discussion
The lifespan of the cocoa plant ranges from more than 15 years. According to the farmers, the planted cacao can produce fruit up to 25 years old but due to lack of maintenance, the cocoa plant productivity at the research location was not optimal and had begun to decline. In the location, cocoa was grown intercroppingly with melinjo, durian, coconut and duku. The spacing between plants was 3 x 3 m.
Harvesting was not carried out regularly with no harvesting schedule. Sanitation and pruning were also not done on the cocoa farm. This can lead to an even bigger CPB attack.

The addition of DEA biosurfactant olein palm to the neem solution makes the solution thicker, does not break down easily, and does not easily fade or drop to the surface so that the neem solution sticks together longer when DEA biosurfactant olein palm is added. This is evidenced by the presence of spray marks, which are dark brown on the cocoa stem a week after application. According to [5] the addition of surfactants to neem oil pesticides will increase the surface tension value. This is presumably due to the positive charge possessed by this type of surfactant which causes instability in the solution because it requires anions (negative ions) to achieve reaction stability. This positive ion will attract oxygen ions in the insecticide solution so that the molecules (negative ions) around it will be attracted. Due to the large surface tension of the solution, the solution is able to stick to the leaf surface and can last longer on the leaves and stems of plants. In addition, surfactants can also preserve pesticides.

Table 2. Percentage of CPB attack, Intensity of CPB attack, and Loss yield

| Treatments | Percentage of CPB attack (%) | Intensity of CPB attack (%) | Loss yield (%) |
|------------|------------------------------|-----------------------------|----------------|
| P0         | 91.67                        | 63.89 e                     | 55.69          |
| P1         | 66.67                        | 37.5 cd                     | 31.82          |
| P2         | 91.67                        | 25.00 b                     | 20.51          |
| P3         | 58.33                        | 17.59 a                     | 13.81          |
| P4         | 75.00                        | 36.11 bc                    | 30.56          |
| P5         | 75.00                        | 30.55 bc                    | 25.54          |
| P6         | 83.33                        | 42.59 de                    | 36.43          |
| P7         | 91.67                        | 52.78 e                     | 45.64          |
| P8         | 50.00                        | 18.52 a                     | 14.65          |

Notes: number followed by the same letter in the same column are not significantly different according to DNMRT at the level of 5%.

3.1. Percentage of CPB attack

The results of variance on the percentage of attacks had no effect in all treatments (Table 2). The high percentage of attacks in each treatment was thought to be due to the poor condition of the garden, lack of pruning and poor sanitation. Based on Table 2, the lowest CPB attack percentage value was in P8 and the highest was in P7 as well as P0 as control. Although the data showed no significant difference, the attack percentage value showed quite different values. According to [7] the treatment given has no significant effect even though the letters differ between treatments. This is due to the high diversity of data. In this study, differences in land conditions in each group and cropping patterns in polyculture caused the data to be highly diverse. This was why the data were not significantly different in the ANOVA test but show different values in the average results.

Fruit size 0.1-2.0 cm showed a lower attack percentage value than the size of 8.0-10.0 cm. According to [8] CPB larvae can attack cocoa fruit starting from 3 cm in size until just before mature. CPB larva damages by grinding the fruit, eating the skin of the fruit, and making a channel to the seeds, so that the seeds stick together, black in color, and difficult to separate as well being smaller in size. Furthermore, [9] adds that PBB can undermine almost all fruit yield. CPB can attack fruits from 3 cm in size, but generally prefer those around 8 cm in size. That is what underlies why the fruit size of 8.0-10.0 cm is still larger to be attacked than the size of 0.1-2.0 cm.
3.2. Intensity of CPB attack

Based on the results of the variance test, it showed that giving a different neem solution and fruit size had a significant effect on the intensity of CPB attacks. Table 2 shows that the neem solution is effective in reducing the intensity of CPB attacks. Neem as natural pesticides have repellent effects, inhibit the growth of pests and contact toxins, thereby reducing CPB attack. According to [10] the primary effect of azadirachtin on insects is in the form of an antifeedant by producing specific stimulants in the form of chemical receptors in the mouth that work together with other chemical receptors that interfere with the perception of stimuli to eat. The secondary effects of azadirachtin on insects are in the form of disturbances in the regulation of their development and reproduction, as a result of direct effects on somatic cells and reproductive tissues and indirect effects that interfere with neuroendocrine processes.

Based on Table 2, the results showed that the most effective treatments were in P3 and P8 treatments. According to [11] the azadirachtin content in neem seeds does not directly kill insects, but through the refuse to eat mechanism, interfering with the growth and reproduction of insects. Salanin works as a barrier to eating insects. Nimbin works as an anti-virus, while meliantriol acts as an insect repellent. Because neem does not immediately kill pests, even at high concentrations it does not have a direct effect on CPB attack, so at a concentration of 15% it was quite effective, with spraying was carried out from fruit measuring 0.1-2.0 cm. Whereas at a concentration of 35%, the fruit can be spraying at a size of 8.0-10.0 cm. [12] stated that the higher the concentration of a chemical, the more active ingredients it contains, so that the more effective the killing power of the neem plant leaf extract will be. Therefore, the application of the neem solution was shorter at a concentration of 35%.

3.3. Loss yield

Based on the results, there was no significant effect on yield loss, but the average loss yield values showed different numbers. it was shown that in P3 and P8 were effective to prevent loss yield. This is because the mechanism of action of the neem solution does not directly kill insects, but through the mechanism of refusing to eat, interfering with the growth and reproduction of insects. Because neem does not immediately kill pests, even at high concentrations it does not have a direct effect on CPB attack, so at a concentration of 15% it can reduce yield losses when applied on fruit sizes of 0.1-2.0 cm and at a concentration of 35% can reduce yield losses on fruit sizes 8.0-10.0 cm. Based on research conducted by [13] the application of several concentrations of neem to British aphid pests on wheat plants in the field showed that the application of neem extract to the formulations of neem leaf extract, neem seed extract, and neem seed oil with different solvents showed the same results on the number of English aphid, however, it was markedly different from the controls. Neem will be more effective if it is digested directly by pests. Neem will kill pests if the pests feed on the plants tissue that the neem has given them. This is why neem does not have a direct effect on CPB pests in the field.

4. Conclusion

Giving a combination of concentrations of young leaves and neem seeds with 5% DEA biosurfactant olein palm and different fruit sizes gave a good effect on attack intensity. The best results were at a concentration of 15% solution of young leaves and neem seeds plus 5% DEA biosurfactant olein palm with fruit size 0.1-2.0 cm and at a concentration of 35% solution of young leaves and neem seeds plus 5% DEA biosurfactant olein palm with size fruit 8.0-10.0 cm. Provision of concentrated solution of young leaves and neem seeds given 5% DEA biosurfactant olein palm and different fruit sizes had not a significant effect on the percentage of attack and loss yield. However, it tended to be better in the combination of 15% solution concentration of young leaves and neem seeds plus 5% DEA biosurfactant olein palm with a fruit size of 0.1-2.0 cm and a concentration of 35% solution of young leaves and neem seeds plus 5% DEA biosurfactant olein palm with a fruit size of 8.0-10.0 cm.
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