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Utilization of Cacao Fruit Peels Waste (*Theobroma cacao L.*) as a Means of Invasive Pest Control *Spodoptera frugiperda*

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Abstract. *Spodoptera frugiperda* is a new type of pest that was first discovered in several countries in the Americas and has spread to several other countries in the African region, detriment agricultural production which is caused by pests *S. frugiperda* in the year 2018 ranged between 4 million tons - 18 million tons every year. This loss is equal to IDR 64,561,000,000 per year. In addition to plant pests, agricultural waste is also a major problem in the agricultural sector. One of the agricultural wastes is cacao peels. Cacao peels waste can reach 74% or equivalent to 2,4 million tons/ year. Cacao peels contain secondary metabolites derived from alkaloid purine, namely xanthine and theobromine (methylxanthine), flavonoids, pectin, tannins, saponins, and triterpenoids which serves as a repellent, an inhibitor of eating (antifeeding) and is poisonous for insects. The purpose of this study was to determine the level of effectiveness of using cacao peels extract in controlling *S. frugiperda* pests. This research method used Completely Randomized Design (CRD) with control treatment, cacao peels extract 1%, 0.75%, 0.5%, 0.25%, and 0.15%. The results showed that the cacao peels extract had a significant effect on the mortality of *S. frugiperda* larvae. The concentration most effectively shutting *S. frugiperda* is 1%, with an average percentage of the highest mortality is 87.8%. The results of the probit analysis to determine the LC50 value also showed an effective concentration level to kill insects as much as 50% is a concentration of 1%.

INTRODUCTION

The attack of Plant Pest Organisms is a major problem for farmers. One of the losses experienced by farmers due to pest attacks is decreased production. Especially in corn, armyworm is one of the main pests that cause the biggest production loss. One species of armyworm that is classified as a new pest is *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae).

S. frugiperda is a new type of pest that was first discovered in several countries in the Americas and has spread to several other countries in the African region. *S. frugiperda* is polyphagous. Some of its main hosts are food crops from the Graminae group, such as corn, rice, wheat, sorghum, chili, and sugar cane, so the existence and development of *S. frugiperda* population needs to be controlled. It is becoming the spotlight of FAO (Food and Agriculture Organization) because of the significant losses caused by *S. frugiperda* [1].

According to The Center for Agriculture and Bioscience International (CABI), in the year 2018, which state that the loss of results of production agriculture as a result of the attack *S. frugiperda* on crops of corn in 12 countries in the region Africa which cause loss of production ranging between 4 million tons to 18 million tons per year. Disadvantage is valued at IDR 64,561,000,000 per year.

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One of the attempts were made by farmers in controlling pests on plants is to carry out the provision of output in the form of pesticides that are made of materials chemistry. However, the provision of pesticides of materials chemistry can cause pollution to the environment. To reduce the use of chemical pesticides, it is necessary to use natural ingredients from plants as an effort to control pest attacks on plants. Natural ingredients are used for the prevention of pests because they can decompose easily in nature and do not contain residues of chemicals. Therefore, the utilization of natural ingredients is environmentally friendly and essential to reducing environmental pollution due to chemical pesticides.

One of the plants that have the potential as an ingredient naturally to control pests *S. frugiperda* is peels of cacao (*Theobroma cacao L*). Cacao peels contain secondary metabolites derived from purine alkaloids, namely xanthine and theobromine (methylxanthine), flavonoids, pectin, tannins, saponins, and triterpenoids obtained from phytochemical screening using the cacao peels extract test method using ethanol. These compounds are secondary metabolites that function as repellents, an inhibitor of eating (antifeeding) and is poisonous for insects. Currently, there is still a lot of cacao peels waste that is underutilized, only left to rot and dumped. The utilization of waste peels fruit cacao should be improved to minimize the buildup of waste agriculture [2].

Peels of cacao contain secondary metabolites that are very useful for the agricultural sector, mainly when used as the material basis of a pesticide plant. Among the compounds of metabolites, secondary skin fruit cacao are alkaloids, flavonoids, tannins, saponins, and triterpenoids. The compounds of these are toxic to insects that have the potential to be used as a controlling pest of plants, especially *S. frugiperda*.

The research is aimed to know the influence of skin extract cacao on mortality *S. frugiperda* and concentration levels are effective in controlling *S. frugiperda*. The results of this study are expected to be an environmentally friendly alternative to pest control by utilizing waste.

MATERIAL AND METHODS

This research was carried out for four months, from May 2021 to August 2021, at the Pesticide and Natural Materials Laboratory and the Entomology Laboratory of the Faculty of Agriculture, Hasanuddin University. *Spodoptera frugiperda* were collected from a corn field in Sanrobone and Galesong, Takalar, South Sulawesi and cacao peels were collected from a cacao plantation in Lilirilau, Soppeng, South Sulawesi.

The tools that are used in this research is Vortex mixer, sprayer, wrap, shaker, water bath, rotary evaporator, blender, oven, hot plate, pole stative, glass chemistry, filter paper, tube flask, rack tubes, tube reaction, measuring cups, strainers, containers, cotton wool, scissors, rice paper, Petri dishes, pans, analytical scales, knives, glass jars, polybags, insulation, funnels, and aluminum foil. Materials that are used in this research is cacao peels, insects test *S. frugiperda*, solvent methanol, Mayer's reagent, Wagner's reagent, Hager's reagent, n-hexane, ethyl acetate, H_2SO_4 concentrated, powdered Mg-HCl, concentrated NaOH, ammoniacal chloroform, gelatin, anhydrous acetic acid, corn seed, distilled water, 70% alcohol, compost, and honey.

This research was conducted experimentally using a completely randomized design (CRD) which consisted of 6 treatments. Each treatment had three replications, with a total of 36 experimental combinations. Among the treatment given is T0: Control (distilled water), T1: 0.15% cacao peels extract, T2: 0.25% cacao peels extract, T3: 0.5% cacao peels extract, T4: 0.75% cacao peels extract, and T5: 1% cacao peels extract. Concentration is obtained from the dilution formula:

$$V_1 M_1 = V_2 M_2 \quad (1)$$

Description:
V_1 = Volume of solution before dissolving
V_2 = Volume of solution after dissolving
M_1 = Molarity of solution before dissolving
M_2 = Molarity of the solution after dissolving

Extraction of Cacao Peels

Extraction of cacao peels was carried out by taking samples of healthy cacao peels and washing them thoroughly. Subsequently, cut thin skin of fruit cacao with a size of ± 3 cm x 3 cm and air-dried during ± 10 days. The skin of fruit cacao was crushed with a blender and sieved using a sieve. It is aimed to obtain powdered peels of fruit cacao that are

smooth and ready to be extracted. After that, weighing the powder peels fruit cacao as much as 25 grams. Then the skin cacao powder was mixed with methanol, and the ratio of powdered peels of fruit cacao and the solvent methanol is 1:10. Next, filter the extract solution and then soak the cacao peels powder again with methanol as solvent. Immersion was carried out for three days with solvent replacement every 24 hours. The filtrate that has been obtained is evaporated using a rotary evaporator to get the pure cacao-peel extract.

Rearing *Spodoptera frugiperda*

Larvae that have been taken in the field were bred in a container and were given feed baby corn. *S. frugiperda* rearing was carried out until it reached the 3rd instar *S. frugiperda* larvae in F1 offspring. Maintenance is carried out for 1 month. Maintenance until F1 offspring is carried out so that the test insects are free from pesticide contact that may be present in the field.

Preliminary Test

Preliminary tests were conducted to determine the range of concentrations of cacao peels extract that would affect the mortality of *S. frugiperda*. Preliminary tests with 5% and 10% concentrations showed a 100% mortality rate of the test insects within 1 minute. This indicates that the concentration used is very high, so a decrease in concentration is carried out.

Plant Extract Test

The contact test was carried out by dripping plant extract on the body part of the test insect as much as 1 mL of the extract/test insect. The test insects used were instar 3 with 10 tails/replication, with a total of 3 replications/treatment. The test insects that had been applied with plant extracts were put into Petri dishes and given feed ingredients.

Phytochemical Screening Test

The phytochemical test of cacao peels extracts aims to determine and prove the secondary metabolite contents in the cacao peels. Before doing the test screening phytochemical, extracts of peels fruit cacao that has been obtained soaked back with methanol: water (2:1) and partitioned successively with n-hexane and ethyl acetate, thus obtaining each partition of the fraction that. The result of the partition of the fractions was evaporated at a temperature of 30–40^{o}C until the obtained extracts from n-hexane, ethyl acetate, and extract water. After that, continued testing with phytochemical tests.

Saponin Test

Extract condensed methanol, which was obtained at the stage of extraction, is weighed as much as 0.1 grams dissolved with water heat as much as 15 ml, then heated for 5 minutes. Subsequently filtered and the filtrate was taken as much as 10 mL and inserted into the tube reaction. The solution was then shaken. Test positive presence of saponins in solution is characterized by the formation of foam/froth.

Flavonoid Test

Extract condensed methanol as much as 0.1 g dissolved in 10 mL of methanol and then divided into the four-tube reaction. Tubes first used as a tube of control, tube second, third, and fourth consecutive added NaOH, H_2SO_4 concentrated, and powders Mg-HCl concentrated. Color in each tube compared with a tube of control if the case changes the color of the positive containing flavonoids.

Alkaloid Test

A total of 0.1 g of methanol extract was dissolved with 10 mL of ammoniacal chloroform, and the results were divided into two tubes. Tubes were first added to the acid sulfate 2 N. Layer acids are separated, divided into two tube reactions, and each tube is done testing by using a reagent Mayer and Wagner. Tubes both conducted tests with reagent Hager. If a precipitate is formed, the sample is positive for alkaloids.

Tannin Test

The powder sample was heated in 100 mL of water for 30 minutes. The extract was filtered, and 5 ml of the solution was added to a solution of gelatin 2% as much as 2 ml. The precipitate white was formed, indicating the presence of tannins.

Triterpenoid Test and Steroid Test

The extract was added 2 ml of acetic acid anhydride, then add 2 ml of acid sulfate of the tube reaction. Layers of chocolate are formed at the meeting of two layers, a layer of top color green which means steroids, and the formation of the color red old, which means triterpenoids.

TABLE 1. Phytochemical Test of Cacao Fruit Peels Extract.

Test	Positive/Negative	Information
Saponin Test	Positive	Foamy
Flavonoid Test	Positive	There is a color change
Alkaloid Test	Positive	A precipitate is formed
Tannin Test	Positive	A precipitate is formed
Triterpenoid Test	Positive	There is a color change
Steroid test	Negative	Not color change

Based on the phytochemical test of cacao peels extract that has been carried out (Table 1.), it shows that the cacao peels extract contains secondary metabolites such as saponins, flavonoids, alkaloids, tannins, and triterpenoids. These compounds are toxic to insects so they can be used as organic insecticides [3].

Observation

Observations were made to determine the number of *S. frugiperda* that died as a result of the treatment. Observations were made every 12 hours until all test insects died. The mortality percentage of *S. frugiperda* larvae can be calculated using the following formula:

$$\frac{Number\ of\ dead\ insects}{Number\ of\ the\ dead + live\ insects}\ x\ 100\% \quad (2)$$

Data Analysis

The data obtained will be analyzed using analysis of variance or ANOVA. If there is a significant difference between treatments, then further tests are carried out using Duncan's test with an error rate of 5%. At the same time, the calculation of Lethal Dose (LD 50) and Lethal Time (LT 50) was carried out by probit analysis using the SPSS application.

RESULT AND DISCUSSION

Mortality of *S. frugiperda*

The results showed that cacao peel extract affected the mortality of *S. frugiperda* larvae. Statistical analysis was performed to determine the effect of treatment on mortality of *S. frugiperda* every 12 hours. The results of observations of mortality of *S. frugiperda* larvae in the treatment of cacao rind extract as a vegetable insecticide can be seen in Table 2.

Observations of mortality of *S. frugiperda* in the first 2 hours after application showed that all treatments of cacao peels extract were significantly different from the control treatment. The highest mortality percentage was found in the 1% treatment of cacao peels extract, which was 66.7%. The 1% treatment of cacao peels extract had a significant effect on the control treatment, and the concentration of the cacao peels extract was 0.15%, 0.25%, 0.5%, and 0.75%.

TABLE 2. Insect Mortality Percentage during Observation.

Treatment	12 HAA	24 HAA	36 HAA	48 HAA	60 HAA	72 HAA
T0	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0
T1	36.7^{bc}	73.3^{b}	83.3^{c}	100^{b}	100^{b}	100
T2	40^{bcd}	73.3^{b}	80^{c}	93.3^{b}	96.7^{b}	100
T3	56.7^{cd}	83.3^{b}	86.7^{c}	93.3^{b}	96.7^{b}	100
T4	26.7^{b}	46.7^{b}	66.7^{b}	93.3^{b}	96.7^{b}	100
T5	66.7^{d}	86.7^{b}	90^{c}	90^{b}	93^{b}	100

Numbers followed by different letters $^{a-d}$ in the same column show a significant difference in Duncan's 5% distance test. HAA : Hours After Application; T0: Control (aquades), T1 (0.15% cacao peels extract), T2 (0.25% cacao peels extract), T3 (0.5% cacao peels extract), T4 (0.75% cacao peels extract), T5 (1% cacao peels extract).

Observation of *S. frugiperda* mortality 24 hours after application showed an increase in mortality in all treatments. The mortality did not show a significant difference between all treatments, but in the application of mortality percentage, the highest was found at a concentration of 1% at 86.7% and lowest in the concentration of 0.75%, amounting to 46.7%. After 36 hours of application, there was a 50% increase in the mortality rate in all treatments. The 1% concentration had the highest mortality rate compared to other concentrations. The size of the insecticide concentration given greatly affects the mortality rate of pests [4].

At 48 hours and 60 hours after application, there was an increase in mortality of *S. frugiperda* at each concentration of cacao peels extract. The results of the analysis did not show significant differences in all treatments of cacao peels extract. Until the end of the observation on 72 hours after application, all insect mortality in all treatments reached 100%, and the control treatment did not affect *S. frugiperda* mortality. Based on the obseravion, it is known that the highest mortality during observation up to 72 hours after application is a concentration of 1%, with an average mortality percentage of 87.8%. This indicates that the cacao peels extract effective in controlling *S. frugiperda*. Botanical insecticides can be effective if they can kill at least 80% of the tested insects [5].

The ability of cacao peel extracts to kill *S. frugiperda* is based on the secondary metabolite content of cacao peels. Cacao peels contain secondary metabolites derived from purine alkaloids, namely xanthine and theobromine (methylxanthine), flavonoids, tannins, saponins, and triterpenoid. Secondary metabolites serve as a repellent, antifeeding toxin for insects. So, the use of secondary metabolites for pest control is an effective way. The combination of secondary metabolites in cacao peels causes a synergistic effect so that the effect to kill insects is higher [2,6].

Probit Analysis of Cacao Peels Extract on Mortality of *S. frugiperda*

Based on probit analysis to determine the effective concentration capable of killing 50% of the tested insects, the LC50 value of cacao peels extract in suppressing the population of *S. frugiperda* was 1.02%. The LC50 value is the concentration of insecticide required to kill 50% of the tested insects under specified experimental conditions [7]. Based on this LC50 value, it can be seen that the effective concentration to kill 50% of the tested insects during observation is the 1% concentration of cacao peels extract. This is also evidenced by the results of research that has been carried out that the mortality rate at a concentration of 1% cacao peels extract showed the highest mortality since the first 12 hours of observation compared to other concentrations (Table 3.).

TABLE 3. LC50 Results of Cacao Peels Extract with Probit Analysis.

LC50	Average (%)
0.5	1.02

Information: The most effective concentration to kill insects as much as 50% is 1.02%.

The Mortality Rate of *S. frugiperda*

The mortality rate of *S. frugiperda* during observations showed the fastest and highest mortality rate was found at a concentration of 1% as many as 20 test insects within 12 hours. This is equivalent to insect mortality of 66.7% within 12 hours. Compared to other concentrations, the 1% concentration treatment was the treatment with the highest mortality rate in the first 12 hours of observation (Fig. 1).

However, it can be seen that cacao peels extract with a concentration of 0.75% had the lowest mortality rate compared to other concentrations. This could be because the developmental phase of the test insects used at this concentration was relatively fast. Therefore it affected the response of the test insects when given treatment. Although the larval instar used was instar 3 in each treatment, some larvae developed faster than other larvae.

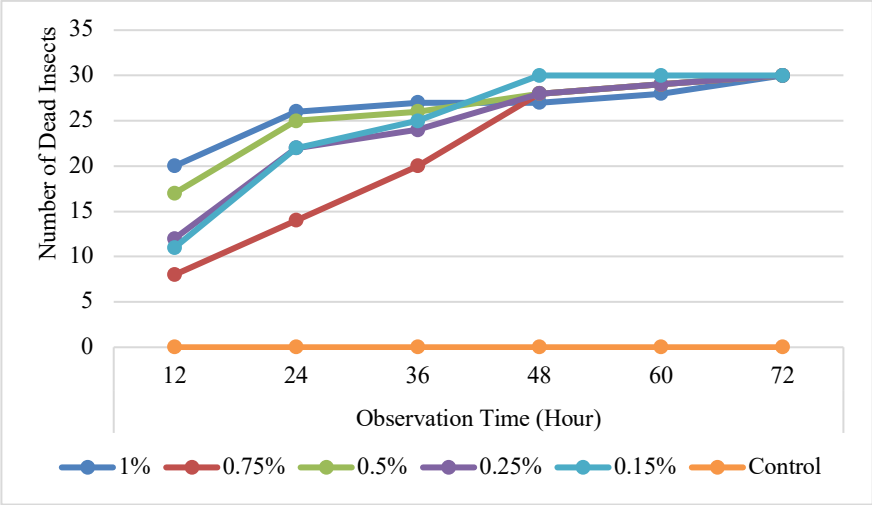

FIGURE 1. Graph of Insect Mortality Rate during Testing.

The existence of larvae whose development phase was faster was due to the imago eggs that hatch gradually so that the F1 offspring of *S. frugiperda* larvae obtained had different growth and development rates within 1-3 days. Larvae that have a faster development have a higher survival ability than other larvae, so the mortality rate is low when treated with insecticides. Various factors can affect the success of insecticides in causing the death of the test insects, including the developmental phase and age of the insect, the type of insecticide, the type of insect, and the method of application of the insecticide [8].

Mode of Action Cacao Fruit Peels Extract

The method of application to test insects is to use a drip system, in which vegetable insecticides are dripped onto all parts of the insect's body. When cacao peels extract was dropped onto *S. frugiperda* larvae, there was a hyperactive behavior change in *S. frugiperda*. This is because *S. frugiperda* responds to the poison given to its body. However, after that, the eating activity of *S. frugiperda* decreased, body movements began to slow down, and finally died. This is because secondary metabolites from cacao peels extract have interfered with the body's metabolism of *S. frugiperda.*

Flavonoid and alkaloid compounds attack the nerves in several vital organs, causing nerve weakness for insects [9]. Alkaloids and flavonoids are compounds that act as stomach poisons so that if these compounds enter the insect's body, they will inhibit the insect's digestive process [10]. Flavonoid compounds and alkaloids can also inhibit taste receptors in the mouth of the larvae so that the larvae cannot recognize their food, and the eating activity of insects is inhibited. The saponin compounds play a role in inhibiting the work of proteolytic enzymes, which cause a decrease in the activity of digestive enzymes and the use of protein. Tannin compounds can reduce the ability to digest food

digestion in insects and reduce digestive activity. Likewise, triterpenoid compounds are toxic and cause death in insects [11].

CONCLUSIONS

Based on the tests that have been carried out, it can be concluded:

1. Cacao peels extract had a significant effect on the mortality of *Spodoptera frugiperda*.
2. Cacao peels extract with a concentration of 1% is the most effective concentration, based on the percentage of the highest mortality is 87.8% and the analysis of probit which shows the value of LC50 at a concentration of 1%.

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