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The Effectiveness of Botanical Insecticides of Four Plant Types and Adjuvants on Nutrition Index of The Fifth Instar Larvae of *Heliothis Armigera* Hubner

Nursal* and Syafruddin Ilyas

Department of Biology, Faculty of Mathematics and Natural Science,
Universitas Sumatera Utara, Jl. Bioteknologi No. 1 Kampus USU Medan 20155

*Email: nursal@usu.ac.id

Abstract. The effectiveness of the botanical insecticide formulations derived from a mixture of the ethanol extracts of the four plants; sweet orange peel (*Citrus sinensis*), Mexican sunflower leaf (*Tithonia diversifolia*), ginger rhizome (*Zingiber officinale*), lemongrass (*Cymbopogon citratus*) with and without miracle adjuvant has been examined on the nutritional index of the fifth instar larvae of *H. Armigera*. Nutritional index parameters were observed using a complete randomized design (CRD) with six treatment concentrations (0.00%, 0.25%, 0.50%, 1.00%, 2.00%, 4.00%) and 15 larvae for the iterations. The observation data were analyzed by variance if there was any difference followed by Duncan's test at the level of 5%. The results showed that all treatment concentrations, both with and without miracle adjuvant, could reduce the larval nutrition index and differ from the control treatments, namely; relative consumption rate (RCR) at 19,506% - 28,457% without adjuvant and at 16,566% - 36,740% with adjuvant; relative growth rate (RGR) at 10,545% - 45,545% without adjuvant and at 22,545% - 62,545% with adjuvant; efficiency of conversion of ingested food (ECI) with and without adjuvant at 14,041% - 24,951% and 16,651% - 57,302% respectively, while the efficiency of conversion of digested food (ECD) is at 14,629% - 30,106% without adjuvant and at 16,651% - 57,302% with adjuvant. On the contrary, the approximate digestibility (AD) increased by 18,195% - 30,656% without adjuvant and 22,223% - 62,579% with adjuvant. The effect caused by botanical insecticide formulation with miracle adjuvant is higher compared to the one without any adjuvant. Effective concentration with adjuvant occurs at 2%, and 4% without adjuvant. The results of phytochemical screening showed that the ethanol extract of sweet orange peel contained 5 secondary metabolites (alkaloid, terpenoid, steroid, tannin, saponin), 2 (Flavonoid and Tannin) in Mexican sunflower leaf, 5 (Flavonoid, alkaloid, terpenoid, steroid, and tannins) in ginger rhizomes, and 3 (terpenoids, steroids, saponins) in lemongrass stems.

1. Introduction

The use of synthetic insecticides to control pests tends to increase and often with irregular usage. This can lead to undesirable effects, such as resurgence, resistance, the killing of natural enemies, environmental pollution, and insecticide residues on crops. The residue found in agricultural commodities can be very detrimental to human health because it can cause cancer, kidney damage, genetic mutations, many more [1]. Vegetables with residual content above the threshold will have decreasing selling value and often do not pass the quality control to be imported which lead to economic loss [2].



The government through the agricultural service intensively conducts socialization to improve farmers' understanding on the impact of the use of synthetic insecticides with the hope that farmers would switch to organic farming systems, since the pest control in this system should use the botanical insecticides. The use of botanical insecticide as alternative insecticide is feasible due to its characteristics; can be combined with other components of pest control (compatibility), relatively safe against natural enemies of pests (selectivity), can slow down the rate of resistance, biodegradable and ensure resilience and sustainability in farming (sustainability) [3].

Pest control in the horticultural organic farming system in Tanah Karo, the farmers use botanical insecticides which come from a mixture of several types of plants, including: sweet orange peel (*C. sinensis*), Mexican sunflower (*Thitonia diversifolia*), ginger rhizome (*Zingiber officinale*) and lemongrass (*Cymbopogon citratus*), and according to [4] this plants have the potential to become botanical insecticides. The application of this insecticide must be evaluated by observing the content of secondary metabolites, the concentration of the mixture of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass (*C. citratus*) and by adding the miracle adjuvant to the nutritional index with the parameters of Relative Growth Rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), efficiency of the conversion of digested nutrient (ECD), and approximate digestibility (AD) of the fifth instar larvae of *H. armigera*.

H. armigera has been widely used as an experimental animal to see the effectiveness of a botanical insecticide [5][6], because it is a pest in various horticultural plants and can cause significant damage. Furthermore, the resistance of *H. armigera* is high against insecticides, and it is reported many to be resistant to pesticides [7]. The concentration of botanical insecticides that have been able to cause the death of *H. armigera* will certainly be more effective and efficient against other horticultural pests.

Based on the analysis, authors conducted research on how the effectiveness of botanical insecticide formulations from four types of plants and adjuvants on the nutritional index of the fifth instar of *H. armigera* Hubner with the aim to analyze the content of secondary metabolites of ethanol extract of the plants and their effectiveness in nutritional index of *H. armigera* Hubner.

2. Methodology

2.1. Maintenance of experimental animals

Mosquitoes larvae from a cornfield were kept in a laboratory with natural foods. The adult mosquitoes were put into the breeding cage. A solution of honey and sugar were inserted into the cage as food, and at the top and three sides of the cage were placed a piece of fabric for the subject to lay eggs. After the eggs hatched, the larvae samples were fed with artificial food in plastic cups to obtain the relatively similar size until they become fifth instar larvae which were ready to be used as test animals [8].

2.2. Preparation of artificial food [8].

Heat the water to boil for the ingredients to be dissolved in 800 ml of distilled water. While boiling, mix 50g of cornmeal ingredients, 50g of soybean juice and 30g of germ wheat, then blend with distilled water. Mix 20g of rice flour, 50g of cornstarch, 50g of sugar and 100 ccs of distilled water. After turning into a paste, add 12g of vitamin, 2g of sorbic acid, 6g of ascorbic acid and 2.5g of nipagin, then stir well. Subsequently, enter ten ccs of corn oil. When the temperature reaches 70°C, combine all the ingredients, then add 15g of yeast and 10 ml of formalin. Furthermore, the food will be put in plastic cups where larvae are kept.

2.3. Procurement of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*) [9]

Powder of sweet orange peel, Mexican sunflower leaves, ginger rhizome, and lemongrass stem was macerated with ethanol with a period of 3x24 hours, repeated until the macerate turns to a transparent

color. A rotary evaporator evaporated the macerate at a temperature of 40°C to produce concentrated ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizome, and lemongrass stem.

2.4. Observation of testing parameter

2.4.1. Test on the chemical content of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaves (*T. diversifolia*), ginger rhizomes (*Z. officinale*), and lemongrass stems (*C. citratus*)[9]

A test was conducted qualitatively on the compounds of saponin, tannin, alkaloid, terpenoid, and flavonoid to determine the chemical contents of concentrated ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stem.

2.4.2. Saponin

Ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stems were diluted ten times with hot water, then filtered, shaken for five minutes, and allowed to stand to see any presence of foam formation. If the foam is formed, the solution contains saponin. Subsequently, a few drops of concentrated sulfuric acid were added into the molded foam. Qualitatively, saponin compound is considered exist if the foam is stable after dripped with concentrated sulfuric acid.

2.4.3. Tannin

Ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stems were diluted ten times with hot water, then filtered. Gelatin was added into the filtrate. Qualitatively, tannin compounds can be identified when the white precipitate is formed after the addition of gelatin.

2.4.4. Alkaloid

Two drops of ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stems were inserted, each, into two plates. The first plate, with an addition of Dragendorff reagent, formed a deposition of orange to red-brown, while the second plate, with Mayer reagents, formed a white precipitate.

2.4.5. Terpenoid

Two drops of ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stem were put on a plate and added 2-3 drops of anhydride acetic acid (AC₂O), while on the other plate added 1-2 drops of concentrated H₂SO₄ as a comparison. A sign of green or bluish green color indicates the existence of terpenoid.

2.4.6. Flavonoid

Two drops of ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stem were inserted into a plate with two extra drops of HCl and Magnesium Powder. Orange color indicates the presence of flavonoids.

2.5. The test on the ethanol extract of sweet orange peel (*C. sinensis*), Mexican sunflower leaves (*T. diversifolia*), ginger rhizomes (*Z. officinale*), and lemongrass stems (*C. citratus*) with or without miracle adjuvant on the nutritional index of the fifth instar larvae of *H. armigera*

Artificial food were prepared in six different treatment concentrations (0%, 0,25%, 0,50%, 1,00%, 2,00%, 4,00%) with testing parameter of nutritional index [10].

- Relative Growth Rate (RGR)

$$RGR = \frac{G}{T.A}$$

- Relative Consumption Rate (RCR)

$$RCR = \frac{F}{T.A}$$

- The efficiency of Conversion of Digested Food (ECD)

$$ECD = \frac{G}{F - E} \times 100\%$$

- The efficiency of Conversion of Ingested Food (ECI) = G/F x 100%.

$$ECI = \frac{G}{F} \times 100\%$$

- Approximate Digestibility (AD) = F-E/F x 100%.

$$AD = \frac{F - E}{F} \times 100\%$$

Where G is larvae weight gain during meal period (initial weight of larvae - final weight of larvae), F represents the amount of the food depleted, E is dry weight of feces, T equals the eating period and A is the average weight of grub during the feeding period (initial weight of larvae + finale weight of larvae/2)]

2.6. Experimental design

This study implemented a complete randomized design (CRD) with six concentration treatments (0%, 0.25%, 0, 50%, 1.00%, 2.00%, 4.00%) with a ratio of 1: 1 on ethanol extracts of sweet orange peel, Mexican sunflower leaves, ginger rhizomes, and lemongrass stem with and without miracle adjuvant and 15 larvae for iterations.

2.7. Data analysis

Data acquired from the observed parameters were analyzed by variance, if there were significant differences, the process would be followed by Duncan test at a 5% confidence level.

3. Results and Discussions

Based on research on the effectiveness of botanical insecticides from a mixture of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*) with and without miracle adjuvant on nutritional index of the fifth instar larvae of *H. Armigera* and phytochemical screening, obtained results as shown in the following Pictures and Tables:

From the results as shown in Figure 1(a) and Figure 1(b), it shows that the entire treatment concentration of ethanol extracts of sweet orange peel, Mexican sunflower leaf, ginger rhizome, and lemongrass stem with and without miracle adjuvant can reduce the relative consumption rate (RCR) and the relative growth rate (RGR).

3.1. The effect of ethanol extracts mixture of sweet orange peel (C. sinensis), Mexican sunflower leaves (T. diversifolia), ginger rhizomes (Z. officinale), and lemongrass stems (C. citratus) with and without miracle adjuvant on the relative consumption rate (RCR) and the relative growth rate (RGR) of the fifth instar larvae of H. armigera.

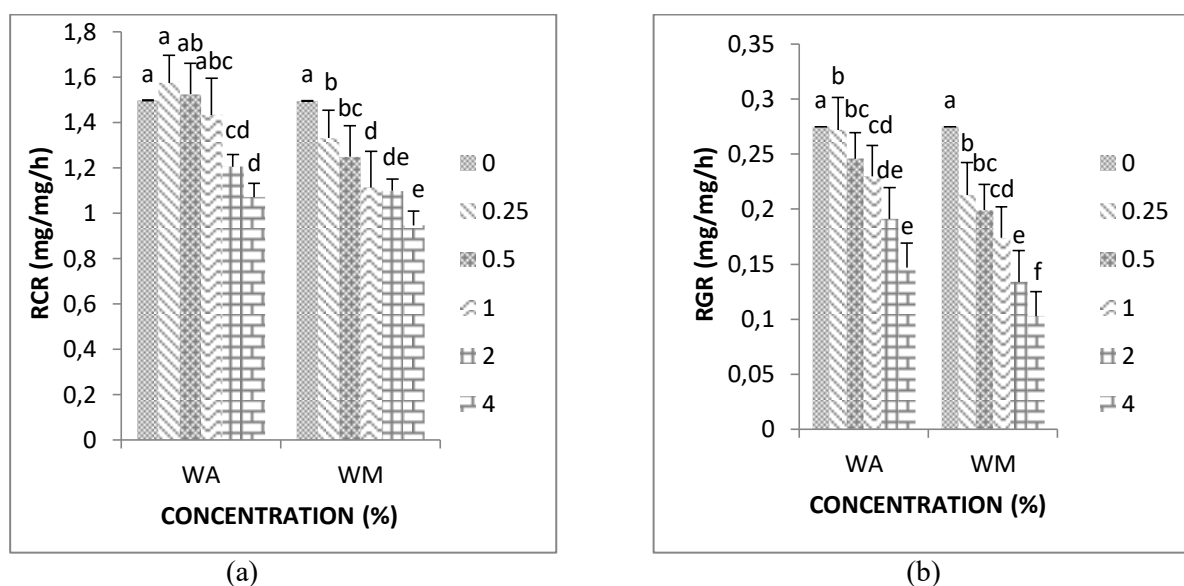


Figure 1. Graphs of "error bar" of RCR and RGR; (a) Relative consumption rate (RCR); (b) Relative growth rate (RGR) of the fifth instar of *H. armigera* larvae on foods treated with a mixture of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*) with and without miracle adjuvant. Details: WA = Without adjuvant, WM = With adjuvant Miracle. Graphs with the same letter notation in the same group and different groups were not significantly different in the Duncan test at the confidence level of 5%.

From Figure 1(a), it can be seen that in the treatment (0.25-1%) without adjuvant, when compared to the control, did not affect the RCR of larvae. This means that the concentration is not toxic, so it does not affect larval feeding activities. As explained in [11], if the insecticide concentration of a plant has not been toxic to insects, then insect feeding activities will not be distraught. In the treatment without adjuvant, RCR of the larvae decreased and differed from the controls at a concentration of 2% - 4%, amounting to 19.506% - 28.457%. In the treatment with additional miracle adjuvant, RCR value decreased to 7.749% - 36.740% at a concentration of 0.25% - 4% and it increased as the treatment concentration upsurged. This phenomenon can be caused by the secondary metabolites contained in the mixture of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*) which are toxic to larvae. The same thing happened to *Azadirachata indica* plants, *Curcuma longa*, *Acorus calamus* [12], *Vicia faba* [13], *Couroupita guianensis* [14], mahogany, neem, tobacco [15], *Nigella sativa*, *Aristolochia*, and *Jatropha curcas* [6] which are lethal to *H. armigera*. The combination of sweet orange peel and neem leaves is toxic to *Spodoptera litura* [16]. The effect caused by the formulation with an adjuvant to the nutritional index of *H. armigera* larvae was better than the one without adjuvant. The comparison between the toxicity level of the treatment with adjuvant compared and with no adjuvant was also obtained in the study [2]. Based on Figure 1(a) and Figure 1(b), it shows that the smaller the RCR value, the lesser the value of RGR. The decrease is even more significant in line with the increasing concentration of treatment. This is a consequence of the lesser amount of ingested food [8].

3.2. The impact of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*), with and without miracle adjuvant, on efficiency of conversion of digested food (ECD), efficiency of conversion of ingested food (ECI), and approximate digestibility (AD) of the fifth instar larvae of *H. armigera*

Based on the conducted test as shown in Figure 2(a), Figure 2(b), and Figure 2(c), all treatment concentrations of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*), with and without adjuvant, has impact on the nutritional index of the larvae, namely: efficiency of conversion of digested food (ECD), efficiency of conversion of ingested food (ECI), and approximate digestibility (AD).

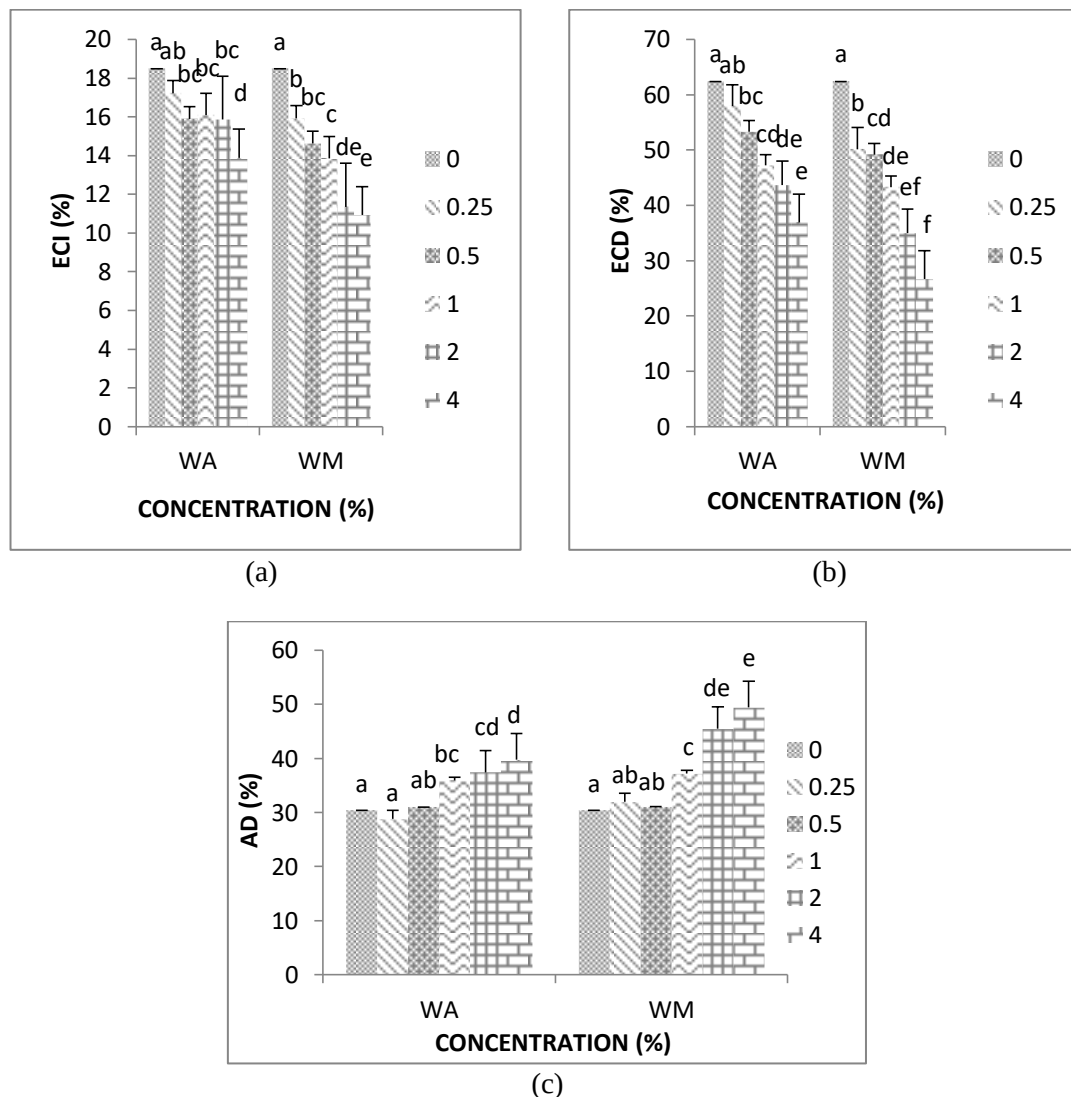


Figure 2. Graphs of "error bar" of RCR and RGR of ; (a) Efficiency of conversion of ingested food (ECI); (b) Efficiency of conversion of digested food (ECD); (c) Approximate digestibility of the fifth instar of *H. Armigera* larvae on the foods treated with a mixture of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*) with and without miracle adjuvant. Details: WA = Without adjuvant, WM = With adjuvant Miracle. Graphs with the same letter notation in the same group and different groups were not significantly different in the Duncan test at a confidence level of 5%

In accordance with Figure 2 (a), (b), (c), ECI decreased and differed from the control (0%) at a concentration of 0.5%-4% with values of 14,041% - 24,951% without adjuvant and at a concentration of 0.25%-4% with values of 13,933% - 40,960% with adjuvant. While for ECD, the values decreased

at a concentration of 0.25% - 4% and 0.5% - 4%, amounting to 16.651% - 57.302% and 14.629% - 30.106%, with and without adjuvant respectively. In the contrary, AD values increased at a concentration of 1% - 4% to 18.195-30.6656% without adjuvant and to 22,223% - 62,579% with adjuvant. The decrease in the value of ECD and ECI, also the increment in the value of AD become greater with the increasing treatment concentration. The effectiveness caused by adjuvant is higher than without adjuvant. Thus it can be said that the given treatment concentration is fairly effective to affect the efficiency of food consumption of the larvae. As an indication, it can be seen from the decreasing RGR value of the larvae. The downturn in larval RGR will be greater in line with the increasing decrease in ECD and ECI of the larvae, while the increase in the AD is a compensation response so the larvae can survive. So if there are toxic compounds in the food, insects will make a compensation response, including an increase in AD values [8].

Furthermore, the combination of *Annona scuaamosa* seeds, *Piper retrofractum* fruit, *Tephrosia vogelii* leaves is envenomed and inhibits the appetite of the vegetable pest of *Crosidolomia pavonang* [2]. The quality of mulberry leaves affects the nutritional index of silkworms [17]. *Azadirachta indica* seed extract and leaves of *Vitex negundo* L. (Lamiales: Verbenaceae) have a significant impact on the medinal *Cnaphalocrocis*' nutrition index [18].

Table 1. Phytochemical Test of Ethanol Extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaf (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*)

Phytochemical Test	sweet orange peel (<i>C. sinensis</i>)	mexican sunflower leaf (<i>T. diversifolia</i>)	ginger rhizome (<i>Z. officinale</i>)	Lemongrass stem (<i>C. citratus</i>)
Flavonoid	-	+	+	-
Alkaloid	+	-	+	-
Terpenoid	+	-	+	+
Steroid	+	-	+	+
Tannin	+	+	+	-
Saponin	+	-	-	+

Note: + means containing; - means not exist

From Table 1, it is shown that there are five contents (alkaloid, terpenoid, steroid, tannin, and saponin) of secondary metabolites in ethanol extract of sweet orange peel, two contents (flavonoid and tannin) in Mexican sunflower leaf, five types in ginger rhizome (flavonoid, alkaloid, terpenoid, steroid, tannin), and three in lemongrass (terpenoid, steroid, and saponin). The secondary metabolite content found in these plants can be toxic and applied as self-defence so that it can be used as a botanical insecticide. As stated that these plants will produce secondary metabolites that differ from one plant to another. Secondary metabolites such as the flavonoid, terpenoid, alkaloid, and the others will have different characteristics such as toxic, inhibiting the food and the growth of an insect [3].

4. Conclusions

Based on the conducted test, it can be concluded as follows:

- The formulation of a mixture of ethanol extracts of sweet orange peel (*C. sinensis*), Mexican sunflower leaves (*T. diversifolia*), ginger rhizome (*Z. officinale*), and lemongrass stem (*C. citratus*), with and without adjuvant can reduce the nutritional index of the fifth instar larvae of *H. armigera*, namely; the decrease of the relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI), the increase in approximate digestibility (AD).
- The impacts caused by formulation with adjuvant are better than the one without adjuvant.
- The effective concentration of the formulation with adjuvant is at 2.0% and a concentration of 4% without adjuvant.

- The contents of secondary metabolites contained in the ethanol extracts are amounted to five contents (alkaloids, terpenoid, steroid, tannin, and saponin) in sweet orange peel, two contents (flavonoid and tannin) in Mexican sunflower leaf, five in ginger rhizome (flavonoid, alkaloid, terpenoid, steroid, tannin), and three in lemongrass (terpenoid, steroid, and saponin).

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