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Bioactivity a mixture of spiked pepper fruit extract and fish-poison bean leaf extract against *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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Abstract. The active compounds on spiked pepper (*Piper aduncum*) fruit extract and fish-poison bean (*Tephrosia vogelii*) leaf extract have a potency used as botanical insecticides as alternative pest control. This study was conducted to determine the activity of mixed extracts of Spiked pepper fruit and Fish-poison bean leaf extract against the new invasive pest *Spodoptera frugiperda* larvae. This study was held from February 2021 to November 2021 at the Insect Bioecology Laboratory, Faculty of Agriculture, Andalas University, Padang. The experiment consists of a preliminary test and an advanced test. The advanced test was conducted using six concentrations and five replications of a mixture extract of Spiked pepper and Fish-poison bean. The parameters observed were larvae mortality, feeding inhibition activity (antifeedant), and larval development time. The result showed that the combination indexes of the mixed extract of Spiked pepper fruit and Fish-poison bean leaves (1:1) were additive with the value of LC₅₀ and LC₉₅ is 0,29% and 0,59%, respectively. Mixed extract of Spiked pepper fruit and Fish-poison bean leaves (1:1) caused larvae mortality, inhibited feeding activity, and inhibited *S. frugiperda* larvae growth. The formulation is needed to increase the mixed extract of Spiked pepper fruit and Fish-poison bean leaves (1:1) activity against *S. frugiperda*.

Keywords: Additive, antifeedant, botanical insecticide, larval development time, mortality

1. Introduction

Spodoptera frugiperda is an insect native to the Americas with tropical and subtropical climates. In 2016, *S. frugiperda* was found in Central and West African countries, and then this pest *S. frugiperda* was reported to attack almost all states of Saharan Africa except Djibouti, Eritrea, and Lesotho in 2018. *S. frugiperda* is a polyphagous pest attacking maize, rice, sorghum, sugarcane, barley, cotton, and sugarcane [1]. In Indonesia, *S. frugiperda* is commonly found in maize. Nelly et al. [2] reported that in West Pasaman District, *S. frugiperda* attacked five maize varieties (NK212, NK7328, Pioneer 32, Pertiwi, and Bisi 18) with attack rates ranging from 6.0 to 96.0%.

Controlling *S. frugiperda* in the field uses synthetic insecticides with active ingredients such as chlorantraniliprole, spinosad, spinetoram, and lambda-cyhalothrin [3]. However, synthetic insecticides can harm the environment and human health. It is necessary to carry out appropriate and environmentally friendly control to avoid the effects of synthetic insecticides. One alternative that can be used is to use botanical insecticides.

One source of botanical insecticides found in nature is spiked pepper (*Piper aduncum*). The n-



hexane and ethyl acetate extract of forest betel fruits contained strong insecticidal activity against *Crociodolomia pavonana* larvae with $LC_{95}\%$ values of 0.26% and 0.32, respectively [4,5]. In another study, Spiked pepper fruit extract with a concentration of 0.5% caused the mortality of *C. pavonana* larvae to reach 100% [6]. Another plant that contains sources of plant-based insecticides is the fish-poison bean (*Tephrosia vogelii*). The leaves of the fish-poison bean are known to have rotenone compounds which can cause physiological disturbances that cause death [7,8]. The fish-poison bean leaf extract at a concentration of 0.1% could cause 88.89% *C. pavonana* larvae mortality [9].

The use of botanical insecticides can be a single extract or a mixture of extracts. Botanical insecticides with mixed extracts can reduce dependence on certain plant species used as raw materials [10]. In another result, the LC_{50} is synergistic with the mixture of the solid hexane fraction of *Piper cubeba* and the hexane fraction of Fish-poison bean (8: 3) [11]. The mixed extract of spiked pepper fruit and fish-poison bean leaves (5:1) was identified to have strong synergistic action with LC_{50} values of 0.014% and LC_{95} of 0.06% against *C. pavonana* pests [9].

Thus, research on the control of *S. frugiperda* larvae needs to be carried out. One of the control methods that can be done is to utilize the mixed extracts of spiked pepper fruit, and fish-poison bean leaves as a botanical insecticide. This study was conducted to find out the activity of mixed extracts of Spiked pepper fruit and Fish-poison bean leaves against *Spodoptera frugiperda* larvae.

2. Methods

This research was carried out at the Insect Bioecology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Andalas University, Padang, from February to November 2021. The study was an experimental method with a Complete Randomized Design (CRD). This test was initiated with a preliminary test using four concentration treatments (0.00%, 0.25%, 0.50%, and 0.75%) and consists of 3 replications for each treatment. The data obtained from the preliminary test were used to determine the concentration level in the further test of single extracts and mixed extracts of spiked pepper and fish-poison bean. For further tests, the concentration of single extracts of Spiked pepper and Fish-poison bean consisted of 0.00%, 0.20%, 0.28%, 0.38%, 0.51%, and 0.70%. The mixed extract concentrations were 0.00%, 0.20%, 0.26%, 0.32%, 0.40%, and 0.50%. Each concentration of the single and mixed extract was carried out five times.

2.1 Procurement of larval feed

Corn plants were used as insect-rearing feed, and tests were grown in polybags. The planting medium used was a mixture of soil and compost (1:1). Two seeds are put into the planting holes ± 2 cm deep and covered with dirt. Maintenance is carried out by watering, weeding weeds, and mechanical pest control. The corn leaves used as feed were $\pm$ one month old.

2.2 Procurement of larvae

S. frugiperda larvae were collected from corn plantations in the Belimbing area, Kuranji District, Padang City. Then the larvae were brought to the laboratory and kept in a plastic container (30 cm diameter and 35 cm height) with a lid made of gauze. Then corn plants are put in a plastic container to feed the larvae of *S. frugiperda*. Every day pupae were collected and placed into gauze cages (50cm $\times$ 50cm $\times$ 50cm). After the imago was formed, they were given 10% honey liquid dripped onto cotton and inserted into the corn plant where the eggs were laid. Eggs that hatch into larvae were transferred to plastic boxes (23cm $\times$ 15cm $\times$ 7cm) containing corn leaves. The instar 2 or 3 larvae were used as test insects in the study.

2.3 Plant ingredients source extract

Plant materials were used as a source of extracts: the fruit of the spiked pepper plant and the leaves of fish-poison bean. Spiked pepper fruit was taken from Bukit Lampu Jalan Padang – Painan, Padang city, and the leaves of fish-poison bean were obtained from the Limau Manis area, Pauh District, Padang.

2.4 Extraction

Spiked pepper fruit and fish-poison bean leaves were cut about 1-2 cm, then placed on a tray lined with newspaper on top, and the material was dried for two weeks without direct sunlight. The dried extract source materials were then mashed using a blender. After the material was smooth, it was sifted using a 0.5 mm sieve to obtain a powder.

Extraction of Spiked pepper and Fish-poison bean was conducted by maceration or immersion method with ethyl acetate as solvent. The extract in the form of powder was taken as much as 50 grams, then placed into an Erlenmeyer flask, and added 500 ml of ethyl acetate solvent was following previous research. Soaking is done for 2 x 24 hours. The extracted liquid was filtered two times, and the first filtering was conducted using a glass funnel with a diameter of 9 cm, lined with ordinary filter paper, then the second filtering using *Whatman* filter paper number 41. The filtering activity was carried out in an isolation room. The results obtained from the filtering are then accommodated in a steam flask and evaporated using a rotary evaporator at a temperature of 50°C and a pressure of 240 mbar. The evaporation results obtained as a solution were then used for re-soaking the pulp from the plant extract, which was carried out four times. The extract obtained was stored in a refrigerator at 4°C until it was used as material for testing and analysis of active compounds [12].

2.5 Extract test

Preliminary and advanced tests were carried out testing spiked pepper fruit extract and fish-poison bean leaf extract. In the initial test, the extracts of Spiked pepper and Fish-poison bean were tested at concentrations of 0.75%, 0.5%, 0.25%, and control with three replications each. Each replicate contained ten 2-instar larvae of *S. frugiperda*. The extract concentration that gave the best results of insecticidal activity in the preliminary test was then used in the advanced test.

Further testing was carried out in 2 stages, namely single extracts and mixed extracts, which were tested at level 5 concentrations and every five replications based on the preliminary tests that had been carried out. Each petri dish contains 15-second instar larvae. The concentration is to be used for further testing with the ratio of the highest and lowest concentrations using the formula X^4 .

2.5.1 Single extract advanced test

The test was conducted after obtaining the previous concentration from the preliminary test. The concentrations of the single extracts of spiked pepper and fish poison bean were 0.00%, 0.20%, 0.28%, 0.38%, 0.51%, and 0.70%, respectively. This single extract further test was conducted to obtain the LC₅₀ of every single extract of spiked pepper and fish-poison bean, which was later used as a reference to get a composition ratio of the two ingredients in the mixed extract.

2.5.2 Mixed extract advanced test

After knowing the LC₅₀ from the further test of spiked pepper and fish-poison bean extracts, the concentration of mixed extracts was 0.00%, 0.20%, 0.26%, 0.32%, 0.40%, and 0.50%. The activity properties of the mixture of spiked pepper fruit extract and fish-poison bean leaf extract were analyzed based on the combined index of LC₅₀ and LC₉₅ levels.

2.6 Analysis of mixed activity properties

All treatments used the leaf dipping method. The corn leaves were cut to a size of 4x4 cm², dipped evenly into the extract solution, and then dried. The extract solution was prepared by mixing its according to the respective concentrations with the parent material (acetone: methanol: tween) in a ratio of 3: 1: 0.2. The parent material was taken as much as 0.3 ml to homogenize the extract, then added with distilled water become 25 ml. For the control, immersed in a solution consisting of a mixture of acetone, methanol, and tween in a ratio of 3: 1: 0.2, adding distilled water becomes 25 ml of. Instar 2 larvae were placed in a petridish lined with tissue with an inverted position of 15 each. Feeding with treated leaves was carried out for two days, and every 1x24 hours, the feed was replaced with untreated until it reached the 6th instar.

For extract mixtures whose components come from different plant species, the activity properties of the mix were analyzed based on other working models by calculating the combination index at the LC50 and LC95 levels. The following formula calculated the combined index (IK) at the LCx level:

$$IK = \frac{LC_{X1}}{LC_{X2}} + \frac{LC_{X2}}{LC_{X1}} + \left(\frac{LC_{X2}}{LC_{X1}} \times \frac{LC_{X1}}{LC_{X2}} \right) \quad (1)$$

CI = Combination Index
 LC_{X1} = Lethal concentrate ingredient 1 (*Piper aduncum*)
 LC_{X2} = Lethal concentrate ingredient 2 (*Tephrosia vogelli*)
 $LC_{X1}^{(cm)}$ = Lethal concentrate mixed 1
 $LC_{X2}^{(cm)}$ = Lethal concentrate mixed 2

LCx1 and LCx2 were LCx extracts of spiked pepper and fish-poison bean fruit extracts, respectively, in separate tests. LCx1 (cm) and LCx2 (cm), respectively, LCx extracts of spiked pepper fruit and fish-poison bean leaves in the mixture resulted in mortality x (e.g., 50% and 95%). The LCx value was obtained by multiplying the LCx of the mix by the proportion of the concentration of spiked pepper fruit extract and Fish-poison bean leaf extract in the mixture.

The category of mixed interaction properties was adapted from Kosman & Cohen [13], namely:

- (1) if the CI < 0.5, the components of the mixture are strongly synergistic;
- (2) if the CI $0.5 \leq 0.77$, the components of the mixture are weak synergistic;
- (3) if CI > $0.77 \leq 1.43$, the components of the mixture are additive;
- (4) if the CI > 1.43, the components of the mixture are antagonistic;

2.7 Observation Variables

2.7.1 Larval mortality.

Observations of *S. frugiperda* larvae mortality were carried out every 1 x 24 hours after testing. The mortality percentage was determined using the following formula :

$$M = \frac{n}{m} 100\% \quad (2)$$

Information:

M = Larval mortality (%)
 n = Number of dead larvae
 N = Number of larvae treated

2.7.2 Antifeedant activity

This observation was made to determine the antifeedant effect of *S. frugiperda* larvae by measuring the area of the treatment leaves for 2 x 24 hours after treatment. It was measured using the index of inhibition of eating was calculated by the following formula:

$$AF = \frac{Cl-Tl}{Cl} \times 100\% \quad (3)$$

Information:

AF = antifeedant effect
 Cl = area of the control leaf eaten by the larva (mm²)
 Tl = area of the treatment leaf eaten by the larvae (mm²)

2.7.3 Duration of larvae development.

This observation aimed to see the effect of spiked pepper fruit extract and fish-poison bean leaf extract on larval development. The length of larval development was observed every day. This observation was carried out by recording the length (days) of larval development from the second instar to the fourth instar during the mixed extract test.

2.8 Probit Analysis

Probit analysis was used to find out the values of LC_{50} and LC_{95} of each treatment concentration in advanced tests using the probit analysis method with a PC POLO program [14].

2.9 Data Analysis

Analysis of variance (ANOVA) was performed using the Statistix 8 application. The data used in this application are mortality of larvae, antifeedant, and larval development duration at a level of 5%. If the results obtained are significantly different, it is continued with the Least Significant Difference (LSD) test at the level of 5%.

3. Results and Discussion

3.1 Mortality

3.1.1 Single Extract

A single extract of spiked pepper at the highest concentration of 0.75% caused larval death to reach 100%. While the single test extract of Fish-poison bean at the highest concentration of 0.75% caused 86.66% of deaths (Table 1).

Follow-up tests were carried out based on preliminary tests conducted using the X^4 formula. The concentrations obtained from further tests for each extract of Spiked pepper fruit and Fish-poison bean leaves were 0.00%, 0.20%, 0.28%, 0.38%, 0.51, and 0.70% (Table 2).

Table 1. Mortality of *Spodoptera frugiperda* larvae after being treated with a single extract of spiked pepper and fish-poison bean during the preliminary test.

Concentration (%)	Mortality (%)			
	Spiked pepper		Fish-poison bean	
0.75	100.00	a	86.66	a
0.50	55.16	b	43.33	b
0.25	20.69	c	13.33	c
0.00	3.33	d	0.00	c

The average value on the same column followed by the same lowercase letter is not significantly different according to the LSD test at a level of 5%.

Table 2. Mortality of *Spodoptera frugiperda* larvae after being treated with a single extract of spiked pepper and fish-poison bean during the follow-up test.

Spiked pepper			Fish-poison bean		
Concentration (%)	Mortality(%)±SD		Concentration (%)	Mortality (%) ± SD	
0.70	98.64 ± 2.98	a	0.70	94.66 ± 5.57	a
0.51	74.32 ± 5.56	b	0.51	72.00 ± 8.60	b
0.38	44.60 ± 5.58	c	0.38	45.34 ± 5.58	c
0.28	25.68 ± 4.71	d	0.28	25.34 ± 5.58	d
0.20	18.91 ± 4.73	e	0.20	17.32 ± 3.66	e
0.00	1.34 ± 2.99	f	0.00	0.00 ± 0.00	f

The average value on the same column followed by the same lowercase letter is not significantly different according to the LSD test at a level of 5%.

3.1.2 Mixed extract

The mixed extract test of spiked pepper fruit and fish-poison bean leaves at all concentrations significantly differed from the control. The mixed extract of spiked pepper fruit and fish-poison bean leaves at concentrations of 0.00%, 0.20%, 0.26%, 0.32%, 0.40%, and 0.50% resulted in mortality of test larvae of respectively 1.34%, 21.61%, 32.42%, 59.46%, 77.03%, and 89.18%. The increasing treatment concentration will result in high larval mortality at the testing time.

Table 3. Mortality of *Spodoptera frugiperda* larvae after being treated with a mixture of *Piper aduncum* and *Tephrosia vogelii* extracts.

Concentration (%)	Mortality (%) ± SD
0.50	89.18 ± 5.96 a
0.40	77.03 ± 7.60 b
0.32	59.46 ± 8.16 c
0.26	32.42 ± 6.65 d
0.20	21.61 ± 5.99 e
0.00	1.34 ± 2.99 f

The average value on the same column followed by the same lowercase letter is not significantly different according to the LSD test at a level of 5%.

The mortality of the test larvae began to be seen on the first day of observation and then increased significantly on the second day of observation. Treatment with a concentration of 0.50% caused mortality to reach 70.66% on the first day of observation. From the third day of observation until the eleventh day after treatment, there was no significant increase in mortality, and this was because the feed given to the test larvae no longer used mixed extracts, so the test larvae ate feed that was free from insecticides.

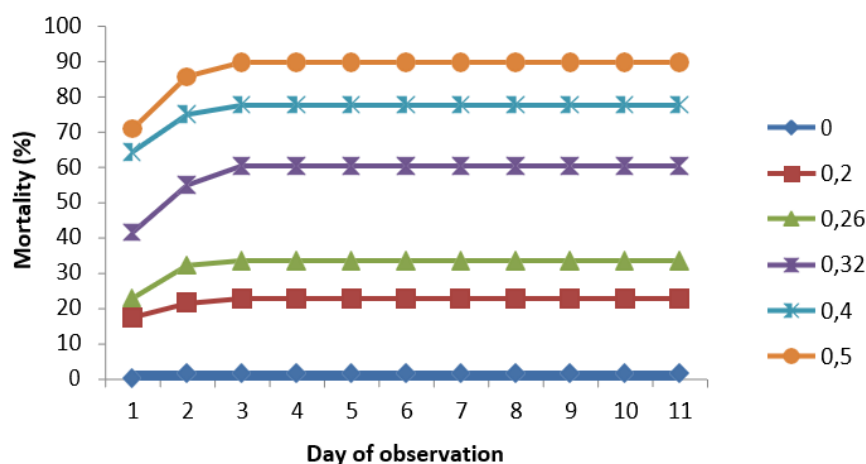


Figure 1. The curve of cumulative observation of larval mortality of *Spodoptera frugiperda* using mixed extracts during 11 days of observation

In Table 4, it can be seen the relationship between test concentration and mortality of test larvae. In the single extract of spiked pepper, the LC_{50} and LC_{95} values were 0.36% and 0.78%, respectively, with a regression slope of 4.88. Then in the single extract of a fish-poison bean, the LC_{50} and LC_{95} values were 0.37% and 0.85%, respectively, with the regression slope value lower than the Spiked pepper single extract, which was 4.55. Meanwhile, in the mixed extracts of Spiked pepper and Fish-poison bean, the LC_{50} and LC_{95} values were 0.29% and 0.59%, respectively, with a regression slope of 5.42, which was higher than the single extracts of spiked pepper and fish-poison bean.

Table 4. Probit regression parameters of the relationship between a single extract and a mixture of spiked pepper fruit and fish-poison bean leaves

Treatment	b $\pm$ SE	LC_{50} (%)	LC_{95} (%)
Spiked pepper	4.88 $\pm$ 0.46	0.36	0.78
Fish-poison bean	4.55 $\pm$ 0.43	0.37	0.85
<i>P. aduncum</i> + <i>T. vogelii</i> 1:1	5.42 $\pm$ 0.57	0.29	0.59

b = regression slope value

The results of the calculation of the combined LC_{50} index value in the mixed extract of spiked pepper and fish-poison bean (1 : 1) was 0.97, with the criteria being additive. While on LC_{95} , the combined index value is 0.86, and the criteria are additive (Table 5).

Table 5. The combined index value of the mixed extract of *Piper aduncum* fruit and *Tephrosia vogelii* leaves (1:1).

Lethal Concentrate Value	Combination Index Value	Criteria
LC_{50}	0.97	Additive
LC_{95}	0.86	Additive

Based on the research results, the spiked pepper fruit single extract, fish-poison bean leaves single extract, and mixed extracts from spiked pepper fruit and fish-poison bean leaves affect the mortality of

S. frugiperda larvae. Increasing the treatment concentration will lead to higher mortality of test larvae. The death of the larvae is indicated by the size of the larvae, which are smaller and dark brown in color. Larval mortality increased with increasing treatment concentration. This is presumably because the active compounds contained in the solution with higher concentrations will increase. The higher concentration given, the more active ingredient content and followed the insect mortality rate [6].

The larval mortality rate from the mixed extract treatment of Spiked pepper fruit and Fish-poison bean leaves (1:1) increased on the first day after treatment. Then there was a significant increase on the second day after treatment. However, on the third to the eleventh day, there was only some increase in mortality in the treatment. It is because feeding with extract treatment is only for 2 x 24 hours. On the third to eleventh day, the test larvae were only fed without treatment (insecticide-free). The death of the test larvae was caused by the presence of the active compound dilapiol in the Spiked pepper extract and the rotenoid compound in the fish-poison bean extract. The active compound in dilapiol has synergistic properties by inhibiting the activity of the enzyme poly substrate monooxygenase (PSMO), which decreases the toxicity of the foreign compound in the larvae body through the oxidation process. The inhibition of this PSMO enzyme could result in the accumulation of toxic compounds in the larvae body, slowly leading to the larvae's death [16].

3.2 Inhibit feeding activity (antifeedant)

The results show that each treatment was significantly different from the control. In control, the average leaf area eaten was 206.8 mm² and did not show any antifeedant effect, while at the highest concentration of 0.5%, the average leaf area consumed was 24 mm² with an antifeedant effect of 88.39%. It indicates that the higher the test concentration, the smaller the average leaf area eaten and the higher the antifeedant effect on the test larvae (Table 6).

Table 6. The antifeedant activity of mixed extract of spiked pepper fruit and leaves of fish-poison bean (1:1) against *Spodoptera frugiperda* larvae at various concentrations.

Concentration (%)	The average area of eaten leaves(mm ²) ± SD		Antifeedant effect (%)
0.00	206.8 ± 22.88	a	0.00
0.20	167.4 ± 09.71	b	19.05
0.26	152 ± 08.15	b	26.50
0.32	116 ± 12.18	c	43.90
0.40	63.4 ± 11.88	d	69.34
0.50	24 ± 05.24	e	88.39

The average value on the same column followed by the same lowercase letter is not significantly different according to the LSD test at 5%.

3.3 Duration of development of larvae

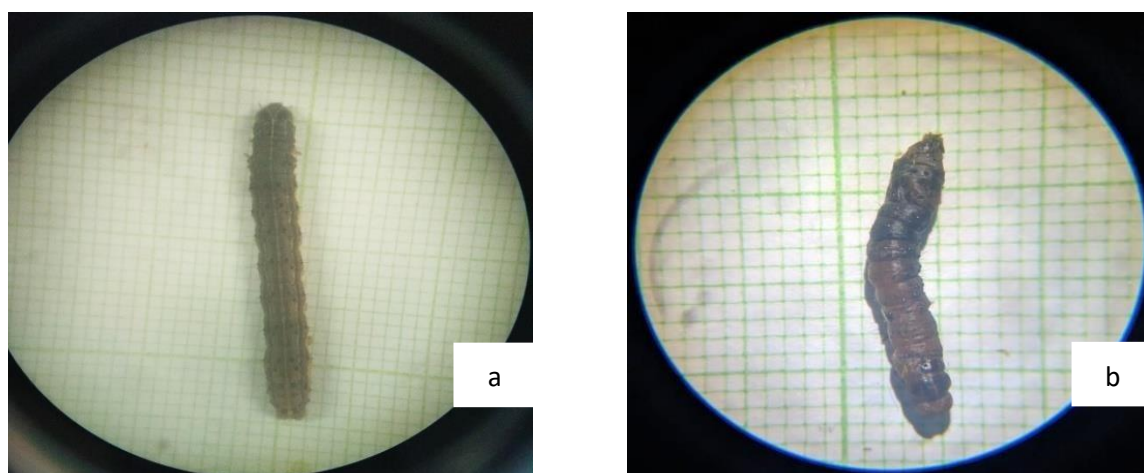
It can be seen that giving the concentration slowed the development of the test larvae when compared to the control. In instars 2-3 with a concentration of 0.50%, larval development was 1.39 days longer than the control. In instars 2-4, the concentration of 0.50% inhibited larval development for 0.66 days compared to the control. In instars 2-5 with a concentration of 0.50% inhibited larval development for one day compared to controls. Then in instars 2-6 with a concentration of 0.50% inhibited larval growth for 2.2 days compared to controls (Table 7).

Table 7. Duration of development of the larva *Spodoptera frugiperda* due to the mixed extract of *Piper aduncum* fruit and *Tephrosia vogelii* leaves (1:1) at several concentration levels

Concentration (%)	Duration of development of larvae (day) ($\bar{X} \pm \text{SD}$)			
	(2-3)	(2-4)	(2-5)	(2-6)
0.50	3.53 ± 0.50	4.80 ± 0.83	7.30 ± 0.44	10.63 ± 0.41
0.40	2.91 ± 0.25	4.76 ± 0.22	6.96 ± 0.29	9.13 ± 0.36
0.32	2.68 ± 0.14	4.73 ± 0.32	6.66 ± 0.09	8.81 ± 0.13
0.26	2.57 ± 0.12	4.50 ± 0.33	6.49 ± 0.17	8.65 ± 0.18
0.20	2.38 ± 0.11	4.29 ± 0.22	6.46 ± 0.17	8.49 ± 0.09
0.00	2.14 ± 0.08	4.14 ± 0.07	6.30 ± 0.09	8.43 ± 0.12

This mixed extract of spiked pepper fruit and fish-poison bean leaves also affected the antifeedant activity of *S. frugiperda* larvae. With the increasing treatment concentration, the less leaf area eaten by the larvae, the greater the antifeedant effect. So that the death of the test larvae was also influenced by the feeding activity of the larvae, which were inhibited by the Spiked pepper and Fish-poison bean active compounds. Overall larval death was caused by the activity of the toxic active ingredients and the activity of the active ingredients, which were anti-feeding. The active ingredients that enter the body of *S. frugiperda* larvae in sufficient doses will kill the test larvae. However, if the active ingredient dose is insufficient to cause the test larvae's death, there will be physiological disturbances in the larvae [8].

Physiological disturbances that occur in *S. frugiperda* larvae that eat feed that has been treated with extracts are the inhibition of larval development. The inhibition is thought to be due to the larvae requiring a large amount of energy to detoxify toxic compounds so that energy allocation occurs, where the energy used is not for the development process but for toxic detoxifying compounds [15].

**Figure 2.** *Spodoptera frugiperda* larvae. a. larvae with control (healthy) treatment b. larvae treated by the mixed extracts of *Piper aduncum* and *Tephrosia vogelii* (dead)

The mixed extract activity was more lethal than the single extract against *C. pavonana* larvae, where the mixture of the three types of extracts of *Brucea javanica*, spiked pepper, and fish-poison bean was strongly synergistic [9]. In contrast to the results of this study, although the LC_{95} value of the mixed extracts of spiked pepper and fish poison bean was lower than the LC_{95} value of every

single extract, the combination was additive. The additive properties are conditions where the effect of toxicity given by mixed extracts with every single extract is relatively the same [17].

In the combined index value criteria, it was obtained that spiked pepper and fish-poison bean fruit extracts were suitable to be mixed because the combination index values obtained were additive. Although the effect of toxicity produced by the mixed extract is relatively the same as the single extract, it can still reduce raw materials' dependence on one type of plant.

4. Conclusion

The mixed extract of spiked pepper fruit and fish-poison bean leaf (1:1) were additive at LC_{50} and LC_{95} , respectively, with combined index values of 0.97 and 0.86. The relationship between the treatment concentration and the percentage of larval mortality showed LC_{50} and LC_{95} values of 0.29 and 0.59, respectively. The treatment with the highest concentration (0.50%) could inhibit the feeding activity of the test larvae by 88.39% and slow the development of the larvae to reach the 6th instar for 2.2 days when compared to the control.

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