



Stability of Anti-Insect Ingredient from Jayanti Plants (*Sesbania sesban*) for Integrated Control of Cabbage Pest

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Abstract: The specific purpose of this study was to determine the stability of *S. sesban* insecticide during storage before application and its stability in water during application. The dry powder of *S. sesban* leaves was extracted using water as the single solvent. Bioassay of *S. sesban* leaf extract was carried out on cabbage caterpillars (*Plutella xylostella* larvae) and *Diadegma semiclausum* imago on cabbage plants using a variation of storage time design. Mortality data of *P. xylostella* larvae and *D. semiclausum* imago were respectively processed by probit analysis to determine LC₅₀. The results showed that the insect repellent content of *S. sesban* leaves was a compound from the saponin group and was unstable during simplicia storage, extract storage and also unstable in water before and during application. Storage of *S. sesban* leaf simplicia from 1 to 3 months only slightly reduced the lethal toxicity of the extract to *P. xylostella* larvae (mortality from 95 to 80% or LC₅₀ from 28.82 to 28.83 ppm), but after 6 to 12 months storage, the lethal toxicity decreased drastically (mortality was 12.5 to 1.25% and LC₅₀ was 247.99 ppm until calculated). Storage of *S. sesban* leaf extract from 7 to 15 days had resulted in a sharp decrease in lethal toxicity to *P. xylostella* larvae (mortality 70 to 40% and LC₅₀ 34.05 to 59.43 ppm) and 30 days storage causes the insect repellent to be inactive. (mortality was only 1.25% and LC₅₀ was unaccounted for). Exposure to a solution of *S. sesban* leaf extract for 24 to 48 hours caused a decrease in lethal toxicity to *P. xylostella* larvae (mortality 32 to 28% and LC₅₀ 62.63 to 64.85 ppm) and after the solution was stored for 72 hours, the insect repellent was almost no active again (mortality was only 1.25 and LC₅₀ was unaccounted for). All storage treatments of insect repellent from *S. sesban* leaves showed no significant difference in the effect of lethal toxicity on *D. semiclausum* imago. In all the results of the bioassays, the mortality of *D. semiclausum* was 0 to 1.25% and each of all LC₅₀(s) was unaccounted for.

Keywords: Anti-insect ingredients from *Sesbania sesban*; *Diadegma semiclausum*; *Plutella xylostella*.

Introduction

Observations on the use of insecticides to control cabbage pests extensively show that since the 1960s cabbage caterpillars have been a serious problem. As was the case in Taiwan, initially there were only two insecticides recommended for cabbage caterpillar pest control, but then the number of chemicals listed as recommended continued to increase from year to year. Every previous chemical that was ineffective was rarely

removed from the list of recommendations, resulting in new problems arising from the use of this insecticide in the form of resurgence and resistance of insect pests, while the population of natural enemies (parasitoids and predators) for these insect pests was decreasing (Solichah et al., 2014). This is because insecticides from synthetic chemical compounds are generally stable, so they remain active as pollutants (toxic) in the post-application environment (Suripto et al., 2020).

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Pollutant chemicals that are stable in the environment, even with low toxicity, are still dangerous along the food chain in ecosystems. This is because stable chemicals, which are not easily and not rapidly degraded, can accumulate at certain tropic levels, especially at the top tropic level of the food chain in the ecosystem. The accumulation of these pollutants will cause an increase in chronic lethal or sub-lethal toxicity to organisms of a certain tropic level, especially at the peak tropic level.

To suppress the emergence of environmental problems in controlling cabbage caterpillars, the use of natural insecticides from the jayanti plant (*Sesbania sesban*) has been studied against cabbage caterpillars as target insects and against their natural enemy, *Diadegma semiclausum* (Suripto *et al.*, 2017; 2021). However, whether the active natural insect repellent from the *S. sesban* plant remains active (toxic) or not after application, it is not yet known. Therefore, it is necessary to study more about the stability of the natural anti-insect active ingredients of this plant, especially for the integrated control of cabbage caterpillars.

Based on the background of the problem above, this study was carried out with the aim of knowing the LC₅₀ of the water-extract of *S. sesban* leaves against *P. xylostella* larvae and *Diadegma semiclausum* imago according to variations in storage time of simplicia before extraction, storage time of extract before application, and length of storage time of extract solution before bioassay.

Method

Material preparation

1. Rearing of *Plutella xylostella* larvae

Plutella xylostella insects were collected from cabbage plantations in Sembalun Village, East Lombok, West Nusa Tenggara. Rearing of *P. xylostella* was carried out at The Biology Laboratory University of Mataram by using leaves of cabbage plants as a place to lay eggs according to the procedure of Wang *et al.* (2014) resulting in sufficient number of larvae (instars 1-4) for all bioassays in this study.

2. Rearing of *Diadegma semiclausum* imago

Pupae of *Diadegma semiclausum* were collected from leaf cabbage plantations in Sayang-sayang Village, West Lombok. Rearing of *D. semiclausum* imago was carried out in cages sized 50cm x 50cm x 40cm of nylon material (Ø mess 2 mm), using pure bee honey solution as feed according to the procedures from AVRDC 2008 (Francis *et al.*, 2012) at The Laboratory of Environmental Science University of Mataram in order to obtain the number of sufficient imago for bioassay.

3. Extraction of anti-insect ingredients from the leaves of *S. Sesban*

To extract the active anti-insect compound from the leaves of *S. Sesban*, extraction (liquid-solid) was carried out in stages, using a series of solvents, the polarity of which increased successively, namely hexane, dichloromethane (DCM), ethanol (EtOH) and water (Suripto *et al.*, 2017).

It was also known that the ethanol fraction of *S. sesban* leaf extract was the most selective in suppressing the population of cabbage caterpillars, which was very toxic to cabbage caterpillars (*P. xylostella* larvae) but very low toxicity to their natural enemies (*D. Semiclausum* imago) compared to other extract fractions (Suripto *et al.*, 2020).

Because ethanol is a very polar solvent (water is the most polar), direct single extraction using water is expected to produce insect repellent active ingredients from the same class of compounds as the ethanol extract fraction, in this case triterpene saponins as active ingredients, as previously reported by Jain *et al.* (2019), which are relatively selective for insect repellent against cabbage caterpillars. Thus, in this study, *S. sesban* leaf extraction was carried out using a single solvent, namely water.

S. sesban leaves were collected from plants that were 1 year old or more (already flowering). The leaves are separated from the stems and then air-dried (not dried or exposed to sunlight) and after drying (after 3 days) then milled. Dry powder (simplicia) leaves were stored at room temperature with variations in storage time of 1 month, 3 months, 6 months and 12 months before being extracted.

Extraction of leaf simplicia of *S. sesban* was carried out by maceration technique using a single solvent of water, as has been done by Suripto *et al.* (2021). This extraction process was carried out at the Chemistry Laboratory University of Mataram. The resulting extract was stored in a refrigerator with variations in storage time of 1 day, 7 days, 14 days and 30 days before being diluted (made a solution) for application. The water-extract solution of *S. Sesban* leaves was prepared in 6 concentration levels, namely 0 ppm, 10 ppm, 15 ppm, 20 ppm, 28 ppm and 30 ppm. The treatments for the extract solution before the bioassay were variations in storage time, namely 1 hour, 24 hours, 48 hours, and 72 hours.

Bioassays

Prior to the bioassay, an initial examination of the active anti-insect content (in this case the content of saponins) was carried out from the leaf extract of *S. sesban*, using the foam test and thin layer chromatography (TLC) with the procedure ever done by Suripto *et al.* (2017).

Bioassays of *S. sesban* leaf extract against *P. xylostella* larvae and on *D. Semiclausum* imago, respectively, was carried out according to variations in the storage time of

insecticidal material from *S. sesban* (leaf dry powder or simplicia, extract, and extract s solution)

The lethal toxicity test of *S. sesban* leaf extract against *P. xylostella* larvae (instar III) was carried out on cabbage leaves that had been grown in experimental pots (10 larvae per pot), using 6 levels of concentration treatment with a modified procedure from Hamburger & Hostettmann (2021). The treatment was given by spraying a solution of *S. sesban* leaf extract according to a predetermined concentration on the surface of cabbage leaves in a petri dish that had been infected with *P. xylostella* larvae (20 larvae per dish). For each concentration treatment, 3 replicates (dishes) were used. The variable observed was mortality of *P. xylostella* larvae after 6 hours of treatment.

The lethal toxicity test of *S. sesban* leaf extract on *D. semiclausum* imago was carried out in cages made of 1000cc keller covered with nylon (mes Ø 2 mm) (40 imago per cage), using pure bee honey solution as feed according to the procedures of AVRDC 2008 (Francis *et al.*, 2012). The treatment was given by spraying the extract solution according to the concentration of each treatment into the test cage. The variable observed was the percentage of dead *D. semiclausum* imago (mortality data) after 6 hours of treatment.

In general, the work flow chart for the stability study of active natural anti-insect ingredients from the jayanti plant for the integrated control of cabbage caterpillars can be seen in Figure 1.

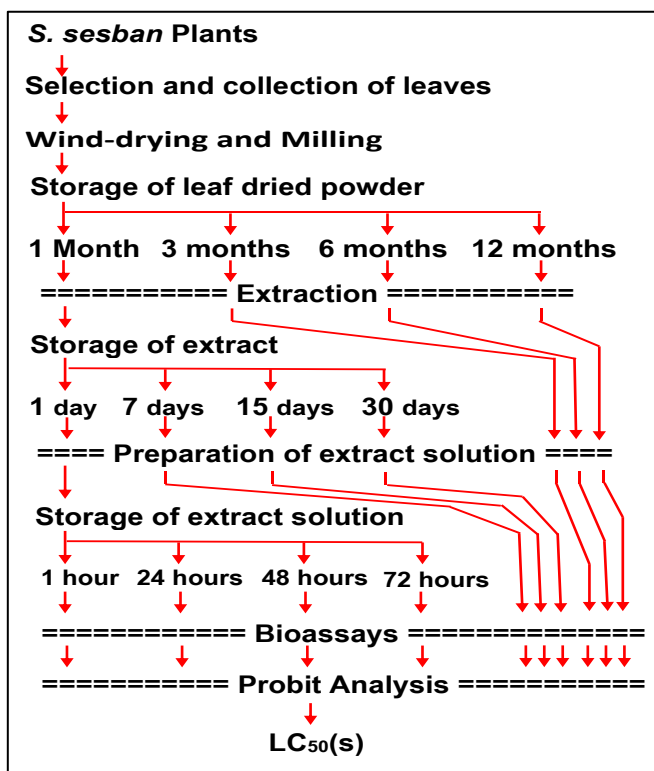


Figure 1. Flow chart of study on the stability of the insecticide power of *S. sesban*

Data analysis

Mortality data of *P. xylostella* larvae and *D. semiclausum* imago were processed by probit analysis, respectively, to produce an output of LC₅₀ (ppm). Prior to processing, the mortality data of the test animals (% mean of 4 replications) and the treatment concentration data (ppm), were first converted into log statistics. This converted data is then used in probit analysis.

Result and Discussion

Stability of anti-insect activity according to variations in storage time of *S. sesban* leaf dried powder (simplicia)

Storage of insecticide raw materials in the form of dry powder (simplicia) from the leaves of *S. sesban* for up to 3 months did not significantly reduce the toxicity of *P. xylostella* larvae. The mortality of cabbage caterpillars treated with the highest concentration of *S. sesban* leaf extract solution (30 ppm) from simplicia 1 month and 3 months, respectively, was 95% and 80%. Simplicia storage for 6 months caused a drastic reduction in the mortality of cabbage caterpillars, namely 12.5%, and storage of simplicia for up to 12 months caused the insect repellent material from *S. sesban* leaves to become inactive (*P. xylostella* larvae mortality 0 to 1.25%) (Figure 2).

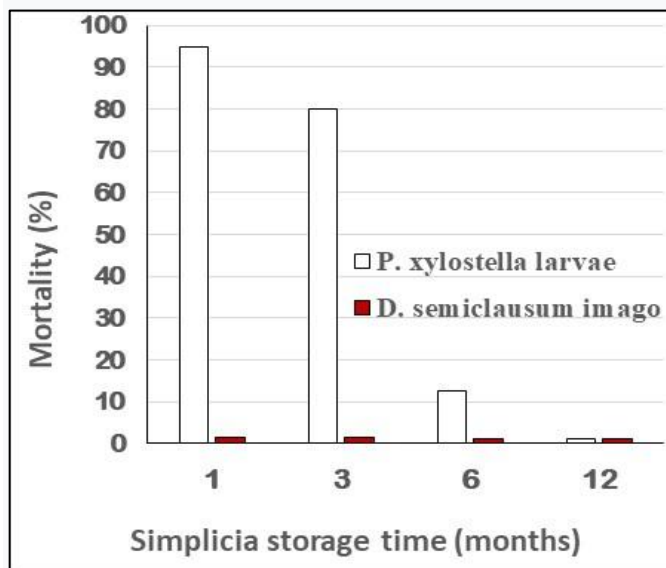


Figure 2. Mortality (%) of *P. xylostella* larvae and *D. semiclausum* imago according to variations in storage time of jayanti leaf simplicia.

The results also showed that the concentration of *S. sesban* leaf extract solution of 1.85 ppm from 1 month simplicia, 1.47 ppm from 3 months simplicia and 1.63 ppm from 6 months simplicia did not cause the death of *P. xylostella* larvae, respectively. The lethal toxicity to cabbage caterpillars from *S. sesban* leaves slightly decreased when simplicia was stored for up to 3 months

(LC₅₀ price slightly increased from 28.82 to 29.83 ppm but decreases drastically when simplicia wa stored for up to 6 months (the price of LC₅₀ increased sharply, to 247.90 ppm) and the LC₅₀ was incalculable after the simplicia was stored for 12 months (Table 1).

Table 1. Lethal concentration (LC)(ppm) of *S. sesban* leaf water-extract against *P. xylostella* larvae according to variations in storage time of anti-insect ingredient in the form of simplicia.

	1 month	3 months	6 months	12 months
LC ₀	1.85	1.47	1.63	Countless
LC ₅₀	28.82	29.83	247.89	Countless
LC ₁₀₀	46.88	50.87	603.53	Countless

The concentrations of *S. sesban* leaf extract solution, 1.85, 1.47, and 1.63 ppm, respectively, came from one month simplicia, three months simplicia and six months simplicia, which did not cause death of *P. xylostella* larvae, respectively. The lethal toxicity to cabbage caterpillars from *S. sesban* leaf extract decreased slightly when simplicia was stored for up to 3 months (LC₅₀ value slightly increased from 28.82 to 29.83 ppm), but decreased drastically when simplicia was stored for up to 6 months (LC₅₀ value). increased sharply, namely to 247.90 ppm) and the LC₅₀ was not calculated after the simplicia was stored for 12 months.

The LC₅₀ against imago *D. semicalusum* in all treatments of storage duration of leaf dried powder (simplicia) of *S. sesban* was not calculated. The mortality of *D. semiclausum* imago at the highest concentration of jayanti leaf extract (30 ppm) was not significantly different from the control (0 ppm), and this occurred in the simplicia bioassays of 1 month, 3 months, 6 months and 12 months.

Stability of anti-insect activity according to variations in storage time of S. sesban leaf extract.

Storage of insecticide materials from *S. sesban* in the form of extracts for a certain time (especially from extracts that have been stored for 15 days or more) can also cause a decrease in their anti-insect power. In this research, the leaf extract of *S. sesban* was packaged in dark bottles (bottles wrapped in carbon paper) and stored in a refrigerator without temperature variations.

The mortality of *P. xylostella* larvae in the treatment of *S. sesban* leaf extract stored for 1 day was 95%, decreased to 70% after the extract was stored for 7 days, and decreased sharply to 40 to 1.25% after the extract was stored 48 hours to 72 hours. Meanwhile, mortality of *D. semiclausum* imago was very low, ie 0 to 1.25%, and was not significantly different according to the variation of storage time of *S. sesban* leaf extract (Figure 3).

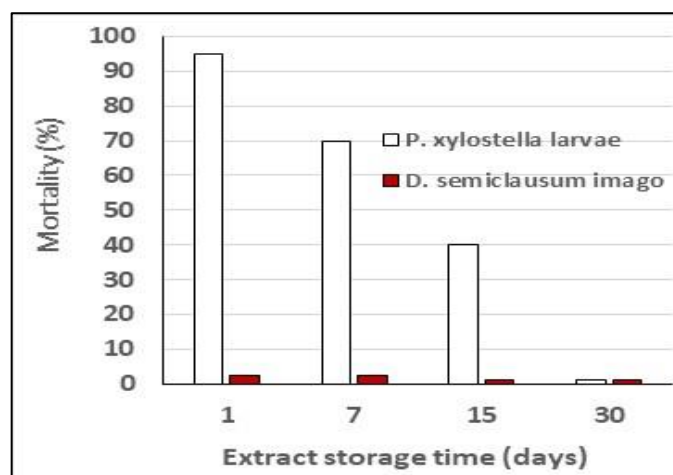


Figure 3. Mortality (%) of *P. xylostella* larvae and *D. semiclausum* imago according to variations in storage time of *S. sesban* leaf extract.

These results indicate that storage of insect repellent from *S. sesban* in the form of an extract for a certain time can also cause a decrease in insect repellent activity. The results of probit analysis showed that storage of insect repellent from *S. sesban* leaves in the form of an extract for 7 days could reduce the lethal toxicity slightly (increase LC₅₀ and LC₁₀₀ slightly) to *P. xylostella* larvae, and after the extract was stored for 15 days, lethal toxicity decreased drastically (LC₅₀ and LC₁₀₀ increased sharply) and were almost non-lethal toxic (LC₅₀ was countless) after the extract was stored 30 days (Table 2).

Table 2. The lethal toxic concentration (results of probit analysis) of *S. sesban* leaf extract against *P. xylostella* larvae according to variations in extract storage time.

	1 day	7 days	15 days	30 days
LC ₀	1.85	2.11	2.08	Countless
LC ₅₀	28.82	34.05	59.43	Countless
LC ₁₀₀	46.88	55.73	107.67	Countless

Stability of anti-insect activity according to variations in storage time of S. sesban leaf extract solution

S. sesban leaf extract solution which was given directly (1 hour storage time) caused 95% death of *P. xylostella* larvae. The mortality of *P. xylostella* larvae decreased drastically to 32.5% in the treatment of extracts stored for 24 hours, and the solution stored for 72 hours was almost no longer lethal toxic to *P. xylostella* larvae (0 to 1.25% mortality) (Figure 4).

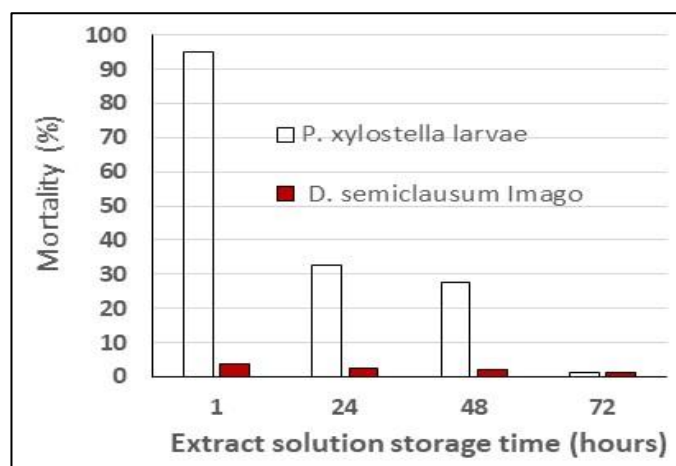


Figure 4. Mortality (%) of *P. xylostella* larvae and *D. semiclausum* imago according to variations in storage time of jayanti leaf extract solution before application.

Similar to the treatment of simplicia storage time and extract storage time, the mortality of *D. semiclausum* imago was very low (0 to 1.25%) and there was no significant difference according to variations in the storage time of the extract solution. The results of the solution storage treatment before application also showed that up to a concentration of 1.85 ppm solution for 1 day, 2.11 ppm solution for 7 days and 2.08 ppm solution for 30 days, each did not cause the death of *P. xylostella* larvae. Death of 50% of *P. xylostella* larvae occurred at a concentration of 28.82 ppm solution for the 1 day treatment, 34.05 ppm solution for the 7 days treatment and 59.43 ppm solution for the 15 days treatment, while the solution stored for 30 days did not cause the death of the larvae of the test animals (Table 3).

Table 3. The lethal toxic concentration of *S. sesban* leaf extract solution against *P. xylostella* larvae according to variations in the storage time of the extract solution before application.

	1 jam	24 jam	48 jam	72 jam
LC ₀	1.85	3.95	2.99	Countless
LC ₅₀	28.82	62.63	64.86	Countless
LC ₁₀₀	46.88	102.21	111.85	Countless

The above results indicate that the storage of insect repellent from the leaves of *S. sesban* plant, either in the form of simplicia, extract or solution for a certain time causes a decrease in lethal toxicity to *P. xylostella* larvae. Storage of simplicia up to 6 months, storage of extract up to 15 days, and storage of extract solution up to 24 hours caused a significant decrease in lethal toxicity to *P. xylostella* larvae.

It is known that the active anti-insect ingredient of *S. sesban* leaves is derived from the class of triterpene saponin compounds, which are polar (Suripto *et al.*, 2021). In this study, it was confirmed that the insecticidal

material from *S. sesban* leaves even though it had been stored for a certain time still contained saponin compounds. The storage of the material referred to is in the form of dry leaf powder, extract, and extract solution. Confirmation of saponin content in insecticidal ingredients from *S. sesban* leaves was determined based on the results of examinations of bioactive ingredients, namely foam test and thin layer chromatography (TLC).

Bioactive examination showed the presence of a triterpene saponin group with relatively higher levels in materials that were stored for a shorter time than those that were stored longer. On shaking the solution of *S. sesban* leaf extract in a test tube, from the material that was stored for a shorter time there was a steady formation (the foam was about 3 cm high, and after being added with 10% HCl, the foam did not decrease). The results of the above examination are supported by the results of the TLC. In TLC using hexane-EtOAc (1:1), the chromatogram showed yellow and brown spots. According to Mahato and Nandy (2021), this phenomenon indicates the presence of a triterpene saponin group. Meanwhile, in TLC with the developer BuOH-H₂O (1:1), the chromatogram showed an absorption (R_f value 0.06) below the 254 nm wavelength which was the same as the absorption of standard triterpene saponins.

Due to its high polarity, these saponins are easily soluble in water. The more this saponin content dissolved in water, the more components of this saponin compound were degraded (Wang *et al.*, 2018; Mahfudh *et al.*, 2021). The degradation of the saponin content may cause a decrease in insecticidal ingredient activity. The content of saponins from other plants such as turi (*Sesbania grandiflora*) is also unstable and degrades during storage so that its activity as an anti-bacterial decreases (Rufaidah, 2021).

There were several advantages of using natural insecticides from *S. sesban* based on the results of this research. By knowing the maximum length of time for storage of insecticidal ingredients (in the form of dry powder or simplicia, extracts and extract solutions), it can be developed to manufacture stocks or supplies of natural insecticides in these forms, and this can be used as a reference for making guidelines on how to manufacture and storage of stocks of natural insecticides prior to application. Making stock of this natural insecticide material can certainly increase the efficiency of its use or increase the effectiveness of its use economically.

The next advantage or benefit was the known instability of the insecticide material from *S. sesban*, namely a sharp decrease in lethal toxicity after application (after the insecticide is dissolved in water for a certain time). In this case, the lethal toxicity of the insecticide from *S. sesban* decreased drastically after 24

hours and was almost non-toxic after 72 hours in water. This means that the use of natural insecticides from *S. sesban* is safe for the environment.

In other words, that the use of insecticides from *S. sesban* will not pollute the environment, that is, it will not cause the accumulation of toxic materials at any tropic level in the food chain in the ecosystem. Thus, the use of *S. sesban* insecticide can be considered as ecologically effective.

It is known that the leaf extract of *S. sesban* only had a lethal toxic effect on *P. xylostella* larvae, as a target insect, but was not toxic or very low in toxicity to its natural enemy, *D. semiclausum* imago, as a non-target insect. Larvae of *D. semicalusum* are the main parasitoids for *P. xylostella* larvae. This means that the insecticidal agent of *S. sesban* has a narrow spectrum of toxic effect or high anti-insect selectivity for the control of cabbage caterpillars.

Another advantage of using *S. sesban* plants as a source of natural insecticides was that it is possible to manufacture and use insecticides with simple technology and low cost, which is certainly feasible for farmers. It is known that the active anti-insect content of *S. sesban* leaves is derived from the triterpene saponin compound group. This compound is classified as very polar and easily dissolved with water, so it is very easy to withdraw using water as the sole solvent (Does not require expensive chemicals). The extraction process of saponin content from *S. sesban* leaves can be done by maceration technique using vessels and other tools commonly owned by farmers (People do not have to use special equipment for extraction, such as Soxhlet, vacuum rotary evaporator and so on which are usually available in laboratories and of course not owned by farmers).

Conclusion

The insect repellent material from the leaves of *S. sesban* was unstable during the simplicia storage time, extract storage, and during exposure to the extract solution prior to the bioassay. Simplicia storage for 6 months to 12 months, extract storage for 15 days to 30 days, and exposure to extract solution for 24 to 72 hours have resulted in a drastic reduction in lethal toxicity to *P. xylostella* larvae. All treatments for storage time of insect repellent from *S. sesban* did not show a significant difference in lethal toxicity to *D. semicalusum* imago. The insect repellent from *S. sesban* from all treatments for the duration of storage of each material was almost non-lethal toxic to *D. semiclausum* imago.

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