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Evaluation of Insecticidal Performances of Jayanti Plant (*Sesbania sesban*) for Integrated Control of Cabbage Caterpillar (*Plutella xylostella*)

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Abstract: The study on the use of local natural materials as a source of insecticides is intended, among other things, to reduce farmers' dependency on using synthetic chemicals. This research was conducted to obtain the most selective insecticidal active ingredient from *Sesbania sesban* plant for controlling cabbage caterpillars, choose a target mode of action that has a safe impact on the environment, and determine insecticidal stability during storage of the material prior to application and its stability in water after application. The dried powder of *S. sesban* leaves was extracted in stages using a series of solvents, namely hexane, DCM, ethanol and water, respectively. Each of the extract fractions produced was tested for its lethal toxicity against *Plutella xylostella* larvae and *Diadegma semiclausum* imago. Each of mortality data were processed by probit analysis to produce LC_{50}(s) to determine their insecticidal selectivity. Subsequent bioassays were carried out using extract-water of *S. sesban* leaves and the data were processed by probit analysis to determine their anti-ovipositor, ovicidal powers and anti-feedant properties against *P. xylostella*. The insecticidal stability of *S. sesban* was also studied according to variations in the storage time of the simplicia before extraction, the storage time of the extract before dissolution, and its stability in water during application. The data was processed using ANOVA. The results show that the insecticidal activity of the ethanol extract fraction of *S. sesban* leaves for controlling cabbage caterpillars was very selective, namely very toxic to *P. xylostella* but very less toxic to *D. semiclausum*. Against *P. xylostella*, *S. sesban* leaf extract has also been proven to significantly prevent oviposition, inhibit egg hatching and feeding activity. The insecticidal power of *S. sesban* did not decrease significantly during storage of the simplicia for less than three months and during storage of the extract for less than seven days. However, the toxicity of the extract solution decreased drastically after 24 hours of application.

Keywords: *Diadegma semiclausum*; Insecticidal performances; *Plutella xylostella*; *Sesbania sesban*

Introduction

Cabbage crop failure is reported to often occur due to attacks by cabbage caterpillars (*Plutella xylostella* larvae) on cabbage leaves. The important impact of this cabbage caterpillar attack is that cabbage crop production can decrease by up to 90%. The widespread use of insecticides to eradicate cabbage pests began in the 1960s, at that time cabbage caterpillar pests were already a serious problem. The use of synthetic chemical insecticides to control this pest in the world reaches more than 1 billion US dollars per year (Isman, 2020; D. Wang et al., 2022).

To reduce dependence on the use of insecticides in controlling cabbage caterpillars, this has actually been done by implementing an integrated pest control strategy, namely developing a system of intercropping both spatially and temporally between cabbage plants and other types of plants which can support the existence and survival of natural enemy organisms, such

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as the parasitoid of the cabbage caterpillar, namely *Diadegma semiclausum* (Li et al., 2023; Ouyang et al., 2023).

However, the practice of controlling pests by using synthetic insecticides excessively creates new problems, namely increasing resistance and resurgence of pests, decreasing populations of natural enemies (parasitoids and predators) or other non-target organisms (Tatiya et al., 2013) and causing pollution environment (Francis et al., 2002). Synthetic chemical insecticides in the environment generally cannot be degraded easily and quickly so they can accumulate at various trophic levels in food chains or food webs in ecosystems (Coats, 2014; Suripto et al., 2023).

This has been done by implementing an integrated pest control strategy, namely developing an intercropping system both spatially and temporally between cabbage plants and other types of plants that can support the existence and survival of natural enemies of cabbage caterpillars, such as *Diadegma semiclausum* (Gassmann et al., 2023; Shakeel et al., 2017).

However, so far it is not known how the application of an intercropping system to cabbage plants can increase the ability (abundance and parasitoidal power) of natural enemy organisms or biological agents, in this case is *D. semiclausum* in controlling pest insect population, in this case is the cabbage caterpillar (Barbosa et al., 2019). Thus, controlling insect pests using insecticides must still be carried out but using materials that do not cause new problems as described above. For that, then studied the use of insecticides from natural materials.

It is known that the jayanti plant (*Sesbania sesban*) especially the organ parts of the leaves contain compounds from the saponin group which have insect repellent properties. The anti-insect performance of natural insecticides from *S. sesban* plant was further studied to evaluate the anti-insect performances, especially for the control of cabbage caterpillars. Evaluation of insecticidal performances in question includes the spectrum of lethal toxic effects or insecticidal selectivity, physiologically, other insecticidal modes of action, namely anti-ovipositor, ovocidal, and anti-feedant powers and their insecticidal stability during material storage and their stability in the environment after application.

To reduce dependence on the use of insecticides from synthetic chemicals, it is necessary to study the use of natural insecticides. Studies need to be directed primarily at obtaining anti-insect active ingredients from *S. sesban* that are selective, do not decrease their insecticidal power during storage of the material for a certain period of time and whose active ingredients are easily and quickly degraded after application.

Based on the background of the problems above, this study was conducted to determine the insecticidal selectivity of various *S. sesban* leaf extract fractions for control of cabbage caterpillars, to determine the anti-ovipositor, ovicidal and anti-feedant properties of *S. sesban* leaf extract-water against *P. xylostella* and the insecticidal stability of *S. sesban* according to variations in material storage time before application and its stability in the environment after application.

Method

Preparation of S. sesban Insecticides

To obtain anti-insect active ingredients from S. sesban leaves, the dry powder or what is often referred to as simplicia of plant leaves is extracted liquid-solid in stages according to a modified method from (El-Emam et al. (2015). Stratified extractions use a series of solvents with increasing polarity as has been done by Hardani et al. (2023), Suripto et al. (2017) and Suripto et al. (2020), namely hexane, dichloromethane (DCM), ethanol and water respectively. In general, the stratified extraction of *S. sesban* leaves can be seen in Figure 1.

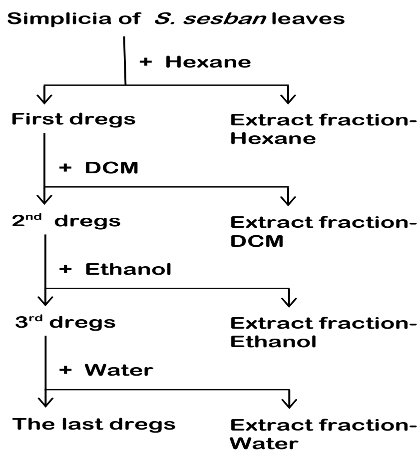

Figure 1. Workflow chart of stratified extraction of *S. sesban* leaves

Each of the *S. sesban* leaf extract fractions produced was then tested for its lethal toxicity against *Plutella xylostella* larvae and *Diadegma semiclausum* imago. This bioassay setting was carried out to determine the spectrum of toxic effects or insecticidal selectivity in controlling insect pests, in this case P. xylostella as the target insect (pest) and *D. semiclausum* as the non-target insect (parasitoid for *P. xylostella* larvae).

In addition to multilevel extraction, *S. sesban* leaves were also extracted directly in one step using water as the sole solvent. The extract-water of *S. sesban* leaves was then used for various biological tests against

P. xylostella, namely anti-ovipositor, ovicidal, larvicidal and anti-feedant tests using modified methods from Nenotek et al. (2022), Peng et al. (2023) and Roy et al. (2023).

The extract of *S. sesban* leaves, especially produced from a single extraction using water as the solvent, was used to test its anti-insect stability according to variations in the length of time of simplicia storage before being extracted, the length of time extract storage before being dissolved, and the length of time of extract solution after application in the environment, as were done by Mossa et al. (2018), Suripto et al. (2020) and Suripto et al. (2023).

Bioassays of S. sesban Insecticidal Performances

The spectrum of toxic effects or insecticidal selectivity of *S. sesban* for controlling cabbage caterpillars was studied by conducting a lethal toxicity test of each fraction of *S. sesban* leaf extract resulting from multilevel extraction on *P. xylostella* larvae as target insects and on *Diadegma semiclausum* imago as non-target insects according to the method used by (Fan et al. (2023), Mossa et al. (2018), Suripto et al. (2017), and Suripto et al. (2020).

Data on the mortality of *P. xylostella* larvae and *D. semiclausum* imago respectively were processed using probit analysis to determine LC_{50}. The ratio of LC_{50} for P. *xylostella* to LC_{50} for *D. semiclausum* was used to express insecticidal selectivity (S). If S<1, it means that the insect repellent being tested is selective and if S>1 means it is not selective (Al-Noor et al., 2023; Z.-J. Wang et al., 2023). The work flow chart of this insecticidal selectivity of *S. sesban* study can be seen in Figure 2.

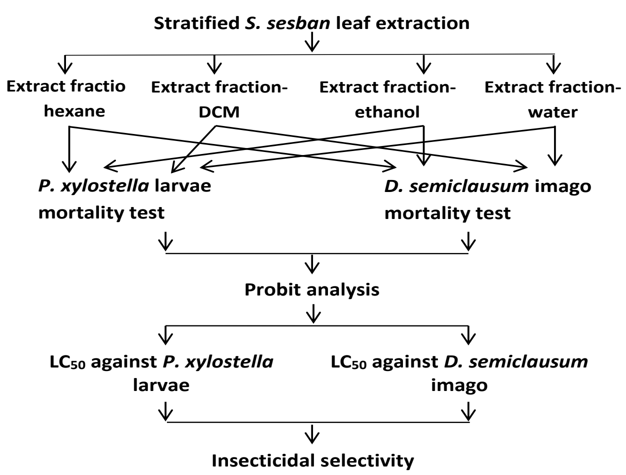

Figure 2. Workflow chart of the study of insecticidal selectivity of *S. sesban*

In particular, the extract-water (the result of a single extraction using water as the sole solvent) of *S. sesban* leaf was used to test various modes of insecticidal action only against *P. xylostella*, namely anti-ovipositor, ovicidal, anti-feedant, and larvicidal actions. Each data from the results of these tests was processed using probit analysis to produce outcomes, namely EC_{50} for anti-ovipositor power, EC_{50} for ovicidal performance, EC_{50} for anti feedant power, and LC_{50} for larvacidal performance, as was done by Suripto et al. (2020). The workflow chart of the insecticidal modes of action research from *S. sesban* leaves against *P. xylostella* can be seen in Figure 3.

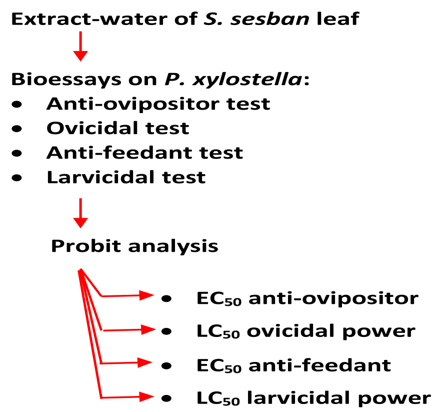

Figure 3. Workflow chart of research on modes of action of *S. sesban* insecticides

The extract-water of *S. sesban* leaves was also tested for its insecticidal stability against *P. xylostella* larvae according to variations in storage time of *S. sesban* insecticidal material, namely 3, 6 and 12 months for storage of simplicia before extraction, 7, 15 and 30 days for storage of the extract before dissolving and 24, 48 and 72 hours for exposure of the solution to the environment after application. The work steps of the insecticidal performance research according to variations in the length of time of storage of insecticides from *S. sesban* can be seen in Figure 4.

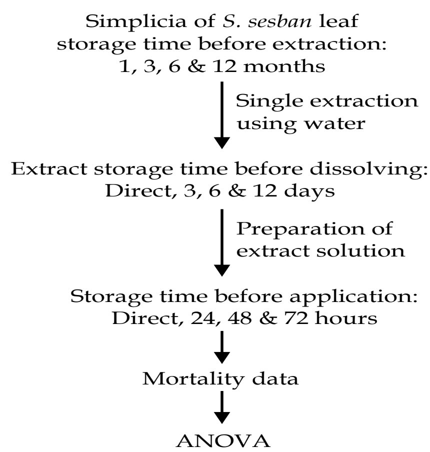

Figure 4. Work flow chart of research on insecticidal performance according to variations in length of time of storage of *S. sesban* insecticides

P. xylostella larvae mortality data from each of the above bioassays were processed with ANOVA to determine the significance of the differences between the treatments being compared, namely variations in storage time for simplicia before extraction, variations in storage time for extracts before dissolving, and variations in the length of solution in water after application. The methods mentioned above were carried out as were done by Suripto et al. (2023).

Result and Discussion

Insecticidal Selectivity of S. sesban Insecticide

Of the four extract fractions of *S. sesban* leaf produced by stratified extraction, only the extract fraction-ethanol has selective insecticidal properties, namely its lethal toxicity is higher against *P. xylostella* (pest insects or target insects) than against *D. semiclausum* (natural enemy insects or non-target insects) (Table 1).

Table 1. Insecticidal Selectivity of Various Extract Fractions of *S. sesban* Leaf against *P. xylostella* as a Target Insect and *D. semoclausum* as a Non-Target Insect

Extract fraction	LC_{50} (ppm)		Insecticidal selectivity	
	Px	Ds	Value	Level
Hexane	343.71	305.55	0.89	Not selective
DCM	294.78	121.56	0.41	Not selective
Ethanol	29.94	37.38	1.25	Selective
Water	1197.10	1043.70	0.87	Not selective

Information: Px = *Plutella xylostella*; Ds = *Diadegma semiclausum*

The extract fraction-ethanol of *S. sesban* leaf was the result of stratified extraction using ethanol as a polar withdrawing solvent. So that at this level of extraction the compounds extracted from the leaves of *S. sesban* are mainly from the polar compound group. Several reports have stated that the active anti-insect content of the *S. sesban* plant is a polar compound from the triterpene saponin group (Hussain et al., 2019; Tatiya et al., 2013; Wulandari et al., 2023).

Previously, Suripto et al. (2017) reported that of the four *S. sesban* leaf extract fractions examined (extract fractions: -petroleum ether, -chloroform, -ethanol, and -water), only the extract fraction-ethanol had a relatively very high saponin content. On shaking the extract solution in the test tube, a steady foam formation occurs (the foam height in the tube is more than 3 cm) even though 10% HCl has been dropped.

In other extract fractions, the height of the foam formed was less than 1 cm and decreased after 10% HCl was added. The results of examining the saponin content were supported by the results of thin layer chromatography (TLC) by Suripto et al. (2020). In TLC with the Hexane-EtOac developer eluent (1:1), the chromatogram showed yellow and brown spots (an indication for the presence of triterpene saponins) which were relatively clear in the ethanol-extract fraction sample compared to other extract fractions. The active anti-insect materials in the form of saponins from *S. sesban* was selective, which was highly lethal toxic to the larval life stage of target insects such as *P. xylostella* but much lower in toxicity to non-target insects, namely *D. semiclausum* imago.

Saponin compounds can affect the membrane permeability of nerve cells of larval stage organisms. Changes in the permeability of the nerve cell membrane can interfere with transmission which results in the transfer of acetolytics that are too fast. This tremendous accumulation of acetololis causes rapid muscle spasm followed by swelling and paralysis of the muscles which eventually leads to the death of the larvae (Francis et al., 2002; Ran et al., 2022).

The modes of anti-insect action of the saponin content of *S. sesban* extract as described above did not occur in the adult or imago phase of the insect as was observed in the *D. semiclausum* imago mortality test (Suripto et al., 2017). Through the larvicidal mode of action test mentioned above, the use of natural insecticides from *S. sesban* was declared physiologically selective for the control of cabbage caterpillars (*P. xylostella*).

However, because the pest control was directed against the larval phase that had attacked the cabbage plants, the cabbage plants were still damaged (Their values of nutritional and taste decreases) even though the cabbage caterpillars were eventually eradicated) (Zhang et al., 2023).

Thus, other insecticidal modes of action were also studied, such as anti-ovipositor, anti-hatching and anti-feedant. Which life phase of the pest insect is selected as the control target will determine the ecological selectivity of the insecticide. The life phase of pest insects that were successfully suppressed with insecticides but did not leave any damage such as decreased nutritional and taste values in cabbage plants deserve to be selected as control targets.

It has been stated previously that the extract fraction-ethanol of *S. sesban* leaves was the most selective compared to other extract fractions for controlling cabbage caterpillars. The insecticidal content of this extract fraction has been confirmed as a polar chemical compound which is a triterpene saponin group. Thus, this polar saponin content can actually also be withdrawn through a single extraction using water as the solvent. The use of water which can extract the active anti-insect content from natural materials has an important meaning for the development of the use of natural materials as a source of pesticides because it is cheap and easy for people to use and is environmentally safe.

Making and using pesticides from natural materials, for example the *S. sesban* plant, which is cheap, easy, proven to have a narrow spectrum of toxic effects and is environmentally safe is not only in accordance with an integrated pest control program, but is also really needed by farmers to increase the quantity and quality of their agricultural production. Another important meaning is that farmers' appreciation for natural sources, such as the *S. sesban* plant, has increased (Munde et al., 2023).

Making and applying natural pesticides that are easy and environmentally safe can also be a means of increasing awareness and active participation of the community and students in maintaining environmental health and making sustainable use of natural resources. These events take place both in the form of community service activities, field and laboratory practicums, as well as daily life activities, as has been reported by Fetiana et al. (2022) and Zulkarnaen et al. (2023).

For this reason, larvicidal, anti-ovipositor, ovocidal and anti-feedant tests against *P. xylostella* were carried out using only extract-water of *S. sesban* leaves. The results of the study showed that Extract-water of *S. sesban* leaf not only killed the larvae but also prevented oviposition, thwarted egg hatching and inhibited the feeding activity of *P. xylostella* in cabbage plants (Table 2).

Table 2. Concentrations of *S. sesban* Leaf Extract Capable of Killing Larvae, Preventing Oviposition, Thwarting Egg Hatching, and Inhibiting the Feeding Activity of *P. xylostella* in Cabbage Plants

Variables of insecticidal power against *P. xylostella*	LC_{50} or EC_{50} (ppm)
Larvicidal power	40.89
Anti-ovipositor	20.52
Ovicidal power	26.77
Anti-feedant	48.24

However, taking into account the results of previous studies, namely that administration of *S. Sesban* leaf extract can also kill *D. Semiclausum* imago (LC_{50}: 37.38 ppm) (Suripto et al., 2017), the insecticidal activity of *S. sesban* is stated to be selective if the target of control is aimed at inhibiting oviposition and inhibiting egg hatching of *P. xylostella* (LC_{50} or EC_{50} to *P. Xylostella* > LC_{50} to *D. Semiclausum*) and not selective, if the target of control is aimed at inhibiting feeding activity (anti feedant) and killing larvae of *P. xylostella* (EC_{50} or LC_{50} against *P. Xylostella* < LC_{50} against *D. Semiclausum*).

Although the use of *S. sesban* leaf extract succeeded in egg thwarting hatching of P. xylostella eggs selectively (without killing its natural enemies insect), the deleterious effects of oviposition that occurred before control had affected cabbage plants. According to Shakeel et al. (2017), Suripto et al. (2022) and Zhang et al. (2023), the effects of laying *P. xylostella* eggs on cabbage plants include damage to the lower epidermal tissue of the cabbage leaf organs and the production of secondary metabolites in these tissues as an allelopathic response of cabbage plants to the presence of *P. xylostella* eggs. This causes a decrease in nutritional value and reduces the taste and visual taste of the cabbage leaves. Thus, control by inhibiting egg hatching (ovicidal agents) is considered ineffective or not ecologically selective.

Based on the above results, the control of *P. xylostella* by inhibiting or preventing oviposition in cabbage plants is considered the most suitable strategy. Cabbage caterpillar control using the extract fraction-ethanol of *S. sesban* leaves as an anti-ovipositor agent proved to be selective, namely a concentration of 20.52 ppm was enough to inhibit 50% of the oviposition of *P. xylostella* (EC_{50}: 20.52 ppm) in cabbage plants (Suripto et al., 2020) but not enough to kill 50% of the *D. semiclausum* imago population (LC_{50}: 37.38 ppm) (Suripto et al., 2017). It has also been reported that administration of extract-water (the result of single extraction using water as a solvent-attractor) of *S. sesban* leaves at a concentration of 478.63 ppm *in situ* can inhibit 50% oviposition of *P. xylostella* in cabbage plants (Suripto et al., 2022).

The results above show evidence of the importance of selective pest control both physiologically and ecologically, and this supports the message of the importance of selective pest control, as reported by (Sun et al. (2023).

After the insecticidal effect spectrum of *S. sesban* for controlling *P. xylostella* was known, its stability during storage before application and its stability in water in the environment after application were studied. It is important to know how long the storage of insecticides does not cause a significant reduction in their insecticidal power before application (prospects for economic advantage) and how long after application causes the insecticides to become non-toxic in the environment (prospects of excellence for environmental safety).

The results of the study showed that storage of *S. sesban* leaf simplicia for up to 3 months did not significantly decrease its insecticidal power against *P. xylostella*, but after 6 months of storage, its toxicity decreased drastically and after storage for more than 12 months, the substance was not toxic (Figure 5).

Based on the results above, it can be recommended that the insecticidal material from *S. sesban* leaf in the form of simplicia (dry powder) can be stored for a maximum of three months before being extracted so that its insecticidal power does not decrease significantly, especially against *P. xylostella*.

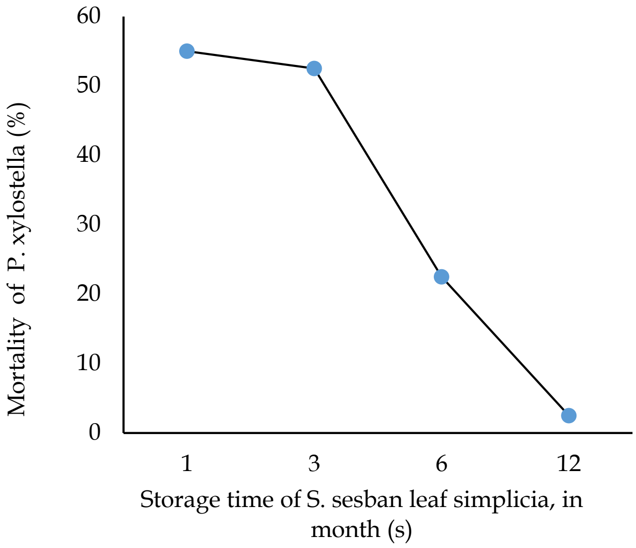

Figure 5. Mortality of *P. xylostella* larvae in 28 ppm treatment of *S. sesban* leaf extract-water according to variations in simplicia storage time before extraction

The active anti-insect content in the form of saponins in simplicia leaves of S. sesban, which is one of the secondary metabolite products, may be stable as long as it is not exposed to sunlight (heat and ultraviolet) and is not in high humidity conditions. According to El-Emam et al. (2015) and Isman (2020), bioactive materials of plant origin are generally thermolabile, the chemical structure of the active groups can change when exposed to ultraviolet light and are biodegradable in high humidity conditions.

The length of storage of natural insecticides of *S. sesban* in the form of leaf extracts before dissolving also affects their insecticidal power against *P. xylostella*. Observations showed that storage of *S. sesban* leaf extract for one day to seven days did not significantly reduce its lethal toxicity to *P. xylostella* larvae, but storage for 15 days significantly reduced its toxicity. Storage of the extract for more than 30 days causes the material to become non-lethal toxic to *P. xylostella* (Figure 6).

The stability of the active insecticide ingredients in the extract can be maintained by preventing it from being exposed to light by storing it in a dark bottle (the walls of the bottle are opaque) and storing it in a dry, non-humid room or it is highly recommended that it be stored in the refrigerator. When compared with extracts, based on the stability of the active ingredient, insecticide ingredients in the form of dry leaf powder or simplicia are more durable. The durability of natural insecticides provides significant economic and practical value both for the agricultural industry and for farmers in general.

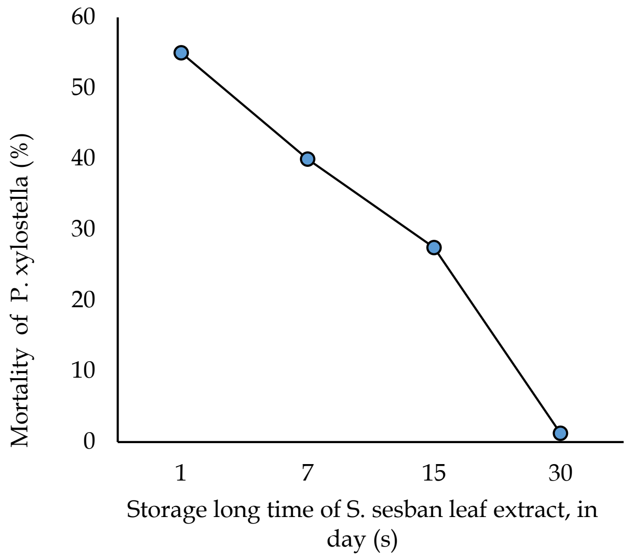

Figure 6. Mortality of *P. xylostella* larvae in 28 ppm treatment of *S. sesban* leaf extract-water according to variations in length of storage of the extract before dissolving

Based on these results, it is suggested that the length of storage of the extract, if necessary, is a maximum of seven days before it is dissolved in water for application. If a solution of *S. sesban* leaf extract has been prepared, it should be applied immediately to control *P. xylostella*. This is because if the solution is stored for up to 24 hours before application, its toxicity to *P. xylostella* decreases drastically, even if it is stored for more than 72 hours, the material becomes non-toxic (Figure 7).

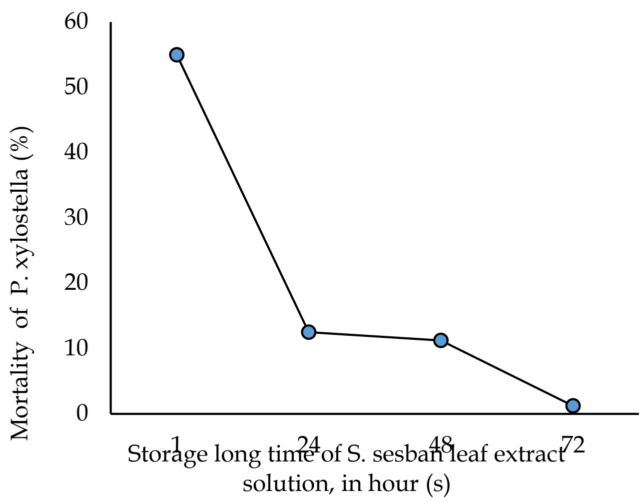

Figure 7. Mortality (%) of *P. xylostella* larvae in 28 ppm treatment of *S. sesban* leaf extract-water according to variation of storage time of extract solution

Based on these results, it can be interpreted that the toxicity of the insecticide from *S. sesban* plant decreased drastically in the environment after 24 hours of application and even became completely non-toxic after more than 72 hours after application. So that the use of *S. sesban* leaf extract to control cabbage caterpillars is safe for the environment.

The advantages of using extract-water of *S. sesban* leaf (single extraction using water as a solvent) compared to the extract fractions (stratified extraction using a series of organic compounds) apart from having high insecticidal selectivity and being safe for the environment, are also feasible (technically easy and economically affordable) for farmers to create and apply it in the field.

The anti-insect performance of the S. sesban plant that has been studied above and the characteristics of the plant itself are in accordance with the criteria for selecting plants as a source of natural insecticide (Gassmann et al., 2023; Priya et al., 2018; Rohyani, 2021; Suripto et al., 2023), i,e, high toxicity to target insects but very low toxicity or non-toxic to natural enemies as non-target organisms, the insecticide source plants are locally available, with high yields of insecticides per plant and per unit area of planted land. The resulting insecticide does not lose its potency during storage for at least one year. Plant type, especially perennials, propagates mainly by seeds, resistant to lack of water for use in dry areas or in the form of aquatic or semi-aquatic plants for direct use in target insect habitats, high growth and propagation speed and easy to cultivate. Adaptability of insecticide source plants are high against changes in local environmental conditions. Active ingredients can be extracted (extracted) with simple equipment using readily available solvents, and the resulting extracts can be dissolved in water for application. Growth habits and requirements, toxicity and some medical properties of insecticide source plants are well known by the local population. Customary considerations, namely source plants that are not considered by the local community to have magical values or are often used in religious ceremonies.

Increasing public appreciation especially from farmers and students, for the *S. sesban* plant can be achieved through the introduction of science and technology regarding other superior benefits of this plant. Apart from being a source of natural insecticide, which has been proven effective for controlling cabbage caterpillars, the *S. sesban* plant is also a source of natural medicines and can be developed as a phytoremediator to restore soil fertility in fields (Abd_Allah et al., 2015; Din et al., 2020; Nohwar et al., 2019; Patra et al., 2020).

Conclusion

It has been proven that *S. sesban* leaf extract is very toxic to *P. xylostella* but very less toxic to its natural enemy, *D. semiclausum*. *S. sesban* leaf extract can also prevent ovipositor activity, inhibit egg hatching, inhibit feeding activity, and kill *P. xylostella* larvae. It has been confirmed that the active anti-insect ingredients from *S. sesban* leaves belong to the triterpene saponin group. The bioactive ingredients from *S. sesban* remained active for three months of storing the dry leaf powder, for seven days of storing the extract, and the toxicity decreased drastically after 24 hours of the extract solution being applied.

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Author Contributions

Conceptualization, S.; methodology, R.N.R. and S.; validation, H.A. and L.J.; formal analysis, R.N.R. and S.; investigation, L. J. , H.A. and S.; resources, H.A., L.J. and S.; data curation, H.A. & L.J. : writing—original draft preparation, L.J., R.N.R. and S.; writing—review and editing, L. J. & R.N.R.; visualization, H.A., R.N.R. and S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interests

The authors declare there are no conflicts of interest.

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