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Anti-Ovipositor Power of Jayanti Plant (*Sesbania sesban*) for Integrated Control of Cabbage Pest (*Plutella xylostella*)

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Abstract: Cabbage harvest failure is often caused by failure to control cabbage pests. Cabbage pest control by eradicating *Plutella xylostella* larvae is considered less effective because the cabbage plant has been damaged due to infection with *P. xylostella* eggs and larvae. Thus, the control target needs to be shifted to prevent *P. xylostella* amago from laying eggs (ova-positioning) on cabbage plants. This study aims to determine the anti-ovipositor power of *Sesbania sesban* leaf extract against *P. xylostella*. The leaves of *S. sesban* were air-dried and then ground. The dry powder (simplicia) of *S. sesban* leaves was extracted with water as the sole solvent. The water-extract of *S. sesban* leaves was tested for anti-ovipositor against *P. xylostella* in situ on cabbage plants according to a completely randomized block design. Experimental groups were made according to variations in extract concentration. Complete randomization was carried out on experimental units in each group according to the variation in the length of exposure to the extract before access to the ovipositor was opened (Cages were opened after 1,2,3,4,5 and 6 days from the time of extract spraying). Each experimental unit consisted of 4 cabbage plants as replicates. The treatment was given by spraying a solution of *S. sesban* leaf extract with a concentration according to the group evenly on the entire leaf surface of each cabbage plant. The observed research variable was the number of *P. xylostella* eggs per cabbage plant after the cage of cabbage plant unit was opened. The results showed that the leaf extract of *S. sesban* could inhibit *P. xylostella* as an ovipositor to lay its eggs on cabbage plants effectively for up to three days. With concentrations of 1.83 to 2.14 ppm, the aqueous extract of *S. sesban* leaves did not inhibit *P. xylostella* egg laying, but with concentrations of 478.63 to 1283.88 ppm it could inhibit 50 to 100%.

Keywords: Anti-ovipositor, cabbage plant, *Plutella xylostella* and *Sesbania sesban*

Introduction

It has often been reported that crop failures for cabbage are generally caused by uncontrolled pest attacks on these plants. Pests that often cause cabbage crop failure are larvae of *Plutella xylostella*. Cabbage caterpillar control by using insecticides from synthetic chemical compounds often creates new problems, such as the emergence of pest resurgence and environmental contamination (Nurfajriani *at al*., 2022). So that later, the use of natural insecticides was also studied to reduce farmers' dependence on the use of synthetic compound pesticides.

It has been reported that natural insecticides from the jayanti plant (*Sesbania sesban*) have been shown to kill cabbage caterpillars or *Plutella xylostella* larvae with a narrow effect spectrum. The narrow spectrum of effect in question is very lethal toxic to *P. xylostella* as insect pests (target organisms) but not toxic or very low toxicity to natural enemies (non-target organisms). According to Suripto *et al*. (2017), the ethanol-extract fraction of *S. sesban* leaves was lethally toxic selectively to *P. xylostella* larvae, but other extract fractions,

namely hexane and dichloromethane (DCM) fractions, was respectively not selective in its toxicity. Each hexane and DCM-extract fraction mentioned above was not only lethally toxic to *P. xylostella* larvae, but also lethally toxic to the natural enemy of the cabbage caterpillar, namely imago *Diadegma semiclausum*. Larvae of *D. semiclausum* are the main parasitoids for *P. xylostella* larvae.

The ethanol-extract fraction is a polar fraction, which means it is easily soluble in water, so that the content of insect repellent active ingredients in this extract fraction can be extracted directly by a single extraction using water as the sole solvent.

The use of natural insecticides is also considered safe for the environment because natural chemicals are generally easily soluble in water and unstable, easily and quickly degraded (Suripto *et al*., 2021; Susanti *et al*., 2015).

Although the use of natural insecticides from *S. sesban* is selective to kill *P. xylostella* larvae in cabbage plants, this is still considered ineffective, because cabbage plants have been damaged by *P. xylostella* larvae before the larvae die. Likewise, the results of controlling the cabbage pest, P. xylostella, which were directed at inhibiting the activity of larvae eating cabbage leaves and inhibiting the hatching of *P. xylostella* eggs were considered less effective, because the cabbage plants remained somewhat damaged and dirty due to infection by the eggs of these pests (Suripto *et al*., 2020). Thus, the control target needs to be directed at preventing female *P. xylostella* imago as ovipositors from laying their eggs on cabbage plants.

The female imago of *P. xylostella* chose cabbage plants to lay their eggs because it was not just instinct, that cabbage leaves could be a source of food for the larvae in the future, however, because it was attracted by the presence of attractant compounds in cabbage plants, especially in the leaves (Herlinda *et al*. (2004). The compounds that attract *P. xylostella* to lay their eggs on cabbage plants are thioglucosides or glucosinolates (Sari, 2016). These attractant compounds are generally volatile so that they can be easily and quickly detected by the ovipositor (Susniahti *et al.*, 2017; Sari *et al*., 2018).

To obscure the attractiveness of attractant compounds produced by the host plant to ovipositor insects, it can be done by inhibiting evaporation or reducing the evaporation power. The rate of evaporation of attractants can be reduced by increasing their polarity by adding more polar compounds such as water and other polar chemical compounds (Solichah *et al*., 2014; Sari *et al*., 2018).

It is suspected that the saponin content of *S. sesban* leaf extract which has a high polarity, which is close to the polarity of water can inhibit the evaporation of attractants in cabbage plants. Thus, the administration of natural insecticides in the form of leaf extract of *S. sesban* is thought to be able to inhibit the attractiveness of the attractants produced by cabbage plants to adult female *P. xylostella*.

The saponin content of S. sesban leaf extract was also thought to be able to reject the presence of *P. xylostella* imago. This is in accordance with the observations made by Suripto *et al*. (2020), that water-extract from *S. sesban* leaves can kill larvae, inhibit egg hatching, and inhibit feeding activity of *P. xylostella* larvae in cabbage plants. However, because the water-extract of S. sesban leaves tends to be polar, there is no report yet on how long it is active as an ovipositor repellent after being exposed to the environment.

From the description of the background of the problems above, the research problems can be formulated as follows:

1) What is the concentration (in ppm) of S. sesban leaf extract that has not been able to reject, 50% and 100% rejected P. xylostella as an ovipositor in cabbage plants?
2) How long (in days) was the anti-ovipositor activity of S. sesban leaf extract after exposure to the environment on cabbage plants?

Based on the formulation of the problem above, the research was carried out with the following objectives:

1) Determination of EC_0, EC_{50} and EC_{100} (respectively in ppm) of anti-ovipositor of *S. sesban* leaf water-extract against *P. xylostella* in cabbage plants

2) Knowing how long (in days) the leaf extract of *S. sesban* was active as an anti-ovipositor against *P. xylostella* in cabbage plants.

Materials and Methods

Material preparation

S. sesban leaves were collected from mature plants, which were 1 year old or more or those that have flowered. According to Hamburger and Hostettmann (2011), bioactive ingredients of plant origin are generally secondary metabolites. These secondary metabolites are produced mainly by plants that are mature or have entered the generative phase which is characterized by the presence of inflorescences.

The leaves were aerated-dried and then ground. The leaf dry powder was extracted using water as the sole solvent by the procedure according to Suripto *et al*. (2017).

The water-extract of *S. sesban* leaves was filtered to be separated from the dregs. The water from the filtrate was evaporated using a vacuum rotary evaporator. The resulting extract paste was then dissolved in water for bioassay in six concentration levels, namely 0, 150, 300, 400, 500, and 600 ppm.

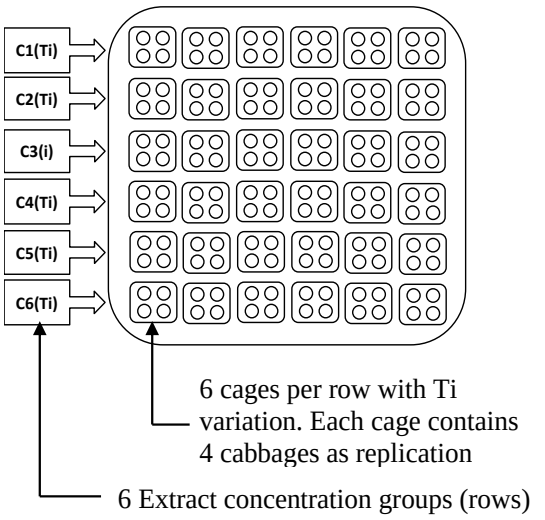

Figure 1. Completely randomized design of the anti-ovipositor test of *S. sesban* leaf extract against *P. xylostella* in situ in a cabbage garden.

Experimental design

The ovipositor rejection test of *S. sesban* leaf extract against *P. xylostella* was carried out *in situ* on cabbage plants according to a completely randomized block design (Figure 1).

Cabbage garden with an area of 12 m x 12 m, divided into 6 treatment groups with extract concentration level (6 rows). Each group consisted of six experimental units or 6 cages (6 levels of time length in days between spraying the extract and when the cage was opened). Each experimental unit (each cage) contained 4 cabbage plants as replicates.

Bioassay procedures

After cabbage transplantation, which was carried out by the procedure previously performed by Marlina ***ET AL***. (2021)**, IN THE MORNING EACH EXPERIMENTAL CABBAGE PLANT UNIT WAS SPRAYED WITH A SOLUTION OF *S SESBAN* LEAF EXTRACT (5 ML PER PLANT) WITH A CONCENTRATION ACCORDING TO THE CONCENTRATION TREATMENT GROUP. AFTER SPRAYING THE EXTRACT, EACH EXPERIMENTAL UNIT (CONSISTS OF 4 CABBAGE PLANTS AS REPLICATION) WAS CONFINED USING NYLON CLOTH.** The cage was opened in the morning on a certain day according to the variation in the length of time (in day) that has been determined between the time of spraying the extract and the time of opening the cage.

Observations were made one day after the cage was opened on the number of *P. xylostella* eggs that infect each cabbage plant. The eggs of *P. xylostella* can be distinguished from the eggs of other insects that are also commonly found in cabbage plants, by the characteristics of an oval shape measuring about 3 mm x 6 mm, greenish yellow from laying up to 3 days turning white (Barbosa *et al*., 2019; Herlinda *et al*., 2004; Susniahti *et al*., 2017)(Figure 2).

Data analysis

Anti-ovipositor power or percentage of egg laying inhibition (I) was calculated using the following formula:

$$I = 100\ \% \times (N_0 - N_i)/N_0$$

Where,
N_0 *is the percentage of egg laying inhibition in the control*
Ni *is the percentage of inhibition of egg laying in treatment i*

(a) (b)

Figure 2. *Plutella xylostella* eggs since laying are greenish yellow (a) and after three days they turn reddish white (b)

Anti-ovipositor power data (Inhibition level in %) from each treatment set (same Ti) was processed by probit analysis to produce outputs that included EC_0, EC_{50} and EC_{100} (In ppm, respectively). EC_x is the concentration of the test substance that causes x percent of the sub lethality (repelling, growth inhibition, etc.) of the test target organism.

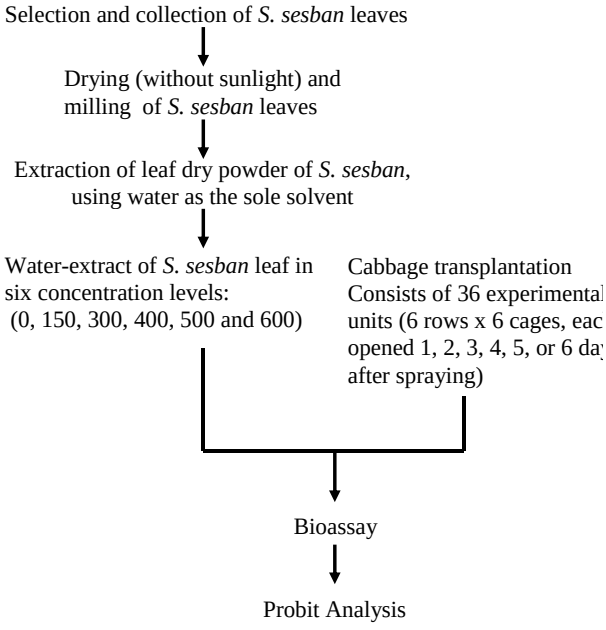

Figure 3. Flowchart of anti-ovipositor test of water-extract of *S. sesban* leaves against *P. xylostella* in cabbage plants

The overall work flow chart of the anti-ovipositor test of the water-extract of *S. sesban* leaves on *P. xylostella* in cabbage plants can be seen in Figure 3.

Results and Discussion

Anti-ovipositor power stability

The anti-ovipositor activity of *S. sesban* leaf extract against *P. xylostella* slightly decreased after the extract was exposed to the environment for 1 to 3 days, especially at high concentrations. For example, the anti-ovipositor power decreased slightly from 100% to 91.67% on the first day to the third day, but decreased drastically after 4 to 6 days, i.e. to 58.33 to 14% (Figure 4).

In the treatment of concentrations of 500 ppm and below, namely 300 and 150 ppm, the anti-ovipositor power of the leaf water-extract of *S. sesban* has decreased drastically since two to three days after spraying. This indicates that the stability of the insect repellent material from the *S. sesban* plant decreases in line with the lower concentration of the extract and the increase in exposure time of the extract to the environment. This is because the active anti-insect ingredients of *S. sesban* are derived from the saponin compound group. According to Suripto *et al.* (2017), the active anti-insect ingredient of *S. sesban* was derived from the triterpene saponin compound group.

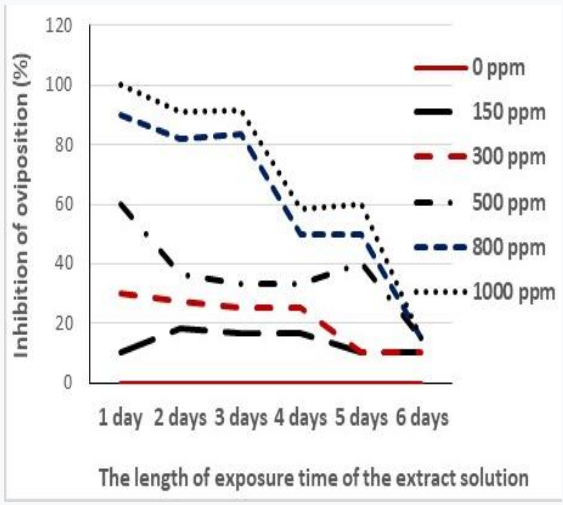

Figure 4. Anti-ovipositor power of *S. sesban* leaf extract for 6 days against *P. xylostella* in cabbage plants.

Evidence of the presence of saponins was indicated by the formation of foam in the extract solution which had been shaken in a test tube, which remained steady even after the drops of 10% HCl solution. The results of the examination of the saponin content of *S. sesban* above were supported by the results of thin layer chromatography (TLC). In TLC with hexane-EtOAs (1:1), the chromatogram showed more distinct yellow and brown spots in the polar extract fraction, which had the highest saponin content than the non-polar extract fraction, which had much lower saponin content.

Saponins are generally polar compounds, which are easily soluble in water so that they are easily and quickly degraded when exposed to the environment, which causes their bioactivity to decrease (Hamburger & Hostettmann; 2011; Mahato & Nandy, 2011; Hossain *et al*., 2007)).

The use of insecticides from natural materials which are generally unstable so that they are easily and quickly degraded in the environment is important and is recommended to be applied in accordance with an integrated pest control program.

Effectiveness of anti-ovipositor power

In line with this decrease in anti-ovipositor power, the concentration that was effective for inhibiting egg laying only increased slightly on the first day to the third day, but increased significantly and sharply on the fourth day to the sixth day (Table 1).

Table 1. Effective concentration (ppm) of *S. sesban* leaf extract for inhibition of *P. xylostella* egg laying

	EC_0	EC_{50}	EC_{100}
1 day	1,83	478,63	1283,88
2 days	1,29	553,49	1621,03
3 days	1,4	584,05	1700,82
4 days	1,07	891,12	2934,46
5 days	2,14	1086,99	3279,56
6 days	0,95	12362,58	66227,14

The results above indicate that the use of *S. sesban* leaf water-extract can significantly reject or inhibit the laying of eggs of *P. xylostella* in cabbage plants for three days. The use of *S. sesban* leaf extract up to 1.83 ppm has not been able to inhibit *P. xylostella* laying eggs (EC_0), but with concentrations of 478.63 ppm (EC_{50}) and 1283.88 ppm (EC_{100}) of *S. sesban* leaf extract has been effective in inhibiting 50 to 100%.

The use of *S. sesban* leaf extract with an effective concentration of anti-ovipositor against *P. xylostella* can be chosen to be applied because it does not interfere with the population of natural enemies (parasitoids), such as *Diadegma semicalusum*. According to Suripto *et al*. (2017), leaf extract of *S. sesban* was very lethally toxic to *P. xylostella* but very low toxicity to *D. semiclausum* imago.

The choice of mode of insecticidal action in the form of inhibition of egg laying (anti-ovipositor power) was considered more important than the inhibitory action of hatching eggs and lethal toxic action on larvae (*Suripto et al*., 2020. If cabbage pest control is carried out on larvae that have infected cabbage plants, then this is not effective because the cabbage will still be damaged, even though the larvae have been controlled and die. It is also certain that the quantity and quality of cabbage yields will be greatly reduced (Suripto *et al*., 2020) (Figure 5).

(a) **(b)**

Figure 5. Cabbage plant is a bit damaged by *P. xylostella* larvae attack, even though the larvae were controlled and 50% were killed (a) and severely damaged by *P. xylostella* larvae attack and too late to control (b) (from Suripto *et al*., 2020).

Likewise, if cabbage pest control is directed at P. xylostella eggs that have infected cabbage plants, it is also considered ineffective, because cabbage has been infected with eggs, its health is disturbed and its nutritional quality

drops drastically, even though the eggs can be controlled and are 100% sterile (Suripto *et al*., 2020).

Although the damage caused by infected cabbage eggs does not cause a decrease in the quantity of the harvest, but the quality will definitely decrease drastically. The decrease in the quality of the cabbage harvest can be in the form of a decrease in nutritional value, poor color and appearance, bad taste and ultimately not being liked by consumers or buyers or not selling well.

Cabbage plants that were damaged in quality due to infection with eggs, even though the eggs had been controlled and were 100% sterile, can be seen in Figure 6.

Figure 6. Cabbage plants were damaged in quality due to infection with P. xylostella eggs, although the eggs were eventually sterile after being given pesticides.

The control strategy with the mode of action in the form of ovipositor rejection was more effective and resulted in smooth cabbage plants or no defects at all.

Thus, the control of *P. xylostella* should be directed to prevent the ovipositor from laying eggs, so that cabbage plants are not infected with eggs and are unlikely to be attacked by larvae. An example of a cabbage plant that was 100% spared from *P. xylostella* visits to lay eggs (EC_{100} anti-ovipositor from the water-extract of *S. sesban* leaves against *P. xylostella* in cabbage = 1283.88 ppm) can be seen in Figure 7.

According to the integrated pest control program, the use of pesticides, including natural pesticides with concentrations that are 100% lethal (LC_{100}) or 100% inhibiting the growth (EC_{100}) of pest populations is prohibited. The recommended pesticide concentration is LC_{50}, namely the concentration of pesticides that kill 50% of the pest population or EC_{50}, namely the concentration that is effective in inhibiting the growth of pest populations (Suripto *et al*., 2021).

Figure 7. The cabbage plant that were 100% protected from *P. xylostella* as ovipositor.

In this study, the anti-ovipositor EC_{50} of the water-extract of *S. sesban* leaves against *P. xylostella* in cabbage was 478.63 to 584.05 ppm, which was effective for up to three days. Cabbage plants that were spared 50% of *P. xylostella* ovipositor visits, even though they were infected by the eggs from the ovipositor looked smooth and fresh because they were only slightly infected (Figure 8).

For the inhibition to be effective for up to a week, a higher concentration of extract is required. In the results of this study, the EC_{50} for up to 6 days still effective at inhibiting was 12362.58 ppm. This concentration is too high so it is not safe for the environment. Administration of *S. sesban* leaf extract with the above concentration can disrupt the population of natural enemies of *P. xylostella*, such as *Diadegma semiclausum*. According to Suripto *et al*. (2017), administration of *S. sesban* leaf extract with a concentration of 1000 ppm or more can kill 50% of the *D. semiclausum* imago.

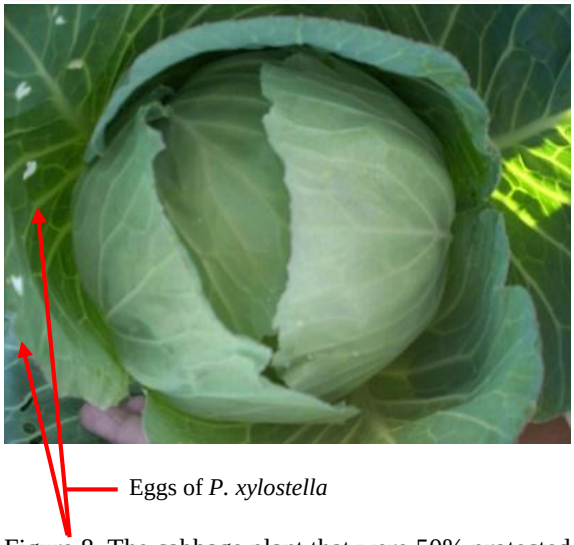

Figure 8. The cabbage plant that were 50% protected from *P. xylostella* visits to lay eggs.

The above results also showed that the insect repellent content of *S. sesban* leaf extract was unstable after four days onwards. This implies that the use of *S. sesban* leaf extract is safe for the environment. It was known that the anti-insect content of the *S. sesban* plant is derived from the triterpene saponin group (Emitaro *et al*., 2020; Suripto *et al*., 2017; Anil *et al*., 2013; Hussain *et al*., 2019).

Compounds from the triterpene saponin group are generally very polar so they are easily soluble in water. Compounds with high solubility in water will be unstable when exposed to the environment for a certain time and this causes their bioactivity, both acute lethal and acute sub lethal toxicity to decrease (Anil *at al.*, 2013; Francis *at al.*, 2012; Hamburger & Hostettmann, 2011; Yanuartono *at al.*, 2017).

The use of natural insecticides must not only be selective, that is, only toxic to insect pests (target insects) but not toxic to their natural enemies (non-targets) such as predators and parasitoids, but also to be environmentally safe after application, i.e. their bioactivity decreases or disappears after application. In the results of this study, the anti-insect activity of the leaf water-extract of *S. sesban* decreased drastically after 4 days and even almost lost its bioactivity after 6 days after being exposed to the environment.

Thus, the use of water-extract of *S. sesban* leaves for the control of cabbage pests such as *P. xylostella* can be declared safe for the environment. Because this natural insecticide from *S. sesban* for cabbage pest control is easily and quickly degraded in the environment, it is not only safe for the food chain in the environment, it is also safe for residents who consume cabbage as their daily food.

Besides being safe for the environment, the use of natural insecticides from *S. sesban* may also have superior economic value, which is relatively cheap both in production and application. The manufacture and application of natural insecticides from *S. sesban* is believed to be easy for farmers to do or feasible for farmers.

According to Suripto *et al*. (2021), the insect repellent active ingredient of *S. sesban* can be extracted using water as the sole solvent and this can be done by farmers easily. The economic advantages of the use of *S. sesban* as a natural insecticide and feasible for farmers are then expected to increase the appreciation of farmers towards *S. sesban* plants.

In fact, the *S. sesban* plant has other benefits, such as the leaves can be used as a mixture for animal feed, a medicine for vaginal discharge in women (Kathiresh *et al*., 2012), and the dregs from the extraction of the leaves can be used as green manure (Emitaro *et al*., 2020; Suripto *et al*., 2021; Yanuartono *et al*., 2017). If the benefits of *S. sesban* as a source of natural insecticides and other benefits as mentioned above are socialized to farmers, then the appreciation of farmers for this plant will increase.

However, it is very unfortunate that most of the farmers do not recognize the *S.sesban* plant as a source of natural insecticides. Even if there are, some farmers recognize the benefits of *S.sesban* as a hedge plant in rice fields where the stem size is small and the leaves, flowers and fruit are not edible, so it is not attractive for farmers to develop it as a source of firewood or additional food sources. These things caused the farmers' appreciation of the *S. sesban* plant to be low. The research results are expected to increase farmers' appreciation of *S.sesban* plants as a source of natural insecticides. In Indonesia, *S. sesban* plants have regional names, such as Jayanti (Sundanese in West Java), Bianti (Cirebon), Gianti (Java), Ganti (Lombok), and

Turi Rawa (Sumbawa) (Suripto *et al*., 2021). The habitus of S. sesban can be seen in Figure 9.

Figure 9. Habitus of *S. sesban* plant

The position of the *S. sesban* plant in Plant Taxonomy is as follows:

Divisio : Magnoliophyta
Class : Magnoliopsida
Family : Fabaceae
Genus : *Sesbania*
Species : *Sesbania sesban* L. (Merr.)

Conclusion

The water-extract of *S. sesban* leaves up to a concentration of 1.83 ppm could not inhibit the activity of *P. xylostella* as an ovipositor but concentrations of 584.05 to 1700.82 ppm were able to inhibit the egg laying of *P. xylostella* by 50 to 100% on cabbage plants until for 3 days. After 4 days of exposure to the environment, the insect repellent ingredients from the water-extract of *S. sesban* leaves are no longer active. The use of water extract of *S. sesban* leaves for population control of *P. xylostella* is recommended to choose an anti-ovipositor method with an effective concentration of EC_{50} for three days and it is not recommended to use effective concentrations for six days.

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