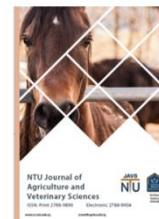




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Efficiency of Alkaloid and Phenolic Extracts of Seeds and Leaves of Sesbania Plant on Larvae of Red Flour Beetle *Tribolium castaneum* Herbest (Coleoptera : Tenebrionidae)

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ABSTRACT

The results study of efficiency of the alkaloid and phenolic extract of the seeds and leaves of the sesban plant in the average percentage of mortality the larvae of the red rusty flour beetle *T. castaneum* showed a variation in the average percentage of mortality insect larvae, are reached 62.08 and 73.75%, respectively. The results of the statistical analysis showed that there was a clear significant difference in the average percentage of mortality the larvae for both extracts. The results of the study showed a variation in the effect of the extract of the plant part of the seeds and leaves of the sesban plant on the average percentage of mortality insect larvae, which reached 67.08 and 68.75%, while the results of the effect of the concentrations of the alkaloid and phenolic extract were 0.25, 0.50, 0.75 and 1%, to the average percentage of mortality insect larvae directly proportional to The studied concentrations increased and reached 50.00, 65.00, 72.50, and 83.33%, respectively. The results of the study showed the LC50 values and the relative toxicity of the alkaloid and phenolic extract of the seeds and leaves of the Sisban plant, indicating that the alkaloid extract of the leaves of the Sisban plant was superior to the rest of the studied extracts. The LC50 value reached 0.284 and the relative toxicity was 151.41 compared to the alkaloid extract of the seeds of the Sisban plant, which gave the lowest toxicity with an LC50 value of 0.430 and a relative toxicity of 100.



Introduction :

The red flour beetle *Tribolium castaneum* Herbst, is considered one of the significant stored-product insects prevalent in most regions worldwide, especially in warm and dry areas where it has the ability to reproduce rapidly in stored materials under favorable conditions. It can cause losses of up to 15% of stored commodities in a single season, in addition to contaminating foodstuffs, rendering them unfit for consumption due to unacceptable odors and their effects on human health through secretions that cause skin irritation and allergies, and may lead to respiratory disorders [1,2]. The difficulty in controlling this insect arises from its presence alongside stored foodstuffs, and using pesticides for its control may lead to contamination of foodstuffs and the emergence of insect resistance to those pesticides. Currently, chemical pesticides are widely used worldwide to control this insect, with approximately 1.0% of those pesticides reaching the intended pests [3,4]. Despite the importance of pesticide use in controlling stored-product insects, they often degrade slowly, causing harm due to human use [5,6]. Realistic alternatives to reduce and rationalize pesticide use include adopting proper methods of use or finding alternatives that yield positive results, such as botanical pesticides or plant extracts. Among these, *Sesbania sesban*, a multipurpose plant widely distributed in tropical and subtropical regions of Africa and Asia, plays a significant role. It improves soil fertility as a source of green manure, exhibits anti-inflammatory activities, nitrogen fixation, and serves as a source of bioenergy. It is also used as an antibacterial, antiparasitic, antioxidant, and mosquito repellent [7]. found that the water extract of *Sesbania sesban* seeds had a significant effect on the eggs of potato tuber moth at 1-2 days more than eggs at 3-4 days old. Increasing the concentration of the water extract inhibited the emergence of adults from treated larvae. Additionally [8], confirmed that the phenolic extract of *Sesbania sesban* fruits and leaves gave a mortality rate of bean beetle eggs ranging from 05.67% to 77.67% for fruit and leaf extracts, respectively, at a concentration of 2% compared to the control, which reached 06.14% and 4.15%. From here, the idea emerged to use phenolic and alkaloid extracts from *Sesbania sesban* leaves and seeds as a botanical insecticide with lethal and repellent effects against flour beetles.

Materials & Methods :

Preparation of the Red Flour Beetles Insects :

Insect colony preparation involved obtaining red flour beetles *Tribolium castaneum* from infested flour. Individuals were transferred to a sterilized wheat-based diet and placed in incubators at a temperature of $28 \pm 2^\circ\text{C}$ and a relative humidity of $70 \pm 5\%$ to obtain sufficient numbers of beetles for use in the study.

Preparation of the Sesbania Plants :

Sesbania plant samples were collected, and phenolic and alkaloid extracts were prepared. Sesbania seedlings and seeds were obtained from the forest nursery of the Forestry Department at the College of Agriculture and Forestry. They were identified by Prof. Haiss and then washed to remove soil particles. The seedlings were spread under shade at room temperature for drying, with occasional turning to prevent mold formation. After drying, plant parts, including leaves and seeds, were separately ground using a grinder and stored in glass jars until extraction [9]. The phenolic extract of Sesbania plant leaves and seeds was prepared according to the method described by [10].

Preparation of Phenolic Extract :

To isolate phenols, 100 grams of Sesbania plant powder (leaves and seeds) is extracted using 250 ml of ethyl alcohol with continuous stirring for 24 hours using an electric stirrer. The process is repeated twice with the same amount of alcohol each time. The filtrate is collected and concentrated using a rotary evaporator. Then, 20 grams of the extract are dissolved in 250 ml of distilled water with gentle heating at 40°C in a water bath. The solution is transferred to a separating funnel, and 100 ml of ethyl acetate is added and mixed well. The mixture is left until two layers separate: the ethyl acetate layer and the aqueous layer. The ethyl acetate layer is separated, while the aqueous portion is subjected to the same process with another 100 ml of ethyl acetate several times. After concentrating the ethyl acetate extract using a rotary evaporator, the extract becomes a thick liquid, known as the ethyl acetate fraction, containing phenolic compounds. It is stored in glass bottles in the refrigerator until use. The aqueous portion is placed in a rotary evaporator to evaporate the water at 40°C , and small amounts of 90% ethyl alcohol are added to aid in water evaporation until a thick liquid is formed. This is also stored in glass bottles until use.

Preparation of Alkaloid Extract :

To extract alkaloids, 50 grams of Sesbania plant powder (leaves and seeds) are placed in 100 ml of glacial acetic acid in alcohol. The mixture is left for 4 hours, then the extract is concentrated to one-quarter of its original volume under reduced pressure. Alkaloids are precipitated by adding drops

of concentrated ammonium hydroxide at a pH of 9.5 to allow the solution to precipitate, and the precipitate is washed.

Toxicity Effect of Alkaloid and Phenolic Extracts of Seeds and Leaves Sesbania Plant on Larvae Red Flour Beetle *Tribolium castaneum* :

The acute toxicity effect of phenolic and alkaloid extracts from the leaves and seeds of Sesbania plant on larvae red flour beetle . To determine the lethal effect of phenolic and alkaloid extracts from Sesbania plant on red flour beetles and their larvae reared on wheat, four concentrations are prepared for each phenolic and alkaloid extract for each plant part (leaves and seeds) after preliminary experiments to determine the concentrations. They are dissolved in water, and the beetles are treated by spraying with a half-liter sprayer at the prepared extract concentrations. The treated beetles are left to dry and then transferred to a Petri dish containing food, with three replicates, each with 10 larvae , while the control group is treated with water only. The mortality rate is calculated after 24 hours of treatment, and the corrected mortality rate is calculated using the Abbotte equation mentioned by [11] which is:

$$\text{Corrected mortality ratio (\%)} = \frac{\text{Mortality in treatment} - \text{Mortality in control}}{100 - \text{Mortality in control}}$$

As well as toxicity lines are plotted and LC values, slope, and confidence limits are calculated using the Probit program according to [12]. LC₅₀ values are used to calculate relative toxicity according to the following equation:

$$\text{Relative toxicity of extract} = \frac{\text{LC}_{50} \text{ for high toxic extract}}{\text{LC}_{50} \text{ for other extract}} \times 100$$

$$\text{Relative efficiency of extract} = \frac{\text{LC}_{50} \text{ for low toxic extract}}{\text{LC}_{50} \text{ for other extract}} \times 100 \text{ [13]}$$

Statistical Analysis :

The data is analyzed using the Completely Randomized Design (C.R.D.) experimental system, employing Duncan's Multiple Range Test to determine differences between means at a significance level of 5% [14].

Results & Discussion :

Table (1) results showed the contrasting effect of alkaloidal and phenolic extract of Sesbania plant seeds and leaves on the average mortality rates of red flour beetle larvae. They reached 62.08% and 73.75% respectively. Statistical analysis indicated a significant difference in the average mortality rates of insect larvae for both extracts. The same table results revealed the variation in the effect of seed and leaf plant part extract of Sesbania on the average insect larvae mortality, reaching 67.08% and 68.75% respectively. Statistical analysis showed no

significant differences in mortality rates between the plant part extracts. However, the same table results demonstrated the effect of alkaloidal and phenolic extract concentrations of 0.25, 0.50, 0.75, and 1%, where the average mortality rates increased proportionally with the concentrations studied, reaching 50.00%, 65.83%, 72.50%, and 83.33% respectively. The average mortality rates of red flour beetle larvae may be attributed to their exposure to phenolic and alkaloidal secondary compound extracts, which hinder gas exchange externally and internally through the association of these substances with cell cytoplasm components. This interference may affect larval developmental processes when treated at an early stage. The increase in insect larval mortality with increasing concentrations of phenolic and alkaloidal compounds may be justified by the toxic entry of these compounds into the larvae, leading to developmental failure or affecting muscular tissues, thus impeding larval development. [15 , 16 , 17].

From Table (1), the interaction effect between alkaloidal and phenolic extracts and their concentrations shows that the highest average mortality rate in insect larvae was observed with the phenolic extract at a concentration of 1%, reaching 86.67%. Conversely, the lowest average mortality for larvae was with the alkaloidal extract at a concentration of 0.25%, reaching 40.00%. The results also demonstrate the interaction effect between the extract type and plant part. The average mortality rate of insect larvae exposed to phenolic extract from Sesbania seeds was higher than the other extracts, reaching 76.67%, significantly differing from the phenolic extract from leaves, which reached 70.83%.

The mortality of immature stages of the South American bean weevil reared on cowpea seed powder may be attributed to direct exposure of larvae to alkaloidal and phenolic extracts or the larvae's sensory perception of toxic substances present in the plant. It may also result from the toxic effects on the cells of the digestive tract responsible for absorption and a decrease in metabolic efficiency. Phenolic compounds are known to form complexes with proteins via hydrogen bonds, making them difficult to digest, or the treated larvae may refuse to feed due to exposure to the extract, leading to their demise [18]. These results are consistent with those reported by [19], where aqueous and alkaloidal extracts of tobacco waste were found to effectively kill developmental stages of the date moth *Ephesia cautella* at concentrations of 1.25%, 2.5%, 5.0%, and 10.0%, reaching 90% mortality at 10% concentration compared to 17.7% in the control treatment. These results are consistent with what [8] demonstrated, that the phenolic extract of Sesbania plant leaves and seeds had a clear effect on killing developmental stages of the South American bean weevil, with average mortality rates of 76.05% and 76.77% respectively.

From Table (2), which confirmed the results of Table (1), the LC₅₀ values and relative toxicity of alkaloidal and phenolic extracts of Sesbania plant seeds and leaves on the average mortality of red flour beetle larvae were obtained. The lowest LC₅₀ value was for the alkaloidal extract of Sesbania plant leaves, reaching 0.284, indicating that the toxicity of this extract exceeded that of the other studied extracts, with a relative toxicity of 151.41, the highest among the studied extracts. The lowest LC₅₀ value for the alkaloidal extract of Sesbania seeds was 0.430, with a relative toxicity of 100, the lowest among the studied extracts.

The results from Table (3) showed the effect factor values and regression equations for the alkaloidal and phenolic extracts of Sesbania plant seeds and leaves on the average mortality of red flour beetle larvae. The highest effect factor was for the phenolic extract of Sesbania plant leaves on insect larvae, reaching 86.50%, while the lowest effect factor was for the phenolic extract of Sesbania seeds on insect larvae, reaching 62.50%. Phenolic compounds have caused two types of physiological effects in insect tissues: an indirect toxic effect that disrupts the nervous secretion system, or a direct effect by impacting the targeted larval tissues. Meanwhile, alkaloidal compounds have been labeled as larvicidal due to their association with enzymes in the digestive tract, leading to the death of treated larvae [20]. Alternatively, they may affect the nervous tissues of the larvae, leading to paralysis and subsequent rapid killing of the treated insect [17]. [21] revealed that feeding South American bean weevil larvae on seeds coated with gallic acid and salicylic acid at a concentration of 8% resulted in complete larval mortality. Conversely, seeds coated with tannic acid and coumarin at a concentration of 3% led to the death of 71.4% of the treated larvae.

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Table 1. Effect of Alkaloidal and Phenolic Extracts of Sesbania Seeds and Leaves on Larvae Red Flour Beetle *Tribolium castaneum* Mortality.

Type of Extract	Con. %	%Mortality		The interaction between the type of extract & the con.	The general average	
		Seeds	Leaves		The con.	Type of extract
Alkaline	0.25	33.33h	46.67gh	40.00d		
	0.50	56.67efg	66.67d-f	61.67c		
	0.75	63.33def	70.00b-e	66.67c		
	1.00	76.67a-d	83.33ab	80.00ab		
Phenolic	0.25	66.67d-f	53.33fg	60.00c		
	0.50	73.33a-d	66.67d-f	70.00bc		
	0.75	80.00abc	76.67a-d	78.33ab		
	1.00	86.67a	86.67a	86.67a		
The interaction between the type of extract & the plant part	Alkaloid	57.50 c	66.67b			62.08 b
	Phenolic	76.67 a	70.83 ab			73.75a
The interaction effect between the con. & the plant part	0.25	50.00d	50.00d			50.00c
	0.50	65.00c	66.67c			65.83b
	0.75	71.67bc	73.33bc			72.50b
		81.67ab	85.00a			83.33a
The general average for the plant part		64.58 b	70.00 a			

* Similar letters in the same column indicate that there is no significant difference between the means at the 5% probability level.

Table 2. values of slope, LC₅₀, confidence limits, and relative toxicity for the alkaloidal and phenolic extract of seeds and leaves of the Sesban plant on larvae red flour beetle *T. castaneum*

Type of Extract	Plant part	Slope values	LC ₅₀	Confidence limits	Relative toxicity	Relative efficacy
Alkaloidal	Seeds	1.84	0.430	0.348–0.506	100	26.98
	Leaves	1.55	0.284	0.184 – 0.360	151.41	40.84
Phenolic	Seeds	1.09	0.116	0.018 – 0.206	370.69	100
	Leaves	1.63	0.243	0.150 – 0.315	176.95	47.74

Table 3. Regression Equations and Effect Coefficients for the Relationship between the Effect of Alkaline and Phenolic Extracts of Sesbania Plant Seeds and Leaves on the Average Mortality of larvae Red Flour Beetles *T. castaneum*

Type of Extract	Plant part	r ²	Regression Equations
Alkaloidal	Seeds	79.4	$^2Y = 10.83 + 104.7 * -40.00X$
	Leaves	63.9	$Y = 30.00 + 78.67X - 26 - 67 X^2$
Phenolic	Seeds	62.5	$Y = 60.00 + 26.67 X - 0.00X^2$
	Leaves	86.5	$Y = 43.33 + 44.00X$