

# Bioactivity of five invasive weed extracts against the fall armyworm pest (*Spodoptera frugiperda*)

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**Abstract.** *Ikawati S, Chen MC, Aviva N, Ahmadjati DA, Nadhifah D, Darmawan SKA, Choliq FA. 2026. Bioactivity of five invasive weed extracts against the fall armyworm pest (Spodoptera frugiperda). Biodiversitas 27 (3): d270310. <https://doi.org/10.13057/biodiv/d270310>. Spodoptera frugiperda (Lepidoptera: Noctuidae) or Fall Armyworm (FAW) is an invasive pest that attacks corn plants. Synthetic insecticides are commonly used for pest control but are not environmentally friendly, prompting the need for alternatives such as botanical insecticides. Sleeping grass (*Mimosa pudica*), nutgrass (*Cyperus rotundus*), Bermuda grass (*Cynodon dactylon*), wild sage (*Lantana camara*), and goatweed (*Ageratum conyzoides*) are invasive weeds in agroecosystems that have the potential to be used as botanical insecticides. This study aims to determine the bioactivity of the invasive weed extracts against FAW. Methods using bioassay in the laboratory. For each type of weed extract, an experiment was carried out with five levels of extract concentration (each extract using a different range) and two controls, which were repeated four times with ten individual 3rd instar larvae of FAW per repetition. The results showed that five invasive weed extracts have the potential to be used as insecticides, caused an increase in larval mortality (>70%), decreased the feeding activity (>50%), caused weight reduction (>50%), and reduced the percentage of successful development of pupae and adults (<15%). The LC<sub>50</sub> values from the smallest to highest at 144 h after application were for *C. dactylon* aerial parts extract (6%), *C. rotundus* tuber extract (16%), *A. conyzoides* leaf extract (22%), *L. camara* leaf extract (49%), and *M. pudica* leaf extract (53%). For LT<sub>50</sub> of *M. pudica* at a concentration of 70%, *C. rotundus* at a concentration of 50%, *C. dactylon* at a concentration 29%, *L. camara* at a concentration of 60%, and *A. conyzoides* at a concentration of 41% were 90, 57, 73, 81, and 42 h, respectively.*

**Keywords:** *Ageratum conyzoides*, botanical insecticide, *Cynodon dactylon*, *Cyperus rotundus*, *Lantana camara*

## INTRODUCTION

The major lepidopteran insect pest species known as the Fall Armyworm (FAW), or *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae), is indigenous to the Americas (Benjamin et al. 2024) and is the most important noctuid pest (Montezano et al. 2018). It is regarded as a super pest based on its host range (at least 353 host plants), its inherent ability to survive in a wide range of habitats, its strong migration ability, high fecundity, rapid development of resistance to insecticides/viruses, and its gluttonous characteristics (Montezano et al. 2018). The fall armyworm, as an invasive alien species in Indonesia, threatens the productivity of cereal crops such as maize (Afandhi et al. 2022). When FAW attacks plants, it causes yield losses ranging from 15% to 73% (Sudihardjo et al. 2023). Multiple applications of monitoring and scouting, agricultural control, chemical pesticides, sex attractants, biological control agents (parasitoids, predators, and entomopathogens), and botanicals have been used in FAW's Integrated Pest Management (IPM). Control with botanical insecticides has several advantages over synthetic chemical control, which farmers often use.

Botanical pesticides, or biopesticides, are considered desirable alternatives to conventional chemical pesticides in agriculture, owing to their favorable profiles for human

health and the environment (Damalas and Koutroubas 2020; Ikawati et al. 2024; Ikawati et al. 2025). This insecticide contains an active ingredient derived from plants. Many plant species in Indonesia have strong potential as botanical insecticides, including various weeds and invasive plants. Several invasive weeds compete with cultivated crops and can reduce agricultural productivity; therefore, their use as botanical insecticides may simultaneously support pest management and invasive weed control.

Previous studies have reported insecticidal activity from different plant preparations, including solvent extracts and essential oils derived from various plant parts. For example, petroleum ether, chloroform, ethyl acetate, and methanol extracts obtained from the leaves, stems, and roots of *Mimosa pudica* have demonstrated insecticidal and repellent activities (Mondol and Islam 2020). Essential oils extracted from *Cyperus rotundus* have been reported as effective alternatives to synthetic pesticides for protecting stored products, primarily due to their volatile bioactive constituents (Janaki et al. 2018). Similarly, methanolic, acetone, and ethoxyethanol extracts of *Cynodon dactylon* leaves and roots significantly reduced growth rate, consumption index, and food conversion efficiency in late instar nymphs of *Schistocerca gregaria*. Essential oils obtained from *Lantana camara* leaves have also shown strong insecticidal activity against several stored-product

pests, including *Tribolium castaneum*, *Lasioderma serricorne*, and *Callosobruchus chinensis* (Aisha et al. 2024). In addition, solvent extracts of *Ageratum conyzoides* have exhibited broad-spectrum insecticidal activity against major field and storage pests such as *C. chinensis*, *Chilo partellus*, *Plutella xylostella*, *Sitophilus oryzae*, *Panonychus citri*, *Sitophilus zeamais*, and *Brevicoryne brassicae* (Rioba and Stevenson 2017). Although some bioactive compounds may be shared among essential oils and solvent extracts, their chemical profiles, polarity, and modes of action differ substantially. Therefore, the present study specifically focuses on the insecticidal potential of ethanolic plant extracts, which are expected to contain both polar and semi-polar bioactive compounds distinct from those dominant in essential oils.

In this study, observations were made on several bioactivity variables of five weed extracts, including toxicity and effects on FAW growth and development. The survival rate, insect weight, and feeding behavior are important variables in insects that are affected by external and internal factors, including those caused by insecticides (Müller 2018; Liu et al. 2025). Based on this background, this study was conducted to examine the toxicity, antifeedant activity, larval weight reduction, and effects on pupal and adult development of ethanolic extracts of five invasive weed species against FAW under laboratory conditions.

## MATERIALS AND METHODS

### Experimental site

The study was conducted at the Laboratory of Plant Pest and the Laboratory of Pesticide Toxicology, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya, in Malang, East Java, Indonesia. Both laboratories had an average temperature of  $27\pm 2^{\circ}\text{C}$  and a relative humidity of  $75\pm 5\%$ . The research was conducted from January, 2023 to June, 2025.

### Insect propagation

First-generation FAW larvae were obtained from corn fields. The larvae were transferred into separate plastic vials (30 mL) for each individual to avoid cannibalism and fed with baby corn. After the first-generation larvae became pupae, the pupae were transferred into mating cages measuring  $30 \times 40 \times 70$  cm in pairs of 10 males and 10 females. At the top of the cage hung cotton soaked in a honey solution, and at the bottom was a container filled with corn leaves for laying eggs. After the adult laid eggs, the corn leaves attached to the eggs were cut and then transferred to a plastic container containing young corn leaves as food for the second-generation larvae, which would later hatch. After the second-generation larvae hatched and entered the second instar, they were transferred to individual plastic vials and fed young corn leaves. The second-generation larvae were maintained until they entered the early third instar, at which point they were ready for use as test insects. Only second-generation larvae were used in the research.

### Weed extraction

Weed extracts were produced in the Laboratory of Pesticide Toxicology by maceration with 96% ethanol. Each extract material (*C. dactylon* aerial parts, *C. rotundus* tuber, *A. conyzoides* leaf, *L. camara* leaf, and *M. pudica* leaf) was washed thoroughly and left at room temperature until dry, then the material was ground with a blender and mixed with 96% ethanol at a ratio of 1:4 (w/v) in an Erlenmeyer flask. 50 g of powder were macerated with 200 mL of ethanol solvent. The Erlenmeyer flask was covered with aluminum foil and macerated on an orbital shaker at 120 rpm at about  $24^{\circ}\text{C}$  for 3 days. The macerated material was filtered through Whatman No. 1 filter paper, then evaporated using a rotary vacuum evaporator at  $78^{\circ}\text{C}$ . The weed extract was collected into a light-tight glass bottle and stored in a refrigerator until ready for use.

### Bioactivity test

This study was conducted using a Completely Randomized Design (CRD). The treatment for each extract type used 5 levels of extract concentration and two controls: distilled water and the semisynthetic insecticide emamectin benzoate. The concentration range for each type of extract is determined through preliminary tests. In the preliminary toxicity test, each extract showed different levels of toxicity, so in the main test, different concentrations were used for each extract. This study was conducted by observing five observation variables: larval mortality (toxicity test), a decrease in feeding activity test, larvae weight reduction, and pupal and adult development test. All observation variables are conducted within the same research unit and simultaneously, not individually.

### Toxicity test

Mortality tests were conducted on third-instar larvae. As much as 1 g (4 cm leaf discs) of corn leaves in the appropriate concentration of extract dissolved in distilled water, including positive controls (0% emamectin benzoate) and negative control (distilled water) for 5 minutes. A semisynthetic pesticide, emamectin benzoate, is used to control a variety of pests (Saeed et al. 2021). The corn leaves were then dried at room temperature for 20 minutes. The corn leaves were placed in a plastic container (300 mL) along with the FAW larvae that were placed in the container and had been fasted for 2 h. Ten individual larvae were used per treatment, with four replicates. Thus, 240 larvae were used per extract treatment, for a total of 1200 larvae across the entire study. If in the negative control treatment, mortality ranges between 5-20%, then the mortality value is corrected using the formula described by Abbott (1925).

$$P = \left[ \frac{X - Y}{X} \right] \times 100$$

Where P is the corrected mortality percentage, X is the percentage of surviving insects in the control, and Y is the percentage of surviving insects in the treatment.

### Feeding activity reduction test

This test was conducted using 4cm leaf discs using the no-choice method. The method used is the same as the

toxicity test method because it is in the same research unit. Observations were made by calculating the remaining leaf area after 24 h using a millimeter block. Observations were made by calculating the remaining leaf area using a millimeter grid. The test results were calculated using a Feeding Deterrence Index (FDI) formula (Jiang et al. 2012).

$$FDI = \left[ \frac{(C - T)}{C + T} \right] \times 100$$

Where C is the consumed leaf area in the control, while T is the consumed leaf area in the tested treatment.

The criteria for eating inhibitors are divided into 4 groups: no inhibitor (0%), low (0-40%), moderate (40-60%), and quite high (60-80%), with high (>80%) as the highest.

#### Weight reduction test

Observations on larval weight were conducted before treatment, 3 days after treatment, and 7 days after treatment for surviving larvae. The surviving larvae from the toxicity test were fed corn leaves without treatment to observe their development into pupae and adults. The weight of the surviving larvae was measured on the 3rd and 6th days after treatment to the nearest 0.1 g. Then, the larval weight data were calculated using the following formula to determine the value of larval weight reduction (Arora et al. 2017).

$$\text{Percent reduction in weight} = \frac{(\text{weight increase at control} - \text{weight increase at treatment})}{\text{weight increase at control}} \times 100$$

#### Pupal and adult development tests

For this test, larval weight-reduction tests were run. Observations of pupal and adult development aimed to determine the effect of weed extracts on the biology of FAW. This test was conducted by observing pupal and adult development (abnormalities and normality). The value of the ability of larvae to reach the pupal and adult stages was calculated as a percentage using the following formula.

$$\text{Percent Larvae to pupae/adult} = \frac{\Sigma \text{Larvae become pupae/adult}}{\Sigma \text{Insect test}} \times 100$$

#### Data analysis

To compare the effects of different extract concentration treatments, the analysis uses ANOVA. Because the treatments are in different concentration ranges, the further test used to determine whether the treatments are significantly different is the DMRT (Duncan's Multiple Range Test) at the 5% level. In addition, probit analysis was also carried out to determine the Median Lethal Concentration (LC<sub>50</sub>) and Median Lethal Time (LT<sub>50</sub>) values. Although the same individuals were followed throughout development, each life stage was analyzed separately to describe stage-specific responses. Potential non-independence among stages is acknowledged and accounted for in the interpretation of the results. All analyses were performed using the SPSS Statistics version 25 application.

## RESULTS AND DISCUSSION

### Toxicity of weed extracts to FAW larvae

The average mortality percentage results show that four of the weed extracts, excluding the *C. dactylon* aerial parts extract, already showed different toxicity rates across concentrations at 24 HAA. When comparing the effectiveness of controlling FAW with weed extracts and emamectin benzoate, emamectin benzoate is faster-acting. However, weed extracts can still cause mortality, albeit over a longer period than semisynthetic pesticides (Table 1). This indicates that its toxicity is lower than that of emamectin benzoate. In IPM, combining various techniques to optimize prevention and control with botanical pesticides remains beneficial because pest control effectiveness does not depend solely on botanical pesticides. Furthermore, with lower toxicity, it is suspected that its toxicity to non-target organisms is also low, allowing it to support other control methods, such as biological control with natural enemies.

The greater effect of weed extracts on increasing FAW larval mortality occurred at a high concentration. It can be seen that there is a positive relationship between concentration and mortality, namely that FAW mortality increases with increasing concentration of weed extracts. In the observation of 72 HAA *M. pudica* leaf extract at the highest concentration (70%), it killed only 35% of FAW larvae. In contrast, the observation of 144 HAA *M. pudica* leaf extract at the same concentration resulted in 77.5% mortality. Meanwhile, in the observation of 144 HAA *M. pudica* leaf extract at the lowest concentration (40%), it killed only 30% of FAW larvae. This differs from the results of research on the effect of *M. pudica* extract on *T. urticae* mites, which required only a 1% concentration to achieve mortality of around 75% even at 48 h after application (Puspitarini et al. 2024). This difference is thought to occur because the target pests differ, and the mites are more sensitive than FAW.

Mortality of FAW larvae in the *M. pudica* leaf extract treatment is suspected to be due to the influence of the bioactive compounds in *M. pudica* leaf extract that enter the larvae's body through their food. A total of 7 compounds were identified in the ethanol extract of *M. pudica*, and several of these have potential as insecticides (Puspitarini et al. 2024). Along with many beneficial secondary metabolites, including tannins, steroids, flavonoids, triterpenes, and glycosylflavones, *M. pudica* also includes the alkaloid mimosine (Muhammad et al. 2016).

*Cyperus rotundus* tuber extract also showed lower mortality against FAW at 9% and 16% concentrations. In contrast, higher mortality was observed at concentrations of 28% and 33%. At a concentration of 16%, the 72 HAA observation time showed a mortality rate of 33%. This differs from the research results for *Helicoverpa armigera*, which showed that, after 72 h of treatment, 90% of the insects died from the tuber ethanolic extract of *C. rotundus* at a 12% concentration (Elhaj et al. 2021). This can occur due to differences in the plant parts extracted and in the types of target pests.

**Table 1.** Mortality (%) of fall armyworm (FAW) larvae treated with different weed extracts and their concentrations

Insecticide	Concentration (%)	Mortality (%) ( $\bar{x}$ ±SD) <sup>1</sup> , in ... HAA					
		24	48	72	96	120	144
<i>Mimosa pudica</i> leaf extract	40	2.5±5.0a	7.5±9.6a	12.5±9.6a	15.0±12.9a	22.5±12.5a	30.0±8.16a
	48	5.0±5.8ab	12.5±12.6a	20.0±8.2ab	22.5±12.6ab	30.0±8.16ab	37.5±9.6ab
	55	10.0±8.2ab	20.0±14.1a	20.0±14.1ab	37.5±9.6bc	45.0±12.9bc	50.0±14.1bc
	63	12.5±12.6ab	15.0±12.9a	32.5±12.6b	40.0±14.1bc	47.5±15.0c	62.5±12.5cd
	70	17.5±9.6b	20.0±8.16a	35.0±12.9b	45.0±12.9c	60.0±8.16c	77.5±9.6d
Emamectin benzoate	0.1	100.0±0.0c	100.0±0.0b	100.0±0.0c	100.0±0.0d	100.0±0.0d	100.0±0.0e
<i>Cyperus rotundus</i> tuber extract	9	10.0±0.8a	20.0±0.81a	23.0±2.1a	25.0±1.3a	28.0±1.0a	28.0±1.0a
	16	15.0±1.9ab	23.0±1.3a	33.0±1.0a	33.0±1.0a	35.0±1.3a	40.0±1.4ab
	23	20.0±0.8ab	25.0±1.3a	35.0±1.7a	38.0±1.7ab	48.0±0.5ab	53.0±2.2bc
	28	25.0±1.3ab	33.0±1.0ab	40.0±2.6a	53.0±3.5ab	58.0±2.1ab	73.0±1.7cd
	33	30.0±0.8b	43.0±1.3b	48.0±3.0b	65.0±2.4b	60.0±2.2b	90.0±0.8de
Emamectin benzoate	0.1	100.0±0.0c	100.0±0.0c	100.0±0.0c	100.0±0.0c	100.0±0.0c	100.0±0.0e
<i>Cynodon dactylon</i> aerial parts extract	12	17.5±0.8a	17.5±0.8a	25.0±0.7a	25.0±0.7a	32.5±1.5ab	68.0±2.5a
	15	15.0±0.2a	15.0±0.2a	22.5±0.1a	22.5±0.1a	30.0±0.8a	70.0±1.8a
	20	27.5±0.3a	37.5±0.6a	55.0±0.6ab	55.0±0.6b	55.0±2.9 bc	75.0±2.1ab
	25	15.0±0.6a	35.0±0.8a	45.0±0.9a	52.5±0.4b	57.5±1.7c	80.0±1.8ab
	29	17.5±0.8a	35.0±0.9a	35.0±0.9a	52.5±0.4b	67.5±1.0c	88.0±1.3ab
Emamectin benzoate	0.1	100.0±0.0b	100.0±0.0b	100.0±0.0b	100.0±0.0c	100.0±0.0d	100.0±0.0b
<i>Lantana camara</i> leaf extract	40	10.0±0.8a	15.0±0.6a	22.5±1.3a	25.0±1.3a	27.5±1.5a	27.5±1.5a
	45	20.0±0.8ab	25.0±0.6b	30.0±0.8ab	32.5±1.0ab	37.5±1.0ab	40.0±0.8a
	50	17.5±1.0ab	32.5±1.0b	37.5±0.5b	45.0±0.6bc	52.5±0.5bc	55.0±0.6b
	55	22.5±1.0ab	30.0±0.8b	37.5±1.0b	45.0±1.3bc	50.0±1.4bc	60.0±0.8b
	60	27.5±1.0b	35.0±0.6b	40.0±0.8b	50.0±1.2c	60.0±1.6c	70.0±1.4b
Emamectin benzoate	0.1	100.0±0.0c	100.0±0.0c	100.0±0.0c	100.0±0.0d	100.0±0.0d	100.0±0.0c
<i>Ageratum conyzoides</i> leaf extract	23	7.50±1.0a	25.0±1.9a	27.5±2.2a	30.0±2.4a	45.0±1.9a	52.5±2.8a
	28	17.5±1.0ab	35.0±1.3ab	40.0±2.4a	42.5±2.5a	52.5±1.3b	70.0±1.4ab
	33	17.5±1.0ab	32.5±2.1ab	42.5±3.3a	45.0±3.1a	62.5±2.1ab	80.0±0.8bc
	37	17.5±1.0ab	32.5±1.7ab	40.0±2.4a	55.0±1.7a	77.5±1.0bc	82.5±1.0bc
	41	22.5±1.0b	50.0±0.8b	82.5±1.7b	87.5±1.0b	90.0±0.8c	90.0±0.8bc
Emamectin benzoate	0.1	100.0±0.0c	100.0±0.0c	100.0±0.0b	100.0±0.0b	100.0±0.0c	100.0±0.0c

Note: <sup>1</sup>Using ten larvae per replication, four replicates of each concentration were made. Numbers followed by different letters in the same column indicate significantly different results based on the DMRT test at a 5% error level. The data shown are pre-transformation data. HAA is hours after application,  $\bar{x}$  is the mean, and SD is the standard deviation

Mortality of FAW larvae is thought to be affected by bioactive compounds in *C. rotundus* tuber extract. Extracts from *C. rotundus* are rich in several phytochemicals; a methanol extract revealed 26 phenolic compounds, including organic acids, flavonoids, and phenolic acids (El-Wakil et al. 2023). Several compounds are suspected to cause this. Key constituents of the essential oil were  $\alpha$ -cyperone (38.46%), cyperene (12.84%), and  $\alpha$ -selinene (11.66%) (Hu et al. 2017).

Treatment with *C. dactylon* aerial parts extract at several concentrations affects FAW larval mortality. In the 120 HAA observation, mortality of FAW larvae was highest at 29% (67.5%), with differences of 12% (32.5%) and 15% (30%) between the concentration treatments. Larval mortality in the 144 HAA observation was also higher at 29% (88%). Mortality of FAW larvae is thought to be due to the presence of bioactive compounds in the *C. dactylon* aerial parts extract. Saponins and terpenoids are known bioactive compounds present in the *C. dactylon* aerial parts extract and are toxic to insects. Alkaloids, cardiac glycosides, terpenoids, steroids, saponins, phenolic compounds, flavonoids, sugars, and protein were among the phytochemicals found in ethanol from this plant extract (Krishnaveni et al. 2024). Active compounds such as

alkaloids, terpenoids, and flavonoids in the *C. dactylon* aerial parts extract certainly play a role in the mortality of FAW larvae. While terpenes have been shown in studies to interact and reduce the activity of Acetylcholinesterase (AChE) (dos Santos Cardoso et al. 2020), flavonoids have also been shown to do the same (Perumalsamy et al. 2015).

FAW larvae that died in the *L. camara* leaf extract treatment also increased at 144 HAA, with the highest concentration (60%) causing the highest mortality (70%). This is also different from the results of research on the effects of *L. camara* extract on different targets, such as *T. urticae* mites, which required only a 1% concentration to achieve mortality of around 73%, even at 96 h after application (Puspitarini et al. 2024).

The pesticidal potential of *L. camara* is attributed to its diverse chemical composition, which includes primary and secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids (Kumar et al. 2024). The main phytochemicals found in the essential oil of *L. camara* leaves were found to be  $\alpha$ -copaene (4.11%), isolodene (12%), and caryophyllene (69.96%) (Aisha et al. 2024). The principal components, notably lantadene A, lantadene B, lantadene C, lantadene D,  $\beta$ -caryophyllene,  $\alpha$ -humulene, and several others, constitute a significant portion of the

essential oil derived from the leaves and flowers (Kumar et al. 2024). Because leaf powder and extracted oil contained bioactive and phytochemical compounds such as phytol, pyrrolone, paromomycin, pyrrolizin, and 1-Eicosano, they affected insect repellency and mortality (Ayalew 2020). *L. camara* extract contained compounds such as  $\beta$ -caryophyllene and linalool that had high binding affinity for AChE, thereby increasing its neurotoxic effects (Kumar et al. 2024).

At 96 HAA at the highest concentration of 41%, a mortality rate of around 87% can be achieved. Mortality of FAW larvae applied to *A. conyzoides* leaf extract is thought to be due to the content of compounds in the extract. This is also different from the results of research on the effect of *A. conyzoides* extract on *T. urticae* mites, which require only a 1% concentration to achieve a mortality of around 87%. The effectiveness of botanical insecticides depends on the target pest (Riyaz et al. 2022). *A. conyzoides* aqueous crude leaf extract contained alkaloids, tannins, cardiac glycosides, phlorotannins, flavonoids, terpenoids, saponins, steroids, sodium, potassium, calcium, and phosphorus (Adelakun et al. 2022). The most prevalent component of the *A. conyzoides* oil group was chromene, followed by the sesquiterpene and monoterpene groups, and the main components of these groups were three substances harmful to insects: precocene I,  $\beta$ -caryophyllene, and precocene II (Pintong et al. 2020).

FAW larvae that died in the weed extract treatment had various symptoms. It is suspected that the initial symptoms will cause the larvae to vomit a green liquid after eating leaves soaked in weed extract, due to the toxic substances in the extract, which cause gastric irritation. Other symptoms observed in dead larvae included softening and rotting of the bodies.

Median Lethal Concentration, or LC<sub>50</sub>, is a calculation used to estimate the concentration of a chemical compound required to cause 50% mortality in a given population of test animals. Whereas Median Lethal Time, or LT<sub>50</sub>, is a calculation used to estimate the time required for 50% of a given population of test animals to die as a result of a given compound. The analysis showed that the five extracts had different LC<sub>50</sub> values (Table 2).

The results of the LC<sub>50</sub> analysis indicate that at 144 HAA, the lower the LC<sub>50</sub> value obtained. Based on the LC<sub>50</sub> value, the most toxic was the *C. dactylon* aerial parts

extract. *C. dactylon* extract has greater toxicity, presumably because the type of bioactive content associated with lethality in this extract is more toxic than others.

For LT<sub>50</sub>, the value is determined by the concentration at which a compound is effective. Therefore, the LT<sub>50</sub> value will depend on the concentration of the extract administered, whether high or low. The LT<sub>50</sub> calculation shows that the higher the concentration used, the higher the active compound concentration in the extract, which is thought to result in faster death.

## The effects of weed extracts on the growth and development of FAW

### The feeding activity reduction

Observations showed that weed extracts reduced FAW larval feeding activity at various concentrations. All weed extracts at their highest concentrations showed a feeding deterrence index well above the criteria. The decrease in feeding activity of FAW larvae in the highest concentration of weeds extracts experienced the highest decrease in feeding activity compared to the lowest concentration of extracts (Table 3).

In the observation, 40% *M. pudica* leaf extract decreased feeding activity by 27%, while 70% *M. pudica* leaf extract decreased feeding activity by up to 78.5%. This trend is also consistent with observations on other extract treatments. The reduction in feeding activity caused by *C. rotundus* tuber extract at 9% and 16% is a low concentration criterion, while at 23, 28, and 33% it is a moderate concentration criterion. It can be said that the increase in the concentration used by the test insecticide is thought to be directly proportional to the increase in toxic substances, thereby reducing feeding activity and increasing larval mortality. The inhibition of eating activity is thought to be due to the extract containing substances such as essential oils and saponins, which can be stomach poisons. Animal feed consumption is greatly impacted by saponins, which also hinder protein digestion and the gut's absorption of vitamins and minerals, leading to hypoglycemia (Francis et al. 2002) and affecting food intake (Thakur et al. 2011). In addition, saponins have a bitter taste, and their concentration was associated with reported bitterness (Heng et al. 2006), which can decrease feeding activity (Singh and Kaur 2018).

**Table 2.** The weed extracts against fall armyworm (FAW) larvae

Extract	Analysis	Observation at	Value	Regression equation	R <sup>2</sup>
<i>Mimosa pudica</i> leaf	LC <sub>50</sub> (%)	144 HAA	53	y = 5.2291x - 4.0029	0.96
	LT <sub>50</sub> (h)	70%	90	y = 2.069x + 0.9491	0.84
<i>Cyperus rotundus</i> tuber	LC <sub>50</sub> (%)	144 HAA	16	y = 2.8803x - 10.018	0.81
	LT <sub>50</sub> (h)	33%	57	y = 1.8602x - 1.7284	0.72
<i>Cynodon dactylon</i> aerial parts	LC <sub>50</sub> (%)	144 HAA	6	y = 1.5401x - 2.392	0.88
	LT <sub>50</sub> (h)	29%	73	y = 2.2844x + 0.7409	0.81
<i>Lantana camara</i> leaf	LC <sub>50</sub> (%)	144 HAA	49	y = 4.5282x - 20.898	0.91
	LT <sub>50</sub> (h)	60%	81	y = 1.3451x + 2.4279	0.88
<i>Ageratum conyzoides</i> leaf	LC <sub>50</sub> (%)	144 HAA	22	y = 4.5444x - 19.268	0.99
	LT <sub>50</sub> (h)	41%	42	y = 4.5444x - 19.268	0.99

**Table 3.** Feeding deterrence index (FDI, %) of fall armyworm (FAW) larvae treated with different concentrations of various weed extracts

Insecticide	Concentration (%)	Decrease in feeding activity of <i>S. frugiperda</i> (%) <sup>1</sup> ( $\bar{x} \pm SD$ )
<i>Mimosa pudica</i> leaf extract	40	27.0 $\pm$ 6.2a
	48	40.4 $\pm$ 8.4b
	55	49.5 $\pm$ 7.3b
	63	61.7 $\pm$ 3.6c
	70	78.5 $\pm$ 5.3d
Emamectin benzoate <i>Cyperus rotundus</i> tuber	0.1	90.0 $\pm$ 4.2e
	9	18.0 $\pm$ 6.6a
	16	36.0 $\pm$ 13.6b
	23	46.0 $\pm$ 6.6bc
	28	49.0 $\pm$ 4.2bc
Emamectin benzoate <i>Cynodon dactylon</i> aerial parts extract	33	58.3 $\pm$ 9.7c
	0.1	84.0 $\pm$ 0.1d
	12	18.2 $\pm$ 1.0a
	15	34.2 $\pm$ 1.2b
	20	38.6 $\pm$ 0.6b
Emamectin benzoate <i>Lantana camara</i> leaf extract	25	45.7 $\pm$ 0.3b
	29	62.5 $\pm$ 0.6c
	0.1	85.9 $\pm$ 0.1d
	40	27.8 $\pm$ 4.9a
	45	34.8 $\pm$ 5.2ab
Emamectin benzoate <i>Ageratum conyzoides</i> leaf extract	50	42.8 $\pm$ 3.3b
	55	56.5 $\pm$ 6.7c
	60	69.8 $\pm$ 8.3d
	0.1	89.0 $\pm$ 3.4e
	23	18.2 $\pm$ 6.6a
Emamectin benzoate	28	28.3 $\pm$ 12.4b
	33	36.4 $\pm$ 6.9b
	37	60.0 $\pm$ 13.0c
	41	72.2 $\pm$ 7.0cd
Emamectin benzoate	0.1	87.0 $\pm$ 0.8d

Note: <sup>1</sup>Using ten larvae per replication, four replicates of each concentration were made. Numbers followed by different letters in the same column indicate significantly different results based on the DMRT test at a 5% error level.  $\bar{x}$ : The mean and SD are the standard deviation

The decrease in larval feeding activity can also be caused by flavonoid and tannin compounds. The flavonoid compounds contained in the *C. dactylon* aerial parts extract reduce larval feeding when consumed orally, leading to numbness around the mouth and an inability to recognize food, thereby decreasing FAW feeding activity. The tannin content in the *C. dactylon* aerial parts extract also causes the inhibition of FAW larvae's feeding activity. Tannin compounds in *C. dactylon* aerial parts extract, when consumed by insects, have an astringency and bitter taste (Soares et al. 2020), thus reducing the appetite of FAW larvae. Through toxicity and or deterrence, tannins can protect leaves from insect herbivores (Barbehenn and Constabel 2011). For *A. conyzoides*, leaves contain tannins, saponins, flavonoids, phenols, tannins, and alkaloids, which are quite high compared to other parts of the *A. conyzoides* plant, such as roots (Lim 2014).

In qualitative observations, the initial evidence of antifeedant activity of all weed extracts is that FAW larvae did not completely consume corn leaves treated with the

extracts. In fact, according to visual observations, some feeds do not have holes or signs of larval bites. Before the test to reduce feeding activity, the larvae were starved for 2 h. The starved larvae will initially eat the treatment leaves because there is no other food available other than the leaves dipped in extracts with varying concentrations. FAW larvae then experienced behavioral changes, such as becoming restless; their sensitive bodies curled up when touched, and they allegedly vomited a green liquid. Consequently, the larvae discontinued feeding and left more leaf residue than the control group.

#### The effect of weed extracts on the weight reduction of FAW

Observations regarding the impact of weed extracts on the growth of FAW larval weight indicated a reduction. Different types of extracts have different abilities to reduce weight. The results obtained demonstrated that providing larvae with treated corn containing weed extracts markedly decreased their feeding activity (Table 4).

The weight loss of insect pests fed insecticide-containing feed increases over time. In observations of 3 DAA, *M. pudica* leaf extract at the highest concentration (70%) caused a greater decrease in FAW larval weight, up to 42.9%; at 6 DAA, the decrease in larval weight increased to 60.7%. In addition, other weed extract concentrations showed increased weight reduction at 6 DAA.

In observations of 6 DAA for all types of extract except *A. conyzoides* leaf extract, the decrease in larval weight for each concentration treatment tended to be different, especially for the highest concentration. The greater concentration of bioactive chemicals in the extracts is assumed to be the cause of the significant weight loss. Larval weight decreased due to saponin and tannin compounds that disrupt larval development, allegedly by disrupting the insect's nervous system and decreasing feeding activity.

#### Development of larvae into pupae and pupae into adults of FAW

After entering the 7th day, the surviving larvae were observed to determine whether they successfully pupated normally and developed into normal adults, or whether they exhibited any abnormal symptoms. The larvae utilized in the toxicity tests were then tracked through pupation and adult emergence, so the same individuals were used to measure survival and developmental parameters across life stages. The results are understood as consecutive life-history reactions to ethanolic weed extracts. However, tracking the same individuals throughout development offers biological information about the delayed and sublethal effects of the extract, which may not be apparent from acute toxicity tests alone. Studies of insect toxicity often use similar methods to record cumulative effects on development and metamorphosis. Studies on insect toxicity have frequently employed similar methods to record cumulative effects on development and metamorphosis. Consequently, the observed patterns consistently show that exposure to ethanolic extracts alters life stages (Table 5). The research results showed that extracts decreased the success of FAW larvae reaching the pupal stage and becoming adults.

**Table 3.** Weight loss (%) of fall armyworm (FAW) larvae at different concentrations of various weed extracts

Extract	Concentration (%)	Weight reduction (%) ( $\bar{x}\pm SD$ ) <sup>1</sup> , at ...DAA	
		3	6
<i>Mimosa pudica</i> leaf	40	14.2±2.4a	22.2±1.0a
	48	17.7±3.79ab	29.7±2.7b
	55	22.6±1.8b	35.8±2.4c
	63	31.2±6.5c	46.6±3.7d
	70	42.9±2.7d	60.7±4.1e
<i>Cyperus rotundus</i> tuber	9	23.6±0.9a	14.5±0.4a
	16	32.5±0.5ab	19.4±0.3ab
	23	50.0±0.3ab	39.0±0.1bc
	28	55.3±0.2ab	55.3±0.1bc
<i>Cynodon dactylon</i> aerial parts	33	67.5±0.3b	74.5±0.1c
	12	46.2±0.3a	40.0±0.4a
	15	46.5±0.2ab	43.3±0.4ab
	20	66.7±0.5bc	51.2±0.5ab
	25	71.8±0.2cd	68.1±0.7b
<i>Lantana camara</i> leaf	29	74.5±0.2d	82.3±0.8c
	40	16.6±2.5a	24.6±2.1a
	45	23.8±3.1b	31.6±2.4b
	50	30.3±2.6c	39.8±4.0c
	55	39.7±3.6d	46.7±4.6d
<i>Ageratum conyzoides</i> leaf	60	43.5±3.9d	56.9±4.8e
	23	29.1±0.4a	12.4±0.2a
	28	33.9±0.5a	23.6±0.2ab
	33	52.1±0.3ab	52.8±0.1ab
	37	63.6±0.1b	55.3±0.1b
	41	78.1±0.1b	78.80±0.1b

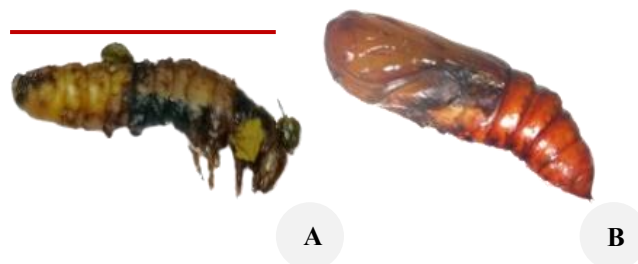
Note: <sup>1</sup>Using ten larvae per replication, four replicates of each concentration were made. Numbers followed by different letters in the same column indicate significantly different results based on the DMRT test at a 5% error level. DAA is days after application,  $\bar{x}$ : The mean, and SD is the standard deviation

The success of FAW larvae becoming pupae and pupae becoming adults at the higher concentrations of weed extracts was lower than at lower concentrations. As an example, in the observation of the success of larvae becoming pupae with 40% *M. pudica* leaf extract treatment, the success of larvae becoming pupae decreased to 60%. In comparison, in the 70% *M. pudica* leaf extract treatment, the success of larvae becoming pupae decreased drastically to only 12.5%. Furthermore, in the observation of the success of pupae becoming adults with 40% *M. pudica* leaf extract treatment, the percentage of success of pupae becoming adults decreased to 47.5%, and in the 63% and 70% *M. pudica* leaf extract treatment, the success of pupae becoming adults also decreased drastically to 10 and 5%.

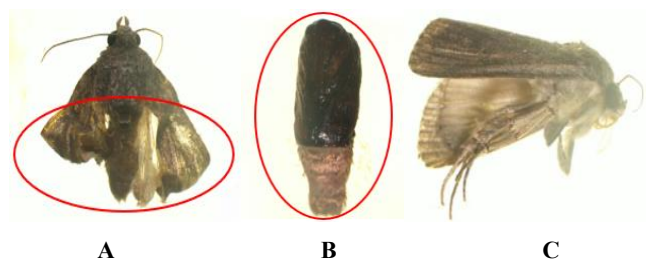
FAW larvae that failed to pupate showed abnormalities that seemed to be covered with exuvium, failed to molt, and died before pupation (Figure 1.A). Among the abnormalities seen in the pupa were slightly damaged cuticles that turned black (Figure 1.B). The presence of slightly damaged cuticles in pupae is assumed to be due to a disrupted molting process. The failure of the larvae to become pupae was due

to contamination of the extracts used in the previous phase with toxins. It is suspected that at the highest extract concentration, the number of toxic chemical compounds to FAW larvae also increased, thereby contributing to the failure of FAW metamorphosis, which undergoes complete metamorphosis. There are substances known as Insect Growth Regulators (IGRs) that can disrupt an insect's endocrine system (Nwonuma et al. 2025). Molting and developmental retardation problems could be induced by saponins (Singh and Kaur 2018).

In some FAW pupae that successfully became adults at the highest and lowest concentrations, symptoms of abnormalities in the form of malformations in the wings, so they could not be stretched perfectly (Figure 2.A). In addition, there are FAW adults that fail to emerge from the puparium (Figure 2.B) compared to normal adults (Figure 2.C). FAW pupae that fail to reach the adult phase show symptoms of abnormal shape, immobility, and even death. This is thought to be due to the effects of the extracts in the previous phase. Several compounds in the extracts are thought to cause this, including one from the alkaloid group. Chitin production is inhibited by IGR compounds, which also mimic or inhibit the Juvenile Hormones (JH) (Nwonuma et al. 2025). Alkaloid compounds can inhibit JH biosynthesis (Ghoneim and Bakr 2018). These results showed that weed extracts can play an important role as an inhibitor of the development phase of FAW.



**Figure 1.** Abnormalities in FAW pupae due to the application of *Cynodon dactylon* aerial parts extract at 29% concentration. A. Larvae covered in exuvium and failed to molt, and B. Slightly damaged and blackened cuticles



**Figure 2.** Abnormalities in the FAW adult caused by *Cyperus rotundus* tuber extract at 33% concentration. A. The red circle indicates wing malformation, B. The red circle indicates an adult that failed to emerge from the puparium, and C. Normal adult

**Table 5.** Development (%) of fall armyworm (FAW) from larval to pupal and pupal to adult stages under different weed extract treatments and concentrations

Extract	Concentration (%)	Larvae reaching pupae (%) ( $\bar{x}\pm SD$ ) <sup>1</sup>	Pupae reaching adult (%) ( $\bar{x}\pm SD$ ) <sup>1</sup>
<i>Mimosa pudica</i> leaf	0	100.0±0.0a	100.0±0.0a
	40	60.0±8.2b	47.5±12.5b
	48	55.0±12.9b	37.5±9.6b
	55	37.5±17.0c	22.5±9.6c
	63	20.0±11.5d	10.0±8.2cd
<i>Cyperus rotundus</i> tuber	0	100.0±0.0a	100.0±0.0a
	9	72.5±1.0b	65.5±2.1b
	16	60.0±1.4bc	45.0±0.6c
	23	47.5±2.2cd	42.5±1.7c
	28	27.5±1.7de	12.5±0.5d
<i>Cynodon dactylon</i> aerial parts	0	100.0±0.0a	100.0±0.0a
	12	32.5±1.3b	22.5±1.7a
	15	30.0±2.2bc	17.5±1.0ab
	20	25.0±1.3bc	15.0±1.0ab
	25	20.0±1.4bc	10.0±0.8ab
<i>Lantana camara</i> leaf	0	100.0±0.0a	100.0±0.0a
	40	72.5±15.0b	62.5±17.0b
	45	60.0±8.1bc	50.0±14.1b
	50	37.5±5.7cd	17.5±9.5c
	55	30.0±14.1d	10.0±8.1cd
<i>Ageratum conyzoides</i> leaf	0	100.0±0.0a	100.0±0.0a
	23	47.5±1.0b	25.0±1.3b
	28	27.5±1.0bc	20.5±0.8c
	33	20.0±0.8bc	7.5±1.0d
	37	2.5±0.5c	0.0±0.0e
41	0.0±0.0c	0.0±0.0e	

Note: <sup>1</sup>Using ten larvae per replication, four replicates of each concentration were made. Numbers followed by different letters in the same column indicate significantly different results based on the DMRT test at a 5% error level.  $\bar{x}$  is the mean, and SD is the standard deviation

In conclusion, the administration of five types of invasive weed extracts can cause increased larval mortality, decreased feeding activity, decreased body weight, and decreased success rates in pupal and adult development. The LC<sub>50</sub> values from the smallest to highest at 144 h after application were for *C. dactylon* aerial parts extract (6%), *A. conyzoides* leaf extract (22%), *C. rotundus* tuber extract (25%), *L. camara* leaf extract (49%), and *M. pudica* leaf extract (53%). This study shows that five invasive weed plant extracts have the potential to be effective insecticides against FAW and can serve as one of the tactics in IPM. Because the toxicity of all extracts is lower than that of synthetic or semisynthetic insecticides, these extracts are not intended to replace them; rather, they can be used in combination to reduce reliance on synthetic insecticides, which are less environmentally friendly. The limitation of this study is that the impact of the five extracts on non-target organisms and research at the land scale has not been studied, so further research on this matter is needed.

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