THE EFFECT OF METHANOL EXTRACT OF Cerbera odollam LEAVES ON LOCOMOTION AND EXPLORATION ACTIVITIES OF Spodoptera frugiperda LARVAE

Pengaruh Ekstrak Metanol Daun *Cerbera odollam* Terhadap Aktivitas Lokomosi Dan Eksplorasi Larva *Spodoptera frugiperda*

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ABSTRACT

The fall armyworm (FAW), Spodoptera frugiperda, is one of the major pests of maize, exhibiting high adaptability and causing significant losses to agriculture in Indonesia. An environmentally friendly approach to controlling FAW infestations involves using local toxic plants, such as Cerbera odollam, also known as Bintaro, which contains cardenolide glycosides, saponins, and alkaloids with potential as botanical insecticides. This study aimed to evaluate the effect of the methanol extract of C. odollam leaves on the locomotion and exploration activities of FAW larvae. The test was conducted using the leaf dip method at concentrations of 0, 1000, 2000, and 4000 ppm, with six replications. The samples were then analyzed and observed using the Organism Video Tracking Application ToxTrac v2.96. Results showed that higher extract concentrations led to a decrease in larval speed, from 17.48 mm/s to 1.83 mm/s, and a reduction in exploration area, from 48 to 9. In addition, larval immobility duration (freeze activity) increased nearly sevenfold compared to the control. These findings indicate that C. odollam shows promise as a natural insecticide that repels FAW larvae by limiting their movement and exploratory behavior.

Keywords: *repellent; organism video tracking; bintaro; freeze activity; insect behaviour*

INTRODUCTION

Maize (*Zea mays* L.) is an important food commodity in Indonesia after rice, serving as a major source of carbohydrate energy, raw material for various industries, and a primary component of animal feed (Sholihat et al., 2022). According to data from the Indonesian Central Bureau of Statistics (2025), the demand for maize continues to increase along with population growth and the expansion of the feed industry, with dry shelled maize production in January 2025 reaching 1.24 million tons and production potential during February–April 2025 estimated at 4.7 million tons. Although national maize productivity remains relatively high, the presence of plant pests and diseases (PPDs) remains a significant constraint for farmers.

One of the major PPDs attacking maize crops is the fall armyworm (*Spodoptera frugiperda*). This invasive pest, commonly known as FAW or Fall Armyworm *(Spodoptera frugiperda)*, was first reported in Indonesia in March 2019 (Sartiami et al., 2020). FAW exhibits high adaptability, rapid life cycle, high reproductive capacity (with females laying more than 1200 eggs), and long-distance flight capability of up to 100 km per night (Montezano et al., 2018; Susanto et al., 2024a). FAW can survive under various environmental conditions in Indonesia, with mortality rates varying depending on the type of plant extract used for control (Subakti Putri et al., 2023; Susanto et al., 2024b). Damage caused by FAW can reach 15–73%, characterized by elongated holes on leaves due to neonate larvae feeding on young leaf tissues, while later instar larvae damage the whorl area, resulting in irregular holes and frass accumulation (Kumar et al., 2020; Sholihat et al., 2021; Subakti Putri et al., 2022). Data from West Java indicates that FAW attacks maize from the early vegetative stage up to tasseling, threatening local maize food security (Maharani et al., 2019).

FAW management in Indonesia still heavily relies on synthetic chemical insecticides, which pose risks of resistance development, non-target toxicity, and environmental contamination (Sinambela, 2024). Therefore, it is essential to seek out safer and more sustainable control strategies, for instance, botanical insecticides derived from toxic plants like *Cerbera odollam* (bintaro). This plant is known as one of the most poisonous species in the world, containing cardenolide glycosides, saponins, flavonoids, and alkaloids with potential insecticidal and antifungal properties (Sahoo & Marar, 2018; Purwani et al., 2014). Purwani et al. (2014) reported that the methanol extract of *C. odollam* leaves was effective in causing mortality of *Spodoptera litura* larvae. Botanical insecticides offer eco-friendly alternatives with multiple modes of action, reducing the likelihood of resistance and minimizing harm to non-target organisms and the environment (Serrão et al., 2022).

The extract of *C. odollam* has been shown to have insecticidal activity against several insect species. However, studies on its behavioral effects, such as movement and exploration activities, remain limited. Movement activity assays are a component of behavioral bioassays that detect changes in insect locomotion resulting from sublethal treatments (Phambala et al., 2020). These assays are important as they measure behavioral parameters such as average speed, exploration, distance traveled, and resting time, which are indicators of potential nervous system disruption or feeding deterrence (Rodriguez et al., 2017). This study aims to evaluate the effect of the methanol extract of *Cerbera odollam* leaves on the locomotion and exploration activities of FAW larvae as a behavioral bioactivity approach to assess the potential of *C. odollam* as a botanical insecticide.

METHODS

Preparation of *Spodoptera frugiperda*

The *FAW* larvae used in this study were collected from maize cultivation areas at Universitas Padjadjaran, Sumedang Regency, West Java, Indonesia. The insects were reared in the insect rearing room under controlled conditions at a temperature of 28±1°C, a relative humidity of 72±15%, and a photoperiod of 12 hours of light and 12 hours of darkness. After the larvae developed into adults and laid eggs, egg masses were transferred to specific hatching containers. Larvae that reached the third instar stage were then individually isolated into plastic cups for subsequent bioassays.

Preparation of *Cerbera odollam* Extract

Fresh green leaves of *C. odollam* were collected along roadsides in the Cileunyi Wetan area, Bandung Regency, West Java. The collected leaves were thoroughly washed under running water to remove dust and other debris, then air-dried. A total of 100 g of dried leaf powder was macerated using analytical-grade methanol as the solvent, with a sample-to-solvent ratio of 100 g:1 L. The maceration process was carried out for 3 × 24 hours (72 hours) at room temperature, with stirring every 24 hours.

The resulting macerate was filtered using Whatman grade 1 filter paper, and the filtrate was concentrated using a rotary vacuum evaporator at approximately 218 mbar to reach a boiling point of ±30°C (with a heating bath set at 50°C) until a thick extract was obtained. The thick extract (*extractum spissum*) was stored in a tightly closed container in a refrigerator (≤4°C) until use.

Locomotion and Exploration Activity Assay

Observations of the movement activity of *Spodoptera frugiperda* larvae were conducted using ToxTrac v2.96 video tracking software by Universisase Da Coruña (Rodriguez et al., 2017). Third instar larvae were starved for 4 hours before treatment and individually placed in a test arena consisting of a 9 cm diameter Petri dish. Treatments were administered using the dip method, in which maize leaves used as larval feed were dipped into methanol extracts of *C. odollam* leaves at concentrations of 0 ppm (control), 1000 ppm, 2000 ppm, and 4000 ppm for approximately 10 seconds, then air-dried until the solution was evenly coated and no longer dripping.

The test was conducted using the leaf dipping method with feeding assays, with six replicates for each treatment, to determine the minimum effective concentration for feeding deterrent activity. The treated leaf was placed at the center of the Petri dish, while the test larva was positioned at the edge of the dish, approximately 3.5 cm from the feed, with consistent initial placement for all treatments.

Larval activity videos were recorded for 10 minutes using a digital camera equipped with a 100 mm macro lens, at a minimum resolution of 720p and a frame rate of 25 frames per second (fps). The camera was positioned perpendicularly (in a top-down view) at approximately 30 cm above the arena surface. The entire recording setup was placed on an anti-vibration table to minimize mechanical disturbances during video acquisition. The room temperature during observation was maintained at 25 ± 2°C with a relative humidity of 70–80% to minimize environmental variables that could affect larval behavior.

Data Analysis

Data obtained from the observations of larval movement activity were statistically analyzed using analysis of variance (ANOVA) at a 95% level. All analyses were conducted using SPSS software version 27.0.

RESULT AND DISCUSSION

The results showed that the average speed and mobile average speed of FAW larvae decreased with increasing concentrations of the methanol extract of *C. odollam* leaf (Table 1). Control larvae recorded the highest average speed (17.48±1.61 mm/s) and mobile average speed (23.40±1.87 mm/s), indicating active movement when untreated (Table 1). In contrast, larvae treated with the highest concentration (4000 ppm) exhibited the lowest speeds, with an average speed of only 1.83±0.33 mm/s. The mobile average speed was 19.57±1.20 mm/s, suggesting suppressed locomotor activity or avoidance behavior under extract exposure.

The decline in locomotor activity suggests that exposure to the extract suppressed larval movement, possibly due to its neurotoxic or repellent effects. Similar results were reported by Phambala et al. (2020), botanical extracts reduced the locomotion and feeding activities of FAW larvae. Likewise, Plata-Rueda et al. (2020)

observed that plant-derived compounds are able to interfere with insect nervous systems, leading to reduced mobility and exploratory behaviour.

Table 1. Speed and acceleration parameters of fall armyworm (FAW) larvae.

Description	Treatment (X±SE)			
	0 ppm (Control)	1000 ppm	2000 ppm	4000 ppm
Average Speed (mm/s)	17.48±1.61	10.74±1.82	6.06±1.02	1.83±0.33
Mobile Average Speed (mm/s)	23.40±1.87	15.12±1.73	19.95±4.84	19.57±1.20
Average Acceleration (mm/s^2)	105.04±17.79	100.77±19.55	68.11±13.97	22.23±4.71

Notes : (X) Average; (SE) Standard Error.

The percentage of FAW larvae mobility rate decreased sharply with increasing extract concentration, from 64±1.47% in the control to only 5.75±1.38% at 4000 ppm, indicating reduced movement activity. On the other hand, the percentage of visibility rate increased from 71.46±5.90% in the control to 96.18±1.90% at 4000 ppm. Invisible time and frames also decreased along the concentration gradient, supporting reduced exploration behaviour.

Although larvae were visible, their mobility was low, and the larvae remained stationary for longer durations. These phenomena indicate that higher extract concentrations significantly impaired larval mobility and exploration. Rodriguez et al. (2017) highlighted that such reductions in mobility parameters reflect sublethal behavioural disruption, potentially interfering with foraging efficiency and survival.

Table 2. Mobility and visibility parameters of fall armyworm (FAW) larvae.

Description	Treatment (X±SE)			
	0 ppm (Control)	1000 ppm	2000 ppm	4000 ppm
Mobility Rate (%)	64±1.47	62.95±2.93	33.59±11.47	5.75±1.38
Visible Frames	10578.15±0.59	11716.88±862.35	13744.66±486.16	14235.75±281.43
Visible Time (m:s)	7:63.1	7:41.02	9:24.51	9:29.37
Invisible Frames	4223.85±62.89	3085.13±862.35	1306.78±304.68	565.25±154.27
Invisible Time (m:s)	2:48.9	1:29.3	0:22.76	0:19.15
Visibility Rate (%)	71.46±5.90	79.16±5.83	92.86±3.29	96.18±1.90
Invisibility Rate (%)	28:54±4.80	20.84±5.83	7.14±3.29	3.82±1.90

Notes : (X) Average; (SE) Standard Error.

The explored areas and exploration rate (%) of FAW larvae declined significantly as extract concentration increased (Table 3). Control larvae explored an average of 47.93±1.73 areas with an exploration rate of 21.93±1.33%, while at 4000 ppm, larvae only explored 9.00±2.00 areas with an exploration rate of 4.05±0.83% (Table 3). Similarly, the total distance travelled decreased from 10150.44±1148.34 mm in the control to 1267.42±240.28 mm at 4000 ppm. This suggests strong suppression of larval locomotion and exploratory behaviour under high extract exposure.

The extract of *C. odollam* significantly reduced the movement and exploration activities of FAW larvae, indicating a repellent effect. Similar results were reported by Akter et al. (2024), who found that 10% castor oil repelled up to 93% of FAW larvae without requiring ingestion. In addition, leaf extracts of *C. manghas* were also reported to reduce *Spodoptera litura* populations through the repellent effects of its secondary metabolites (Susilo et al., 2020). These findings support the potential of *C. odollam* as a botanical insecticide with strong repellent properties.

Table 3. Exploration and distance parameters of fall armyworm (FAW) larvae.

Description	Treatment (X±SE)			
	0 ppm (Control)	1000 ppm	2000 ppm	4000 ppm
Explored Areas	47.93±1.73	47.75±1.54	31.21±7.78	9.00±2.00
Number of Areas	219.22±5.18	221.25±6.27	125.00±3.55	91.25±6.60
Exploration Rate (%)	21.93±1.33	21.61±1.13	11.43±2.18	4.05±0.83
Total Distance Travelled (mm)	10150.44±1148.34	6089.51±1550.27	3459.03±624.92	1267.42±240.28

Notes : (X) Average; (SE) Standard Error.

Control larvae exhibited the fewest freezing events (4.13±1.14) with a total freezing time of only 1:21.84 minutes, while larvae treated with 4000 ppm showed significantly more frequent freezing (11.39±0.35) and the longest total freezing duration (8:65.03 minutes) (Table 4). This suggests that higher concentrations induce immobilization or stress-related freezing, which is potentially due to neurotoxic or deterrent effects.

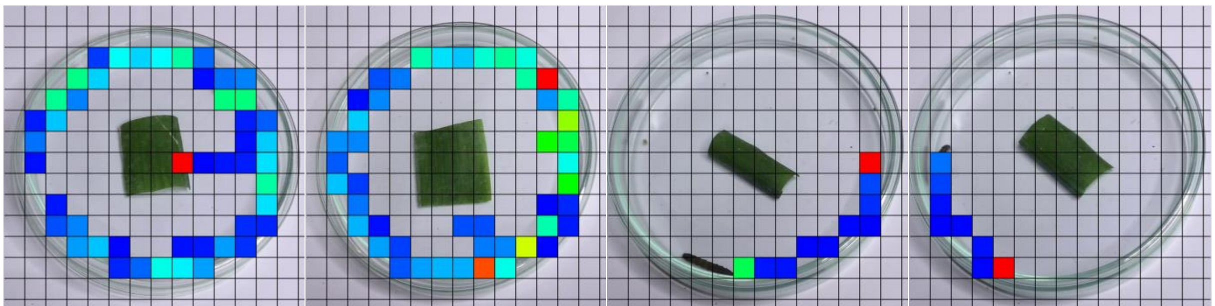
Moreover, Figure 1 shows that control larvae explored widely and settled on the maize leaf, indicating feeding activity (red points on the feed). At 1000 ppm, larvae moved around the arena with some approaches but did not remain on the feed, suggesting mild repellency. At 2000 ppm and especially at 4000 ppm, larvae exhibited minimal movement. They exhibited prolonged freezing periods at the arena edges, as indicated by the concentration of red points away from the feed. This illustrates a shift from active feeding in the control to avoidance and immobility at higher extract concentrations.

Previous studies have demonstrated that *C. odollam* contains potent insecticidal compounds, including cardenolides, saponins, and alkaloids (Saxena et al., 2022; Winarni et al., 2022). Purwani et al. (2014) reported that methanol extracts of *C. odollam* leaves caused significant mortality in *S. litura* larvae, while its larvicidal activity against mosquitoes has been linked to neuromuscular disruption and feeding inhibition (Sahoo & Marar, 2018). Additionally, Subakti Putri et al. (2023) found that methanol extracts of *Nerium oleander*, another toxic plant rich in cardiac glycosides like *C. odollam*, reduced FAW feeding and mobility, resulting in prolonged larval duration and lower survival. These findings support the potential of *C. odollam* as a botanical insecticide with both strong repellent and toxic effects.

Table 4. Freeze and transition parameters of fall armyworm (FAW) larvae.

Description	Treatment (X±SE)			
	0 ppm (Control)	1000 ppm	2000 ppm	4000 ppm
Frozen Events	4.13±1.14	5.88±2.37	11.01±3.22	11.39±0.35
Total Time Frozen (m:s)	1:21.84	2:31.53	5:40.23	7:15.03
Average Time Frozen (m:s)	0:3.74	0:6.07	1:12.99	1:49.25

Notes : (X) Average; (SE) Standard Error.



Control	1000 ppm	2000 ppm	4000 ppm

Figure 1. Distribution maps of locomotion and exploration zones of fall armyworm (FAW) larvae recorded over a 10-minute behavioral assay. The color gradient represents the duration the larvae spent in each zone, ranging from dark blue (shortest) to red (longest).

CONCLUSION

The application of the methanol extract of Cerbera odollam at concentrations ranging from 1000 to 4000 ppm resulted in a substantial reduction in FAW larval speed by up to 90%, along with a sharp decline in explored areas from 48 in the control to just 9 at the highest concentration. Additionally, freezing duration increased nearly sevenfold compared to control larvae. These results demonstrate its substantial behavioural disruption, indicating both neurotoxic and repellent effects. Therefore, *C. odollam* methanol extract shows potential as an effective, plant-based insecticide for managing FAW. However, further field testing is necessary to ensure its safety and practical application.

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