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EFFECT OF SOME *AJUGA IVA* LEAF EXTRACTS ON CERTAIN BIOLOGICAL ASPECTS OF THE GREATER WAX MOTH (*GALLERIA MELLONELLA* L.).

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ABSTRACT

This study was conducted to determine the effect of treating the food of third-instar larvae of the greater wax moth (*Galleria mellonella*) with ethanol and hexane extracts of *Ajuga iva* leaves at concentrations of 5%, 7.5%, and 10%, and their impact on the biological aspects of the insect. The results showed that the 10% concentration achieved the highest larval mortality rate, with an average of 51.84% for both extracts, while the lowest mortality rate was 19.17% at the 5% concentration, and 3.33% in the control treatment. Additionally, there was a reduction in the percentage of larvae that pupated, with the ethanol extract being more effective in reducing the number of pupae its reached average 61.68% and 82.53% for the ethanol and hexane extracts, respectively, compared to 96.70% in the control treatment. The highest rate of pupal deformities was observed when treated with the 10% ethanol extract. It was also noted that the ethanol extract significantly prolonged the larval and pupal stages. The results also indicated that the 10% ethanol extract had the highest impact in reducing the adult longevity, reduced the number of eggs and laid by females reduced the egg hatch rate.

Key words: **Egg hatch, Fertility, Larvae, mortality rate.**

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INTRODUCTION

The greater wax moth (*Galleria mellonella*) is one of the significant pests that affect the wax frames of beehives. The damage is primarily caused by larvae feeding on the wax, honey residues, and stored pollen within the wax frames. The larvae also bore into the hive's wood to settle in before pupating, in addition to secreting silk threads, which lead to the destruction of the hexagonal cells, ultimately causing the collapse of weak colonies and weakening active ones (Al-Joranyet al.,2004; Kurdi,1996). The greater wax moth is also a widespread pest wherever honey bees (*Apis mellifera*) are found. Its larvae burrow into the edges of capped cells containing pollen, bee brood, and honey, often reaching the center of the honeycomb. The larvae of greater wax moth cause significant damage in tropical and subtropical regions and are a major nuisance to honey bee colonies (Kwadha et al., 2017). Therefore, it is one of the pests that affect honey bee colonies, causing considerable losses to beekeeping (Sohal et al., 2017; Vijayahumar et al., 2019). Various methods have been used to control the insect, including chemical pesticides and fumigation with Phostoxin and Methyl bromide. Researchers are seeking biologically active and non-toxic substances to control pests, which has led them to explore plant-based sources. Plants contain natural compounds that can serve as anti-feedants, repellents, toxic substances, or growth regulators. Plants possess numerous compounds that can be utilized to develop environmentally sound methods for increase

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the plant yield and insect control (Ismael and Nasralla, 2013; Jbilou et al., 2009; Szaboet al., 1997). Phytoecdysteroids (pE) are polyhydroxylated compounds derived from plant sterols that are naturally produced in plants. They are also found in algae, ferns, and fungi and have a chemical structure similar to the molting hormone in insects. Over 500 different pE compounds have been identified in more than 100 plant species, with the most common type being 20-hydroxyecdysone (B-ecdysone). These compounds help alleviate both biotic and abiotic stresses by playing a significant role in enhancing antioxidant enzyme defense systems. They also stimulate the biosynthesis of proteins, lipids, and carbohydrates in plants, as well as enhance plant resistance against insects, fungi, salinity, and other stress factors (Arif et al., 2022; Zibareva et al., 2009). Phytoecdysteroids provide protection to plants from insects by altering the natural levels of molting hormone in insects, leading to significant disruption in their growth and development. Therefore, these compounds can be used against insects and can be integrated into pest management programs reported that phytoecdysteroids contain a wide range of biologically active steroids similar to the molting hormone in insects, where they are distributed as secondary metabolites that offer protection against insects (Chaubey, 2018; Das et al., 2021). Ecdysteroids are steroid hormones that regulate the molting process in insects and control many transformations in arthropods. The same phytoecdysteroids have been found in various plant species, including the *Ajuga* genus. One such compound, β -7-ene-6-one, controls physiological activity in insects (Aly et al., 2023). The leaves of *Ajuga iva* contain various chemical compounds, which were obtained through methanolic extraction, including alkaloids, tannins, flavonoids, glycosides, coumarins, steroids, saponins, and terpenoids (Ismael and Nasralla, 2013). These compounds have multiple effects on the life cycle of many insects, such as the cotton leafworm (*Spodoptera littoralis*), the Indian meal moth (*Plodia interpunctella*), the mosquito (*Culex pusillus*), and other insect pests (Kazem and Mohsen, 2011; Rharrabe et al., 2010). Given the economic importance of the greater wax moth (*Galleria mellonella*) on honey bees, this study was conducted to use safe and environmentally friendly substances to control the insect and examine their effects on its biological aspects.

MATERIALS AND METHODS

Laboratory Rearing of the Insect

Wax frames infested with the greater wax moth were collected from various beehives in Abu Ghraib District, Baghdad Governorate. The wax was cut into pieces and placed in a plastic container, which was then kept in an incubator at a temperature of 27°C and 5% humidity in the laboratory to maintain a continuous life cycle of the insect. The larvae were fed continuously with dark beeswax, which was placed in the freezer for three days to ensure it was free from infestation (Al-Jubouri, 2020).

Preparation of *A. iva* leaves extracts by ethanol and hexane: The leaves of *Ajuga iva* were obtained from commercial markets due to their high content of chemical compounds. The leaves were ground using a high-speed multifunctional crusher (German-made, *Germany*).

Extraction Process of *A. iva* Leaves

The extraction process was carried out in the Plant Extracts Laboratory at the Plant Protection Directorate. A total of 500 grams of *Ajuga iva* leaf powder was divided into two parts, each weighing 250 grams. Each part was placed in a 2-liter plastic container, and 1 liter of 99.9% ethanol was added the last part was added to 1 liter of hexane. The container was sealed tightly and left for 48 hours on a rocker shaker (German-made) operating at 70 shakes per minute to obtain the crude extract (raw material). The extract was then filtered using a Buchner funnel with *Whatman No. 1* filter paper to remove impurities. The filtrate was concentrated using a rotary evaporator with a vacuum pump at 40°C and a rotation speed of 100 rpm to obtain the crude extract (Al-khazraji et al., 2016). The extract was stored in sterile sample containers with all relevant plant sample information labeled, and the containers were placed in a freezer until the experiment was conducted. The same steps were followed to prepare 500 grams of hexane extract.

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Preparation of Ethanol and Hexane Extract Concentrations from *A. iva* Leaves: Concentrations of 5%, 7.5%, and 10% for both the ethanol and hexane extracts were prepared by dissolving 5 grams of the extract in water and adjusting the volume to 100 ml to obtain the 5% concentration. Similarly, 7.5 grams and 10 grams of the extract were dissolved in the same way to prepare the 7.5% and 10% concentrations, respectively. A few drops of *Dimethyl sulfoxide (DMSO)* were added to aid in dissolving and homogenizing the extract. For the control treatment, the larvae were treated with water only (Baday et al., 2015).

Treatment of Third-Instar Larvae : Eleven-day-old larvae were selected and distributed at a rate of 10 larvae per replicate, placed in plastic dishes with a height of 7 cm and a diameter of 12 cm. The experiment was conducted with three replicates for each treatment. Each dish was provided with a piece of wax measuring 10 x 10 cm. The wax pieces were treated using a 14 ml manual sprayer from a distance of 5 cm, applying 14 ml of the solution for each replicate. The wax pieces were left for 10 minutes to dry before being placed in the dishes. Vaseline was applied to the edges of the dishes to prevent the larvae from escaping, and the dishes were covered with a tight, perforated lid secured with a rubber band. The dishes were then placed in an incubator at 27°C. The dishes were monitored daily to assess the effect of the extracts on various biological aspects of the insect, including larval mortality rates, duration of the larval stage, pupation rates, duration of the pupal stage, adult emergence rates, adult longevity, number of eggs laid, hatch rates, and insect deformities.

Statistical Analysis : The results of the laboratory experiments were analyzed using a Completely Randomized Design (CRD). Data were statistically analyzed through an Analysis of Variance (ANOVA) table, employing the Least Significant Difference (LSD) test to compare treatment means at a significance level of 0.05 (Al-Rawi and Khalaf Allah, 2000). The statistical analysis was conducted using the Genstat 2011 software. Mortality rates were corrected using the Orall and Schnider equation, a modification of Abbott formula referred to in (Al-khazraji et al., 2016).

$$\text{Corrected Mortality rate} = \frac{(\%\text{ treatment mortality} - \text{Control mortality}\%}{100 - \text{Control mortality}\%} \text{x} 100$$

RESULTS AND DISCUSSION

Effect of *A. iva* extracts on the percentage mortality of *G. mellonella* larvae: The results shows that the plant extracts had a significant impact on larval mortality **(Table 1)**. The results demonstrated a direct relationship between the extract concentration and mortality rate, with the 10% concentration causing the highest mortality rate of 51.84%, while the lowest mortality rate was 19.17% for the 5% concentration. The overall mortality rates were 49.00% and 19.78% for the ethanol and hexane extracts, respectively. Statistical analysis revealed significant differences between the concentrations used in the study. The 10% concentration resulted in the highest mortality rates of 72.67% and 31% for the ethanol and hexane extracts, respectively, while the 5% concentration caused the lowest mortality rates of 27.67% and 10.67%, respectively. The control treatment showed a mortality rate of only 3.33%. Furthermore, statistical analysis indicated highly significant differences between the extracts. The larval mortality of *G. mellonella* may be attributed to the presence of toxic compounds in the extracts, which likely act directly on the insect's nervous system. It has been observed that phenolic compounds act as inhibitors of the enzyme acetylcholine esterase, leading to the excessive release of acetylcholine, causing its accumulation at nerve endings, followed by paralysis and eventually death (Al-Marmadhi, 2014;Burhlmann et al., 2004). Al-Jubouri (2020) indicated that the ethanol extract of *Dodonaea* leaves had a lethal effect on the larvae of the greater wax moth using concentrations of 2%, 5%, and 10%, with third-instar larval mortality rates of 38.1%, 60.2%, and 67.2%, respectively, and 31.1%, 45.2%, and 55.2% for fifth-instar larvae. Rharrabe et al. (2009) reported that the effect of phytoecdysteroid on larvae treated may be due to the toxicity of compounds and their effect on the midgut cells.

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Table 1. The percentage of mortality in *G. mellonella* larvae treated with *A. iva* plant extracts

Extract	Corrected Mortality (%) Concentration %			
	%5	%7.5	%10	Mean
Ethanolic *A. iva*	27.67	46.67	72.67	49.00
Hexane *A. iva*	10.67	17.67	31.00	19.78
LSD 0.05		5.35**		2.68**
Mean	19.17	32.17	51.84	
LSD 0.05		3.78**		

Effect of *A. iva* extracts on the larval duration of *G. mellonella* larvae: The results in **(Table 2)** indicate that treating third-instar larvae with ethanol and hexane extracts of *Ajuga iva* leaves led to an increase in larval duration. This was evident by the presence of larvae of different ages and sizes at the same time. The larval duration was 39.33, 55, and 64 days for the 5%, 7.5%, and 10% ethanol extracts, respectively, while it was 33.67, 37, and 40.33 days for the hexane extracts at the same concentrations. The control treatment showed a larval duration of 30.33 days. Statistical analysis of the results revealed that the ethanol extract significantly outperformed the hexane extract, with the larval duration averaging 47.17 days for the ethanol extract and 35.33 days for the hexane extract, showing highly significant differences. The prolonged larval duration could be attributed to the presence of plant-based phytoecdysteroids, which affect insect behavior and are found in many plant extracts. These compounds are known to delay molting by influencing the molting hormone. Chaubey (2018) indicated that plant extracts contain chemical compounds that extend larval development by inhibiting the synthesis of the molting hormone *ecdysone*. Rahmani and Ouahrani (2022) stated that *Ajuga iva* leaves contain various chemical compounds, such as alkaloids, tannins, flavonoids, glycosides, coumarins, steroids, saponins, and terpenoids, which have repellent and anti-feedant effects.

Effect of *A. iva* extracts on the pupal duration of *G. mellonella* larvae: The results in **(Table 3)** show that treating third-instar larvae with ethanol and hexane extracts of *Ajuga iva* leaves led to an increase in the pupal duration. The pupal durations were 13, 10.67, and 7 days for the 5%, 7.5%, and 10% ethanol extracts, respectively, while they were 14.67, 9.67, and 9 days for the

Table 2. Larval duration of *G. mellonella* larvae treated with *A. iva* plant extracts

Extract	Larval duration (day) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	30.33	39.33	55.00	64.00	47.17
Hexane *A. iva*	30.33	33.67	37.00	40.33	35.33
LSD 0.05			2.10**		1.05**
Mean	30.33	36.50	46.00	52.17	
LSD 0.05			1.48**		

hexane extracts at the same concentrations. Statistical analysis showed that the ethanol extract significantly outperformed the other treatments, with an average pupal duration of 12.00 days. Additionally, the analysis revealed significant differences between the concentrations. The prolonged pupal duration is attributed to the presence of certain compounds in *Ajuga* leaves that interfere with molting. These compounds act as growth regulators and mimic the action of the molting hormone (juvenile hormone, JH). These findings are in line with Al-Jubouri (2020), who reported that the ethanol extract of *Dodonaea* leaves extended the pupal duration to 8.75, 10.16, and 10.25 days for concentrations of 2%, 5%, and 10%, respectively. Rharrabe et al. (2010) studied the effect of the methanol extract of *Ajuga iva* (L.) Schreiber against the Indian meal moth *Plodia*

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interpunctella Huber (Lepidoptera: Pyramidal). Their results showed that using a concentration of 200 ppm affected the pupation rate, which was 8%, 43.4%, and 35% due to the effects of the compounds Ponasterone A, Polypodine B, and 20-hydroxyecdysone, respectively. The compound Makisterone A inhibited larval pupation, with a rate of 79%. Additionally, a study confirmed the effect of the ethanol extract of *Adhatoda vasica* on third-instar larvae of the Mediterranean fruit fly (*Ceratitis capitata*), using 5% and 7.5% concentrations, which inhibited the molting hormone, leading to an extended pupal duration (Mohmoud et al., 2019).

Effect of *A. iva* extracts on the pupation rate of *G. mellonella* larvae: The results in **(Table 4)** reveal the effect of ethanol and hexane extracts from *Ajuga iva* leaves on the pupation rate of larvae treated with concentrations of 5%, 7.5%, and 10%. The pupation rates were 70%, 53.30%, and 26.70% for the ethanol extract, respectively, while the hexane extract showed rates of 86.70%, 80%, and 66.70%, respectively. The control treatment had a pupation rate of 96.70%. There was an inverse relationship between the pupation rate and the concentration used, with the lowest rate of 46.70% at the 10% concentration and the highest rate of 78.35% at the 5% concentration. Statistical analysis revealed significant differences between the concentrations. The ethanol extract resulted in the highest inhibition of pupae formation from treated larvae, while the hexane extract caused the least inhibition in the pupation of the greater wax moth larvae. Statistical analysis also showed significant differences between the extracts. A study on the effect of black pepper (*Piper nigrum*) fruit extract on the cotton leafworm (*Spodoptera littoralis*) showed that the pupation rate of second-instar larvae was 54.9%, and 91.6% for sixth-instar larvae, while the control treatment resulted in 91.5% and 100% pupation rates for the second and sixth instars, respectively (Al-khazraji et al., 2016).

Table 3. Pupal duration of the greater wax moth treated with *A. iva* plant extracts

Extract	Pupal duration (day) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	6.67	9.67	13.33	14.00	10.92
Hexane *A. iva*	6.67	6.67	10.00	10.67	8.50
LSD 0.05		1.30**			0.65**
Mean	6.67	8.17	11.67	12.34	
LSD 0.05		0.92**			

Table 4. Pupation rates of *G. mellonella* larvae treated with *A. iva* plant extracts

Extract	Pupation rates (%) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	96.70	70.00	53.30	26.70	61.68
Hexane *A. iva*	96.70	86.70	80.00	66.70	82.53
LSD 0.05		11.91**			5.96**
Mean	96.70	78.35	66.65	46.70	
LSD 0.05		8.42**			

Effect of *A. iva* Extracts on the Deformity Rates of *G. mellonella* Pupae: The results in **(Table 5)** show the effect of treating third-instar larvae with ethanol and hexane extracts on the deformities resulting from the treatments. These deformities manifested as intermediate stages between the larval and pupal phases, along with the presence of dried and hardened pupae, and delayed adult emergence. The deformity rates for the ethanol extract were 37.10%, 34.17%, and 8.33% at concentrations of 5%, 7.5%, and 10%,

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respectively, while the hexane extract showed rates of 65%, 62.10%, and 45.30%, respectively. Statistical analysis revealed significant differences in deformity rates between the extracts and concentrations used. The 5% concentration caused the highest deformity rate at 51.05%, while the lowest deformity rate of 26.82% occurred at the 10% concentration. The control treatment showed a 0.00% deformity rate. The hexane extract outperformed the ethanol extract in terms of causing deformities. The difference in deformity rates can be attributed to the number of surviving larvae in each treatment, as the 10% concentration achieved the highest mortality rate, resulting in fewer deformities. Statistical analysis also revealed significant differences between the average concentrations and plant extracts. Al-Khazraji et al., (2016) reported that treating sixth-instar larvae of the cotton leafworm with black pepper fruit extracts caused pupal deformities of 6% and 8.5% at concentrations of 1.25% and 2.5%, respectively. These deformities appeared as intermediate stages between the larval and pupal phases. Salah et al., (2002) explained that the deformities were due to active compounds in plants that affect hormonal neural secretions. Terpenoids, which are present in plant extracts, act as secondary compounds that function as juvenile hormone (JH) antagonists, due to their similarity to insect growth regulators (Abdul-Karim, 2012).

Table 5. The effect of *A. iva* extracts treatment on the deformity rate in *G. mellonella* pupae

Extract	Deformity rate in *G. mellonella* pupae (%) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	0.00	37.10	34.17	8.33	19.90
Hexane *A. iva*	0.00	65.00	62.10	45.30	43.10
LSD 0.05		6.69**			3.35**
Mean	0.00	51.05	48.14	26.82	
LSD 0.05		4.73**			

Effect of *A. iva* extracts on the adult emergence rates of *G. mellonella*: The results in **(Table 6)** show that treating third-instar larvae with ethanol and hexane extracts of *Ajuga iva* leaves at concentrations of 5%, 7.5%, and 10% affected the adult emergence rates from the pupae of treated larvae. The emergence rates were 53.17%, 29.17%, and 26.67% for the ethanol extract, and 35.00%, 33.60%, and 30.16% for the hexane extract, respectively. The control treatment showed a 100% emergence rate. The effect of the different concentrations on adult emergence rates revealed an inverse relationship between concentration and emergence rate. The 5% concentration resulted in the highest adult emergence rate at 44.09%, while the 10% concentration resulted in the lowest rate at 28.42%. Statistical analysis indicated no significant differences between the extracts, but there were significant differences between the concentrations. Al-Jubouri (2020) reported that the ethanol extract of *Dodonaea* leaves affected the adult emergence rate of the greater wax moth, with an average rate of 86.20% using concentrations of 2%, 5%, and 10% in treated third-instar larvae. The adult emergence rate for treated fifth-instar larvae averaged 88.6%.

Table 6. The effect of *A. iva* extracts treatment on the adult emergence rate *G. mellonella*

Extract	Adult emergence rates (%) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	100.00	53.17	29.17	26.67	52.25
Hexane *A. iva*	100.00	35.00	33.60	30.16	49.69
LSD 0.05		7.08**			N.S
Mean	100.00	44.09	31.39	28.42	
LSD 0.05		5.01**			

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Effect of *A. iva* plant extracts on the longevity of *G. mellonella* adults: The results in (**Table 7)** indicate that treating third-instar larvae with ethanol and hexane extracts of *Ajuga iva* leaves at concentrations of 5%, 7.5%, and 10% reduced the longevity of male moths. The longevity of males were 13, 10.67, and 7 days for the ethanol extract, and 14.67, 9.67, and 9 days for the hexane extract, respectively. For females, the longevity were 6.67, 6, and 3.67 days for the ethanol extract, and 7.67, 4.67, and 5 days for the hexane extract at the same concentrations. In the control treatment, the longevity was 17.33 days for males and 9 days for females. Statistical analysis revealed that the ethanol extract significantly outperformed the other treatments, with highly significant differences. There was an inverse relationship between adult longevity and the concentrations used. The shortest male longevity was observed at the 10% concentration, with an average of 8 days, while the longest longevity was at the 5% concentration, averaging 13.84 days. For females, the shortest longevity was at the 10% concentration, averaging 4.33 days, and the longest was at the 5% concentration, averaging 7.17 days. The results also indicated that the size of emerging adults was smaller, especially when treated with the 10% concentration. Statistical analysis showed highly significant differences between the extracts. A study on the effect of the ethanol extract and the nanomaterial preparation of *Dodonaea* on male moths resulting from the treatment of third-instar larvae with concentrations of 2%, 5%, and 10% showed that the male longevity averaged 15.50, 16.00, and 12.00 days, respectively, compared to 17.00 days for the control (Al-Jubouri, 2020).

Effect of *A. iva* plant extracts on the average number of eggs laid by *G. mellonella* Adults: The results in **(Table 8)** show a decrease in the average number of eggs laid by adult females as a result of treating third-instar larvae with concentrations of 5%, 7.5%, and 10% of *Ajuga iva* leaf extracts. The number of eggs laid was 349.70, 265, and 200.70 eggs per female for the ethanol extract, respectively, and 546.70, 490.30, and 300 eggs per female for the hexane extract, respectively. In comparison, the control treatment resulted in 686.30 eggs per female. Statistical analysis indicated highly significant differences between the concentrations and their averages, as well as between the types of extracts. The ethanol extract significantly reduced the number of eggs laid, showing highly significant differences. There was an inverse relationship between the number of eggs laid and the concentrations used, with the lowest average of 250.35 eggs per female at the 10% concentration, and the highest

Table 7. The longevity of adult *Galleria mellonella* treated with *A. iva* extracts

Extract	longevity of males (days) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	17.33	13.00	10.67	7.00	12.00
Hexane *A. iva*	17.33	14.67	9.67	9.00	12.67
LSD 0.05		1.07**			0.54**
Mean	17.33	13.84	10.17	8.00	
LSD 0.05		0.76**			
Extract	longevity of females (days) Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	9.00	6.67	6.00	3.67	6.33
Hexane *A. iva*	9.00	7.67	4.67	5.00	6.58
LSD 0.05		0.68**			N.S
Mean	9.00	7.17	5.33	4.33	
LSD 0.05		0.48**			

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average of 448.20 eggs per female at the 5% concentration. The smaller size of the pupae resulted in smaller adults, which in turn reduced the size of the ovaries in females due to poor nutrition, leading to a lower number of eggs. Galvan et al. (Galvan et al., 2005) explained that the decrease in female fertility is due to the natural compounds present in the extracts, which act as anti-feedants for larvae, reducing the weight of adult insects. Al-Jorany et al. (2004) found that treating 8-day-old larvae of the greater wax moth with *Eucalyptus camaldulensis* extracts reduced the average number of eggs laid, with results of 592.1, 591.22, and 498.55 eggs per female, compared to 678.52 eggs per female in the control treatment. Sahi (2019) reported that the ethyl alcohol extract of *Sesbania sesban* seeds had an effect on adult females of the khapra beetle (*Trogoderma granarium*), showing that the extract contains phytoecdysteroid compounds, which reduce or prevent females from laying eggs. These compounds resemble the molting hormone (*ecdysone*) found in insects, which plays a role in reducing female productivity and egg maturation. Al-Jubouri (2020) found that treating third-instar larvae of the greater wax moth with ethanol extract of *Dodonaea* reduced the average number of eggs laid by adult females resulting from the treatment of fifth-instar larvae, with an average of 494.25 eggs per female, compared to 630 eggs per female in the control treatment.

Table 8. The average number of eggs laid by *G. mellonella* treated with *A. iva* extracts

Extract	Average number of eggs laid Concentration %				
	Control	%5	%7.5	%10	Mean
Ethanolic *A. iva*	686.30	349.70	265.00	200.70	375.43
Hexane *A. iva*	686.30	546.70	490.30	300.00	505.83
LSD 0.05		35.74**			17.87
Mean	686.30	448.20	377.65	250.35	
LSD 0.05		25.27**			

Effect of *A. iva* extracts on the hatch rate of *G. mellonella* eggs: The results in **(Table 9)** show that treating the food of third-instar larvae of the greater wax moth with ethanol and hexane extracts at concentrations of 5%, 7.5%, and 10% reduced the hatch rate of eggs laid by females. The hatch rates were 68.86%, 71.03%, and 23.42% for the ethanol extract, and 94.56%, 80.17%, and 30.92% for the hexane extract, respectively. In comparison, the hatch rate for the control treatment was 98.09%. Statistical analysis confirmed significant differences between the treatments, concentrations, and extract types. There was also an inverse relationship between the hatch rate and the concentrations used, where increasing the concentration resulted in a lower hatch rate. The highest average hatch rate was 81.71% at the 5% concentration, while the lowest hatch rate was observed at the 10% concentration. Al-Jourany and Al-Khazraji (2022) found that the hexane extract of *Withania somnifera* leaves at a concentration of 5% inhibited the hatch rate of *Spodoptera littoralis* eggs by 75.00%.

Table 9. The hatch rate of *G. mellonella* eggs treated with *A. iva* extracts

Extract	Hatch rate of *G. mellonella* eggs Concentration %				
	Control	5%	7.5%	10 %	Mean
Ethanolic *A.iva*	98.09	68.86	71.03	23.42	65.35
Hexane *A.iva*	98.09	94.56	80.17	30.92	75.94
LSD 0.05		3.64**			1.82**
Mean	98.09	81.71	75.60	27.17	
LSD 0.05		2.57**			

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CONCLUSION

The current study demonstrated that *Ajuga iva* extracts, both ethanol and hexane, significantly impacted various biological aspects of the greater wax moth (*Galleria mellonella*). The extracts effectively reduced larval and pupal duration, increased deformity rates, decreased adult emergence, and reduced the number of eggs laid and their hatchability. The ethanol extract was more effective than the hexane extract in most cases, particularly at higher concentrations. These findings suggest that *A. iva* extracts can be a promising, eco-friendly alternative to chemical pesticides for controlling *G. mellonella* populations, with the potential to disrupt the insect's life cycle and reduce its reproductive capacity. Further research into field applications and long-term effects is recommended to validate these results in practical beekeeping environments

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us.

Author/s signature on Ethical Approval Statement.

Ethical Clearance and Animal welfare

Funds: Not found

AUTHOR'S CONTRIBUTION STATEMENT

Conceptualization, H.I.A; methodology, A.N.A; software, A.N.A.; validation, H.I.A., A.N.A.; formal analysis, H.I.A. and A.N.A.; investigation, A.N.A. and H.I.A

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تأثير بعض مستخلصات أوراق نبات ألاجوجا *Ajuga iva* على بعض ألجوانب ألحياتية لحشرة دودة ألشمع ألكبرى *Galleria mellonella* L.

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المستخلص

أجريت هذه الدراسة لمعرفة تأثير معاملة غذاء يرقات ألطور ألثالث لدودة ألشمع الكبرى *Galleria mellonella* بمستخلصات ألايثانول وألهكسان لاوراق نبات ألاجوجا *Ajuga iva* بالتراكيز 5 ، 7.5 و 10 % وتأثيرها على الجوانب الحياتية للحشرة وبينت النتائج أن ألتركيز 10% حقق أعلى نسبة قتل لليرقات أذ بلغ معدلها 51.84 % للمستخلصين واقل نسبة قتل بلغت 19.17 % عند ألمعاملة بألتركيز 5 % للمستخلصات وبلغت 3.33 % لمعاملة ألمقارنة، بينما لوحظ تقليل نسبة اليرقات المتعذرة أذ تفوق المستخلص الايثانولي في تقليل ألعذارى اذ بلغ معدلها 61.68 و 82.53 % لمستخلص الأيثانول والهكسان بينما في ألمقارنة بلغت 96.70 % ، كما أن أعلى نسبة للتشوهات في العذارى أذ بلغ معدلها 26.80% عند المعاملة بالتركيز 10 % للمستخلص الايثانول ، ولوحظ أيضاً تفوق ألمستخلص ألايثانولي في حدوث اكثر أطالة في مدة ألدورين أليرقي والعذري ، واعلى في خفض معدل عمر البالغات وتقليل عدد ألبيض الموضوع من قبل ألاناث ونسبة فقسه.

الكلمات المفتاحية: فقس البيض، الخصوبة، اليرقات، نسبة القتل.