



Laser-enhanced baobab seed oil (*Adansonia digitata*): A novel eco-friendly biopesticide for effective control of *Spodoptera frugiperda*

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Abstract

This study explores the chemical composition, insecticidal activity, and biochemical effects of *Adansonia digitata* (baobab) essential oil extracted from laser-irradiated seeds, with a focus on its efficacy against the fall armyworm (*Spodoptera frugiperda*), a key pest in worldwide agriculture. Essential oils were extracted from laser-treated and untreated seeds with ethyl acetate and xylene solvents, and analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The research demonstrated a considerable concentration of beneficial chemicals, including tributyl acetylcitrate and 1,2,3-propanetricarboxylic acid, in laser-treated extracts (LTE) compared to untreated samples. Four extracts were tested for their effects on second-instar *S. frugiperda* larvae using bioassays. Ethyl acetate-based LTE was the most dangerous, with LC₅₀ of 31.33 ppm after 20 days, whereas xylene-based LTE had LC₅₀ of 479.75 ppm. The study found substantial mortality rates and considerable developmental changes, including as larval deformities, dwarfism, unsuccessful molting, and pupal abnormalities. Histological dissection of adult moths subjected to laser-treated extracts showed defective reproductive organs, such as undifferentiated ovarioles, emphasizing their ability to restrict pest reproduction and disturb population dynamics. The results suggest that laser irradiation improves the bioactivity of baobab seed oil by increasing the content of essential phytochemicals. These findings showed that laser-treated baobab oil had the potential to be an environmentally benign biopesticide, providing a novel and long-term alternative to chemical pesticides for managing *S. frugiperda* and other agricultural pests.

Keywords: Baobab plant, extracts, He-Ne, bioassay, fall armyworm

Introduction

Fall armyworm, *Spodoptera frugiperda*, is a major pest that causes damage to various kinds of economically important crops, including maize, sorghum, rice, cotton, wheat, sugarcane, peanut, soybean, cabbage, alfalfa, onion, tomato, potatoes, and other plant species (Mohamed *et al.*, 2022) [1]. It attacks more than 350 plant species and causes yield losses more than 70% (Montezano *et al.*, 2018^[2], and Lee *et al.*, 2020^[3] and Kandil *et al.*, 2023) [4]. The greatest hazard posed by this destructive insect is its vast distribution, high fertility rate (> 2000 eggs), long-distance migration (> 500 km), yearly generational continuity, adaptation to climate change, and tremendous flight ability up to 100 miles per night (Ammar *et al.*, 2024) [5].

Since the pest was first discovered in Egypt on maize crops in 2019 in Aswan Governorate, Upper Egypt (Dahi *et al.*, 2020) [6], numerous strategies for management have been developed to combat it. Although farmers continue to rely on traditional chemical pesticides, the excessive and continued use of the main classes of these chemicals has not provided a long-term solution for pest management. Furthermore, chemical management has resulted in substantial pest resistance to such pesticides, as well as negative consequences for natural enemies, pollinators, and biodiversity, environmental pollution, minor pest recurrence, and human health. According to Van den Berg and du Plessis, 2022 [7], alternative strategies that combine biological management, cultural practices, and targeted

chemical use are increasingly being recommended as environmentally friendly alternatives because this pest is currently resistant to a wide range of pesticides, including pyrethroids, organophosphates, and even novel chemical classes like diamides.

The application of biopesticides in pest control has recently attracted plenty of attention (Duke, 2018 and Gupta *et al.*, 2023) [9, 8]. Biopesticides are compounds with specific biological activity that can be used to manage agricultural pests (Gupta *et al.*, 2023) [9]. Plants, nematodes, bacteria, viruses, and fungus are all suppliers of these chemicals. Among these several groupings, the use of botanicals (plant-based biopesticides) in pest control is increasing global popularity in order to reduce the entry of synthetic chemicals into the environment (Shawer *et al.*, 2022 and Gupta *et al.*, 2023) [9, 10].

Essential oils (EOs) derived from such plants have been found to have beneficial ecotoxicological properties, such as low human toxicity, further breakdown, biodegradability, and environmental safety (Visakh *et al.*, 2024) [11]. Furthermore, they have been shown to exhibit a wide range of bioactivities, including insecticidal, virucidal, fungicidal, bactericidal, and antiparasitic properties. The usefulness of several plant-derived EOs for pest management has been extensively studied, with encouraging results (Sundar *et al.*, 2021) [12]. EOs from diverse plant families, specifically Piperaceae, Lamiaceae, and Verbenaceae, were examined for their efficacy against *S. frugiperda*. However, the

essential oils of *Ocimum basilicum*, *Piper marginatum*, and *Lippia alba* were the most assessed, while *Ageratum conyzoides*, *Piper septuplinervium*, *Ocimum gratissimum*, and *Siparuna guianensis* were shown to be the most effective against *S. frugiperda*. EOs from diverse plant families, specifically Piperaceae, Lamiaceae, and Verbenaceae, were examined for their efficacy against *S. frugiperda*. However, the essential oils of *Ocimum basilicum*, *Piper marginatum*, and *Lippia alba* were the most evaluated, while *Ageratum conyzoides*, *Piper septuplinervium*, *Ocimum gratissimum*, and *Siparuna guianensis* were the most effective against *S. Adansonia digitata* L. (Malvaceae) is a multifunctional seasonal tree widespread throughout Africa, including Botswana, Namibia, Zimbabwe, Malawi, Mozambique, South Africa, and other countries. Traditional African medicine treats a wide range of ailments, including malaria, toothache, anemia, fever, diarrhea, microbiological infections, and TB, with various baobab tree components (Adebayo *et al.*, 2014) [13].

There are still restrictions on the activity and importance of EO of *A. digitata* in agricultural pest management, despite the fact that the pharmacological and medicinal effects of the plant's many sections have been well studied. The chemical composition, insecticidal, and biochemical effects of *Adansonia digitata* (baobab) essential oil (EO) isolated from laser-irradiated seeds, as well as its activity on *Spodoptera frugiperda* second larval instars, were assessed in the current study.

Materials and methods

Insect rearing

The *Spodoptera frugiperda* culture utilized in this experiment was maintained for several generations at the Plant Protection Research Institute (PPRI) within the Agriculture Research Center (ARC) in Sabahia, Alexandria, Egypt, under controlled laboratory conditions. These conditions included a temperature of $25 \pm 2^\circ\text{C}$, relative humidity of $70 \pm 10\%$, and a photoperiod of 12 hours of light followed by 12 hours of darkness (Adebayo *et al.*, 2014) [13]. The larvae were fed fresh leaves of castor (*Ricinus communis* L.) in large glass jars until they reached the pupal stage and subsequently emerged as adults. Fresh castor leaves were provided daily throughout this period. Newly emerged adults were placed in glass jars containing a 10% sugar solution absorbed by cotton tissue to facilitate mating (Ibrahim *et al.*, 2023) [14]. Each jar was also supplied with branches of *Tafila (Nerium oleander)* to encourage egg-laying. The eggs were collected daily, surface sterilized using a 10% formaldehyde solution for 2-5 minutes, rinsed with double distilled water, and allowed to dry before hatching. The emerging larvae were examined, and second instar larvae were selected for the bioassay study.

Laser treatment

Seeds of *Adansonia digitata* were obtained from a local market in Khartoum, Sudan. The seeds were thoroughly cleaned, rinsed under running tap water for several minutes, and air-dried at room temperature for two hours prior to laser pre-illumination. A total of 150 grams of seeds were subjected to pre-illumination in the laboratory of the National Institute of Laser Enhanced Science (NILES) at Cairo University. This process utilized a He-Ne laser operating at a wavelength of 630 nm (Equipment

Whitening, Laser II, DMC Equipment Ltd.) (Hassan *et al.*, 2024) [15]. Each seed was individually irradiated for five minutes at a power of 1400 mW, with the laser beam positioned perpendicular to the seeds and maintained at a distance of 1 cm from the source. A control group was established that did not receive any laser treatment.

Crude Extract Preparation

Following the laser treatment, both irradiated and non-irradiated seeds of *Adansonia digitata* were thoroughly dried at room temperature and then ground using an electric mill. Approximately 50 grams of seeds underwent oil extraction via a Soxhlet apparatus (Kavalier, Sázava, Czech Republic), utilizing ethyl acetate and xylene as solvents (Dasari and Goud, 2014) [16]. The solvents were subsequently removed under vacuum using a rotary evaporator (HS-2005S-N, HAHNSHIN, Korea), and the remaining extracts were stored at 4°C in a refrigerator for future applications.

GC-MS analysis of baobab seeds extract

The chemical composition of *Adansonia digitata* seed extracts was analyzed using a Thermo Scientific Gas Chromatography (GC Trace 1300) system coupled with a Mass Spectrometer (ISQ 7000, Thermo Scientific, USA). The analysis utilized a Thermo TR-50 MS capillary column with dimensions of 30 m in length, 250 μm in diameter, and a film thickness of 0.25 μm (Farah *et al.*, 2021) [17]. The GC-MS detection employed an electron ionization system operating at an energy level of 70 eV, with both the MS transfer line and ion source temperatures maintained at 300°C . Pure helium gas (99.995%) served as the carrier gas, flowing at a rate of 1 mL/min. The temperature program commenced at 60°C for 2 minutes, followed by an increase to 100°C at a rate of $10^\circ\text{C}/\text{min}$, which was held for 5 minutes. The temperature was then raised to 150°C at the same rate and held for another 5 minutes, followed by an increase to 200°C , again maintained for 5 minutes, and finally elevated to 250°C at the same rate for duration of 20 minutes. A volume of one microliter of the prepared extracts was injected in a partless mode.

Contact bioassay

Four bioassay trials were conducted to evaluate the contact activity of the yielded four *A. digitata* seed extracts (laser-treated seeds in ethyl acetate, laser-treated seeds in xylene, non-laser treated seeds in ethyl acetate and non-laser treated seeds in xylene) against the 2nd instar of army cotton worm, *S. frugiperda* (Visakh *et al.*, 2022) [11]. Each trial included six treatments; five serial concentrations of each extract (250, 500, 750, 1000 and 1250 ppm) and a control treatment. The emulsion of the tested four extracts were diluted at in the solvent that used in its extraction process (ethyl acetate or xylene). Same sized castor leaves (7x4 cm²) were dipped in the prepared extract emulsions for 15 sec, and allowed for air dring for 30 m (Sharma *et al.*, 2022) [18]. Castor leaves were put in the Petri dishes (9 cm diameter, 1.5 cm height) and then five 2nd-instar larvae *S. frugiperda* (same age, size and weight) were transferred in each Petri dish. Castor leaves dipped in solvent only were used in the control treatments. All treatments were replicated five times. Larvae were investigated for mortality and judged dead if they failed to move when gently pushed by a camelhair needle. Larvae mortality was recorded at 1,

5, 10, 15 and 20 days after treatment (DAT) and corrected using Abbott's formula (Abbott, 1925) [19]. The LC₅₀ values (concentration causing 50% larvae mortality compared with the control) expressed as ppm were calculated (Finney, 1952) [20].

$$\text{Corrected mortality\%} = \frac{(\text{Mortality\% of treated larvae} - \text{Mortality\% of control larvae})}{(100 - \text{Mortality\% of control larvae})} \times 100$$

Reproduction tract dissection

A laboratory trial was carried out at the Applied Entomology and Zoology Department, Fac. of Agric. (El-Shatby), Alexandria University to study the histological effects of the irradiated and non-irradiated *A. digitata* seed extracts using either ethyl acetate or xylene solvents on the reproduction tract of adult *S. frugiperda* emerged from the treated 2nd instar larvae with the determined LC₅₀ values at 1 DAT. Ten second-instar larvae (same age, size and weight) were transferred in wide glass jars and feed on castor leaves were previously dipped in the extract emulsions prepared at concentrations of LC₅₀ values at 1 DAT for 15 sec. Larvae

were checked daily and dead ones were removed. Malformation caused in the treated larvae was observed for all treatments. Puppated larvae were followed, and the emerged adults were used for the reproduction tract dissection. The reproduction tract of these adults was dissected with a binocular microscope (10×magnification) in Ringer's solution (0.42 g KCl, 0.2 g NaHCO₃, 9.0 g NaCl, 0.48 g CaCl₂ in 1000 mL DW) as described by (Hadley, 2007) [21], and then classified to normal and abnormal tracts.

Results and Discussion

1. Gas chromatography–mass spectrometry analysis from *Adansonia digitata*

GC–MS analysis of extracts is considered as an essential step in determining the main components and active compounds of plant extracts. As a result, chromatographic analysis was performed on each solvent used in essential oil extraction, as well as on laser-treated and untreated plant seed samples (Figure, 1).

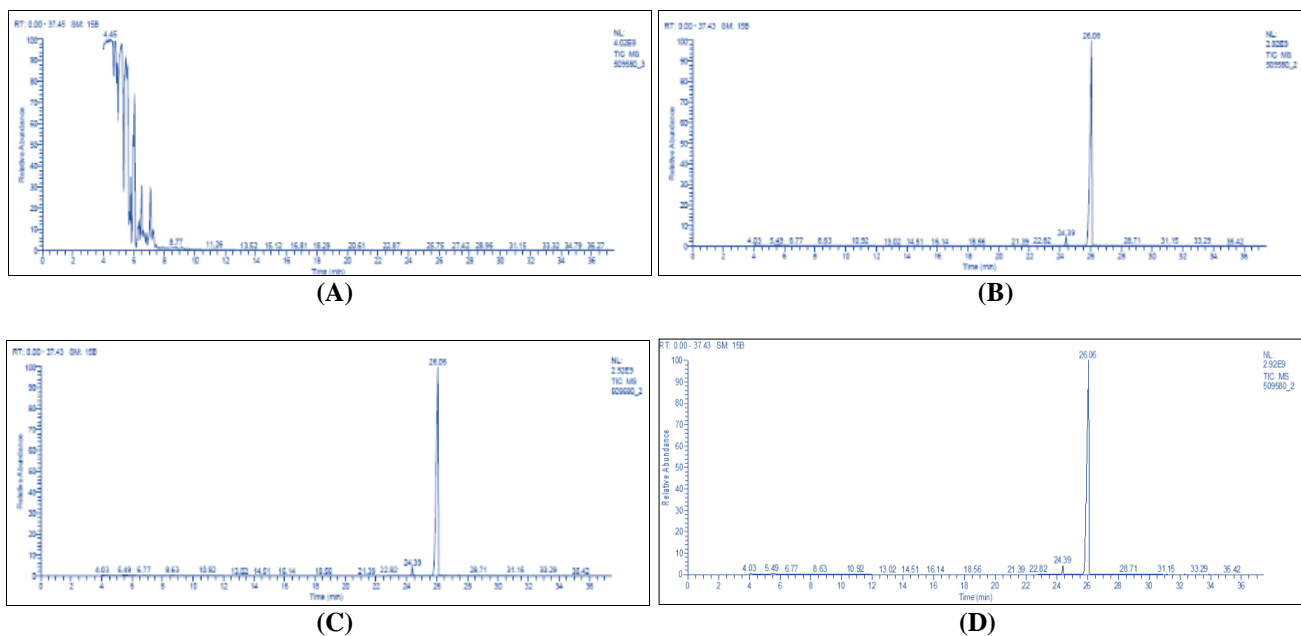


Fig 1: Gas chromatography–mass spectrometry analysis from *Adansonia digitata*

A: GC- MS of *A. digitata* seeds extract using Ethyl acetate solvent with laser

B: GC- MS of *A. digitata* seeds extract using Ethyl acetate solvent without laser

C: GC- MS of *A. digitata* seeds extract using Xylene solvent with laser

D: GC- MS of *A. digitata* seeds extract using Xylene solvent without laser

2. Effects of *A. digitata* seeds extract irradiated with and without laser on *Spodoptera* larvae

2.1. Effect of *A. digitata* seeds extract using ethyl acetate solvent

Study of the toxicity effect of *Adansonia digitata* plant extract using two solvents after exposing the seeds of plant to laser make a comparison between laser treated and non-treated seed extract Calculation of the reduction percentage and the effect of toxicity on the larvae of the 2nd larval instar of the army worm after feeding on the castor plant

treated in laboratory conditions according to Khalil *et al.* 2022 [22].

Data in Table (1) showed that the effect of using extract seeds of *Adansonia digitata* plant with Ethyl acetate solvent, and clarifying the rates of death, laser treated extract (LTE) was more toxic than non-laser treated extract (Non- LTE) at 1 day post treatment, recording low LC₅₀ (315.938 and 952.565 ppm/l), respectively.

The LC₅₀ values for (LTE) at 5 and 10 days were 189.874 and 62.586 ppm/l, respectively, while LC₅₀ values for (Non-LTE) were 508.825 and 315.518 ppm/l, LTE at 10, 15 and 20 days had same slop values, Table (1). This study agree with Acheuk and Doumandji-Mitiche, 2013[23].

The 2nd larval instar treated with 1250 ppm/l of the tested extract laser, had a significant effects on the duration of 2nd larval instar for adansonia oil extracts non-laser treated comared with 250 ppm/l concentration. LET Oil exhibited the highest reduction percentages against 2nd larve instar of army worm comared with non-LTE Oil due to tributyl

acetylcitrate (>97%) and 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Tributyl Ester (>97%) in ethyl extract of *A.*

digitata seeds irradiated with laser, which exhibited insecticidal activity against *S. frugiperda* larvae.

Table 1: Efficacy of laser-treated and non-laser treated *Adansonia digitata* seeds extract using ethyl acetate solvent against 2nd instar of army cotton worm *Spodoptera frugiperda*

Non-laser treated <i>Adansonia digitata</i> seeds extract (Non-LTE)									
Days after treatment	Mortality % at indicated concentration (ppm)					LC ₅₀ (ppm)	Conf. Limits		Slope
	250	500	750	1000	1250		Lower	Upper	
1	27.5	35	45	57.5	72.5	952.565	752.477	1397.721	1.117
5	32.2	40	57.5	77.5	82.5	508.825	253.751	709.45	2.057
10	47.5	75.5	70	80	87.5	315.518	226.741	389.371	1.646
15	57.5	67.5	80	87.5	87.5	203.782	115.023	278.576	1.489
20	60	72.5	82.5	90	90	182.832	101.214	253.02	1.567
Laser treated <i>Adansonia digitata</i> seeds extract (LTE)									
Days after treatment	Mortality % at indicated concentration (ppm)					LC ₅₀ (ppm)	Conf. Limits		Slope
	250	500	750	1000	1250		Lower	Upper	
1	50	55	60	77.5	82.5	315.938	199.015	408.689	1.283
5	60	65	77.5	80	90	189.874	90.582	273.643	1.29
10	72.5	75	77.5	85	90	62.586	2.235	152.028	0.845
15	75	77.5	82.5	87.5	90	47.721	0.848	129.966	0.841
20	75.5	82.5	87.5	90	90	31.33	0.11	104.415	0.819

2.2. Effect of *A. digitata* seeds extract using xylene solvent

Based on 10-day- LC₅₀ values, the (LTE) extract was more effective against 2nd larval stage of army worm, LC₅₀ =761.89 ppm. At 20 days post treatment the 2nd larval instar treated with 1250 ppm of the tested extracts with laser have a significant effect on the duration of 2nd larval instar for seed oil *Adansonia digitata* compared with (Non-LTE) reach to 75% compared with 67.5% respectively (Table, 2). Data was recorded at 1,5,10,15 and 20 days after treatment and LC₅₀ values were shown in Table (2).

LC₅₀ values for laser treated extract (LTE) with xylene solvent was more toxic than no-laser treated (Non-LTE) with the same solvent after 1 day recording 1303.91 and 1777.7 ppm/l, respectively (Kedia *et al.*, 2015)^[24]. From of *A. digitata* seeds extract using xylene solvent GC MS analysis, the xylene extract of *A. digitata* seeds irradiated with laser consists of more than 15% of Benzene, 1-ethyl-2,3-dimethyl; Benzene, 1-ethyl-2,4-dimethyl and Benzene, 2-ethyl-1,3-dimethyl, and more than 11% of Benzene, 4-ethyl-1,2-dimethyl; Benzene, 2-ethyl-1,4-dimethyl; Benzene, 1,2,4,5-tetramethyl-; Benzene, 1-ethyl-2,3-dimethyl- and Benzene, Methyl(1-Methylethyl).

Table 2: Efficacy of laser-treated and non-laser treated *Adansonia digitata* seeds extract using xylene solvent against 2nd instar of army cotton worm *S. frugiperda*

Non-laser treated <i>Adansonia digitata</i> seeds extract (Non-LTE)									
Days after treatment	Mortality % at indicated concentration (ppm)					LC ₅₀ (ppm)	Conf. Limits		Slope
	250	500	750	1000	1250		Lower	Upper	
1	22.5	27.5	32.5	37.5	47.5	1777.7	1457.328	3160.527	4.079
5	25	30	32.5	50	52.5	1260.576	955.595	2196.592	1.108
10	17.5	25	32.5	40	54.5	1156.789	989.631	1549.435	2.059
15	20	30	40	50	60	980.295	820.606	1264.819	1.552
20	32.5	37.5	42.5	55	67.5	758.738	611.145	986.491	1.203
Laser treated <i>Adansonia digitata</i> seeds extract (LTE)									
Days after treatment	Mortality % at indicated concentration (ppm)					LC ₅₀ (ppm)	Conf. Limits		Slope
	250	500	750	1000	1250		Lower	Upper	
1	20	25	37.5	42.5	52.5	1303.91	1015.91	2053.216	1.305
5	25	30	40	47.5	62.5	985.103	804.737	1340.702	1.347
10	30	35	42.5	55	70	761.895	632.045	949.526	1.392
15	35	40	47.5	60	75	736.871	628.319	893.38	1.204
20	40	45	57.5	65	75	479.748	358.137	588.888	1.28

3. Effects of ethyl and xylene extracts of *A. digitata* seeds irradiated with laser on *S. larva*

The LC₅₀ of ethyl and xylene extracts of *A. digitata* seeds irradiated with laser was calculated after 20 days from treatment and applied on newly 2nd instar larvae that caused larval mortality with 31.33 and 479.748 ppm, (Tables, 1 and 2), respectively. Malformation of larvae in different times after feeding on fresh castor leaves treated by ethyl and xylene, when compare with normal larva Abnormalities died larvae (dwarfing, failed to molt, ...) resulted after 15 and 20 days from treatment, when the

larval fed on fresh castor leaves treated by xylene and ethyl extracts of *A. digitata* seeds irradiated with laser on newly 2nd instar larvae of *S. frugiperda*. Conversely, the daily observations of the development of the treated larvae with ethyl extract of *A. digitata* seeds irradiated with laser proved that there are many larval-pupal intermediates were found and some pupae were reduced in size when comparative that by control. It was clearly noticed that the weights and volume of pupae were decreased when applied the LC₅₀ of ethyl and xylene extracts of *A. digitata* seeds irradiated with laser on newly 2nd instar larvae of *S. frugiperda* by feeding.

Effects of ethyl extract of *A. digitata* seeds irradiated with laser on reproduction tract

By dissection, the undifferentiated ovarioles and only one ovary malformed of adult were observed when the larvae fed on fresh castor leaves treated by LC₅₀ of ethyl extract of *A. digitata* seeds irradiated with laser. The fecundity was strongly affected, when applied the LC₅₀ of ethyl extract of *A. digitata* seeds irradiated with laser on newly 2nd instar larvae of *S. frugiperda* that due to reduce in the next generation population dynamics of *S. frugiperda*

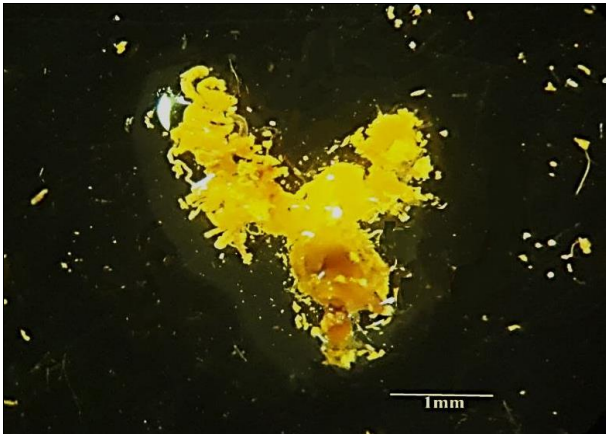


Fig 2: Atrophied ovarioles and only one ovary malformed of adult resulted when the larvae fed on fresh castor bean leaves treated by LC₅₀ of ethyl extract of *A. digitata* seeds irradiated with laser

The study observed abnormalities in the larvae (such as dwarfism) and prepupae, along with undifferentiated ovarioles in adults, attributed to the presence of tributyl acetylcitrate (>97%) and 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Tributyl Ester (>97%) found in the ethyl extract of *A. digitata* seeds that were subjected to laser irradiation. In contrast, the xylene extract from the same seeds exhibited a composition of over 15% of various benzene derivatives, including Benzene, 1-ethyl-2,3-dimethyl; Benzene, 1-ethyl-2,4-dimethyl; and Benzene, 2-ethyl-1,3-dimethyl. Additionally, it contained more than 11% of other compounds such as Benzene, 4-ethyl-1,2-dimethyl; Benzene, 2-ethyl-1,4-dimethyl; and Benzene, 1,2,4,5-tetramethyl. These extracts demonstrated insecticidal properties against both larvae and adult stages of *S. frugiperda*. These findings align with previous studies by El-Sabrou (2013) [25] and Shaker *et al.* (2022) [26], who assessed the impact of various crude extracts and essential oils on *S. frugiperda* through feeding trials (Figure, 2).

Phytochemicals and secondary metabolites in plants are thought to act as juvenile hormone (JH) mimics (Bede and Tobe, 2000)[27], disrupting the normal hormonal balance necessary for metabolic processes during developmental stages (Gaur and Kumar, 2019 [28] and Kannan *et al.*, 2017) [29]. Juvenile hormone analog (JHA) insecticides mimic the biological structure of JH, which is crucial for insect development. The toxic and developmental effects of JHA insecticides such as methoprene, fenoxycarb, and pyriproxyfen were evaluated on the larval and pupal stages of *S. frugiperda*. Bioassays indicated that fenoxycarb exhibited the highest toxicity and quickest lethality in second instar *S. frugiperda*. JH esterase (JHE), an enzyme essential for regulating JH levels during insect development, was also highlighted in this context (El-Sheikh *et al.*, 2016) [30].

3.1. Effects of ethyl extract of *A. digitata* seeds without laser on reproduction tract

In terms of the effects of ethyl extract from *A. digitata* seeds without laser treatment on the reproductive tract, undifferentiated ovarioles in adults were observed following exposure to the LC₅₀ of this extract in newly molted second instar larvae of *S. frugiperda* through a feeding assay using fresh castor leaf discs. The presence of tributyl acetylcitrate (>98%) and 1,2,3-Propanetricarboxylic Acid, 2-(Acetyloxy)-, Tributyl Ester (>98%) in the ethyl extract was noted when larvae fed on treated castor leaves at the LC₅₀ level (Figure, 3).

Phytoecdysteroids, which are analogs of the insect molting hormone ecdysteroid, are found across various plant groups and serve as a defense mechanism against herbivory. These compounds have been identified in at least 27 families of Pteridophyta, 10 families of Gymnospermae, and 74 families of Angiospermae. Chemically classified as triterpenoids—including triterpene saponins and phytosterols—phytoecdysteroids are present in significant concentrations in many plants, comprising 0.001-3% of their dry weight. They are isolated from all plant parts in quantities exceeding those found in insects. Consequently, plants represent a superior source of ecdysteroids compared to insects. Ecdysteroids regulate insect development throughout all life stages by modifying the normal levels of ecdysteroid hormones in both adults and larvae. Thus, phytoecdysteroids offer a promising alternative to synthetic insecticides within integrated pest management strategies (Chaubey, 2018)[31].

Schmidt *et al.* (1998)[32] found that the mean JH-II titer in the hemolymph of *S. littoralis* and *Agrotis ipsilon* larvae given a diet treated with *Melia* extract was greater than that of control larvae. The findings demonstrate that a fruit extract from *M. azedarach* affects the insects' neuroendocrine regulation. The impact on hemolymph protein levels is examined in relation to alterations in the morphology and physiology of the reproductive system (abnormalities of treated larvae and undifferentiated ovarioles of adults).



Fig 3: Undifferentiated ovarioles of adult resulted when the larval fed on fresh castor bean leaves treated by LC₅₀ of ethyl extract of *A. digitata* seeds without laser

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