

Chemical constituents and ovicidal effects of mahlab, *Prunus mahaleb* L. kernels oil on cotton leafworm, *Spodoptera littoralis* (Boisd.) eggs

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Abstract: The carried out investigations evaluated ovicidal activity of mahlab, *Prunus mahaleb* L. kernel oil against cotton leafworm, *Spodoptera littoralis* (Boisd.). The chemical constituents of the fixed oil of mahlab were analyzed using gas-liquid chromatography (GLC). Timnodonic (33.07%), oleic (28.71%) and linoleic (24.35%) were the basic fatty acids, while the major hydrocarbon and sterol were found to be heneicosane (62.57%) and β -sitosterol (10.57%). The LC₅₀ values for the one-day-old egg masses were found to be more susceptible than 3-day-old ones. Moreover, the leaf dip technique occurred to be more efficient than spraying technique. The results also showed abnormalities in the external morphology of egg shell, chorion surface, shell imprints and aeropyles of *S. littoralis* eggs treated with mahlab and KZ oils as compared to a control by using scanning electron microscope. Generally, the tested oils significantly reduced the activities of transaminase enzymes (AST and ALT), acid and alkaline phosphatases and total soluble protein except mahlab oil on acid phosphatase as compared to a control. Additionally, the oils of both mahlab and KZ oil affected some biological aspects such as incubation period, larval duration, larval mortality and pupal weight comparing to a control.

Key words: electron microscope, enzymes, mahlab, *Prunus mahaleb* L., *Spodoptera littoralis* eggs

Introduction

The noctuid *Spodoptera littoralis* (Boisd.) (Lipodoptera: Noctuidiae) is a major polyphagous pest, widely distributed throughout Africa, Mediterranean Europe, and several parts of Asia (Hosny and Isshak 1967). Today, Insect Pest Management (IPM) has to deal with the economic and ecological consequences of the use of pest control measures. Though highly effective, application of synthetic insecticides often has resulted in secondary effects, which include mammalian toxicity, insect resistance and ecological hazards. The diversification of the approach inherent in IPM is necessary for better environmental protection. Among the alternative strategies, the use of plants insecticidal allelochemicals appears to be promising, and many of these compounds are secondary plant substances, including alkaloids; quinones and oils (Regnault-Roger 1997).

Oils from plants may have minimal direct and/or indirect effects on natural enemies for ecological equilibrium. For these reasons, oils are currently under investigation for their broad-spectrum pest control properties. Mahlab, *Prunus mahaleb* L. (Rosales: Rosaceae) is a large perennial shrub or deciduous small tree that is found through Mediterranean countries. Due to the special fragrance, previous studies focused on the seed kernels, which have a high protein content and fixed oil (Mariod *et al.* 2009). Özçelik *et al.* (2012) found that seed

kernels of *P. mahaleb* have strong antimicrobial, antifungal and antioxidant activities and may be important for pharmaceutical and industrial purposes. Also, these seed kernels are used as sedatives and vasodilators in Arabic countries (Mariod *et al.* 2010). In Egypt, the mineral oils of various qualities are traditionally been used for insect pest management as ovicidal agents (Helmy *et al.* 2012). Therefore, the main goal of this paper was to utilize the fixed oil of mahlab kernels to control *S. littoralis* eggs, whereas KZ oil (mineral oil) was used as a reference. The chemical composition of mahlab oil components were also analyzed and identified. Furthermore, the ovicidal activities, changes in the morphological structure of eggs; biochemical and biological features induced by mahlab and KZ oils on *S. littoralis* were studied.

Materials and Methods

Tested oils

Prunus mahaleb oil

A sample of about 200 g of kernels of mahlab, *P. mahaleb* was bought at a local market, Sharqia Governorate, Egypt.

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Extraction technique of fixed oil

Extraction of *P. mahaleb* oil was performed at room temperature using electric grinder. About 200 g of ground kernels were soaked in 300 ml petroleum ether (60–80°C) for 3 days.

The pooled extract was filtered and evaporated under vacuum at 50°C to yield about 80 ml oil residue, that stored at 4°C until use.

Determination of fatty acids

Free fatty acids were separated from crude oil as methylated at the Food Technology Research Institute, Center Laboratory, Egypt according to the method by Cunniff (1995) and determined using gas liquid chromatography analysis (Macherey-Nagel, Düren, Germany) according to the procedure reported by Arens *et al.* (1994).

Isolation of unsaponifiable fractions

About 10 ml of crude oil was refluxed with 100 ml of 10% alcoholic potassium hydroxide for 6 h, and the obtained residue was diluted with ether (5 × 300 ml). The combined ether extract was washed several times with water to remove alkalinity, dried over anhydrous sodium sulphate and let to evaporate. The sample of 2 g of unsaponifiable matter was collected. Analysis of unsaponifiable compounds was carried out in gas-liquid chromatography (GLC) at the National Research Center, Cairo, Egypt according to the method described by Ramadan and Mörserl (2003).

KZ mineral oil® (95% EC)

A petroleum-derived oil, produced by Kafr El-Zayat Co. for Pesticides & Chemicals, Egypt was used in the study at the rate of 15 ml · l⁻¹ water.

Culture of the cotton leafworm, *Spodoptera littoralis* rearing technique

A laboratory (susceptible) strain of *S. littoralis* was reared away from any insecticidal contamination, at the Plant Protection Research Institute Zagazig, Sharqia Governorate, Egypt. Egg-masses were reared on leaves of castor bean, *Ricinus communis* L. according to El-Defrawi *et al.* (1964) at 26±1°C and 70±5% relative humidity (RH).

Susceptibility of two developmental egg stages of *Spodoptera littoralis*

Two different techniques were used to study the susceptibility of eggs at the two developmental ages (1- and 3-day-old). Six concentrations of mahlab oil were prepared using petroleum ether as a solvent (10.0, 5.0, 2.5, 1.25, 0.625, and 0.312%) (v/v). As for KZ oil, six serial concentrations starting with the recommended one (1.5 l · 100 l⁻¹ water) were used. Egg-masses were collected from laboratory reared population and divided into two groups; the first one was the 1-day-old eggs and the second group contained

3-day-old eggs. Two different techniques were used; leaf dip technique (dipped for 20 s) and spraying technique. Five egg masses were used for each tested concentration of mahlab and KZ oil in both tested groups. The treated egg-masses were left to dry in air, and then transferred to Petri dishes (5 egg-masses/dish). The same number of egg-masses was dipped and sprayed with distilled water as a control. Daily inspection for all treatments was performed until the untreated eggs hatched.

Two controls were used in this experiment. Petroleum ether alone (solvent) was the positive (+ve) control to ensure that the effect against egg-masses attribute to only the active compounds in the mahlab oil, while distilled water was used as a negative (-ve) control for KZ oil. The mortality percentages of egg-masses were recorded as average mortality percentages of each tested concentration using Abbott's formula (1925). To estimate the LC₅₀ values, the corrected mortality percentages were subjected to probit analysis according to Finney (1952).

Concerning the susceptibility of the two ages of egg-masses, the LC₅₀ values of mahlab treatments and KZ oils against 3-day-old egg-masses of *S. littoralis* using leaf dip technique were evaluated. As for scanning electron microscopy and biochemical analysis, the data was recorded 48 h post treatment with the previous described LC₅₀s, to ensure the formation of embryos inside treated eggs.

Scanning electron microscopy examination

Scanning electron microscopy of egg shell (chorion) of *S. littoralis* using the most potent LC₅₀s of both mahlab and KZ oil and petroleum ether treatment were used.

Preparation of tissue samples for scanning electron microscopy examination

The tested egg-masses were fixated by glutaraldehyde 2.5% and dehydrated by serial dilutions of ethanol using automatic tissue processor (Leica EM TP), then the samples were dried using CO₂ critical point drier (Tousimis Audosamdri-815). Next the samples were coated by gold coater (SPI-Module) and examined by scanning electron microscopy (JEOL-JSM-5500 LV) using high vacuum mode at Regional Center of Mycology and Biotechnology, Cairo, Egypt.

Biochemical studies

Samples preparation

Egg-masses used for biochemical assays were collected 48 h after treatment with potent LC₅₀s of tested oils plus positive control. Five mg of egg-masses per treatment were homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 min. Homogenates were centrifuged at 3,500 rpm for 10 min at 5°C to remove haemocytes. The supernatants were used directly for the biochemical analysis. Three replicates were used for each assay.

Total soluble protein

Colorimetric determination of total soluble protein in the total homogenate of larvae was carried out as described by Gornall *et al.* (1949).

Transaminase enzymes

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities were determined calorimetrically according to the method of Reitman and Frankel (1957).

Acid and alkaline phosphatase

The activities of acid and alkaline phosphatase were determined using the method of Powell and Smith (1954).

Latent effect of tested oils on the successive stages of *Spodoptera littoralis* resulted from treated eggs

This experiment was designed to evaluate the latent effect of the most potent LC₅₀ of tested mahlab and KZ oils and the two controls against 3-day-old eggs. The treated and untreated eggs were kept in Petri dishes until hatching, and then an incubation period was calculated. Fifty newly hatched larvae from each concentration were selected randomly and separately transferred into the glass rearing jars. The larvae were supplied daily with fresh leaves of castor bean and checked daily. The rearing jars were kept under laboratory conditions as mentioned earlier. Larval mortality, larval duration, pupal duration and pupal weight were represented by the parameters of long-term bioactivity of each treatment.

Statistical analysis

The significance differences were determined by analysis of variance (ANOVA). The significance of various treatments was evaluated by Duncan's multiple range test

($p < 0.05$) (Snedecor and Cochran 1980). Data were subjected to statistical analyses using the software package Costat® Statistical Software (2005) a product of Cohort Software, Monterey, California, USA.

Results

Fatty acid composition

The fatty acid composition of *P. mahaleb* using GLC analysis, contain 14 hydrocarbons that are presented in Table 1 and Figure 1. The highest three fatty acids were timnodonic (33.07%) followed descendingly by oleic acid (28.71%) and linoleic (24.35%), while heptadecanoic and heptadecenoic were detected with the same least value (0.06%).

Identification of unsaponifiable constituents

Table 2 and Figure 2 summarized the unsaponifiable constituents resulting from mahlab kernels oil. Mahlab oil contained 14 compounds: 11 hydrocarbons (74.48%) and 3 sterols (25.52%). In case of hydrocarbons, heneicosane was major hydrocarbon (62.57%) while tetracosane was the minor one (0.09%). Steroles were identified as β -sitosterol (10.57%), cholesterol (10.31%) and stigmasterol (4.64%).

Susceptibility of two egg ages of *Spodoptera littoralis* to tested oils

Both mahlab oil and KZ oil have ovicidal effects on two tested developmental ages of *S. littoralis* eggs using two methods, leaf dip technique and spraying technique (Table 3). Based on LC₅₀ values, the dipping technique was more toxic than spraying method. LC₅₀ values recorded for mahlab oil applied on 3-day-old eggs and 1-day-old eggs were 6.58%, 4.09% respectively while for KZ oil 0.50%, 0.34%, respectively, compared to the second method (7.01%, 5.35%) for mahlab oil and (0.70%, 0.69%) for KZ oil, respectively.

Table 1. Fatty acids profile of *Prunus mahaleb* kernels oil

Peak number	Fatty acid	Carbon number	Retention time [min]	Area [%]
1	palmitic acid	C16:0	10.23	2.74
2	palmiloleic acid	C16:1	10.66	0.17
3	heptadecanoic	C17:0	11.79	0.06
4	heptadecenoic	C17:1	12.22	0.06
5	stearic	C18:0	13.67	1.73
6	oleic	C18:1	14.09	28.71
7	linoleic	C18:2	14.84	24.35
8	linoleic	C18:3	16.25	0.37
9	arachidic	C20:0	17.12	0.73
10	gadoleic	C20:1	17.56	0.41
11	timnodonic	C20:5	21.25	33.07
12	behenic	C22:0	21.73	0.72
13	erucic	C22:1	22.39	6.74
14	lignoceric acid	C24:0	29.74	0.14

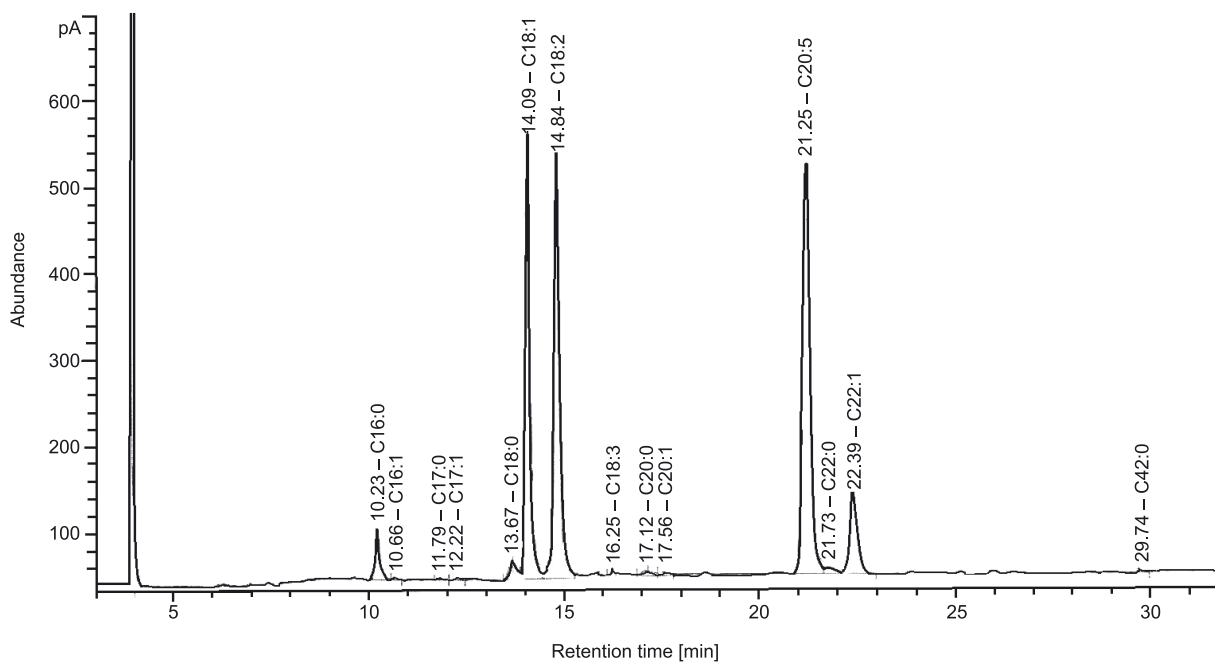


Fig. 1. Gas-liquid chromatography (GLC) analysis of saponifiable components (fatty acids) of petroleum ether fraction of *Prunus mahaleb*

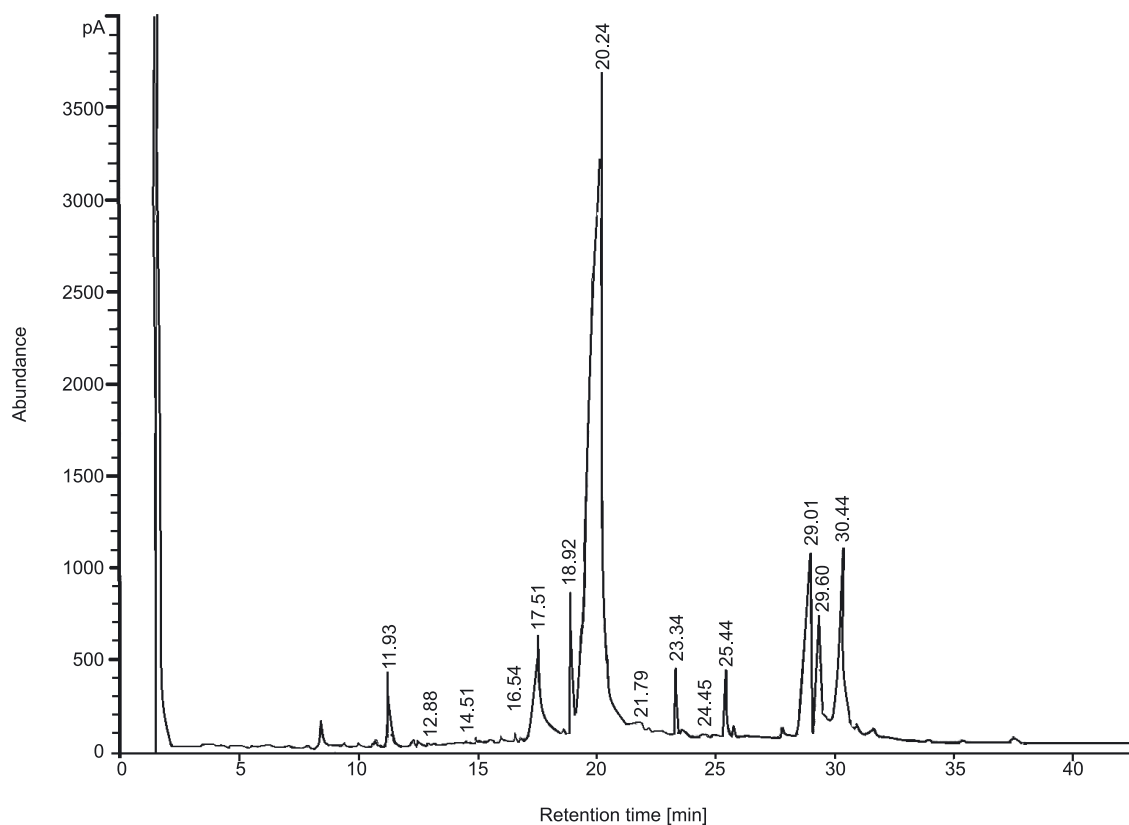


Fig. 2. Gas-liquid chromatography (GLC) analysis of unsaponifiable components of petroleum ether fraction of *Prunus mahaleb*

The medial lethal concentrations (LC_{50}) values of mahlab and KZ against 3-day-old eggs masses of *S. littoralis* using leaf dip technique were used in the subsequent studies. As for scanning electron microscopy and biochemical studies, data were recorded at 48 h post treatment to ensure the formation of embryos and before hatching process.

Ultrastructure studies of *Spodoptera littoralis* eggs

The external morphology of egg-shell and chorion of *S. littoralis* have been inspected using scanning electron microscope. Generally, the normal shape and chorionic structure of *S. littoralis* eggs are illustrated in Figure 3A. The egg-shell has a highly decorated chorion by follicular

Table 2. Common names and chemical structure of unsaponifiable components identified by gas-liquid chromatography (GLC) analysis of *Prunus mahaleb* kernels oil

Compound	Common name	Empirical formula	Structure formula	Retention time [min]	Relative%
Hydrocarbons	Pentadecane	C ₁₅ H ₃₂		11.19	0.79
	Hexadecane	C ₁₆ H ₃₂	H ₃ C CH ₃	12.88	0.03
	Heptadecane	C ₁₇ H ₃₆		14.51	0.22
	Octadecane	C ₁₈ H ₃₈		16.54	0.30
	Norphytane	C ₁₉ H ₄₀		17.51	5.58
	Eicosane	C ₂₀ H ₄₂		18.92	2.72
	Heneicosane	C ₂₁ H ₄₄		20.24	62.57
	Docosane	C ₂₂ H ₄₆		21.79	0.16
	Tricosane	C ₂₃ H ₄₈	H ₃ C CH ₃	23.34	0.89
	Tetracosane	C ₂₄ H ₅₀	H ₃ C CH ₃	24.45	0.09
	Pentacosane	C ₂₅ H ₅₂	H ₃ C CH ₃	25.44	1.13
Steroids	Cholesterol	C ₂₇ H ₄₆ O		29.01	10.31
	Stigmasterol	C ₂₉ H ₄₈ O		29.61	4.64
	β-Sitosterol	C ₂₉ H ₅₀ O		30.44	10.57

Table 3. Susceptibility of different ages of *Spodoptera littoralis* eggs to tested oils

Technique used	Tested age	Treatments			
		mahlab oil		KZ oil	
		LC ₅₀ (lower-upper)	LC ₉₀ (lower-upper)	LC ₅₀ (lower-upper)	LC ₉₀ (lower-upper)
Dipping	1-day-old	4.09 (2.67–5.20)	16.25 (12.91–24.19)	0.34 (0.18–0.48)	1.91 (1.43–3.44)
	Slope	2.15±0.37		1.75±0.3	
	3-day-old	6.58 (5.19–7.81)	26.11 (19.71–42.51)	0.50 (0.31–0.67)	3.60 (3.32–9.67)
	Slope	2.14±0.33		1.61±0.28	
Spraying	1-day-old	5.35 (4.17–6.33)	17.10 (13.95–23.66)	0.69 (0.55–0.81)	2.70 (2.03–4.40)
	Slope	2.54±0.37		2.21±0.32	
	3-day-old	7.01 (5.33–8.51)	35.77 (24.57–73.93)	0.70 (0.51–0.85)	3.85 (2.56–8.73)
	Slope	1.81±0.32		1.80±0.25	

cell imprints (Fc) especially at the upper zone, this decoration decreased gradually until it disappeared toward the egg base. Additionally, the micropyle (Mi) aperture is located at the anterior pole of the egg. The eggs treated with mahlab oil showed abnormal granular protrusions (P) or projections on the egg surface polygons (Figs. 3B–C), rupturing of chorionic surface (Fig. 3D). Application of KZ oil caused reduction in the egg surface area by increasing the number of folds of external chorionic surface (Fig. 3E).

In the upper pole of normal egg, there is a special structure, micropyle rosette (Mr) that consisting of the primary petal shaped cells. The rosette pattern made by about 8–11 petals which longitudinal ridges radiate and start from the equator and extended downward (Fig. 4A), primary petals are followed by secondary petal shaped cells (SMr) of unequal sizes (Fig. 4D). Reversely, in eggs treated with mahlab oil the arrangement of petals was encircled, in addition to the micropyle regions, the petals showed many deformations such as irregular shape, deformed follicular cell imprints (DFc), loss of its vertical columns and opening canals at micropyle not distinct (Figs. 4B–C). Treatment with KZ oil showed many differentiations that ranged between narrower micropyle opening (NMP), irregular plate concavities or even loss of the outline of the concavity (Figs. 2E–F).

Our results showed the normal chorionic surface covered with polygonal reticulations honey comb-like with concave surface (Fig. 3A and Fig. 5A), while the eggs treated with mahlab oil showed alternation in its demarcating outline orientation (Fig. 5C) as well as KZ oil revealed many minute microscopic bodies widely spread inside the concavities (Fig. 5B). Respiratory aeropyles (A), responsible for oxygen entrance are distributed on the borders of the reticulations of follicular cell imprints. The number of aeropyles of shell imprints adjacent to the micropyle zone are less in number than those which are far from micropyle zone (Fig. 5A). Eggs treated with mahlab oil suffer from closing of aeropyles by granular protrusions (P) like structure formed as a result of oil administration (Fig. 5C).

Biochemical changes

The changes in the effect of total soluble protein and enzymatic activities on 3-day-old eggs of *S. littoralis* as response of treatment with the potential LC₅₀ of both mahlab and KZ oils as well as positive control after 48 h of treatment were detected.

Total soluble protein (TSP)

In general, all the tested oils caused significant decrease in the effect of total soluble protein compared to a control (60.40±1.76 mg · g⁻¹). KZ oil revealed the highest significant reduction (19.40±1.66 mg · g⁻¹) followed by mahlab oil (35.40±2.57 mg · g⁻¹), $p = 0.0000$ (Fig. 6A).

Transaminase enzymes (AST and ALT)

Generally, all the tested oils caused highly significant decrease in the activities of both AST and ALT as compared to a control (118±2.31 and 64.21±1.96 µg pyruvate · min⁻¹ · g⁻¹, respectively) (Fig. 6B). Mahlab oil significantly reduced the activities of both enzymes (33.10±1.48 and 20.41±1.37 µg pyruvate · min⁻¹ · g⁻¹, respectively), $p = 0.0000$ for both enzymes.

Acid and alkaline phosphatases

All the used oil significantly reduced the activities of acid and alkaline phosphatase as compared to a control with the exception of effect of mahlab oil on the acid phosphatase activity (569.50±17.75 µg phenol · min⁻¹ · g⁻¹) (Fig. 6C), a control gave (1634.50±172.66 and 542.32±21.35 µg phenol · min⁻¹ · g⁻¹, respectively), $p = 0.0011$ and 0.0001 , respectively. The previously LC₅₀ values of mahlab and KZ oils against 3-day-old egg masses of *S. littoralis* were used to evaluate some biological parameters that occurred in the successive stages.

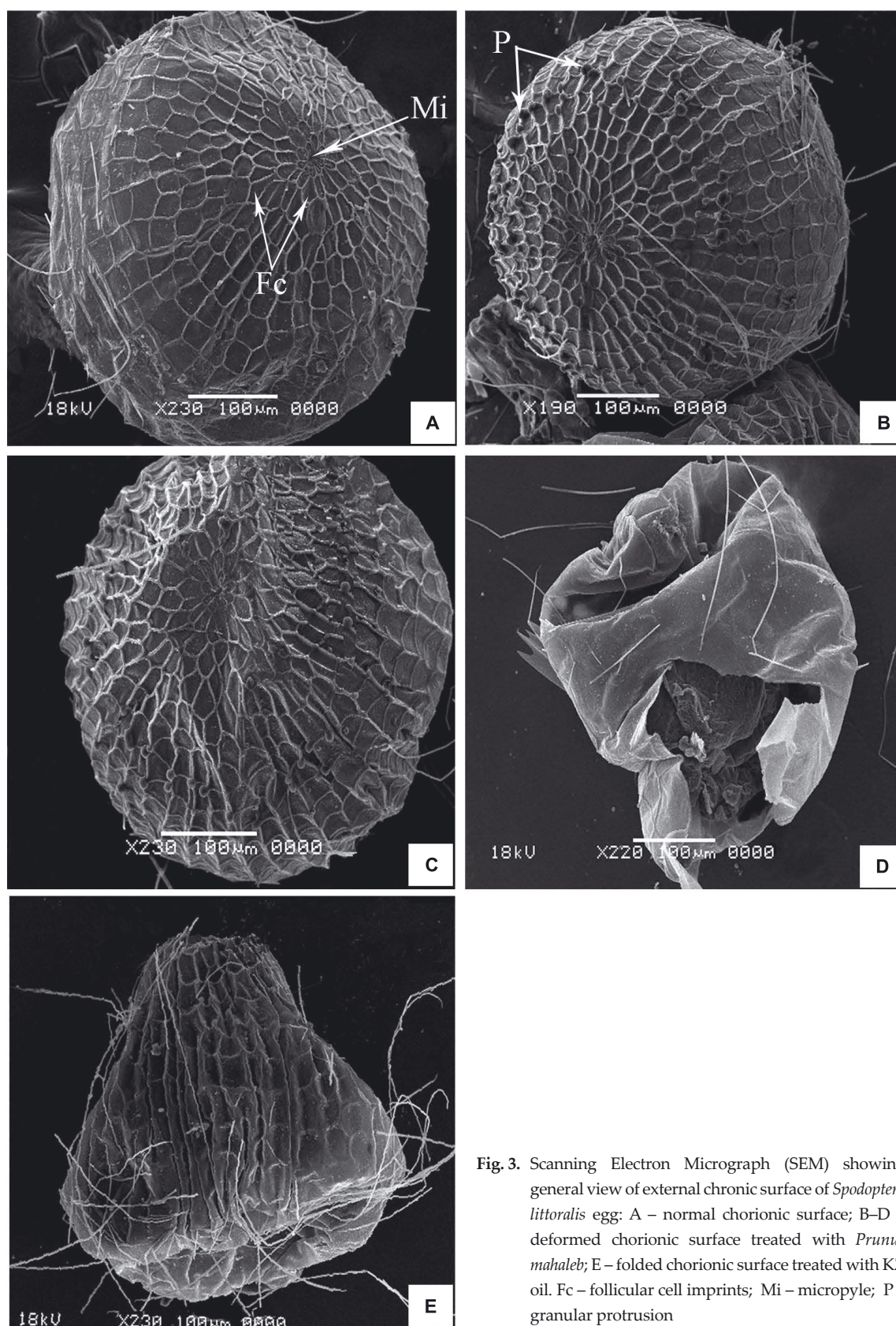


Fig. 3. Scanning Electron Micrograph (SEM) showing general view of external chorionic surface of *Spodoptera littoralis* egg: A – normal chorionic surface; B–D – deformed chorionic surface treated with *Prunus mahaleb*; E – folded chorionic surface treated with KZ oil. Fc – follicular cell imprints; Mi – micropyle; P – granular protrusion

Biological aspects of both tested oils against *Spodoptera littoralis*

Incubation period

The incubation period of the 3-day-old eggs treated by both mahlab and KZ oils were retarded from 2 days in controls and 4 days for the tested oils (Fig. 7A).

Larval duration

Data of Figure 7B indicated that the mahlab and KZ oil caused elongation of the larval duration from (16.15±0.89 and 17.48±1.43 days) for (-ve) and (+ve) controls, respectively to (19.82±0.85 and 18.45±1.13 days) for mahlab and KZ oils, respectively without any significant difference, $p = 0.1953$.

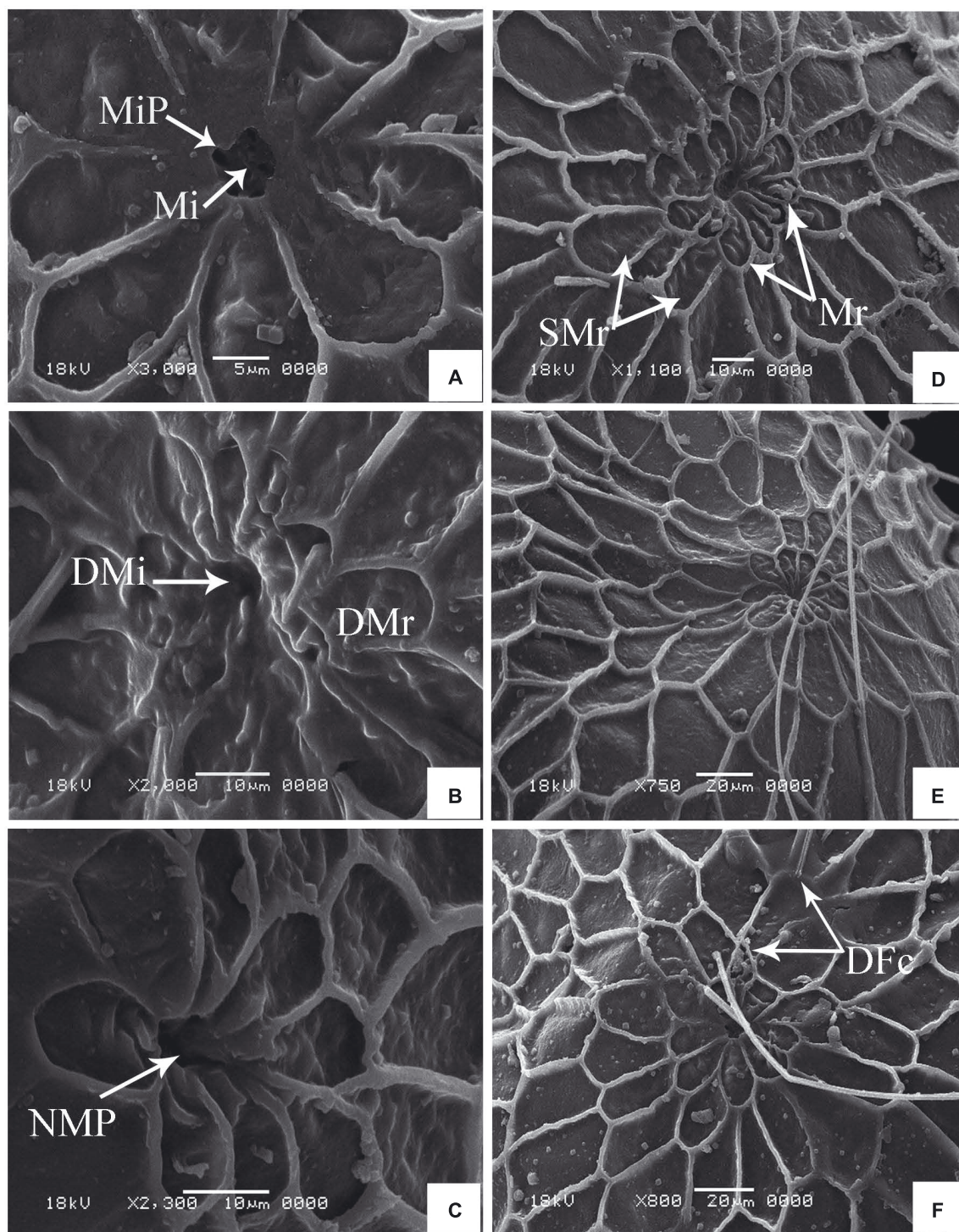


Fig. 4. Scanning Electron Micrograph (SEM) showing general view of anterior pole of *Spodoptera littoralis* egg: A–B – normal micropyle area; B–C – deformed micropyle area treated with *Prunus mahaleb*; E–F – deformed micropyle area treated with KZ oil. Mi – micropyle; MiP – micropylar opening; Mr – primary rosette petal; SMr – secondary rosette petal; DMi – deformed micropyle; DMr – deformed primary rosette petal; NMP – narrow micropyle; DFc – deformed follicular cell imprints

Larval mortality

Cumulative mortality percentages up to the prepupal period were recorded 22.30 ± 0.90 and $14.60 \pm 0.75\%$ for mahlab oil and KZ oil, respectively. Whereas (–ve) and (+ve) control recorded the same value (0.00%), $p = 0.0000$ (Fig. 7C).

Pupal weight

The obtained results indicated that mahlab oil reduced significantly the average weight of pupae developed from treated 3-day-old egg masses than both KZ oil and control experiments. Mahlab oil recorded pupal weight of 0.2860 ± 0.02 g, while KZ oil, (+ve) and (–ve) controls gave (0.2910 ± 0.01 , 0.3124 ± 0.01 and 0.3345 ± 0.01 g, respectively), $p = 0.0441$ (Fig. 7D).

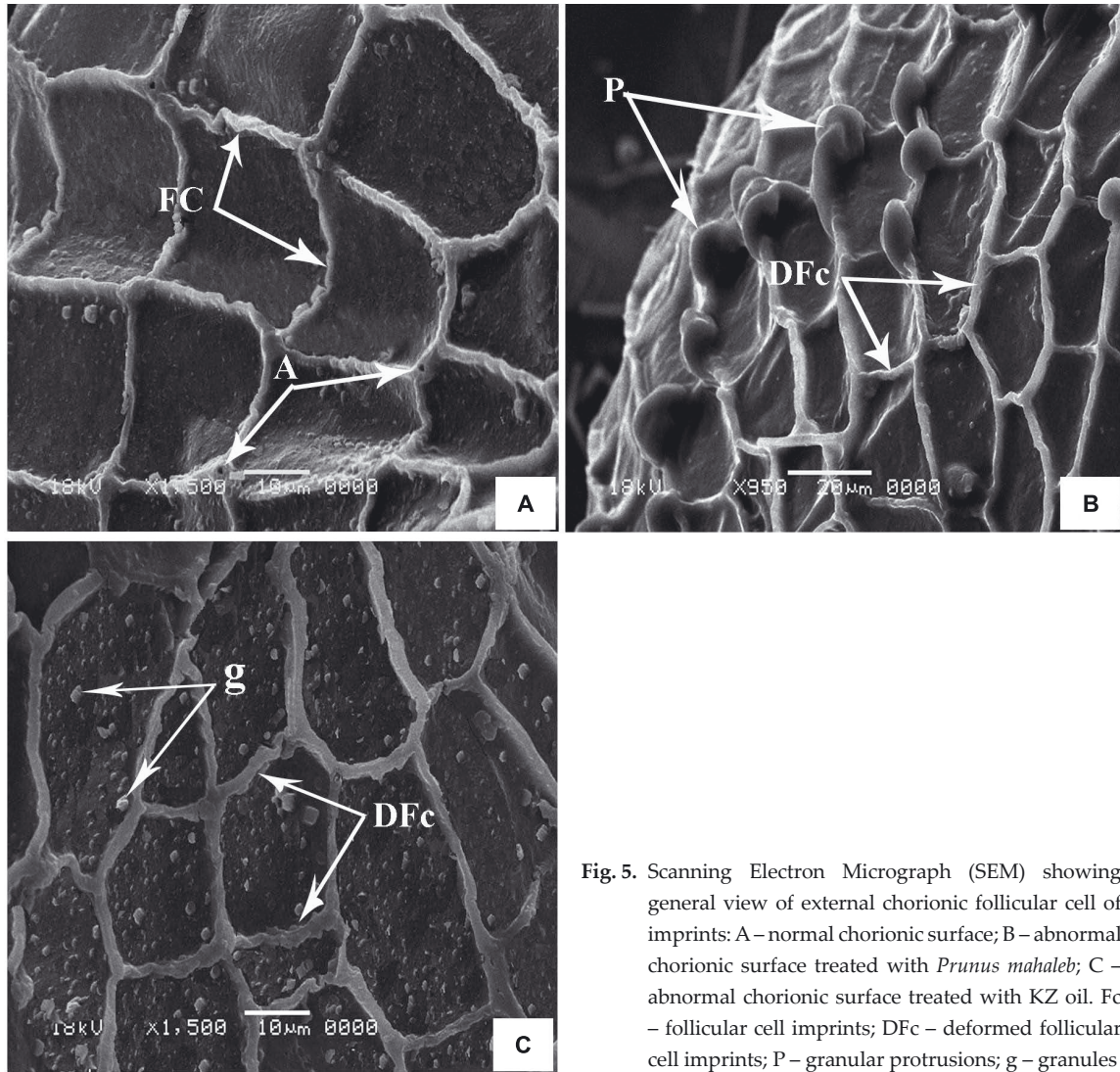


Fig. 5. Scanning Electron Micrograph (SEM) showing general view of external chorionic follicular cell of imprints: A – normal chorionic surface; B – abnormal chorionic surface treated with *Prunus mahaleb*; C – abnormal chorionic surface treated with KZ oil. Fc – follicular cell imprints; DFc – deformed follicular cell imprints; P – granular protrusions; g – granules

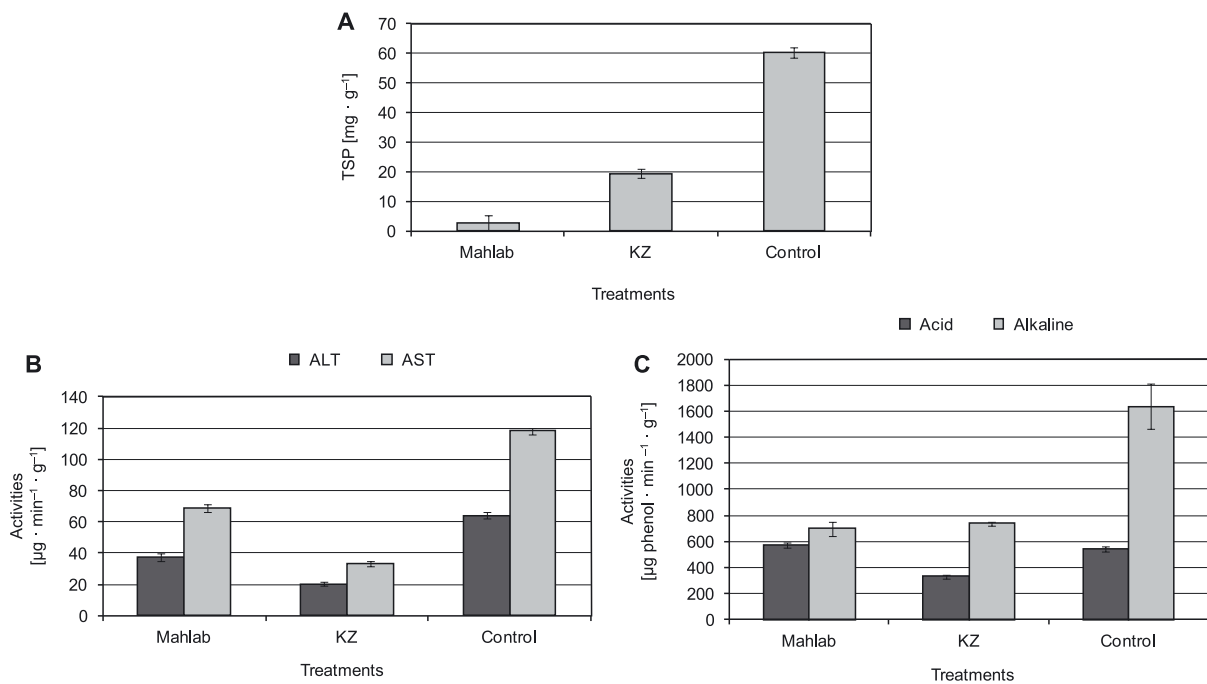


Fig. 6. Changes in the measured biochemical parameters: A – total soluble protein (TSP); B – aspartate transaminase (ALT) and alanine transaminase (AST); C – acid and alkaline phosphatases

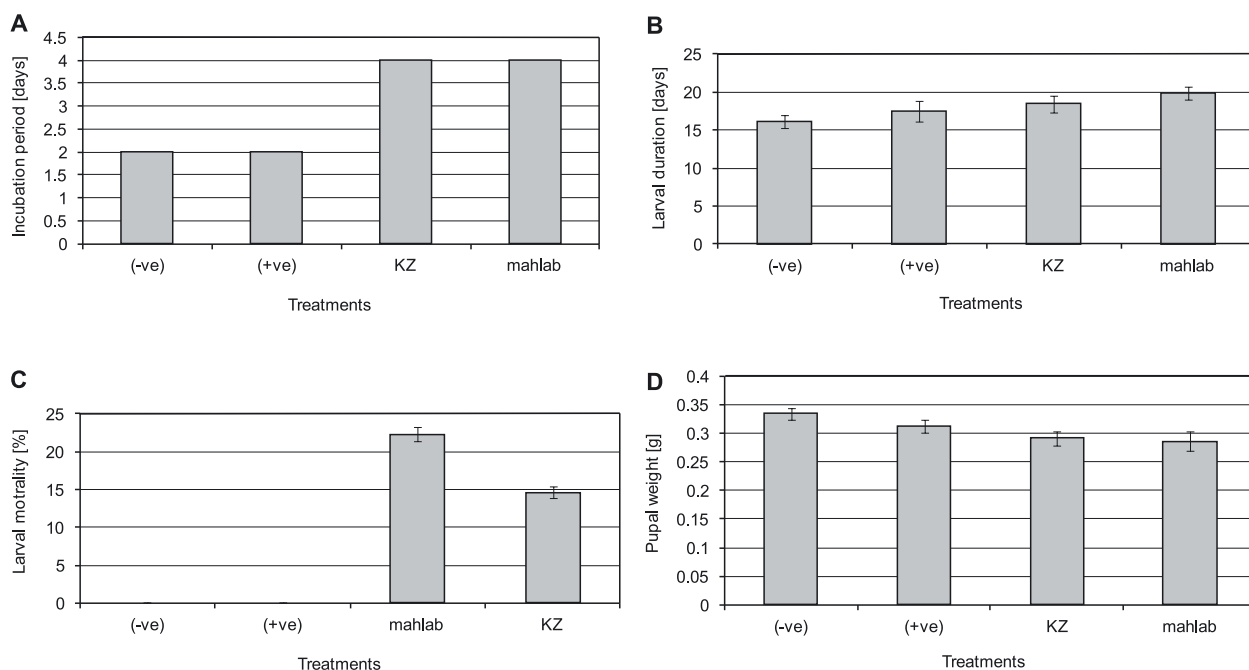


Fig. 7. Incubation period and some biological aspects (A–D) of two tested oils – mahlab and KZ oil – against *Spodoptera littoralis*. (-ve) and (+ve) – the controls

Discussion

In the current study, the analysis of oil from mahlab kernels using GLC found that, this oil was rich in unsaturated fatty acids i.e. timnodonic, oleic, linoleic and erucic acids and that they were the major constituents and represented as 92.87%, while the main saturated fatty acids were palmitic acid (2.74%) and stearic acid (1.73%). These results are in agreement with Shams and Schmidt (2007) and Mariod *et al.* (2009) who found that the oleic and linoleic are major constituents of unsaturated fatty acid of mahlab oil and palmitic acid was the major saturated one. It was also proved that more than 85% of mahlab fatty acids consisted of unsaturated fatty acids.

Additionally, the sterol fraction comprises of β -sitosterol, cholesterol and stigmasterol in mahlab oil. Similar results were obtained by Shams and Schmidt (2007) and Kamel (2010) who analyzed mahlab and moringa oils.

According to LC_{50} and LC_{90} values, fixed oil of mahlab and KZ oil (mineral oil) proved to possess highly pronounced ovicidal activity using leaf dip technique as compared to the spraying method. Furthermore, the younger eggs are more susceptibility to tested oils than older ones. The results indicated the important role played by age of eggs that determined the ovicidal activity of two tested oils against *S. littoralis* eggs. The younger eggs are more susceptible than the old ones, and their susceptibility might be due to occurrence of acetylcholine esterase enzyme, which plays an important role on ovicidal action in insect eggs (Chino and Yushima 1953). Moreover, Mehrotra (1960) reported that this enzyme was not present in early stages of eggs. Khedr and El-Kawas (2013) who tested coriander essential oil against eggs of *S. littoralis* and *Tetranychus urticae* reached the same conclusion.

The mortality effect of the oil on eggs and the developmental stages (larvae and pupae) could be a result of toxic components of mahlab oil. Certain unsaturated and saturated fatty acids have been reported to have ovicidal effects, especially oleic, linoleic, palmitic and stearic acids (Malek and Wilkins 1994) or as larvicidal activity (Perumalsamy *et al.* 2015). Kamel (2010) added that, the mortality of larvae and pupae caused by fixed oil of moringa might be due to the effect of sterol and fatty acids on the cuticle of *Spodoptera frugiperda* or the disturbance of the hormonal regulation caused by sterols. Helmy *et al.* (2012) mentioned that the mineral oil (KZ oil) create a layer on the plant parts that prevents the settlement of the newly hatched individuals of insects. In addition, Helmy *et al.* (1992) found that mineral oils act as ovicides on *Lepidosaphes beckii* and *Parlatoria ziziphus*.

In general, oil induced reduction or inhibition of oviposition of *S. littoralis* female and mortality of the developmental stages has been reported by number of workers (Marei *et al.* 2009; Khedr and El-Kawas 2013).

Our study on eggs surface treated with mahlab and KZ oil using scanning electron microscopy showed many deformations in chorion sculpturing as compared to a control; narrower micropyle opening, irregular plate concavities or even loss the outline sculpturing of the concavity and closing the aeropyles by granular protrusions. Additionally, the exochorion layer dried and shrunk leading to its separation from the endochorion and subsequently death of embryo due to the leakage of oxygen and water vapour uptake. Thus, the mode of action of fixed oil of mahlab on *S. littoralis* eggs revealed two possible mechanisms. The ovicidal effect of oil could be due to its ability to penetrate the chorion of eggs through the micropyle that would result in mortality of the embryo or affect the respiratory activity by formation granular protrusion

on the chorionic surface that blocking aeropyles and causing respiratory impairments, which probably affected metabolism and consequently other systems that would lead to egg mortality. Similar results were reported when fixed vegetable oils were tested on the eggs of *Callosobruchus maculatus* (Don-Pedro 1989; Adedire and Ajayi 2003).

As for KZ oil, it creates a thin layer on the eggs surface that stops the gas exchange or acts as poison, interfering with normal metabolism (Helmy *et al.* 2012). Chapman (1998) added that, the shape modifications in follicular cell are reflected in the chorion morphology. However, changes in the cell constituents may also be responsible for modification in the chorion surface, since the proteins synthesized by follicular cells behave as basic material to the chorion formation. These proteins may also affect the formation of aeropyle, micropyle and other chorion pores, causing many deformations.

Generally, protein is among the most important compounds of insect that bind with foreign compounds. The decrease of total soluble protein in treated larvae might reflect the decrease in the activity of various enzymes (El-Kordy *et al.* 1995). The building block for protein synthesis comes from the amino acids pool maintained mainly by transaminase enzymes (Meister 1957). Transaminase enzymes – AST and ALT – in insects are the most active transaminase enzymes (Crabtree and Newsholme 1970).

In the present study, the level of protein, AST and ALT activities were significantly reduced by both mahlab and KZ oils due to the harmony between protein synthesis and transaminase activities (Abdel-Hafez *et al.* 1988). Also, AST and ALT serve as a strategic link between the carbohydrate and protein metabolism, which are known to be altered during various physiological and pathological conditions (Etebari *et al.* 2005). Thus the disturbance in AST and ALT will be closely related to metabolism of proteins and amino acids causing disrupt of many physiological functions and leads to mortality of eggs.

As a general trend, both mahlab and KZ oils decreased the activities of alkaline and acid phosphatases as compared to the control, with the exception of mahlab oil on acid phosphatase. The increase or decrease in activity of enzymes during development is reflected in decrease or increase in the acid soluble phosphorus content (Sridhara and Bhat 1963). Acid and alkaline phosphatases have been shown to be associated with insect development, especially in relation to nutrition and egg maturation (Tsumuki and Kanehisa 1984). Alkaline phosphatase is an important synthesizing enzyme of tyrosine which is known to take part in the control of levels of insect developmental hormones (Rauschenbach *et al.* 2007).

These findings are in harmony with El-Sweerki (2002) who tested garlic extract on *S. littoralis* eggs. Accordingly, the prolongation in the incubation period of *S. littoralis* eggs and larval duration, reduction in pupal weight and increase in larval mortality induced by the toxic components of mahlab oil greater than KZ oil and the controls might be attributed with the changes in biochemical and morphological characters of eggs that prevent it to hatch and leading to mortality. Adedire and Ajayi (2003) and Kamel (2010) studied the effect of fixed oils of sandbox seeds and moringa against *C. maculatus* and *S. frugiperda*.

The results revealed that all the tested oils were found to be toxic to the successive developmental stages but by variable degrees. In the same connection, Khedr *et al.* (2015) found that, the reduction in growth rate prolonged in time taken for larval and pupal duration, reduction in the weight of resulting pupae compared to a control accompanied with reduction in key metabolic components such as total soluble protein when fed larvae of *S. littoralis* on different cotton varieties.

Conclusions

The presented data showed the importance of naturally origin compounds especially those derived from plants. It also indicates probability of using oils, such as mahlab oil that has the possibility to play an important role as bio-rentional pesticide in insect control as ovicidal agent against *S. littoralis* eggs to break life cycle of this pest. The necessity of carrying out further studies to evaluate the feasibility and economic sides of mahlab oil on wider range of pests needs to be emphasized.

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