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# CHEMICAL CONSTITUENTS OF JOJOBA OIL AND INSECTICIDAL ACTIVITY AGAINST *Schistocerca gregaria* AND BIOCHEMICAL EFFECT ON ALBINO RATS

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## ABSTRACT

The present work deals with chemical constituents, insecticidal and biological studies of jojoba oil. Its saponifiable and unsaponifiable matters of the lipid were studied against *Schistocerca gregaria*. In addition, the effect of supplementation of jojoba oil and its defatted meal on albino rats. The chemical analysis of saponifiable matter showed that alpha-linolenic acid was found to be the major constituents, in fatty acids (37.1%) followed by nervonic acid (13.8%). The main constituents of unsaponifiable matter were fatty alcohols, represent 53.84% and 9-octadecen-1-ol was detected with high quantity (41.35 %). The hydrocarbons were also detected in high quantity, 1, 21-docosadiene was the main hydrocarbons (20.64 %). The higher level of jojoba oil induced the lowest eaten area percentage (30.3%) of *S. gregaria*, followed by the unsaponifiable and saponifiable matters (39.5% and 47.4%), respectively. Antifeedant and protection activity percentage were increased by increasing the concentration. The highest mortality percentage (100%) of *S. gregaria* nymphs was recorded at 10% jojoba oil while it was (93.3%) in the case of unsaponifiable matter. The biochemical evaluation on albino rats showed that supplementation of 4 % jojoba oil to an atherogenic diet (containing 1% cholesterol, 10 % animal fat and 0.25% cholic acid) led to a reduction in serum cholesterol and triacylglycerols levels after three weeks; the decrease amounted to 33.1% and 20.8%, respectively. The remainder after oil extraction is referred to defatted jojoba meal. Rats fed diet supplemented with 10% defatted jojoba meal had (after 14 days) a significant reduction in mean values of body weight, food intake and faecal excretion compared to control group but showed no significant change in serum alanine and aspartate aminotransferases activity and creatinine levels.

**Key words**: Jojoba oil, fatty acids, fatty alcohols, *Schistocerca gregaria*, toxicity, biological effect, atherogenic diet, serum cholesterol, defatted jojoba meal, alanine and aspartate aminotransferases

## INTRODUCTION

Jojoba, *Simmondsia chinensis* L. is native to the desert southwestern, United States and northern Mexico. It is also grown in Australia, Brazil, Argentina and some Middle East countries. Jojoba has become an attractive alternative crop because of the promising commercial applications for its seed oil in cosmetics. Many countries are looking toward developing jojoba culture to solve overproduction and low price for their food and other traditional crops (Ayerza, 1996).

The oil of jojoba is composed mainly of straight chain monoesters in the range of $C_{20}$–$C_{22}$ as alcohols and acids, with two double bonds (Wisniak, 1987 and Wisniak *et al.*, 1987). In Egypt, several laboratory studies were done on seed crude extracts of jojoba dealing with its pesticidal effect on various economic pests such as *Pectinophora gossypiella, Bemisia tabaci, Empoasca discipiens, Agrotis ipsilon, Sesamia cretica* and *Ostrinia nubilalis* (Rofail *et al.*, 2000; Salem *et al.*, 2003 and Yacoub, 2006) stated that Jojoba oil has the effect as toxic, antifeedant, growth and development inhibitors and oviposition inhibitors. The desert locust (*Schistocerca gregaria*) has threatened agricultural production in Africa, the Middle East and Asia for centuries. The livelihood of at least one-tenth of the world's human population can be affected by this hungry insect. The desert locust is potentially the most dangerous of the locust pests because of the ability of swarms to fly rapidly across great distances. It has two to five generations per year (Showler, 1995).

On the other hand, in New Zealand white rabbits ingestion of jojoba oil as a 2% supplement to an atherogenic diet containing 1% cholesterol resulted in a 40% reduction of blood cholesterol and an altered lipoprotein pattern (Clarke and Yermanos, 1981). Jojoba

liquid wax caused reduction of carrageenan-induced rat paw edema, in addition to diminishing prostaglandin $E_2$ level in the inflammatory exudates (Habashy *et al.*, 2005). The remainder after oil extraction, referred to defatted jojoba meal. Defatted jojoba meal contains relatively large amounts of the unique cyanoglucosides, simmondsin, simmondsin ferrulate and other analogs. Several studies have attributed decreasing food intake and weight loss to these compounds when ~10% defatted jojoba meal was included in the diet of rats (Cokelaere *et al.*, 1992) and broiler hens (Arnouts *et al.*, 1993). The hypothesis that defatted jojoba meal induces its food intake reduction by stimulating satiety (Cokelaere *et al.*, 1995).

The objectives of the present work were mainly: (1) to identify the chemical constituents of saponifiable and unsaponifiable matters of jojoba oil. (2) to determine the efficiency of jojoba oil and its saponifiable and unsaponifiable matters for their insecticidal and biological activities to *Schistocerca gregaria* (3) to study the effect of jojoba oil supplementation to hyperlipidemic diet on lipid parameters in rats; (4) to study the effect of defatted jojoba meal on some biochemical parameters in rats.

# MATERIALS AND METHODS

## 1. Plant material:

The seeds of jojoba were obtained from Oils Press Unit in National Research Center. The oil was obtained by cold mechanical pressing. Air-dried fine powder of the defatted seeds was kept also for biological studies.

## 2. Separation and identification of unsaponifiable matter and fatty acids of jojoba oil:

### 2.1. Separation of unsaponifiable matter and fatty acids:

About 20 ml of jojoba oil was saponified with 10% methanolic KOH (140 ml) at 80°C for 3 hr under reflux. The unsaponifiable matter was extracted with ether (10 x 100 ml), washed several times with distilled water, dried over anhydrous sodium sulphate. Then, the solvent was evaporated and unsaponifiable matter was weighed and kept for further analysis.

The soap solution was acidified with HCl 10%. The liberated fatty acids were extracted with ether (3 x 150 ml), washed several times with distilled water till acid free, dried over anhydrous sodium sulphate. The solvent was evaporated (Van Boden *et al.*, 1997 and 2000).

### 2.2. Identification of the unsaponifiable matter:

The unsaponifiable matter constituents (fatty alcohols, sterols and hydrocarbons) were identified by using GC-MS. The chromatographic conditions were: GC model 2000 produced by Thermo, equipped with DB-5 (5%-phenyl) Methyl poly siloxane capillary column (30mm x 0.25µm), and flame ionization detector (FID), gases flow rate were nitrogen as a carrier gas, hydrogen and air gases were set at flow rate 30, 30 and 300 ml/min., respectively. Oven temp. was programmed from 50-300 at rate at 5°C/min., temperatures of detectors and injector were 300 and 250 C, respectively. Mass Spectrophotometer model SSQ produced by Finnigan (Van Boden *et al.*, 1997 and 2000).

The hydrocarbons and sterols compounds were identified by comparing the separated components with those of library entry and authentic samples. These analyses were carried out in the Central Services Laboratory, National Research Center.

### 2.3. Preparation of fatty acid methyl esters:

Methyl esters of fatty acids were prepared by refluxing 10 mg of the liberated fatty acids with 10 ml (2%) of $H_2SO_4$ in anhydrous methanol for 5 hr on a water bath at 90°C. The fatty acid methyl esters were extracted with petroleum ether (10 ml each). The petroleum ether extract was treated with dillluted sodium bicarbonate solution to remove the acidity, washed several times with distilled water, dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure (Maestri and Guzmán, 1995).

### 2.4. Identification and quantitative determination of fatty acids by GLC:

The fatty acid methyl esters were identified by using GLC under the following conditions: HP-6890 GC mode apparatus equipped with capillary column BPx70, cyanopropyl polysilphenylene-siloxane and flame ionization detector. Nitrogen as a carrier gas, hydrogen and air gasses flow rates at 30, 30 and 300 ml/min. Oven temp. was programmed from 70°C to 240°C increased by 10°C min. Temperature of detector and injector were 300 and 250 °C, respectively. Fatty acids were identified by comparing the relative retention time of each peak with those of standard fatty acid methyl esters. These analyses were carried out in the Central Services Laboratory, National Research Center.

## 3. Insecticidal activity:

### 3.1. Tested insect:

The newly-moulted nymphs of 4$^{th}$ instar were segregated from the gregarious stock of *S. gregaria* which had been maintained under the crowded conditions of Hunter-Jones (1961) for three years in the Department of Plant Protection, Faculty of Agriculture Moshtohor, Benha University. Hoppers were kept in wooden cages with glass sides (50x50x50 cm) at a rate of 100 per cage. All cages were incubated at 32±2°C and 65±5% R.H. The leguminous plant *Sesbania aegyptiaca*, was daily provided as feeding material.

### 3.2. Bioassay for feeding activity:

For testing feeding activity fourth instar nymph of laboratory culture of *S. gregaria* left to starve for 24 hrs before testing for antifeedant properties. Tested nymph

were divided into three replicates (10 nymphs for each). Discs of 39.12 $mm^2$ area of lettuce leaves were impregnated with the jojoba oil, saponifiable and unsaponifiable matters for about two minutes after which the treated discs were left in shade for about 10 minutes to dry. Five concentrations (1.25, 2.5, 5, 7.5 and 10%, w/v) were used. The control test was conducted using the same technique of nymph feeding on lettuce dipped in water and tween 80. The treated and untreated discs were fixed in a vertical position by the sand in the bottom of cylindrical glass container (12 cm height x 12 cm diameter). Two discs were offered to each tested nymph. The eaten area was estimated after 24 hr. by Planimeter.

The percentage of feeding reduction over control was used as factor for determining the presence of feeding deterrent effect. The antifeedant activity was calculated by using the formula of Saleh *et al.*, (1986).

### 3.3. Toxicity and Biological effects:

Thirty 4th instars nymphs of *S. gregaria* for each concentration, five concentrations (1.25, 2.5, 5, 7.5 and 10%, w/v) of jojoba oil and their fractions (saponifiable and unsaponifiable matters) in a three replicates were used. Each replicate was 10 nymphs and each nymph transferred into the bottom of cylindrical glass container (12 cm height x 12 cm diameter). All nymphs were allowed to feed for three days on the treated leaves then complete their life cycle on fresh lettuce leaves. Percentage of total mortality was recorded after the end of the nymphal stage. Percentage mortalities were corrected according to Abbott's formula (Abbott, 1925).

$LC_{50}$ and $LC_{90}$ values for *S. gregaria* (5%) confidence limits and slope regression lines represented and interpreted, using probit analysis statistical method of Finney (1971). Also, nymphal and adult durations, percentages of adult emergence, malformation and sex ratio were all recorded.

### 3.4. Statistical analysis:

Statistical analysis of nymphal and adult durations was carried out also by using a computer software package, "Costat", a product of Cohort Software Inc., Barkeley, California, USA. Duncan's multiple range test (Duncan, 1955) was used to differentiate between means.

## 4. Biochemical studies:

### 4.1. Effect of jojoba oil on hyperlipidemic diet:

#### 4.1.1. Animals and diet

Adult albino rats were obtained from Animal House of National Research Center, Egypt, weighing 150-180 g at the start of the experiment. They were housed in plastic cages under normal laboratory conditions and fed on a basal diet. The basal diet was in the form of dry powder ration, made up of 80% ground wheat, 6% bran, 3% dry milk, 1% yeast, 1% sodium chloride, 1% vitamin D and 8% fish powder (Egyptian Company for Oils and Soaps).

#### 4.1.2. Experimental design

The rats were divided into three groups (each group contained six rats). Group one was fed a basal diet (normal group), group two was fed the same diet supplemented with 1% cholesterol, 0.25% cholic acid, 10% animal fat and 4% jojoba oil (jojoba oil group), while group three was fed the basal diet supplemented with 1% cholesterol, 0.25% cholic acid and 10% animal fat (hyperlipidemic group). The experiment duration was three weeks, at the end of which rats were deprived of food overnight. Blood samples were withdrawn from the retro-orbital venous plexus with capillary tubes. Centrifugation was done at room temperature for 15 min., sera were separated to be used for the determination of biochemical evaluations.

#### 4.1.3. Biochemical evaluations

Serum total cholesterol was measured according to the method described by Allian *et al.* (1974) while serum triacylglycerols were measured according to the method of Fossati and Princioe (1982). The results were expressed as mean ± SD. Statistical analyses were performed using one-way ANOVA test followed by Duncan test.

### 4.2. Effect of defatted meal on some biochemical parameters

#### 4.2.1. Animals and diet

Albino rats weighing about 160 g at the beginning of the experiment were kept in plastic cages under normal laboratory conditions and were fed on a basal diet.

#### 4.2.2. Experimental design

The rats were divided into two groups (A and B), each group contained six rats. Group A (control) was fed a basal diet and group B was fed a basal diet supplemented with 10% defatted jojoba meal for two weeks. Food intake was measured every day and faeces were collected and weighed every day for each group. Body weight of rats was recorded at beginning and end of experiment. After 14 days, blood samples were withdrawn as before. Sera were separated to be used for the determination of biochemical parameters.

#### 4.2.3. Biochemical evaluations

Colorimetric determinations of alanine and aspartate aminotransferases (ALT and AST) activities were accomplished according to the method of Reitman and Frankel (1957), while serum creatinine levels were determined according to the method of Faulkner and King (1976). The results were expressed as mean ± SD; the difference between the means was evaluated with student's t-test (Kurtz, 1983).

# RESULTS AND DISCUSSION

## 1. GC and GC/MS analysis of jojoba oil:

Table (1) represents the fatty acids constituents of jojoba oil. The saponifiable matter of the oil contains different fatty acids. For instance, alpha-linolenic acid was found as major (37.1%) followed by nervonic acid (13.8%). Moderate amounts of caprylic and palmitic acids (8.7 and 8.4%) was found followed by behenic acid (6.6%). Oleic acid and lauric acid were detected with the same quantity (5.7%). Erucic acid was found in low quantity (2.7%). It can be concluded that the unsaturated fatty acids represent 59.3%. The ratio between saturated and unsaturated fatty acids is 1: 1.48.

Table (2) represents the unsaponifiable matter of jojoba oil. The main constituents of unsaponifiable matter were fatty alcohols, represent 53.84%, 9-octadecen-1-ol was detected with high quantity (41.35%) followed by 1,22-docosanediol (9.21%). The hydrocarbons were also detected in high quantity, 1,21-docosadiene was the main hydrocarbons (20.64%) followed by 1,19-eicosadiene (3.43%). Many authors studied the different contradictive constituents of jojoba oils (Miwa, 1984 and Van Boden *et al.*, 1997). They concluded that as much as 97% of jojoba liquid wax consists of a mixture of esters of long chain fatty alcohols and long chain fatty acids. More than 60% of this mixture of esters contains *cis*-11-eicosenoic (jojobenoic) acid ($C_{20}$). Perillo *et al.* (2005) found that jojoba oil (JO) was exceptionally rich in *cis*-11-eicosenoic acid. Besides this fatty acid, oleic and *cis*-13-docosenoic acids were detected in similar amounts. Monounsaturated fatty acids were almost 99% of the fatty acids present in JO. Eight fatty alcohols were found in JO: *cis*-11-eicosenol, *cis*-13-docosenol and *cis*-15-tetracosenol were the major components; *cis*-9-octadecenol, hexadecanol, octadecanol, eicosanol and docosanol were found in small proportions. The combination between acids and alcohols in JO leads to esters of 38, 40, 42, 44 and 46 total number of carbon atoms representing 4.4, 24.1, 55.1, 13.1 and 2.0% of the wax esters, respectively.

## 2. Antifeedant activity against *S. gregaria*:

The obtained data in Table (3) showed that the higher concentration of jojoba oil reduced eaten area 30.3% followed by unsaponifiable and saponifiable matters (39.5 and 47.4%), respectively. Both antifeedant and protection activity percentage were increased by increasing the concentration at all treatments. Here again, it can be concluded that the presence of fatty acids, fatty alcohols, hydrocarbons and sterols in jojoba oil play a synergistic effect as antifeedant on the tested insect. Generally, the fatty alcohols, hydrocarbons and sterols were more active than fatty acids as antifeedant activity. Many authors studied the secondary metabolites (triterpenes, sesquiterpene lactones, alkaloids, cucurbitacins, quinines and phenols) as a natural antifeedant (Nawrot *et al.*, 1986; Norris, 1986; Van Beek and Grood, 1986; Grood and Van Beek, 1987; Mwangi and Rembold, 1988 and Wieczorek, 1996).

## 3. Toxicological effect against *S. gregaria*:

The insecticidal activity of jojoba oil and their fractions were studied and summarized in Tables (4 and 5). The obtained data showed that $LC_{50}$ of jojoba oil, unsaponifiable and saponifiable matters were 2.2, 3.4 and 7.96% respectively. These results revealed that jojoba oil and the unsaponifiable matter were more effective than saponifiable matter. The highest mortality percentage was recorded at 10% with jojoba oil (100%) and unsaponifiable matter (93.3%).

The mortality of target insects may be due to the effect of fatty alcohols as well as hydrocarbons and sterols on the cuticle and/or may be due to the disturbance of the hormonal regulation caused by sterols. The mortality was increased with exist of the fatty acids. Al-Sharook *et al.* (1991) mentioned that the death of treated insects caused by plant extracts may be due to the inability of the moulting bodies to swallow sufficient volumes of air to split the old cuticle and expand the new one during ecdysis, or to a metamorphosis inhibiting effect of the plant extract, which is possibly based on the disturbance of the hormonal regulation. Abd El-Rahman (2003) mentioned that jojoba oil caused 83.8 and 90.8% mortality against *Liriomyza trifolii* larvae at 0.5 and 1% respectively. In the same subject, Salem *et al.* (2003) revealed that jojoba oil formulation was the potent agent against both white fly and leafhopper species where the $LC_{50}$ was 5.4% for *Bemisia tabaci* and 6.4% for *Empoasca discipiens*, respectively.

## 4. Biological activity against *S. gregaria*:

### 4.1. Effect on nymphal development:

As shown in Table (4) jojoba oil prolonged the nymphal duration up to 25.3 days, which ranged from 14 to 25.3 days compared to 13 days in the control. These results may be due to the delaying of moulting process. Many authors studied the effect of many substances on 5 $^{th}$ instar nymphs of *S. gregaria* (Sieber and Rembold, 1983; Bernays and Woothead, 1984; Jagannadh and Nair, 1992 and Ghoneim *et al.*, 2000). They found that treatment with azdirachtin induced juvenilizing effects on 5$^{th}$ instar nymphs of *S. gregaria* and play the same regulatory role of juvenile hormone.

As well as, gas chromatography/mass spectroscopy (GC-MS) of unsaponifiable matter indicated that plant sterols amounted to 0.46%. Insects metabolize phytosterols to cholesterol, which is the dominant sterol found in cell membranes and serves as the precursor for the insect moulting hormone 20- hydroxyecdysone (Grieneisen, 1994). Sitosterol is the most common phytosterol found in plants and is readily metabolized to cholesterol in most phytophagous insects (Svoboda and Thompson, 1985 and Ikckawa *et al.*, 1993). For grasshoppers, however, phytosterols that possess a double bond at position 7 ($\Delta^7$), or at position 22 ($\Delta^{22}$), cannot be metabolized to cholesterol; these sterols will not support normal growth and development (Behmer, 1998).

**Table (1): Chemical composition of fatty acids of jojoba oil identified by using GC.**

| Common Name | Scientific name | Retention time (RT) | (%) of total fatty acids |
|---|---|---|---|
| Caprylic acid | Octanoic acid (8:0) | 7.543 | 8.7 |
| Capric acid | Decanoic acid (10:0) | 8.432 | 4.3 |
| Lauric acid | Dodecanoic acid (12:0) | 11.089 | 5.7 |
| Myristic acid | Tetradecanoic acid (14:0) | 12.73 | 4.3 |
| Palmitic acid | Hexadecenoic acid (16:0) | 18.217 | 8.4 |
| Stearic acid | Octadecanoic acid (18:0) | 20.572 | 0.5 |
| Oleic acid | 9-octadecenoic acid (18:1) | 22.285 | 5.7 |
| Alpha-Linolenic acid (ALA) | 9,12,15-octadecatrienoic acid (18:3) | 25.88 | 37.1 |
| Arachidic Acid | Eicosanoic acid (20:0) | 26.605 | 2.2 |
| Behenic acid | Docosanoic acid (22:0) | 28.552 | 6.6 |
| Erucic acid | 13-docosenoic acid (22:1) | 29.540 | 2.7 |
| Nervonic acid | *Cis*-tetracos-15-enoic acid (24:1) | 35.446 | 13.8 |

**Table (2): Chemical composition of the unsaponifiable matter of jojoba oil using GC-MS analysis.**

| | Compound | (%) of total unsaponifiable matter |
|---|---|---|
| 1. | 5-decanol | 0.01 |
| 2. | 1-docosanol | 0.06 |
| 3. | 1,19- eicosanediol | 3.05 |
| 4. | 1,22-docosanediol | 9.21 |
| 5. | 1-hexadecanol | 0.16 |
| 6. | 9-octadecen-1-ol | 41.35 |
| 7. | Heptane | 0.42 |
| 8. | Octane | 0.59 |
| 9. | Nonane | 0.03 |
| 10. | Decane | 0.01 |
| 11. | 1,5-heptadien-4-one, 3,3,6-trimethyl | 0.01 |
| 12. | Undecane | 0.02 |
| 13. | Dodecane | 0.03 |
| 14. | Butane1,1-dibutoxy | 0.03 |
| 15. | Tetradecene | 0.02 |
| 16. | Tetradecane | 0.03 |
| 17. | Pentadecane | 0.01 |
| 18. | Octadecane | 0.04 |
| 19. | 1-octadecene | 0.01 |
| 20. | Docosane | 0.01 |
| 21. | Hexadecane | 0.01 |
| 22. | Hexadecanoic acid methyl ester | 0.04 |
| 23. | Hexatriacontane | 0.01 |
| 24. | Heptadecane | 0.01 |
| 25. | 1-eicosene | 0.02 |
| 26. | 9-octadecenal | 0.01 |
| 27. | 14,17-octadecadienoic acid, methyl ester | 0.97 |
| 28. | 1,21-docosadiene | 20.64 |
| 29. | 14-tricosenyl | 0.05 |
| 30. | 1,19-eicosadiene | 3.43 |
| 31. | 14-tricosenyl formate | 2.84 |
| 32. | Stigmast-5-en-3-ol | 0.43 |
| 33. | Stigmasta-5-22-dien-3-ol | 0.03 |
| Total | | 83.59 |

**Table (3): Antifeedant activity of jojoba oil, saponifiable and unsaponifiable matters against *Schistocerca gregaria.***

| Treatments | Conc. | Consumed area in $(mm)^2$ treated for 24 hrs ± SE | (% ) of eaten area | (%) of protection | Antifeedant activity |
|---|---|---|---|---|---|
| Jojoba oil | 1.25 | $37.9 \pm 0.9^{c}$ | 52.7 | 47.3 | 16.2 |
| | 2.5 | $36.3 \pm 0.2^{cd}$ | 50.5 | 49.4 | 19.8 |
| | 5 | $35.7 \pm 1.4^{cd}$ | 49.7 | 50.3 | 21.0 |
| | 7.5 | $31.3 \pm 0.5^{de}$ | 43.6 | 56.4 | 30.8 |
| | 10 | $21.77 \pm 0.6^{f}$ | 30.3 | 69.7 | 51.8 |
| Mean | | $32.58^{C}$ | | | |
| Saponifiable matter | 1.25 | $44.7 \pm 0.5^{ab}$ | 62.3 | 37.7 | 2 |
| | 2.5 | $43.4 \pm 1.0^{ab}$ | 60.5 | 39.6 | 3.9 |
| | 5 | $43.2 \pm 0.6^{ab}$ | 60.0 | 39 | 4.5 |
| | 7.5 | $41.6 \pm 0.9^{b}$ | 57.9 | 42.0 | 7.9 |
| | 10 | $34.0 \pm 0.4^{de}$ | 47.4 | 52.6 | 24.7 |
| Mean | | $41.4^{B}$ | | | |
| Unsaponifiable matter | 1.25 | $38.6 \pm 0.2^{bc}$ | 35.8 | 46.2 | 14.5 |
| | 2.5 | $36.1 \pm 0.6^{cd}$ | 50.3 | 49.7 | 20.1 |
| | 5 | $35.2 \pm 0.9^{cd}$ | 49.0 | 51.0 | 22.1 |
| | 7.5 | $30.7 \pm 1.3^{e}$ | 42.7 | 57.3 | 32.2 |
| | 10 | $28.4 \pm 0.8^{e}$ | 39.5 | 60.5 | 37.2 |
| Mean | | $33.8^{C}$ | | | |
| Control | 0 | $45.2 \pm 1.3^{a}$ | 62.9 | 37.1 | — |

The small and the capital letters indicate significant difference between concentrations and between extracts, respectively. LSD (5%) between concentrations was 3.1, and between extracts was 1.4.

**Table (4): Insecticidal and biological activities of jojoba oil, saponifiable and unsaponifiable matters against *Schistocerca gregaria.***

| Treatments | Concentration (%) | Nymphal stage | | Adult stage | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Corrected mortality (%) | Duration (Days ± SE) | Corrected mortality (%) | Malformed adults (%) | Increasing than control (%) | Adult emergence (%) | Sex Ratio ♂ | Sex Ratio ♀ |
| Jojoba oil | 1.25 | 33.3 | $25.3 \pm 0.5^{a}$ | 0.0 | 60 | 79.6 | 66.7 | 1 | 1 |
| | 2.5 | 60 | $23 \pm 1.1^{ab}$ | 16.7 | 50 | 64.6 | 40 | 2 | 1 |
| | 5 | 66.7 | $14 \pm 0.5^{ghi}$ | 33.3 | 40 | 49.7 | 33.3 | 2 | 1.5 |
| | 7.5 | 80 | $14 \pm 0.3^{ghi}$ | 33.3 | 0.0 | 0.0 | 20 | 2 | 1 |
| | 10 | 100 | $0.0 \pm 0.0^{j}$ | 0.0 | 0.0 | 0.0 | 0.0 | - | - |
| Mean | | | $15.3^{B}$ | | | | | | |
| Saponifiable matter | 1.25 | 13.3 | $14.4 \pm 0.6^{ghi}$ | 0.0 | 23.1 | 24.5 | 86.7 | 1.6 | 1 |
| | 2.5 | 26.7 | $19.8 \pm 0.8^{cd}$ | 0.0 | 28.6 | 32.7 | 73.3 | 1.2 | 1 |
| | 5 | 33.3 | $21.3 \pm 0.6^{bc}$ | 0.0 | 33.3 | 39.7 | 66.7 | 1.5 | 1 |
| | 7.5 | 46.7 | $16 \pm 0.4^{efg}$ | 0.0 | 40 | 49.7 | 53.3 | 1.4 | 1 |
| | 10 | 60 | $16 \pm 0.8^{efg}$ | 16.7 | 50 | 64.6 | 40 | 1 | 1 |
| Mean | | | $17.5^{A}$ | | | | | | |
| Unsaponifiable matter | 1.25 | 26.7 | $13.5 \pm 1.1^{ghi}$ | 0.0 | 27.3 | 30.7 | 73.3 | 1.2 | 1 |
| | 2.5 | 33.3 | $15.5 \pm 0.2^{fgh}$ | 0.0 | 33.3 | 39.7 | 66.7 | 2.3 | 1 |
| | 5 | 46.7 | $17.5 \pm 0.9^{def}$ | 22.2 | 50 | 64.6 | 60 | 0.8 | 1 |
| | 7.5 | 66.7 | $18.3 \pm 0.4^{de}$ | 20 | 60 | 79.6 | 33.3 | 1.5 | 1 |
| | 10 | 93.3 | $12 \pm 0.0^{i}$ | 100 | 0.0 | 0.0 | 6.7 | - | - |
| Mean | | | $15.4^{B}$ | | | | | | |
| Control | 0.0 | | $13 \pm 1.3$ | 0.0 | 6.7 | 0.0 | 100 | 0.8 | 1 |

The small and the capital letters indicate significant difference between concentrations and between extracts, respectively. LSD (5%) between concentrations was 2.35, and between extracts was 1.05.

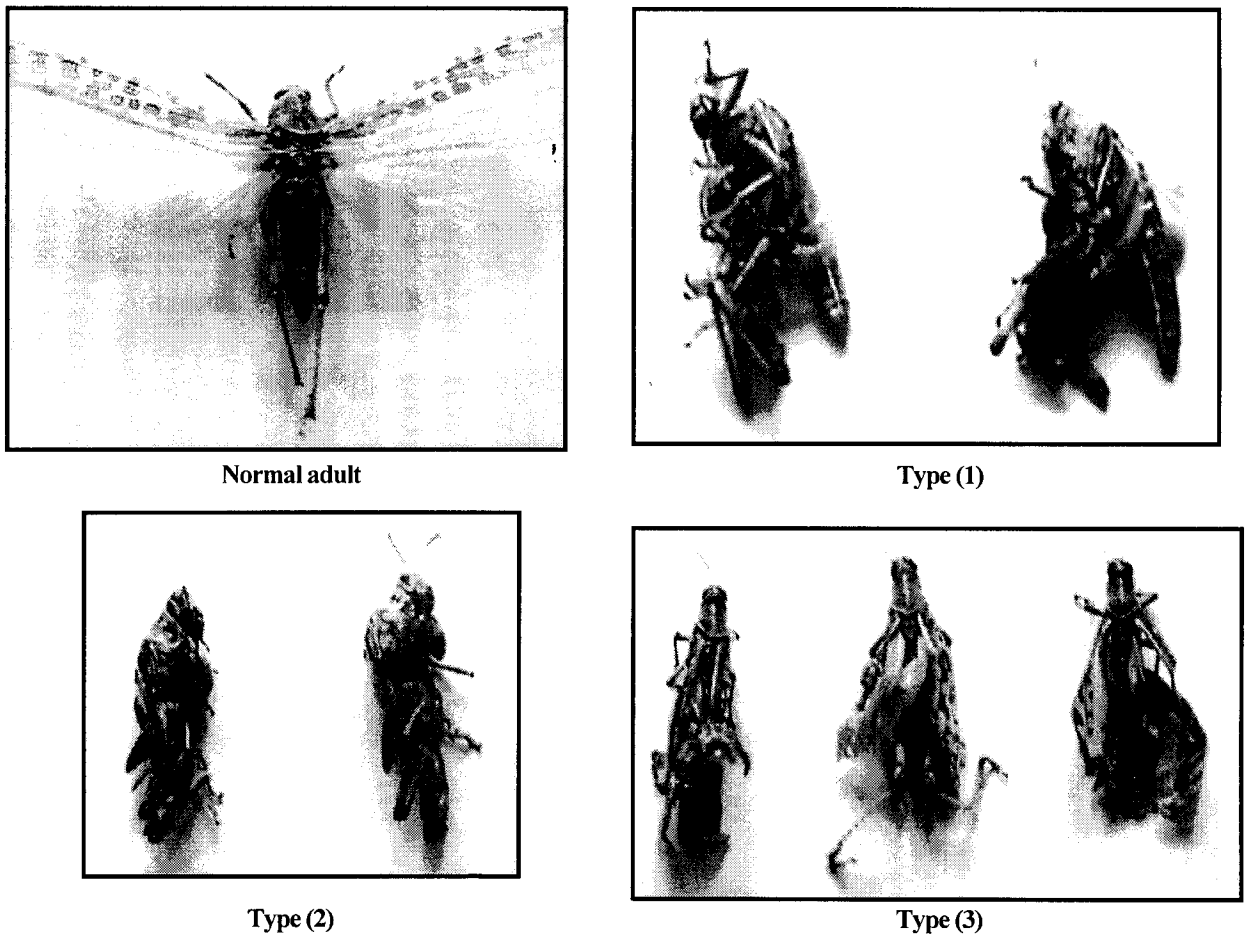


**Fig. (1): Different categories of deformations of *Schistocerca gregaria* adult emerged from nymph of normal adult treated with jojoba oil, saponifiable and unsaponifiable maters.**
Where: 1. Adult failed to emerge from nymphal cuticle,
2. nymphal cuticle covers all the adult bodies accept thoracic,
3. Adult with both legs and curled wings
4. Adult with both legs and curled wings

All these factors may cause a disturbance in hormonal balance which affecting development and metamorphoses of treated insect.

**Table (5): $LC_{50}$ and $LC_{90}$ of the jojoba oil, saponifiable and unsaponifiable matters on 3rd instar nymph of *S. gregaria* after three days of exposure.**

| Treatments | Jojoba oil (%) | Saponifiable matter (%) | Unsaponifiable matter (%) |
|---|---|---|---|
| LC50 | 2.2 | 7.96 | 3.4 |
| LC90 | 11.5 | 65.8 | 13.8 |
| Slope±SE | 1.8 ± 0.5 | 1.4 ± 0.5 | 2.1 ± 0.5 |

### 4.2. Effect on adult development:

Table (4) shows that the jojoba oil, saponifiable and unsaponifiable matters caused the higher percentages of malformed adult compared with control. The malformation was increased by increasing the concentrations (Figure 1). Jojoba oil was the most efficient on the adult emergence followed by the unsaponifiable matter where the adult emergence in the treatment with the higher concentration of both jojoba oil and unsaponifiable matter were 0.0% and 6.7%, respectively. The six ratio was affected by the different treatments and by concentrations. It is clear that male ratio was greater than female; it may be due to the chemical structure of jojoba oil, unsaponifiable and saponifiable matters that alter the normal ratio.

## 5. Biochemical impacts of jojoba oil and defatted meal on albino rats:

Hyperlipidemia is a common metabolic disorder that is considered as a risk factor for many diseases especially cardiovascular disease (Carpentier, 1997). Dietary fats have a major role in the prevention and treatment of atherosclerosis. Dietary cholesterol, by increasing plasma cholesterol levels or by modifying the composition of lipoprotein, can influence the progression of atherosclerosis. Again, epidemiological and clinical studies have clearly demonstrated a relationship between intake of saturated fatty acids and atherosclerosis. On the other hand, both polyunsaturated fatty acids (PUFA) and monosaturated fatty acids (MUFA) could affect the lipoprotein metabolism with a hypocholesterolemic effect (Mancini and Parillo, 1991). Table (6) demonstrates the effect of jojoba oil supplementation on serum total cholesterol (TC) and triacylglycerols (TAG) in rats fed hyperlipidemic diet (atherogenic diet). Serum TC and TAG levels were highest in rats fed hyperlipidemic diet (group 3) but addition of jojoba oil (4%) to the hyperlipidemic diet significantly decreased serum TC and TAG levels down almost to control values. The decrease amounted to 33.1 and 20.8%, respectively.

**Table (6): Effect of jojoba oil (4%) supplement with hyperlipidemic diet on serum cholesterol and triacylglycerols in rats**

| Groups | Cholesterol (mg/dl) | Triacylglycerols (mg/dl) |
|---|---|---|
| Group 1 (normal) | 125.5±15.48[a] | 43.6±2.50[c] |
| Group 2 (jojoba oil) % of change† | 137.1±17.67[a] 33.1% | 42.6±9.11[c] 20.8% |
| Group 3 (hyperlipidemic) | 205.1±12.76[b] | 53.8±8.70[d] |

Means with different letters superscripts a,b,c... for columns with a significant difference (p value) at ≤0.05.
†Percentage of change compared to hyperlipidemic group

As mentioned before, jojoba oil contains alpha-linolenic acid (α-LA) that amounted to 37.1% as the major PUFA, followed by nervonic acid as the major MUFA, which reached 13.8%. Jojoba oil is characterized by the presence of alpha-linolenic acid (18:3, n-3), an omega-3 fatty acid. Flaxseed oil supplementation rich in alpha-linolenic acid significantly lowered the increase in body weight gain, liver weight, plasma TC, TAG, phospholipids, free fatty acids, lipoproteins in rats fed high fat diet (Vijaimohan *et al.*, 2006). Nervonic acid is an essential fatty acid for the growth and maintenance of the brain; it is an important ingredient in nervous cell membranes because it is vital in regulating the ion channels and receptors. It plays a part in the biosynthesis of myelin (the white matter insulating the nerves- that allows the conduction of impulses from one part of the body to another). It can regulate the function of brain cell membranes and has a neuroprotective effect, which is important to hard training individuals, (Sargent *et al.*, 1994). Hypocholesterolemic effect of PUFA has been thought to be mediated via redistribution of cholesterol from blood to the tissue pools, (Grundy and Ahrens, 1970). In this study jojoba oil had a hypolipidemic effect; this may be due to the high percentage of α-LA (37.1%) and the presence of plant sterols. Gas chromatography/mass spectroscopy for unsaponifiable matter indicated that plant sterols amounted to 0.46%. Plant sterols are compounds that can be found in vegetables, fruits, legumes, nuts, grains and certain oils. These compounds compete with the absorption of dietary cholesterol as well as inhibit the reabsorption of endogenous cholesterol in the gastrointestinal tract. Supplementation with plant sterols can significantly lower blood cholesterol levels (Nestet *et al.*, 2001). Additional studies by Grundy and Moke, (1976) suggested that even smaller amounts of plant sterols in diet would produce maximum blockage of cholesterol absorption and blood TC reduction.

Colorimetric determination of alanine and aspartate aminotransferases (ALT and AST) activities revealed no significant changes in serum ALT, AST and creatinine levels in animals fed with 10% defatted meal as compared to control group after 14 days, (Table 7). This proves that the defatted jojoba meals do not affect the liver enzymes and kidney functions, and its use has no toxic effects on tissues. The mean values of body weight changes, food intake and faecal excretion in rats fed with 10% defatted jojoba meal are shown in Table (8). The mean initial body weight of each group was 160.6-162.6 g; after two weeks, rats fed with 10% defatted meal showed a significant reduction in weight as compared to control group. The decrease in weight of group fed with 10% defatted meal was 14 g from the initial weight. Also, rats fed with 10% defatted meal resulted in a significant reduction after 14 days in food intake and faecal excretion in comparison to control group. The remainder after oil extraction (referred to as defatted jojoba meal) contains relatively large amounts of the unique cyanoglucosides, simmondsin, simmondsin ferrulate and other analogs. These glycosides, mostly simmondsin and simmondsin ferrulate, decreased food intake in rats due to induction of satiation (Flo *et al.*, 1999). Several studies have attributed decreased food intake and weight loss to these compounds when ~10% defatted jojoba meal was included in the diet of rats (Cokelaere *et al.*, 1992) and broiler hens (Arnouts *et al.*, 1993). However, compounds other than simmondsin are present in the meal, including polyphenols, phytic acid, trypsin inhibitors and bitter substances, that may contribute to an impaired food intake and energy balance of jojoba fed animals (Vermaut, 1998). The demonstrated mechanism for food intake inhibition in rats fed with defatted jojoba meal is via stimulation of the cholecystokinin satiation, (Cokelaere *et al.*, 1995) or may be caused by its bitter taste due to presence of simmondsin ferrulate and tannins (simmondsin itself is tasteless) (Medina *et al.*, 1988).

**Table (7): Effect of defatted jojoba meal (10%) on biochemical parameters in rats**

| Groups | ALT (U/L) | AST (U/L) | Creatinine (mg/dl) |
|---|---|---|---|
| Group A | 13.1±3.54 | 31.3±2.33 | 1.16±0.12 |
| Group B | 11.3±1.03 | 29.5±2.07 | 1.18±0.18 |
| P value | N.S | N.S | N.S |

N.S: Non- significant
$p \leq 0.05$ is considered significant

**Table (8): Body weight changes, food intake and faecal excretion of rats fed a basal diet supplemented with 10% defatted jojoba meal and rats fed with the same basal diet**

| Parameters | Group A | Group B |
|---|---|---|
| Body weight *(g)* | | |
| Day 0 | 160.6±16.16 | 162.6±7.31 |
| Day 14 | 168.3±8.59 | 148.6±8.20* |
| Alteration *(g)* | 7.7 | -14 |
| Food intake *(g/day)* | 11.1±0.48 | 7.9±1.8* |
| Faecal excretion *(g/day)* | 4.01±0.14 | 2.5±0.33* |

*significant at $p \leq 0.05$.

Generally, from the previous results it is clear that jojoba oil and its saponifiable and unsaponifiable matters exhibited biological activity against nymph of *S. gregaria*. Therefore, jojoba oil could be use as a pest control agent.

As well as supplementation of jojoba oil could affect on serum cholesterol and triacylglycerols levels as hypolipidemic agent in rats fed high fat diet, also defatted jojoba meal relatively safe and can be used in animals feeding.

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