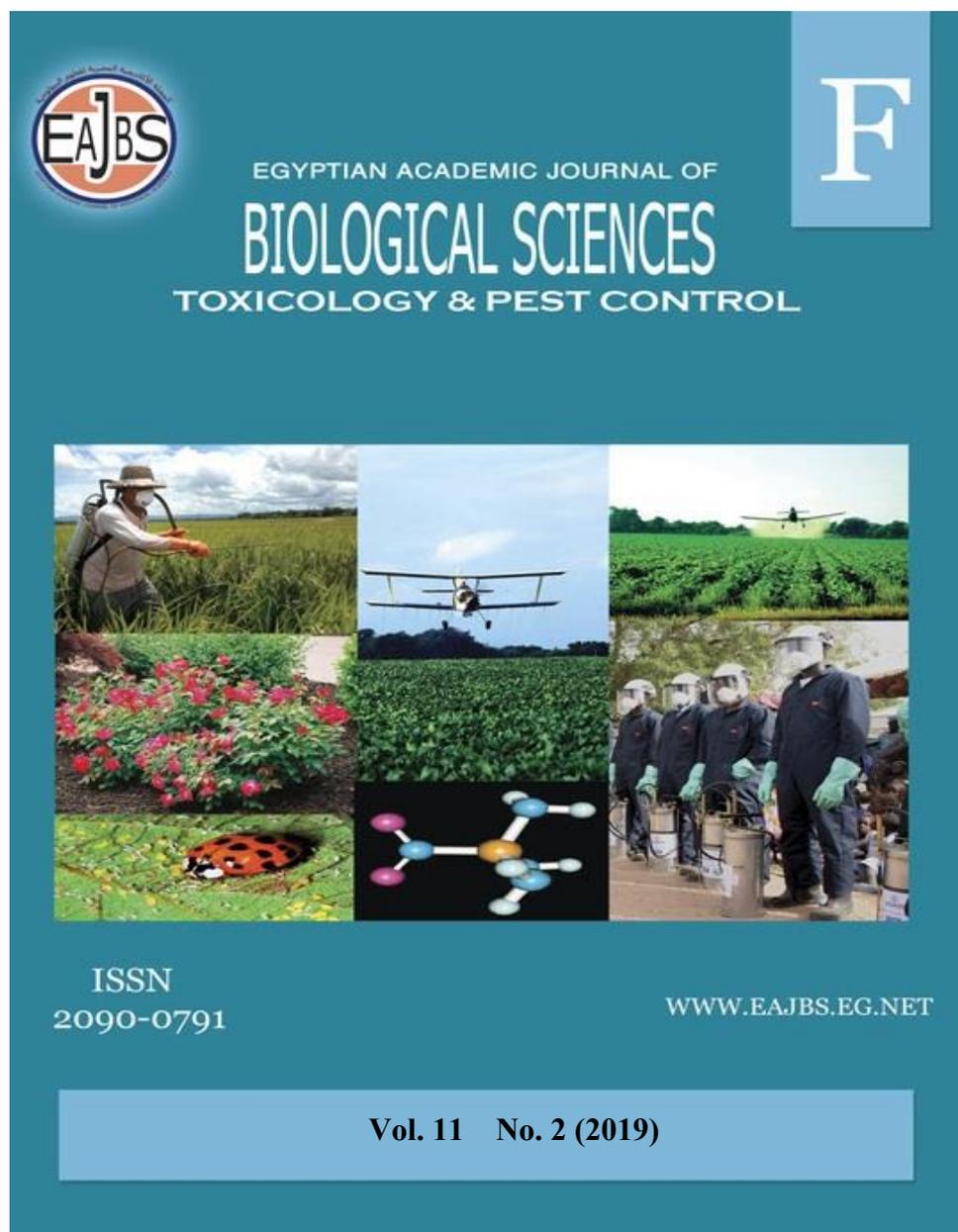


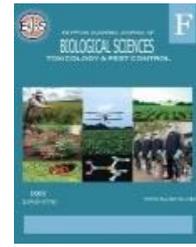
**Provided for non-commercial research and education use.  
Not for reproduction, distribution or commercial use.**



The journal of Toxicology and pest control is one of the series issued twice by the Egyptian Academic Journal of Biological Sciences, and is devoted to publication of original papers related to the interaction between insects and their environment.

The goal of the journal is to advance the scientific understanding of mechanisms of toxicity. Emphasis will be placed on toxic effects observed at relevant exposures, which have direct impact on safety evaluation and risk assessment. The journal therefore welcomes papers on biology ranging from molecular and cell biology, biochemistry and physiology to ecology and environment, also systematics, microbiology, toxicology, hydrobiology, radiobiology and biotechnology.

[www.eajbs.eg.net](http://www.eajbs.eg.net)



**Comparative between Two Eco-Friendly Botanical Oils through Studies Toxicological, Biological and Molecular Impacts on the Cotton Leafworm, *Spodoptera littoralis* (Boisd.)**

**Marwa M.A. EL-Sabagh; Sara M. I. Abd El-Kareem; El Shaimaa N. I. Abd El Mageed and Nashwa S. Amin**

Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza

E-mail: [saraelkhateeb148@gmail.com](mailto:saraelkhateeb148@gmail.com)

**ARTICLE INFO**

**Article History**

Received:10/8/2019

Accepted:6/9/2019

**Keywords:**

botanical oils , Jojoba and Jatropha oils, *Spodoptera littoralis* (Boisd.), Toxicological, biological and molecular effects

**ABSTRACT**

Toxicological, biological and molecular effects of two eco-friendly botanical oils named Jojoba and Jatropha oils against the newly molted 2<sup>nd</sup> instar larvae of the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.), were investigated under laboratory conditions. Results generally revealed a decrease in the mean larval, pupal, and adult durations for the 2<sup>nd</sup> instar larvae surviving treatment with the LC<sub>50</sub> value 1.905 % and 1.793 %, for the two tested oils Jojoba oil and Jatropha respectively. Also, plant extracts caused a reduction in all the other biological impacts (pupation, adult emergence percentage, the mean number of eggs/female and the mean number of hatched eggs). Molecular studies have been carried out on 6<sup>th</sup> instar larvae of *S. littoralis* which treated in 2<sup>nd</sup> larval instars with LC<sub>50</sub> of the tested botanical oils. Seven random primers were used in this study to generate a fragmenting pattern as a tool to investigate the molecular differences between treated samples and control. The numbers of unique and common fragments generated by using these primers (O4, O7, O5, O14, C10, C13 and C15) were recorded. It has been found that primer C13 was the most powerful one in generating a unique informative fragmenting pattern; it gives five specific unique fragments. While the primer O14 was the poorest one in generating an informative fragmenting pattern.

**INTRODUCTION**

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) is considered as one of the deleterious and eradicated insects as it has a broad range of host plants. This pest causes high economic loss in many field crops, vegetables, and fruits. Therefore, it demands many strategies to be managed. In Egypt, the management of *S. littoralis* was limited to the extensive use of conventional insecticides leading to the rise of high resistance to many chemical pesticides, resurgence, and residues of chemical pesticides in the environment (Metayi *et al.*, 2015 and Eldesouky *et al.*, 2019). As a result, finding new alternative, effective, safer for humans and less toxic to our ecosystem, is requisite (Korrat *et al.*, 2012). Of those groups, plant-based pesticides which are extracted from natural plant sources and tested on a wide range of insect species (Ebadah *et al.*, 2016 and El-Seedi *et al.*, 2017). Due to their high level of safety for humans, animals, and fish, plant oils are considered hopeful tools for controlling insect pests. Moreover, they have

a minimal influence on natural pest predator or parasitoid as well as pollinating insects (Moawad *et al.*, 2015; Nollet and Rathore, 2017). They also are used as toxicants, repellents, synergists, growth regulators and Antifeedant for cotton leafworm (Abd El-Zaher, 2017). There are more than 2400 plant species belonging to 189 plant families which are rich sources of bioactive organic compounds (Rao *et al.*, 2005 and Gaaboub *et al.*, 2012). Jojoba oil is a natural compound obtained from the jojoba crop, *Simmondsia chinensis* L. Previously published researchers have proved the pesticidal effect of crude extracts of jojoba against various economically important insect pests belonging to order lepidoptera and orthoptera (Rofail *et al.*, 2000; Salem *et al.*, 2003; Yacoub, 2006; Halawa *et al.*, 2007; and Gaaboub *et al.*, 2012). Treatment with jojoba extract caused toxic, antifeedant, growth and development and oviposition inhibition. *Jatropha curcas* L. is a multi-purpose shrub, traditionally used as a medicinal plant and currently as a source of vegetable oil for biodiesel. Diverse studies report that the leaves extract present anti-diabetic and anthelmintic properties along with insecticide, antibacterial, and nematicidal activities (Pabón and Hernández-Rodríguez, 2012). A phytochemical analysis identified the presence of flavonoids, steroids, saponins, alkaloids, tannins, triterpenoids, carbohydrates in the leaves, in ethanolic extracts  $\beta$ -stigmasterol and phytol were identified (Ahirrao *et al.*, 2011 and Ma *et al.*, 2011).  $\beta$ -stigmasterol has acaricidal activity while phytol has antimicrobial, anti-inflammatory, anticancer, and diuretic activities (Rajeswari *et al.*, 2012). Some studies reported that aqueous and methanolic extracts had insecticidal activity specifically against dipteran insects (Kovendan *et al.*, 2011; Tomass *et al.*, 2011; Juliet *et al.*, 2012; Reichel *et al.*, 2013; Chauhan *et al.*, 2015; Khattak *et al.*, 2015; and Soto-Armenta *et al.*, 2019).

The present study is aiming to evaluate the toxicological, biological, and molecular influence of jojoba and jatropha oils against the 2<sup>nd</sup> instar larvae of the cotton leafworm, *Spodoptera littoralis*, as safe alternatives for conventional chemical insecticides under laboratory conditions.

## MATERIALS AND METHODS

### Tested Insects:

A laboratory strain of the cotton leafworm, *Spodoptera littoralis* (Lepidoptera: Noctuidae), was obtained and reared in the cotton leafworm Department, Plant Protection Research Institute, Dokki, Giza under constant laboratory conditions as described by El-Defrawi *et al.* (1964). Rearing conditions were a 12 h photo regime at 25±1°C and 65±5% relative humidity (RH).

### Tested Oils:

Two Egyptian oils, obtained from Al-Gomhuria Company for Drugs, Chemical and Medical Supplies, Al-Ameria, Cairo, Egypt, approved for human use from the Egyptian Ministry of Health. They were jojoba oil, *Simmondsia chinensis* L. (Simmondsiaceae) and Jatropha oil, *Jatropha curcas* L. (Euphorbiaceae).

### Insecticidal Activity of Essential Oils:

The leaf-dipping technique, similar to that described by Tabashnik *et al.* (1990), was used to determine the toxicity of essential oils against the 2<sup>nd</sup> instar larvae using concentrations of 1, 1.5, 2, 2.5, 3 and 3.5 % (v/v) in 100 ml of distilled water with 0.002% of Tween 80. Castor leaves were dipped for 5 s in each solution, and then the treated leaves were left for natural air-drying. Three replicates each with 20 larvae of 2<sup>nd</sup> instar larvae and were allowed to feed on treated leaves for 24 h. Three replicates of 20 larvae were fed on water-tween treated leaves for 24 h to serve as the control. Larval mortality was recorded after 24 h. Mortality was calculated using the Abbott formula (Abbott, 1925) and subjected to probit analysis according to Finney (1971) using "LdPLine<sup>®</sup>" software.

**Biological Studies:**

The 2<sup>nd</sup> instar larvae were treated with determined LC<sub>50</sub> of jojoba and jatropha oils to estimate the following biological parameters; mean larval and pupal duration of treated larvae and percentage of pupation. Resultant pupae were sexed and coupled in separate glass globes allowed for moth eclosion and mating. Consequently, the percentage of adult emergence, longevity of moths and the fecundity and fertility of eggs/female were calculated. A set of untreated larvae was considered as a control group.

**Molecular Studies:**

The DNA was extracted according to the method of Sambrook *et al.* (1989). Before any analysis, it was important to determine the concentration and purity of isolated DNA; this was carried out by estimating UV absorbance at a wavelength of 260 and 280 nm using a spectrophotometer. DNA was subjected to PCR in order to generate the fragmenting profile. The random primers used were O4, O7, O5, O14, C10, C13 and C15 (Table 1). Reactions were carried out in a thermocycler (Progeny 30, Techno, Cambridge Ltd. Dux ford Cambridge, UK). The PCR profile was as follows: 94 °C for 5 min, 94 °C for 1 min, 40 °C for 1min, 72 °C for 2 min, and a final extension at 72 °C for 7 min. Then the PCR reaction was kept at 4 ° C over-night, till migration on agarose gel occurred.

**Table (1):** Sequence of used random primers

No.	Primer	Sequence
1	O4	5'-TCA GGG TGT T-3'
2	O7	5'-CAC GA TGA C T-3'
3	O5	5'- CCC AGT CAC T-3'
4	O14	5'- AGC ATG GCT C-3'
5	C10	5'- TGT CTG GGT G -3'
6	C13	5'- AAG CCT CGT C-3'
7	C15	5'- GAG TCA GTA A-3'

The gel was prepared with wells into which the DNA fragments are added and submerged under an electrolyte buffer solution between a positive and a negative electrode. The DNA fragments are negatively charged so the wells containing them are placed closest to the negative electrode. When the current is turned on the DNA moves through the pores in the gel towards the positive electrode. PCR- DNA marker was used to determine the molecular weight of each fragment. The shorter fragments move faster because they are able to move through the pores of the gel more easily, whereas the longer DNA fragments move more slowly through the pores (Hurlbert, 1999). DNA sequences were analyzed using version 6 of the Gel-Pro Analyzer package of a genetics computer program.

**RESULTS AND DISCUSSION****Insecticidal Activity of Tested Oils:**

The insecticidal activity of jojoba and Jatropha oils against the 2<sup>nd</sup> instar larvae are summarized in Table (2). Results showed that both oils exhibited nearly the same toxic effect against the 2<sup>nd</sup> instar larvae as determined from LC<sub>50</sub> and LC<sub>90</sub> values obtained. The percent mortality of treated larvae was increased by increasing the concentration. These results were agreed with Abdel-Baky and Al-Soqeer (2017) who reported the insecticidal activity of jojoba extract against 2<sup>nd</sup> instar larvae of tomato leaf miner, *Tuta absoluta* Meyrick. Furthermore, obtained results were in context with results obtained by Ingle *et al.* (2017) on the 3<sup>rd</sup> instar larvae of *S. litura*. The toxic activity of jatropha oil is due to the presence of hydrolates which were reported previously for their toxicological effect against *S. littoralis* larvae besides their antioxidant and antibacterial activities

(Calvo, 2012 and Soto-Armenta *et al.*, 2019). In addition, it was reported that *Jatropha* contains high amounts of phenolics, esters, and flavonoids, which proved to have high larvicidal activity (Oskoueian *et al.*, 2011). Moreover, obtained results agreed with Abbassy *et al.* (2007) who reported the insecticidal activity of jojoba extracts against larvae of *S. littoralis* due to the presence of two glucosides; simmondsin and simmondsin 2'-ferulate.

**Table (2):** Susceptibility of *S. littoralis* 2<sup>nd</sup> instar larvae to Jojoba and *Jatropha* oil

Tested oils	Lethal concentration (%)		Slope $\pm$ S. E.
	LC <sub>50</sub>	LC <sub>90</sub>	
<b>Jojoba</b>	1.905	5.285	2.819 $\pm$ 0.303
<b>Jatropha</b>	1.793	5.108	2.892 $\pm$ 0.305

### Biological Impacts of Tested Oils:

Results presented in table (3) showed the effect of compounds at LC<sub>50</sub> on the mean larval duration, pupation percentage, pupal duration, and percentage of adult emergence. Treatment of the 2<sup>nd</sup> instar larvae with tested oils led to a variable effect on the mean larval duration. As shown in table (3), treatment with Jojoba oil increased the mean larval duration. On the other hand, treatment with *Jatropha* oil decreased the mean larval duration compared to the untreated check. Treatment of 2<sup>nd</sup> instar larvae with all tested oils at LC<sub>50</sub> level caused an obvious reduction in pupation percentage. Both tested oil had reduced the percentage of pupation to nearly half compared to the control, pupation percentage of 41% was the lowest recorded when *Jatropha* oil was tested compared to the control. A significant decrease in pupal duration has also been observed after the treatment of the 2<sup>nd</sup> instar larvae with both tested oils compared to the control Table (3). Meanwhile, the percentage of adult emergence was slightly decreased than the control, adult emergence of 85% and 83.2% was the lowest recorded after treatment for both treatment Jojoba and *Jatropha* oil respectively, less than the control. These are shown in previous studies with Marei *et al.* (2009), Ismail, and Shaker (2014).

**Table (3):** Effect of Jojoba and *Jatropha* oil on larval duration, pupation rate and duration of 2<sup>nd</sup> instar larvae of *S. littoralis*

Tested oil	Larval duration (days $\pm$ S. E.)	Pupal duration (days $\pm$ S. E.)	%Pupation	%Adult emergence
<b>Jojoba oil</b>	16.3 $\pm$ 0.5**	12.0 $\pm$ 1.6*	45	85
<b>Jatropha oil</b>	12.6 $\pm$ 0.3**	12.3 $\pm$ 0.1*	41	83.2
<b>Control</b>	14 $\pm$ 1.1	14.0 $\pm$ 1.7	100	100

\*: Significant at  $P > 0.05$

\*\* : highly significant at  $P > 0.01$

\*\*\*: Very highly significant at  $P > 0.001$ .

Table (4) showed the latent effect of treatment of 2<sup>nd</sup> instar larvae with the LC<sub>50</sub> level of used tested oils on adult longevity, the mean number of laid and hatched eggs/female. Both tested oils have significantly shortened the mean adult longevity for both males and females compared to the control.

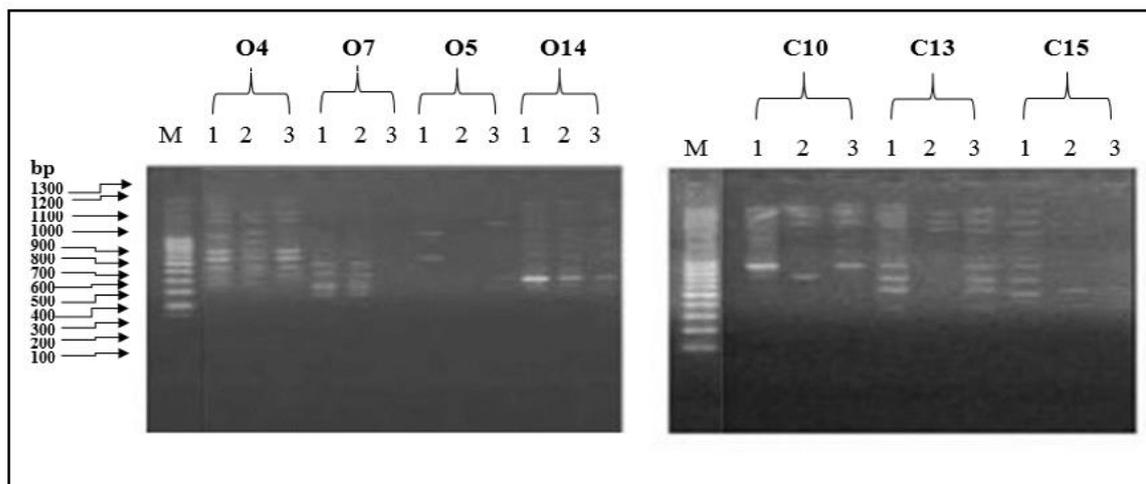
Both the tested plant oils were caused a very high significantly decrease in the mean number of eggs/females. *Jatropha* oil was the most effective oil, followed by Jojoba oil. There was a significant reduction in the mean number of hatched eggs/females.

**Table (4):** Effect of Jojoba and Jatropha oil on adult longevity, fecundity, and fertility of 2<sup>nd</sup> instar larvae of *S. littoralis*

Treatment	Mean adult longevity (days ± S.E.)		Mean no. of eggs/female± S.E.	Mean no. of hatched eggs/female± S.E.
	♂	♀		
Jojoba oil	13.6±1.2**	11.0±1.7**	750±14.2***	681±15.3***
Jatropha oil	12.7±0.3***	11.3±0.1**	552±13.38***	478±21.3***
Control	15.0±1.0	13.6±0.57	2265±15.1	1913±12.11

\*:Significant at  $P > 0.05$ \*\*\*: highly significant at  $P > 0.0$ \*\*\*: Very highly significant at  $P > 0.001$ .**Molecular Impacts of Tested Oils:**

This study has been carried out on 6<sup>th</sup> instar larvae of *S. littoralis* which treated in 2<sup>nd</sup> instar larvae with LC<sub>50</sub> of Jojoba oil and Jatropha oil at 1.905 % and 1.793 %, respectively. Seven random primers were used (O4, O7, O5, O14, C10, C13 and C15) to generate the specific by which an informative conclusion could be summarized. The seven primers used are shown in tables (5 & 6) and in figure (1) along with their sequences. RAPD-PCR technique clarified the DNA diversity among the 6<sup>th</sup> instar larvae of *S. littoralis* which was treated with LC<sub>50</sub> of Jojoba oil and Jatropha oil. 75 DNA fragments were detected using seven random primers. 23 fragments were common in treated and untreated larvae of *S. littoralis*; they represent 30.6 % of all detected fragments. On the other hand, the RAPD-PCR technique shows 32 polymorphic amplified fragments represented 42.6%. This ratio is due to treatment with Jojoba oil and Jatropha oil. Treated and untreated larvae showed 19 unique fragments that represented 25.3 % of all detected fragments (Table 5 and 6). Finally, this study confirmed that Jatropha oil was more effective in DNA generated than Jojoba oil. The previous results showed that the number of the primers (C13) was the powerful one in generating a unique informative fragmenting pattern; it gave five specific unique fragments. While the primer O14 was the poorest one in generating an informative fragmenting pattern, it gives one specific unique fragment. Our results were agreed with those reported by El-Gohary *et al.* (2000) who reported that the DNA fragments varied in intensity and ranged in size from (140-1500 bp) and (196 -1060 bp), respectively. Abd EL- Aziz, (2006) reported in his study that both proteins and RAPD-PCR markers could be used to give estimations of genetic variation and differentiation of different treated and untreated *S. littoralis* larvae with the selected bacterial strains MVPII and the best primers that can be used for developing a genetic marker to differentiate between the different strains were OPB-3 and OPA-18. Abdel-Ghany (2011) generates a banding pattern as a tool to investigate the molecular differences between different treatments botanical extracts castor oil, gossypol on *S. littoralis* larvae. The numbers of unique and common bands generated by using these primers (C1, C4, C17, C13, C15, O6, O7, O15, and O13) were recorded. It has been found that primers O13, C4 was the most powerful one in generating a unique informative banding pattern. Molecular genetic fingerprinting was carried out using 5 random primers on 2<sup>nd</sup> instar larvae of *S. littoralis* which treated with *Bt* and IGR. The obtained data suggested that primer OPO2 was the most powerful primer regarding generating a specific unique band. While the primer OPO4 was the poorest one in generating an informative banding pattern. Abdel-Wahed *et al* (2013) and El-Sabagh (2015).



**Fig. (1):** Molecular fingerprinting using RAPD DNA for pattern for samples treated with Jojoba oil, Jatropha oil and control

M=Marker            1=Control            2= Jojoba oil            3= Jatropha oil

**Table (5):** RAPD-PCR Products in the 6<sup>th</sup> instar larvae of *S. littoralis* after treatment with Jojoba oil and Jatropha oil compared control using random primers

Lanes	Primer 1 : O4									Primer 2 : O7									Marker	
	Control			Jojoba oil			Jatropha oil			Control			Jojoba oil			Jatropha oil				
Rows	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	Amount
r1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1300	3.87
r2	1220	7.01	0.11	1180	10.4	0.12	1220	9.11	0.11	-	-	-	-	-	-	-	-	-	-	-
r3	1140	7.15	0.14	-	-	-	1160	8.47	0.13	-	-	-	-	-	-	-	-	-	1200	9.39
r4	1075	11.7	0.16	1075	10.4	0.16	1075	12.2	0.16	-	-	-	-	-	-	-	-	-	1100	3.79
r5	-	-	-	956	12.6	0.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
r6	933	14.7	0.22	900	10.9	0.24	-	-	-	-	-	-	-	-	-	-	-	-	1000	6.11
r7	767	14.3	0.29	783	11.3	0.28	783	18.9	0.28	-	-	-	-	-	-	-	-	-	-	-
r8	717	11.8	0.31	717	9.42	0.31	717	18.5	0.31	733	10.5	0.3	750	6.36	0.29	-	-	-	900	6.31
r9	-	-	-	-	-	-	-	-	-	633	22.6	0.33	633	8.82	0.33	-	-	-	-	-
r10	-	-	-	575	13.8	0.34	575	16.8	0.34	-	-	-	575	6.67	0.34	-	-	-	800	15.1
r11	550	15.9	0.35	483	8.82	0.37	483	16.1	0.37	483	22.6	0.37	483	26.9	0.37	-	-	-	-	-
r12	450	10.1	0.39	-	-	-	-	-	-	-	-	-	417	33.8	0.4	-	-	-	700	5.3
r13	400	7.35	0.41	400	12.5	0.41	-	-	-	367	27.5	0.42	367	7.08	0.42	-	-	-	600	7.11
r14	-	-	-	-	-	-	-	-	-	288	16.8	0.45	288	10.6	0.45	-	-	-	500	8.57
r15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400	7.93
r16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
r17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	10
r18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200	9
r19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	7.74

Continued table (5):..

Lanes	Primer 3 : O5									Primer 4 : O14									Marker	
	Control			Jojoba oil			Jatropha oil			Control			Jojoba oil			Jatropha oil				
Rows	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount
r1	-	-	-	-	-	-	-	-	-	1260	11.5	0.09	1280	12.2	0.09	1320	9.01	0.07	1300	3.87
r2	-	-	-	-	-	-	-	-	-	-	-	-	1180	6.96	0.12	1220	9.24	0.11	-	-
r3	-	-	-	-	-	-	-	-	-	1140	9.4	0.14	1120	8.63	0.14	1120	9.01	0.14	1200	9.39
r4	-	-	-	-	-	-	1050	8.12	0.16	1025	9.37	0.17	-	-	-	-	-	-	1100	3.79
r5	956	16.6	0.21	-	-	-	-	-	-	944	7.51	0.21	978	6.9	0.19	978	9.13	0.19	-	-
r6	-	-	-	-	-	-	-	-	-	911	5.68	0.24	933	8.87	0.22	900	13.2	0.24	1000	6.11
r7	-	-	-	-	-	-	-	-	-	800	7.78	0.27	850	7.18	0.26	-	-	-	-	-
r8	717	15.2	0.31	-	-	-	-	-	-	733	9.8	0.3	733	12.7	0.3	733	15.1	0.3	900	6.31
r9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
r10	-	-	-	-	-	-	-	-	-	575	10.4	0.34	575	9.29	0.34	575	7.35	0.34	800	15.1
r11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
r12	-	-	-	-	-	-	450	41.7	0.39	433	14.4	0.39	450	12.9	0.39	450	11.9	0.39	700	5.3
r13	400	68.4	0.41	-	-	-	367	50.1	0.42	383	8.68	0.41	383	7.47	0.41	350	9.26	0.34	600	7.11
r14	-	-	-	-	-	-	-	-	-	317	5.6	0.44	317	6.85	0.44	288	6.71	0.45	500	8.57
r15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	400	7.93
r16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
r17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	10
r18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200	9
r19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	7.74

Continued table (5):...

Lanes	Primer 5 : C10									Primer 6 : C13									Marker	
	Control			Jojoba oil			Jatropha oil			Control			Jojoba oil			Jatropha oil				
Rows	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	Amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	amount
r1	1340	6.2	0.08	1340	12.6	0.08	1400	10.8	0.05	1380	0.69	0.06	-	-	-	-	-	-	1300	3.87
r2	1280	17.6	0.1	1280	8.5	0.1	1300	17.7	0.09	1280	8.99	0.1	1300	23.3	0.09	1320	0.26	0.09	-	-
r3	-	-	-	1188	11.3	0.14	1200	16.5	0.13	1220	5.39	0.12	1200	19.4	0.13	1200	8.43	0.13	1200	9.39
r4	1163	9.6	0.15	-	-	-	-	-	-	1175	8.55	0.14	1150	18.9	0.16	1125	1.64	0.17	1100	3.79
r5	1071	11.2	0.2	1100	8.03	0.19	-	-	-	-	-	-	-	-	1071	4.3	0.2	-	-	
r6	-	-	-	-	-	-	1043	13.3	0.22	1000	1.67	0.24	-	-	-	1029	4.4	0.22	1000	6.11
r7	991	8.97	0.25	991	9.7	0.25	982	11.8	0.25	964	4.75	0.27	-	-	-	982	4.51	0.25	-	-
r8	-	-	-	927	11.8	0.3	936	9.9	0.29	936	5.02	0.29	927	38.4	0.3	936	9.57	0.29	900	6.31
r9	883	17.5	0.33	-	-	-	900	19.8	0.32	900	12.9	0.32	-	-	-	883	10.6	0.33	-	-
r10	800	8.8	0.36	767	13.7	0.37	-	-	-	733	11.4	0.38	-	-	-	-	-	-	800	15.1
r11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	700	12.5	0.39	-	-
r12	600	10.4	0.41	560	12.5	0.43	-	-	-	560	14.6	0.43	-	-	-	560	10.5	0.43	700	5.3
r13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	600	7.11
r14	450	9.9	0.47	450	11.9	0.47	-	-	-	-	-	-	-	-	-	-	-	-	500	8.57
r15	-	-	-	-	-	-	-	-	-	383	10.5	0.5	-	-	-	383	8.48	0.5	400	7.93
r16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	256	7.14	0.57	-	-
r17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	300	10
r18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	200	9
r19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	100	7.74

Continued table (5):..

Lanes	Primer 7 : C15									Marker		
	Control			Jojoba oil			Jatropha oil					
Rows	M.w	amount	Rf	M.w	amount	Rf	M.w	amount	Rf	M.w	Amount	
r1	-	-	-	-	-	-	-	-	-	-	1300	3.87
r2	1300	9.83	0.09	1280	11	0.1	-	-	-	-	-	-
r3	1188	10	0.14	-	-	-	-	-	-	-	1200	9.39
r4	-	-	-	1175	10.9	0.14	-	-	-	-	1100	3.79
r5	1113	9.59	0.18	1113	8.8	0.18	-	-	-	-	-	-
r6	1029	6.15	0.22	1043	8.52	0.22	-	-	-	-	1000	6.11
r7	991	7.93	0.25	964	10.7	0.27	-	-	-	-	-	-
r8	936	8.21	0.29	909	10.8	0.31	-	-	-	-	900	6.31
r9	883	10.3	0.33	-	-	-	850	25.6	0.34	-	-	-
r10	817	4.84	0.36	800	10.8	0.36	-	-	-	-	800	15.1
r11	700	8.9	0.39	-	-	-	-	-	-	-	-	-
r12	-	-	-	560	11.5	0.43	580	17.1	0.42	700	5.3	
r13	520	13.9	0.44	-	-	-	520	17.4	0.44	600	7.11	
r14	433	4.75	0.48	467	5.31	0.46	450	4.26	0.47	500	8.57	
r15	383	5.49	0.5	383	12.1	0.5	383	19.2	0.5	400	7.93	
r16	-	-	-	-	-	-	-	-	-	-	-	-
r17	-	-	-	-	-	-	-	-	-	-	300	10
r18	-	-	-	-	-	-	-	-	-	-	200	9
r19	-	-	-	-	-	-	-	-	-	-	100	7.74

Table (6): DNA diversity among *S. littoralis* treated with Jojoba oil and Jatropha oil using RAPD-PCR

Primers	Polymorphism				Genetic markers (bp)*		
	TAF	MAF	PAF	Unique	Control	Treated with jojoba oil	Treated with Jatropha oil
O4	10	5	3	2	450	956	-
O7	7	-	5	2	-	575 - 417	-
O5	5	-	1	4	956 - 717	-	1050 - 450
O14	12	9	2	1	1025	-	-
C10	12	3	7	2	1163	-	1043
C13	14	4	5	5	1380 - 733	-	1071-700 - 256
C15	14	2	9	3	1188 - 700	1175	-
Total	75	23	32	19	9	4	6

bp-----size of genetic marker (unique).  
 TAF-----total amplified fragments.  
 MAF-----monomorphic amplified fragments (common).  
 PAF-----polymorphic amplified fragments.

**REFERENCES**

Abbassy, M. A.; Abdelgaleil, S. A.; Belal, A. S. H.; and Rasoul, M. A. A. (2007).

- Insecticidal, antifeedant and antifungal activities of two glucosides isolated from the seeds of *Simmondsia chinensis*. *Industrial Crops and Products*, 26(3): 345-350.
- Abbott, W.S. (1925). A method for computing the effectiveness of an insecticide. *Journal of economic entomology*, 18: 265- 277.
- Abd EL-Aziz, H. S. (2006). Toxicology and genetics of resistance to some bacterial formulations in the cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). Ph. D. Thesis, Fac. Science., Ain Shams Univ.
- Abd El-Zaher, T. R. (2017). Biological activity of four plant oils in the form of nano products on the larvae of cotton leaf worm. *Sciences*, 7(02): 239-249.
- Abdel-Baky, F. N. and Al-Soqeer, A. A. (2017). Controlling the 2nd instars larvae of *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) by simmondsin extracted from Jojoba seeds in KSA. *J. Entomol*, 14: 73-80.
- Abdel-Ghany, S. E. (2011). Assessment of environmental hazardous and molecular characterization of controlling of *Spodoptera littoralis* (Boisd.) by some plant extracts. M. Sc. Thesis, Institute of Environmental Studies and Research, Ain Shams University.
- Abdel-Wahed, M. S.; El-Bermawy, S. M.; El-DougDoug, K. A.; Mabrouk, A. M.; and Abdel-Aziz, M. M. (2013). Evaluation of the efficacy of one bioagent and an insect growth regulator against the cotton leafworm, *Spodoptera littoralis* (Boisd.), using molecular characterization technique. *Egypt, J. Agric. Res*, 91(2): 525-538.
- Ahirrao, R. A.; Patel, M. R.; Pokal, D. M.; Patil, J. K.; and Suryawanshi, H. P. (2011). Phytochemical screening of leaves of *Jatropha curcas* plant. *Int. J. Res. Ayurveda Pharmacy (IJRAP)*, 2(4): 1324-1327.
- Calvo, I. L. (2012). *Lippia graveolens*. In: *Plantas Aromáticas de Yucatán*. Mérida, Yucatán, México: Centro de Investigación Científica de Yucatán, pp. 60-65.
- Chauhan, N.; Kumar, P.; Mishra, S.; Verma, S.; Malik, A.; and Sharma, S. (2015). Insecticidal activity of *Jatropha curcas* extracts against housefly, *Musca domestica*. *Environ. Sci. Pollut. Res.*, 22(19): 14793-14800.
- Ebadah, I. M.; Shalaby, S. E.; and Moawad, S. S. (2016). Impact of certain natural plant oils and chemical insecticides against tomato insect pests. *J. Entomol.*, 13(3): 84-90.
- El-Defrawi, M. E.; Topozada, A.; Mansour, N.; and Zeid, M. (1964). Toxicological studies on Egyptian cotton leaf worm *Prodenia litura* (F.). I: Suceptibility of different larval instars to insecticides. *J. Econ. Entomol.* 57: 591-593.
- Eldesouky, S. E.; Khamis, W. M.; and Hassan, S. M. (2019). Joint action of certain fatty acids with selected insecticides against cotton leafworm, *Spodoptera littoralis* and their effects on biological aspects. *J. Bas. Env. Sci.*, 6: 23-32.
- El-Gohary, E. A.; Salama, M. S.; Abu Zeid, A. S.; and Kaschef, A. H. (2000). Molecular Approach for cast differentiation of the termite *Kaloterms flavicollis* (Fab.) *J. Egypt. Ger. Soc. Zool.*, 33(E): Entomol. 357-377.
- El-Sabagh, M. M. A. (2015) Evaluation of the efficacy of two bioagents against the cotton leaf worm, *Spodoptera littoralis* (Boisd.), using molecular characterization techniques. *Egypt. J. Agric. Res.*, 93(4): 1127- 1137.
- El-Seedi, H. R.; Azeem, M.; Khalil, N. S.; Sakr, H. H.; Khalifa, S. A.; Awang, K.; Saeed, A.; Farag, M. A.; Alajami, M. F.; Pålsson, K.; and Borg-Karlson, A. K. (2017). Essential oils of aromatic Egyptian plants repel nymphs of the tick *Ixodes ricinus* (Acari: Ixodidae). *Exp. App. Acarol.*, 73(1): 139-157.
- Finney, D. J. (1971). Probit Analysis, A statistical treatment of the sigmoid response curve 7<sup>th</sup> Ed., Cambridge Univ. Press, Cambridge, England.
- Gaaboub, I.; Halawa, S.; and Rabiha, A. (2012). Toxicity and biological effects of some insecticides, IGRs and Jojoba oil on cotton leafworm *Spodoptera littoralis*

- (Boisd.). J. App. Sci. Res., (October): 5161-5168.
- Halawa, S. M.; Kamel, A. M.; and Abd El-Hamid, S. R. (2007). Chemical constituents of jojoba oil and insecticidal activity against *Schistocerca gregaria* and biochemical effect on albino rats. J. Egypt. Soc. Toxicol, 36: 77-87.
- Hurlbert, R. E. (1999) Genetic engineering. Microbiology internet text. Science Hall Room 440 CA.
- Ingle, K. P.; Deshmukh, A. G.; Padole, D. A.; Dudhare, M. S.; Moharil, M. P.; and Khelurkar, V. C. (2017). Bioefficacy of crude extracts from *Jatropha curcas* against *Spodoptera litura*. J. Entomol. Zool. Stud., 5(1): 36-38.
- Ismail, S. M. and Shaker, N. (2014). Efficacy of some essential oil against the immature stages of *Spodoptera littoralis*. Alex. J. Agri. Res., 59(2): 97-103.
- Juliet, S.; Ravindran, R.; Ramankutty, S. A.; Gopalan, A. K. K.; Nair, S. N.; Kavillimakkil, A. K.; Bandyopadhyay, A.; Rawat, A. K. S.; and Ghosh, S. (2012). *Jatropha curcas* (Linn) leaf extract—a possible alternative for population control of *Rhipicephalus (Boophilus) annulatus*. Asian Pacific Journal of Tropical Disease, 2(3): 225-229.
- Khattak, A.; Ullah, F.; Wazir, S. M.; and Shinwari, Z. K. (2015). Allelopathic potential of *Jatropha curcas* L. leaf aqueous extracts on seedling growth of wheat. Pak. J. Bot., 47(6): 2449-2454.
- Korrat, E. E. E.; Abdelmonem, A. E.; Helalia, A. A. R.; and Khalifa, H. M. S. (2012). Toxicological study of some conventional and nonconventional insecticides and their mixtures against cotton leaf worm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noectudae). Ann. Agri. Sci., 57(2): 145-152.
- Kovendan, K.; Murugan, K.; Vincent, S.; and Kamalakannan, S. (2011). Larvicidal efficacy of *Jatropha curcas* and bacterial insecticide, *Bacillus thuringiensis*, against lymphatic filarial vector, *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol. Res., 109(5): 1251-1257.
- Ma, H.; Lang, N.; He, L.; Yu, Z.; Zheng, K.; Peng, M.; ZhenYong, X.; JiJun, K.; and Yuan, R. (2011). Volatile components from plant parts of *Jatropha curcas* in Jianshui, Yunnan Province. J. Zhejiang A&F Uni., 28(4): 674-679.
- Marie, S. S.; Amr, E. M.; and Salem, N. Y. (2009). Effect of some plant oils on biological, physiological and biochemical aspects of *Spodoptera littoralis* (Boisd.). Res. J. Agri. Biol. Sci., 5(1): 103-107.
- Metayi, M. H.; Ibrahim, M. A.; and El-Deeb, D. A. (2015). Toxicity and Some Biological Effects of Emamectin Benzoate, Novaluron and Diflubenzuron against Cotton Leafworm. Alex. Sci. Exch. J, 36(4): 350-357.
- Moawad, S. S., El Behery, H. H., & Ebadah, I. M. (2015). Effect of volatile oils on some biological aspects of *Galleria mellonella* L. and its parasitoid species, *Bracon hebetor* Say. (Hymenoptera: Braconidae). Egypt. J. Biol. Pest Control, 25(3): 603-607.
- Nollet, L. M. and Rathore, H. S. (2017). Green pesticides handbook: Essential oils for pest control. CRC Press.
- Oskoueian, E.; Abdullah, N.; Ahmad, S.; Saad, W. Z.; Omar, A. R.; and Ho, Y. W. (2011). Bioactive compounds and biological activities of *Jatropha curcas* L. kernel meal extract. Int. J. Mol. Sci., 12(9): 5955-5970.
- Pabón, L. C. and Hernández-Rodríguez, P. (2012). Importancia química de *Jatropha curcas* y sus aplicaciones biológicas, farmacológicas e industriales. Revista Cubana de Plantas Medicinales, 17(2): 194-209.
- Rajeswari, G.; Murugan, M.; and Mohan, V. R. (2012). GC-MS analysis of bioactive components of *Hugonia mystax* L. (Linaceae). Res. J. Pharma. Biol. Chem. Sci., 3(4): 301-308.

- Rao, N. V.; Maheswari, T. U.; and Manjula, K. (2005). Review on botanical pesticides as tools of pest management. Narosa Publishing House Pvt., Ltd. Pp: 1-16.
- Reichel, T.; Barazetti, J. F.; Stefanello, S.; Paulert, R.; and Zonetti, P. D. C. (2013). Allelopathy of leaf extracts of jatropha (*Jatropha curcas* L.) in the initial development of wheat (*Triticum aestivum* L.). IDESIA (Chile), 31(1): 45-52.
- Rofail, M. F.; Nada, M. A.; El-Sisi, A. G.; and Rashad, A. M. (2000). Time of spraying some natural oils as a limiting factor for controlling cotton bollworm, *Pectinophora gossypiella* (Saunders). Egypt. J. Agri. Res., 78(4): 1499-1507.
- Salem, H. E. M., Omar, R. E. M., El-Sisi, A. G., & Mokhtar, A. M. (2003). Field and laboratory use of environmentally safe chemicals against whitefly *Bemisia tabaci* (Gennandius) and leafhopper *Empoasca discipiens* (Paoli): Annals Agric. Sci., Moshtohor, 41 (4): 1737-1741.
- Sambrook, J. M.; E. F. Fritsch and T. U. Maniatis (1989) Molecular Cloning: a laboratory manual. 2nd ed. N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, 1659 p. ISBN 0-87969-309-6.
- Soto-Armenta, L.; Sacramento-Rivero, J.; Gonzalez-Coloma, A.; Acereto-Escoffie, P.; Aguilera-Cauich, E. R. I. C. K.; Martinez-Sebastian, G.; and Rocha-Urbe, J. A. (2019). Extraction and bioactivity from *Jatropha curcas* L. leaves by steam distillation. Pak. J. Bot., 51(2): 567-572.
- Tabashnik, B. E.; Cushing, N. L.; Finson, N.; and Johnson, M. W. (1990). Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econom. Entomol. 83(5): 1671-1676.
- Tomass, Z.; Hadis, M.; Taye, A.; Mekonnen, Y.; and Petros, B. (2011). Larvicidal effects of *Jatropha curcas* L. against *Anopheles arabiensis* (Diptera: Culicidae). MEJS, 3(1): 52-64.
- Yacoub, S. S. (2006). Efficacy of some plant extracts in controlling *Sesamia cretica* Led. and *Ostrinia nubilalis* (Hubn.) in miaze fields. Ph.D. Thesis, Fac. Agric., Benha Univ., pp 289.

#### ARABIC SUMMARY

مقارنة بين اثنين من الزيوت النباتية الصديقة للبيئة من خلال دراسة التأثيرات السمية والبيولوجية والجزيئية على دودة ورق القطن (*Spodoptera littoralis* (Boisd.))

مروة محمد محمود عبد العزيز الصباغ، سارة محمد ابراهيم عبد الكريم، الشيماء نجيب ابراهيم و نشوي سعيد امين

معهد بحوث وقاية النباتات-مركز البحوث الزراعي-دقي-جيزة-مصر.

تمت دراسه الاثار السمية والبيولوجية والجزيئية لاثنتين من الزيوت النباتية الصديقة للبيئة الجوجوبا والجنروفا حيث عوملت يرقات العمر الثاني لدوده ورق القطن بالتركيز النصف مميت لكلا من الزيوت النباتية المختبرة الجوجوبا والجنروفا 1.905 و 1.793% علي التوالي. وادت المعامله بصفة عامة الي خفض في متوسط العمر اليرقي والعذري وانخفاض في نسبه التعدير ونسبه خروج الفراشات مقارنة باليرقات غير المعاملة، كما أدت أيضا إلى خفض متوسط عمر الطور البالغ مقارنة بالكنترول. كما أدت المعاملة أيضا إلى خفض الكفاءة التناسلية للفراشات الناتجة من اليرقات المعاملة بالزيوت النباتية محل الدراسة حيث ظهر ذلك في انخفاض متوسط عدد البيض ومتوسط الفقس الناتج مقارنة بالكنترول. كما تم استخدام سبع بادئات عشوائية (O4، O5، O7، O14، O10، C13 و C15) لإنتاج نموذج حزمي مميز كأداة لدراسة التباينات الجزيئية بين الزيوت المعاملة. وتم حصر وعد الحزم المميزة والحزم المشتركة التي تم انتاجها بواسطة استخدام هذه البادئات السبعة، وقد وجد أن البادئ C13 هو أقوى البادئات في انتاج حزم مميزة معبرة، حيث أعطى 5 حزم مميزة متفردة بينما كان البادئ O14 أقل البادئات المستخدمة قدرة على انتاج حزم معبرة.