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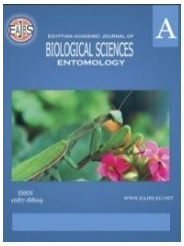

Insecticidal and Biochemical Effects of Jatropha Oil on Cotton Leaf Worm, *Spodoptera littoralis*, Larvae and F1 Larvae Produced from Irradiated Parent Males

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ABSTRACT

When fourth instar larvae of *Spodoptera littoralis* or when F_1 fourth instar larvae (resulted from irradiated parent male pupae) of *Spodoptera littoralis* treated with four concentrations of jatropha oil (2.5, 5, 7.5 and 10%) the %mortality increased with the increase of both concentration and the time of exposure to the oil. effect of Lc_{50} of jatropha oil on acetylcholinesterase activity and lactate dehydrogenase of normal and F1 larvae (resulted from irradiated parent male pupae), acetylcholinesterase activity decreased non-significantly in *S. littoralis* larvae treated with jatropha oil compared to normal larvae the same results obtained in F1 *S. littoralis* larvae where acetylcholinesterase activity decreased in F1 *irradiated S. littoralis* larvae treated with jatropha oil compared to untreated F1irradiated larvae. while lactate dehydrogenase activity increased significantly in *S. littoralis* larvae treated with jatropha oil compared with normal larvae. The same results were obtained in F1 *S. littoralis* larvae where lactate dehydrogenase activity increased in F1 irradiated *S. littoralis* larvae and treated with jatropha oil compared to untreated F1 irradiated larvae. Consequently, it can be concluded that a combination of jatropha oil and gamma rays could serve as an eco-friendly control program for cotton leafworms.

INTRODUCTION

Jatropha curcas L. is a shrub of the Euphorbiaceae family, which originated in Central America. This succulent plant is highly resistant to drought. The Jatropha genus is widespread in tropical countries (Heller, 1996). In several West and Central African countries, *J. curcas* is used as a means of delimiting fields, in order to protect cereal crops against the wind and grazing by animals (Henning, 2008). *J. curcas* seeds are rich in oil used as a biofuel, which makes this plant an important subject for research into renewable energies. In addition to its use as a biofuel, Jatropha oil can also be used as a bio-pesticide (Solsoloy and Solsoloy, 1997). In fact, several authors have tested the use of oil emulsions against insects that attack stored maize grains, *Sitophilus zeamais*, and mung beans, *Callosobruchus chinensis*, at concentrations of 0.5, 1, 2.5 and 10%. After being stored for 2 months, damage to the grains was reduced to 10% when doses of 10% and 5% were applied to *S. zeamais* and *C. chinensis*, respectively. In 2000, the same authors also tested the effect of Jatropha oil on various aggressive bio-agents affecting cotton plants (*Amrarsca biguttula*, *Aphid gossypii* and *Helicoverpa armigera*). Doses of 800 ml and 250 ml/ha were compared against commonly used insecticides (profenofos at 400 g/ha and

deltamethrine at 12.5 g/ha). Jatropha oil showed itself to be more effective than deltamethrine on *A. gossypii*, while the opposite effect could be observed on *A. biguttula*. For *H. armigera*, synthetic insecticides were more effective than Jatropha oil at the start of treatment, as the oil affects only insect growth and its effect is, therefore, slower (Solsoloy and Solsoloy, 2000)

The extracts of jatropha showed nematicidal, fungicidal (Sharma and Trivedi, 2002), antifeedant (Meshram *et al*., 1996), molluscicidal (Liu *et al*., 1997) and insecticidal activities against moths, butterflies, aphids, bugs, beetles, flies, and cockroaches (Wink *et al*., 1997). The toxicity of *J. curcas* seeds is attributed to several components, including saponins, lectins (curcin), phytates, protease inhibitors, curcalonic acid and phorbol (Makkar *et al*., 1997).

Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) is a dangerous pest of many field crops and vegetables in the world (Ghoneim *et al.*, 2020) Although Egyptian cotton leafworm Spodoptera littoralis (Boisd.) (Lepidoptera: Noctuidae) is a native pest to Africa (Shonouda and Osman, 2000; El-Khawas and Abd El-Gawad, 2002), it is distributed in many European countries (Pineda et al., 2007; Lanzoni *et al*., 2012; EPPO, 2019), and the Middle East countries (El-Aswad, 2007; El-Sabrout, 2013; Azzouz *et al*., 2014). Economically, S. littoralis is a dangerous pest of many field crops and vegetables in North Africa, and Middle East countries including Egypt (Kandil *et al*., 2003) In Egypt, cotton cultivation is one of the main resources for the economy. *S. littoralis* represents a key pest of this crop (Raslan, 2002; Ibrahim and Ali, 2018). In addition, it is considered the most destructive pest of more than 60 other crops, ornamentals and vegetables of economic importance (Sannino, 2003; Dahi, 2005; Amin, 2007; Lanzoni *et al*., 2012; Abd El-Razik and Mostafa, 2013).Different control measures have been applied for controlling *S. littoralis*, such as the hand-picking of egg patches by children (Abd-El-Aziz and Sayed, 2014). Some physical control measures have been applied to control this pest, such as gamma irradiation (Rizk *et al*., 2019). However, most farmers prefer using chemically synthetic pesticides for obtaining fast results (Ghoneim *et al*., 2012; Fetoh *et al*., 2015).

The discriminatory uses of many synthetic insecticides lead to the destruction of natural enemies (like parasites, and predators), allowing an increase in pest populations (Naqqash *et al*., 2016) and serious toxicological hazards to humans (Costa *et al*., 2008). Over the past 50 years, the intensive and continuous use of broad-spectrum insecticides against S. littoralis had led to the development of its resistance against many registered insecticides and some insect growth regulators Mosallanejad and Smagghe, 2009; Rizk *et al*., 2010). Soit is important to search for new effective and safer ways with negligible effects on the ecosystem (Dubey *et al*., 2010). In Egypt, numerous attempts have been done to assess the insecticidal activities of different plant products against *S. littoralis* (Abdel-Eltawab, 2016; Sammour *et al*., 2018).

Acetylcholine (Ach) is one of the major molecules by which nerve impulses are transmitted from a nerve cell or involuntary muscle (Lopez and Pascual-Villalobos, 2010). Acetylcholinesterase AChE is an enzyme that breaks down the neurotransmitter acetylcholine at the synaptic cleft (the space between two nerve cells) so the next nerve impulse can be transmitted across the synaptic gap (Fields and Burnstock, 2006) The phosphine, organophosphates, and carbamates act by interfering with the passage of impulses in the insect nervous system (Dua and Gill, 2001) Organophosphate insecticides are generally regarded as irreversible inhibitors of the enzyme acetylcholinesterase. The inability of phosphorylated AChE to hydrolyse acetylcholine, the build-up of concentration of the acetylcholine in the synapse and excessive neuron excitation are the results of prolonged binding of ACh to its postsynaptic receptor. The signs of intoxication include restlessness, hyperexcitability, tremors, convulsions and paralysis leading to death (Fukuto,

1990; Lionetto *et al.*, 2015).

Dehydrogenases are very important tools for the investigation of insect metabolic activities during the course of development. The relative activities of the insect dehydrogenases may be related to the function and energy-yielding demands of the tissues (Dickinson and Sullivan, 1975). Lactate dehydrogenase (LDH) is an important glycolytic enzyme that is present in virtually all tissues (Kaplan and Pesce, 1996). It is involved in carbohydrate metabolism and has been used as an indicative criterion of exposure to chemical stress (Diamantino *et al*., 2001). LDH is, also, a parameter widely used in toxicology and in clinical chemistry to diagnose cell, tissue, and organ damage. However, the potential of this enzyme as an indicative criterion in invertebrate toxicity tests has been scarcely explored (Ribeiro *et al*., 1999; Senthil *et al*., 2006 a & b).

The main objective of the present study was to evaluate the insecticidal efficacy of Jatropha oil against cotton leaf worm *Spodoptera littoralis*, larvae and F1 larvae produced from irradiated parent males and its effect on the activity of acetylcholinesterase and lactate dehydrogenase enzymes.

MATERIALS AND METHODS

Rearing Technique of Insect Culture:

The laboratory strain of the cotton leaf worm *Spodoptera littoralis* (Boisd.) was obtained from the Cotton Leafworm Department, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt. The pest was reared under laboratory conditions without contamination with insecticides for more than ten generations at 27+-2^{o}C and 65-75% R.H. and fed daily on fresh castor bean leaves, *Ricinus communis* (L.) according to the method described by El- Defrawi (1964).

Irradiation Process:

Full-grown male pupae of *S. littoralis* were irradiated with the sub-sterilizing dose 100Gy using Cobalt- 60 gamma cell, located at the National Centre for Radiation Research and Technology, Cairo (NCRRT) with a dose rate of 0.766KGy/h. The 4th larval instar of F1 generation was used if the experiment.

Jatropha Oil:

Jatropha seed oil, *Jatropha curcas* L. (Euphorbiaceae) was 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.

Bioassay:

The leaf-dipping technique, similar to that described by Tabashnik *et al*. (1990), was used to determine the toxicity of different concentrations of jatropha oil (2.5, 5, 7.5 and 10% (v/v) in 100 ml of distilled water with 0.002% of Tween 80) against the 4th instar larvae. Castor leaves were dipped for 1min. in each concentration, and then the treated leaves were left for natural air drying. Four replicates each with 6 larvae was allowed to feed on treated leaves for 24 h. water-tween treated leaves were used as the control. Fresh leaves were added to the replicates daily. 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®" software.

Biochemical Analysis:

To compare the effect of jatropha oil on the biochemical aspects, normal and F1 larvae (resulting from irradiated parent male pupae) were treated with Lc_{50} after 24 h. then homogenized in dista. Water. 6 replicates were conducted for each treatment.

Acetylcholinesterase Activity Determination:

AchE (acetylcholinesterase) activity was measured according to the method

described by Simpson *et al*. (1964), using acetylcholine bromide (AchBr) as a substrate. The reaction mixture contained 200µl enzyme solution, 0.5 ml 0.067 M phosphate buffer (pH7) and 0.5 ml AchBr (3 mM). The test tubes were incubated at 37 ºC for exactly 30 min. 1 ml of alkaline hydroxylamine (equal volume of 2 M hydroxylamine chloride and 3.5 M NaOH) was added to the test tubes. Then 0.5 ml of Hcl (1 part of conc. Hcl and 2 parts of ΔH_2O) was added. The mixture shaken vigorously and allowed to stand for 2 min. 0.5 ml of ferric chloride solution (0.9 M $Fecl_3$ in 0.1M Hcl) was added and mixed well. The decrease in AchBr resulting from hydrolysis by AchE was read at 515 nm.

Lactate Dehydrogenase Estimation:

The method described here is derived from the formulation recommended by the German Society for clinical chemistry (DGKC, 1972). Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate, NADH is oxidized to NAD in the process. The rate of decrease in NADH is directly proportional to the LDH activity and is determined photometrically. The reaction mixture consisted of phosphate buffer; 68mmol/L, pH 7.5, pyruvate 0.73mmol/L, and 1.1 mmol/L NADH. A hundred microliters sample were mixed with 2.5 ml of the reaction mixture that was preincubated at 37˚C. Then they were poured into spectrophotometer cuvette, and the initial absorbance was read. The timer was started simultaneously, and the absorbance was read again after 1,2 and 3 min. Zero adjustments were against air. LDH activity was calculated according to the following equation:

$$\textbf{LDH activity} = \textbf{Factor} \times \Delta \textbf{A} \quad \textbf{340 nm/min}$$

Where:

Factor = 4468 (as recommended by the used kit; Randox, United Kingdom).

Δ A = the change in absorbance/min.

Statistical Analysis*:*

The Minitab program was used to statistically analyze the data by Tukey Pairwise Comparisons test to examine the significant differences between the treatments and to statistically compare the LC values.

RESULTS

Fourth instar larvae of *Spodoptera littoralis* were treated with four concentrations of jatropha oil (2.5, 5, 7.5 and 10%) and mortality was recorded daily. From Table (1) it was noticed that %mortality increased with the increase of concentration land the percent accumulative mortality was 50, 70.84, 87.49, 91.67 and 120.39 compared to 0 in the control. Statistical analysis showed a significant difference between the control and all doses in accumulated mortality and between different concentrations except between the concentrations 7.5 and 10%.

Table 1: Daily and Accumulative mortality of normal *Spodoptera littoralis* 4th instar larvae

Time (Hrs.) / Conc. (%)	24	48	72	96	Accumulative
Control	0 ± 0^{C}	0 ± 0^{B}	0 ± 0^{A}	0 ± 0^{B}	0 ± 0^{D}
2.5	16.67 ± 6.8^{BC}	12.5 ± 4.1^{AB}	8.33 ± 4.8^{A}	12.50 ± 4.1^{AB}	50 ± 0^{C}
5	25 ± 4.8^{AB}	12.5 ± 4.1^{AB}	12.5 ± 4.1^{A}	20.83 ± 4.1^{A}	70.84 ± 4.1^{B}
7.5	29.16 ± 4.1^{AB}	16.67 ± 6.8^{AB}	16.65 ± 9.6^{A}	25 ± 4.8^{A}	87.49 ± 4.1^{A}
10	45.83 ± 15.9^{A}	20.83 ± 4.1^{A}	12.5 ± 4.1^{A}	12.5 ± 4.1^{AB}	91.67 ± 4.8^{A}
F-value	9.40	3.09	1.33	6.12	120.39
P-value	0.001	0.048	0.305	0.004	0.000

- Values represent the mean ± S.E of 4 replicates.
- Means in the same column that do not share a letter are significantly different (Tukey Pairwise Comparisons).

F1 fourth instar larvae of Spodoptera littoralis were also treated with the four concentrations of jatropha oil (2.5, 5, 7.5 and 10%) and mortality was recorded after 24 and 48 hrs. From Table (2) it was noticed that %mortality of F1 fourth instar larvae increased with the increase of the concentration and the percent mortality on the second day were less than the first day in most of the concentration and the percent accumulative mortality were 37, 58.33, 70.83.and 100 compared to 0 in the control. Statistical analysis shows a significant difference between the control and all doses in accumulated mortality and between different concentrations except between the concentrations 7.5 and 10 %.

Table 2: Daily and Accumulative mortality of F1 4th instar larvae of *Spodoptera littoralis*

Time (Hrs.) / Conc. (%)	24	48	Accumulative
Control	0 ± 0^{C}	0 ± 0^{B}	0 ± 0^{D}
2.5	20.83 ± 4.1^{BC}	16.67 ± 0^{AB}	37.5 ± 4.1^{C}
5	25 ± 4.8^{B}	33.33 ± 6.8^{A}	58.33 ± 4.8^{B}
7.5	41.65 ± 8.3^{B}	29.16 ± 7.9^{A}	70.83 ± 7.9^{B}
10	70.84 ± 4.1^{A}	29.16 ± 4.1^{A}	100 ± 0^{A}
F-value	27.48	7.30	67.25
P-value	0.000	0.002	0.000

- Values represent the mean ± S.E of 4 replicates.
- Means in the same column that do not share a letter are significantly different (Tukey Pairwise Comparisons).

Table (3) showed LC_{50}/LC_{90} levels, Resistance ratio, slope values, and index of unirradiated and F1 4th larval instar larvae of *S. littoralis* treated after 2 days of feed on leaves treated with different concentrations of jatropha oil. The data in Fig. (1) exposed that F1 4th larval instar larvae were more sensitive than unirradiated larvae and that LC_{50} of jatropha oil for F1 larvae was significantly reduced that that of unirradiated larvae (table 3). Moreover, the toxicity index in unirradiated larvae was lower than in F1 larvae. In contrast, the resistance ratio was higher in unirradiated larvae than in F1 larvae.

Table 3: LC_{50}/LC_{90} levels, Resistance ratio, slope values, and index of unirradiated and F1 4th larval instar larvae of *S. littoralis* treated after 2 days feed on leaves treated with different concentrations of jatropha oil;

Treatment	LC_{50}	P-value	LC_{90}	Slope	Resistance ratio	Toxicity Index
Unirradiated larvae	6.88	0.037	51.01	1.47±0.29	1.88	53.17
F1 larvae	3.65*		10.27	2.85±0.31	1.00	100

Toxicity index and resistance ratio compared with accumulative mortality on F1 larvae.
* is significantly different (2 sample t-test Comparisons).

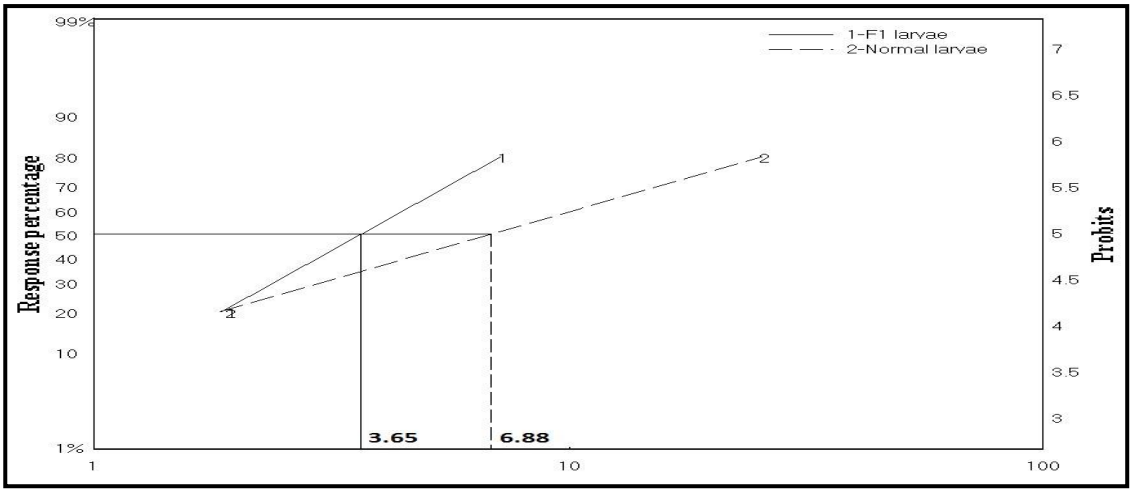

Fig. 1: LC_{50} of jatropha oil after 2 days**.**

Table (4) showed the effect of Lc_{50} of jatropha oil on the acetylcholinesterase activity and lactate dehydrogenase of normal and F1 larvae (resulting from irradiated parent male pupae) *S. littoralis* treated with Lc_{50} of jatropha oil. Results of table (4) revealed that acetylcholinesterase activity was non-significantly decreased to 2.40 in larvae treated with jatropha oil, while it significantly declines in F1 larvae and F1 larvae treated with LC_{50} to 2.06 and 1.04, respectively as compared to the control (unirradiated) 2.48 (ug AchBr/min/mg protein). However, lactate dehydrogenase showed a significant increase in all treated larvae, that being 39.68, 36.41 and 44.65 (mU/mg protein) in unirradiated larvae treated LC_{50}, F1 larvae and F1 larvae treated with LC_{50} to be 2.06 and 1.04, respectively as compared to 30.93 in control (unirradiated) larvae.

Table 4: Effect of Lc_{50} of jatropha oil on the acetylcholinesterase activity and lactate dehydrogenase of normal (unirradiated) and F1 *S. littoralis* larvae.

Treatment	Acetylcholinesterase activity (ug AchBr/min/mg protein)	Lactate dehydrogenase (mU/mg protein)
Normal *S. littoralis* larvae (unirradiated, control)	2.48 ± 0.02^{A}	30.93 ± 0.73^{D}
***S. littoralis* larvae treated with Lc_{50} jatropha oil**	2.40 ± 0.12^{A}	39.68 ± 0.21^{B}
F1 *S. littoralis* larvae	2.06 ± 0.04^{B}	36.41 ± 0.27^{C}
F1 *S. littoralis* larvae treated with Lc_{50}jatropha oil	1.04 ± 0.04^{C}	44.65 ± 0.18^{A}

- Values represent the mean ± S.E of 6 replicates.
- Means in the same column that do not share a letter are significantly different (Tukey Pairwise Comparisons).

DISCUSSION

Our results show that Fourth instar larvae of *Spodoptera littoralis* were treated with four concentrations of jatropha oil (2.5, 5, 7.5 and 10%) and mortality was recorded daily from the table it was noticed that %mortality increased with the increase of the concentration and also %mortality of F_1 fourth instar larvae increased with the increase of the concentration and the percent mortality in the second day were less than the first day in most of the concentration and the percent accumulative mortality were 37.5, 8.33, 70.83 and 100 compared to 0 in the control These results are comparable to those obtained by Solsoloy and Solsoloy (1997) who tested oil emulsions against insect pests attacking stored maize grain, *Sitophilus zeamais*, and bean weevils, *Callosobruchus chinensis*.

Also, Ratnadass *et al*. (1997) focusing on *Busseola fusca* and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae) on sorghum have shown that the raw oil extracted from *Jatropha curcas* seeds has a larvicidal effect, at concentrations of 0.01% and 1% on *B. fusca* and *S. calamistis*, respectively. Our data is also in agreement with those obtained by Amr *et al*. (1995) Also ElNaggar and Abdel-Fattah (1999) found that larval mortality of *S. littoralis* increased with increasing the concentration of Eucalyptus oil.

Many studies have shown that the toxicity of *J. curcas* oil is due to the presence of phorbol esters (Makkar *et al*., 1997). These natural organic compounds are taglines from the diterpene family. *J. curcas* oil has long been implicated in traditional medicine and is also used as an insect repellent. The oil significantly reduced the number of alive Sitophilus granaries. All the Jatropha oil concentrations tested totally were antifeeding, indicating that the oil exhibited mortality. Mortalities were probably due to suffocation and or lethal chemical poisoning of the oil. (Azzaz and Khalifa 2012; Jadhua and Jadhua 1984). The insecticidal effect of this seed oil could be due to the presence of several sterols and terpene alcohols which have been known to exhibit insecticidal properties (Heftmann, 1970).

Hwa-Jeong *et al*. (2015) also agrees with our results where a fumigant toxicity test, tarragon oil exhibited 100% and 90% fumigant toxicity against adult male German cockroaches at 5 and 2.5 mg/filter paper, respectively. Fumigant toxicities of *Artemisia arborescens* and santolina oils against adult male German cockroaches were 100% at 20 mg/filter paper but were reduced to 60% and 22.5% at 10 mg/filter paper, respectively. In addition, Hossain *et al*. (2014) showed that the effectiveness of γ-irradiation against *Sitophilus oryzae* was enhanced by a combination treatment with basil EO. Their study found that *S. oryzae* was 5.3 times more sensitive to irradiation with exposure to basil EO. Ahmadi *et al*. (2013) found that mortality increased by 3-6 times when γ-radiation was combined with *Rosemarinus officinalis* EO than when either of the treatments was applied alone.

Also, our results show that from LC_{50}/LC_{90} levels of unirradiated and F1 4^{th} larval instar larvae of *S. littoralis* it was found that F1 4^{th} larval instar larvae were more sensitive than unirradiated larvae. The LC_{50} values for larvae were also in agreement with Elsinary *et al*. (2008) who mentioned that there was a positive correlation between the concentration of Morus alba extract and their insecticidal effect against both first and fourth larval *S. littoralis* instar.

Our results showed that jatropha oil affects the acetylcholinesterase activity and lactate dehydrogenase of normal and F1 larvae. Hwa-Jeong *et al*. (2015) agree with our results that contact toxicity tests, tarragon and santolina oils showed potent insecticidal activity against adult male German cockroaches. Components of active oils were analyzed using gas chromatography, gas chromatography-mass spectrometry, or nuclear magnetic resonance spectrometer. Among the identified compounds from active essential oils, estragole demonstrated potent fumigant and contact toxicity against adult German cockroaches. β-Phellandrene exhibited inhibition of male and female German cockroach acetylcholinesterase activity with LC_{50} values of 0.30 and 0.28 mg/mL, respectively. Alterations in the lactate dehydrogenase activity of the desert locust *Schistocerca gregaria* by the wild plant *Fagonia bruguieri* (Zygophyllaceae). Also, Hamadah *et al.* (2010) found that all *F. bruguieri* extracts prohibited LDH activity along the nymph's instar, irrespective of the concentration level. The most dramatically reduced activity was expressed in (9166.4±119.0 U/L vs. 22839.5±289.1 U/L of control congeners).

CONCLUSION:

The abovementioned results discovered that jatropha oil induced a physiological disorder in *Spodoptera littoralis* larvae that lead to larval death. Moreover, that disturbance was more obvious in F1 larvae (resulting from irradiated male parent pupae) and the larval

mortality was faster. Consequently, we concluded that a combination of jatropha oil and gamma radiation may work as an integrated program to control *S. littoralis* larvae after extra field experiments.

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ARABIC SUMMARY

التأثير السمى والبيوكيميائى لزيت الجاتروفا على يرقات دودة ورق القطن الغير معاملة واليرقات الناتجة من أباء ذكور مشعة

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عند معاملة الطور اليرقى الرابع لدودة ورق القطن الغير مشعة أو الناتجة من تزاوج ذكور مشعة (F1) بالتركيزات المختلفة لزيت الجاتروفا (2.5 و5 و7.5 و10%) زادت نسبة الموت مع زيادة كل من التركيز وزمن التعرض للزيت. كما أدى معاملة الطور اليرقى الرابع لدودة ورق القطن الغير مشعة أو يرقات الجيل الأول (الناتج من تزاوج ذكور مشعة) بالتركيز الذى يقتل 50% من اليرقات لوحظ أن نشاط إنزيم الأستيل كولينستيريز يقل بينما زاد نشاط إنزيم لاكتات ديهيدروجينيز فى اليرقات المعاملة بالزيت وكذلك فى اليرقات الناتجة من اباء مشعة ومعاملة بالزيت عنها فى اليرقات المشعة والغير معاملة بالزيت. ومن ذلك يمكن إستنتاج إمكانية إستخدام زيت الجاتروفا بالإتحاد مع أشعة جاما لمكافحة دودة ورق القطن.