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Joint action of certain fatty acids with selected insecticides against cotton leafworm, *Spodoptera littoralis* and their effects on biological aspects

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Abstract

Laboratory and semi-field experiments were carried out to investigate the joint action of oleic, linoleic and stearic acids with each of emamectin benzoate, lufenuron and indoxacarb against the 4th instar larvae of cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae). The sub-lethal effects of the selected insecticides and fatty acids on some biological parameters of *S. littoralis* were also evaluated. Results showed that, toxicity of emamectin benzoate (LC_{50} = 0.001 mg L^{-1}) was 80 times more toxic than indoxacarb (LC_{50} = 0.08 mg L^{-1}), 1400 times more toxic than lufenuron (LC_{50} = 1.4 mg L^{-1}). Linoleic acid was the most toxic fatty acid with LC_{50} = 2435.8 mg L^{-1} compared to oleic acid (LC_{50} = 3654.6 mg L^{-1}) and stearic acid (LC_{50} = 4645.2 mg L^{-1}). Potentiating effect was obtained when linoleic acid at concentration equivalent to LC_{10} was mixed with emamectin benzoate or indoxacarb or lufenuron at concentration equivalent to LC_{50} after 48, 72 and 96 hrs of exposure with co-toxicity factors (CTFs) ranged between 27.78 and 58.33. Mixtures of oleic acid with each of tested insecticides resulted in additive effects. Whereas, mixtures of each tested insecticides with stearic acid led to antagonistic effects. All treatments with tested insecticides and fatty acids were significantly reduced pupal mean weight (mg/pupae), percentage of pupation, percentage of adult emergence, fecundity (no. eggs laid/female), fertility (% egg hatch) and adult longevity (days) compared in control treatment. Semi-field data revealed that mixture of indoxacarb (0.5 FR) with the LC_{10} of oleic or linoleic acids had the highest residual toxicity compared to mixtures of the other insecticides against the 4th instar larvae during the cotton seasons of 2017 and 2018. Each of oleic and linoleic acids extended the residual toxicities of the tested insecticides at 0.5 FRs compared to 0.5 FRs of each insecticide alone.

Keywords: cotton leafworm, fatty acids, insecticides, insecticidal activity, binary mixtures, biological parameters.

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1. Introduction

Cotton plant is considered as a unique host in the broad nature of the insect pests attack including cotton leafworm; *S. littoralis* which is one of the most severe polyphagous insect pests that cause serious damage for many of the important vegetables and crops [1]. The difficulty of combating *S. littoralis* is due to its high resistance to most traditional insecticides [2, 3].

In addition, the intense and frequent applications of insecticides adversely affect the environment, non-target organisms and human health. Therefore, prompted search for alternative approaches of pest control may minimize the use of these synthetic chemicals [4].

In this respect, one of the most accepted approaches to increase the residual toxicity of the conventional insecticides is to mix photo-protective substances, suitable additives or adjuvants with their formulations [5-8]. These may contribute to the enhancement of insecticide performance, raise insecticides persistence, limitation of frequent uses of insecticides spray and consequently reduce costs of the field applications.

Eventually, several researches were carried out on structure-activity relationship (SAR) in binary combinations with synthetic insecticides that willing to increase insecticides effectiveness, reduce their rate of application, minimize the environmental hazards, and consequently delay the resistance development to many of these insecticides. Fatty acids of plant origin could be one of the candidate compounds in this respect [9-13].

Meanwhile, the trend towards activating the use of environmental safer insecticides that varied in mode of action has become a new awakening attention and unabated challenge in controlling cotton insect pests. Among these insecticides, emamectin benzoate is a second-generation avermectin analog with exceptional activity against lepidopteran pests. Emamectin benzoate acts as a chloride channel activator, which decreases the excitability of neurons [14]. In addition, oxadiazine insecticide indoxacarb exhibits a new mode of action as it works as a sodium channel blocker resulting in paralysis and death of targeted pests. This insecticide has been reported to have a good field activity against a number of Lepidoptera as well as certain

Homoptera and Coleoptera insects with low mammalian toxicity [15, 16]. Moreover, insect growth regulators (IGRs) include juvenile hormone (JH) mimics and chitin synthesis inhibitors (CSIs). CSIs benzoylphenylureas insecticides such as lufenuron interfere with chitin biosynthesis in insects and thus prevent moulting or produce an imperfect cuticle [17]. Furthermore, it has low mammalian toxicity [18].

Therefore, laboratory and semi-field studies were conducted to investigate the joint action of oleic, linoleic and stearic acids with each of emamectin benzoate, lufenuron and indoxacarb against the 4th instar larvae of *S. littoralis*. In addition, the sublethal effects of the selected insecticides and fatty acids on some biological parameters of *S. littoralis* were evaluated. Such studies could formulate the outlook of a contemporary strategy towards synthetic insecticides represented in enhancing their performance and persistence as well as minimizing the environmental hazards in control program in the future.

2. Materials and methods

2.1. Experimental insect:

A susceptible strain of the *S. littoralis* has been reared for several generations away from any contamination in the Plant Protection Research Station, Alexandria, Egypt. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) under laboratory conditions (25 ± 2 °C, RH 65 ± 5%) according to the method of [19].

2.2. Tested insecticides and fatty acids:

Emamectin benzoate (Emperor® 0.5% EC) was produced by Shandong Jingbo Agrochemicals Co., Ltd., China, with field rate is 40 ml/100 liter water. Indoxacarb (Avaunt ® 15% EC) was produced by Dobon de Nemorez, America, with field rate is 25 ml/100 liter water. Lufenuron (Match ® 5% EC) was produced by Syngenta Agro, Switzerland, with field rate is 40 ml/100 liter water. Oleic acid (98%) was produced by Lobachemie Co. Linoleic and stearic acids (99%) were produced by Sigma Aldrich.

2.3. Bioassay technique and determination of sublethal concentrations:

Toxicity of emamectin benzoate, indoxacarb, lufenuron and tested fatty acids (oleic, linoleic and stearic acids) were evaluated using the leaf dip bioassay method [19]. Six serial concentrations of each insecticide or fatty acid were prepared in distilled water. Homogenous castor bean leaf pieces were immersed in each prepared concentration for 10 sec. and dried at room temperature. In the case of fatty acids, Triton X-100 (0.01 %) had been added to the solution as an emulsifier. Treated castor bean leaf pieces were introduced to ten of the 4th instar pre-starved larvae of *S. littoralis* (46.6 ± 0.4 mg/larvae). Untreated larvae were fed on castor bean leaf pieces immersed in solution containing distilled water and Triton X-100 (0.01 %). Each treatment was replicated four times. Mortality was recorded after 96 hrs and analyzed by probit analysis [20].

2.4. Joint toxic action test:

Toxicity of the binary combinations of emamectin benzoate or indoxacarb or lufenuron with oleic, linoleic and stearic acids against the 4th instar larvae of *S. littoralis* were investigated. Nine combinations (insecticide at concentration equivalent to LC_{50} with fatty acid at concentration equivalent to LC_{10}) were prepared. Homogenous castor bean leaf pieces were immersed in each mixture for 10 sec., and mortality percentages were recorded after 48, 72 and 96 hrs of treatment. Each insecticide alone and fatty acid alone at the same level of concentration used in combination was also tested. Three control groups were subjected to calculate the expected mortalities. The joint action of the different combinations was expressed at the co-toxicity factors (CTFs), estimated by the equation as follows [21]:

$$\text{Co}-\text{toxicity factor} = \left(\frac{\text{Observed\% mortality} - \text{Expected\% mortality}}{\text{Expected\% mortality}}\right) \text{x}100$$

Where:

Co-toxicity factors ≥ +20 meant potentiation; co-toxicity factors < - 20 meant antagonism; and co-toxicity factors between -20 and +20 meant additive effect.

Expected (%) mortality = Sum of % mortalities of each insecticide alone and fatty acid alone at the same level of concentration used in combination.

Observed (%) mortality = % mortality of a combination.

2.5. Sub-lethal effects of the selected insecticides and fatty acids on some biological parameters of *S. littoralis*:

The LC_{10} and LC_{25} equivalent concentrations of the selected insecticides and the LC_{10} equivalent concentration of the fatty acids were prepared. Castor bean leaves were immersed in each concentration and left to dry. One hundred newly 4th instar larvae for each replicate were placed in glass jar (1 liter), left to feed on treated castor bean leaves. Each treatment was replicated four times. After 24 hrs of exposure, survival larvae were transferred to jars with untreated leaves and observed until the pupation and adult emergence. Pupal weight (mg/pupae), pupal duration (days), percentage of pupation and percentage of adult emergence were evaluated. Two adult males and one adult female from each replicate were placed separately in clean jar to maximize the probability of successful mating. Adults feed on 10 % sugar solution, fresh green leaves of tafla, *Nerium oleander* (L.) were provided for egg laying. These eggs on tafla leaves were placed in plastic cups with fresh castor leaves for the newly hatched larvae. The cumulative number of eggs per female laid daily during the first four days after the onset of oviposition, and the percentage of eggs hatched from those collected in the first oviposition were used to evaluate fecundity and fertility. Adult longevity (days) was also evaluated.

2.6. Semi-field trials:

Two field experiments were conducted at cotton plant variety (Giza 86) during 2017 and 2018 seasons at Housh Essa, El-Behira Governorate. All treatments were assigned to plots (about 44 m^2) in a randomized complete block design. Insecticides treatments of emamectin benzoate, indoxacarb and lufenuron were used at the half-field (0.5 FR) and field recommended rates (FR) alone. In addition, the selected combinations of these insecticides at their 0.5 FR with each of the tested fatty acids (oleic and linoleic acids) at rates equivalent to their LC_{10} (R_{10}) values that possessed additive and potentiation effects on the 4th instar larvae of *S. littoralis* in the laboratory. Control treatment was sprayed by water only and each treatment was replicated four times. Foliar sprays on cultivated cotton plant were achieved by using Knapsack sprayer equipment (CP3) at the rate of 10 liter / treatment (4 micro plots) at 19 and 17 July during 2017 and 2018, respectively. Cotton leaves from treated and untreated (control) plots were collected from three levels of plants in perforated bags at 0, 4, 8, 12 and 16 days after foliar spray treatments and transferred to the laboratory. Four leaves of each sample were ready to introduce for ten 4th instar larvae of *S. littoralis* in plastic cups under controlled laboratory conditions (25 ± 2 °C, RH 65 ± 5%). Four replicates were used for each treatment. Mortalities were recorded after 96 hrs of exposure. The treatments were compared with each other using one-way ANOVA with LSD $_{0.05}$ [22].

3. Results

3.1. Toxicity of emamectin benzoate, indoxacarb, lufenuron and selected fatty acids against the 4th instar larvae of *S. littoralis*:

Data presented in (Table 1) demonstrated the LC_{10}, LC_{25} and LC_{50} values, their confidence limits and slope ± SE for the selected insecticides and fatty acids against the 4th instar larvae of *S. littoralis* after 96 hrs of exposure. Results showed that, toxicity of emamectin benzoate (LC_{50} = 0.001 mg L^{-1}) was 80 times more toxic than indoxacarb (LC_{50} = 0.08 mg L^{-1}), 1400 times more toxic than lufenuron (LC_{50} = 1.4 mg L^{-1}). Indoxacarb was 17.5 times more toxic than lufenuron. In addition, linoleic acid was the most toxic fatty acid with LC_{50} = 2435.8 mg L^{-1} compared to oleic acid (LC_{50} = 3654.6 mg L^{-1}) and stearic acid (LC_{50} = 4645.2 mg L^{-1}). The LC_{10} and LC_{25} values after 96 hrs of exposure were 0.02×10^{-3} and 0.097×10^{-3} mg L^{-1} for emamectin benzoate, 0.002 and 0.01 mg L^{-1} for indoxacarb, 0.05 and 0.24 mg L^{-1} for lufenuron, 228.5 and 913.7 mg L^{-1} for oleic acid, 152.4 and 608.9 mg L^{-1} for linoleic acid and for stearic acid were 324.6 and 1142.3 mg L^{-1}, respectively (Table 1).

Table (1): Toxicity of selected insecticides and fatty acids against the 4th instar larvae of *S. littoralis* after 96 hrs of exposure:

Compounds	LC_{50} (mg L^{-1}) (95% CL)	LC_{25} (mg L^{-1}) (95% CL)	LC_{10} (mg L^{-1}) (95% CL)	Slope ± SE*
Emamectin benzoate	0.001 (0.5-1.7) $\times10^{-3}$	0.097×10^{-3} (0.02-0.2) $\times10^{-3}$	0.02×10^{-3} (0.01-0.04) $\times10^{-3}$	0.68 ± 0.11
Indoxacarb	0.08 (0.04-0.14)	0.01 (0.003-0.022)	0.002 (0.2-5) $\times10^{-3}$	0.74 ± 0.11
Lufenuron	1.4 (0.86-2.2)	0.24 (0.1-0.4)	0.05 (0.01-0.1)	0.89 ± 0.13
Oleic acid	3654.6 (2943.5-4837.2)	913.7 (645.2-1285.6)	228.5 (165.3-382.4)	1.82 ± 0.39
Linoleic acid	2435.8 (1576.2-3642.8)	608.9 (452.3-882.6)	152.4 (134.2-182.9)	2.34 ± 0.38
Stearic acid	4645.2 (3866.4-5872.3)	1142.3 (987.2-1345.4)	324.6 (234.7-456.8)	2.26 ± 0.41

* SE means standard error

3.2. Joint action of selected fatty acids with each of emamectin benzoate, indoxacarb and lufenuron against the 4th instar larvae of *S. littoralis* in the laboratory:

Joint action of oleic, linoleic and stearic acids (at LC_{10}) with each of emamectin benzoate, indoxacarb and lufenuron at their LC_{50} after 48, 72 and 96 hrs of exposure was shown in Tables (2, 3 and 4). It is clear that, the higher potentiation effects were obtained when linoleic acid was mixed with emamectin benzoate after 48, 72 and 96 hrs of exposure with CTF values of 58.33, 53.87 and 46.66, respectively (Table 2). Likewise, indoxacarb/linoleic acid and lufenuron/ linoleic acid mixtures produced potentiation effects. Mixture of indoxacarb/linoleic acid had CTFs values of 42.85, 43.77 and 41.17 as well as lufenuron/linoleic acid had CTF values of 35.70, 31.26 and 27.78, respectively, after 48, 72 and 96 hrs of exposure (Table 3 and 4). All mixtures of oleic acid with each of emamectin benzoate, indoxacarb and lufenuron resulted in additive effects with CTF values ranged between 12.50 and 17.65 at all exposure times. On the other hand, mixtures of stearic acid with each of emamectin benzoate, indoxacarb and lufenuron resulted in antagonistic effects. CTF values were -28.58, -26.66 and -22.22 for emamectin benzoate/stearic acid mixture and -29.42, -26.31 and -25.00 for indoxacarb / stearic acid mixture. Moreover, CTF values were -21.43, -23.54 and -22.72 for lufenuron / stearic acid after 48, 72 and 96 of exposure, respectively (Tables 2, 3 and 4).

Table (2): Joint action of selected fatty acids at LC_{10} with emamectin benzoate at LC_{50} against the 4th instar larvae of *S. littoralis* after different times of exposure:

Combinations	Time (hrs)	Expected mortality (%)	Observed mortality (%)	Co-toxicity factor	Action type
Emamectin benzoate + Oleic acid	48	40.00	46.67	16.68	Additive
	72	46.67	53.33	14.27	Additive
	96	53.33	60.00	12.51	Additive
Emamectin benzoate + Linoleic acid	48	40.00	63.33	58.33	Potentiation
	72	43.33	66.67	53.87	Potentiation
	96	50.00	73.33	46.66	Potentiation
Emamectin benzoate + Stearic acid	48	46.67	33.33	-28.58	Antagonism
	72	50.00	36.67	-26.66	Antagonism
	96	60.00	46.67	-22.22	Antagonism

Table (3): Joint action of selected fatty acids at LC_{10} with indoxacarb at LC_{50} against the 4th instar larvae of *S. littoralis* after different times of exposure:

Combinations	Time (hrs)	Expected mortality (%)	Observed mortality (%)	Co-toxicity factor	Action type
Indoxacarb + Oleic acid	48	53.33	60.00	12.51	Additive
	72	60.00	70.00	16.67	Additive
	96	66.67	76.67	14.99	Additive
Indoxacarb + Linoleic acid	48	46.67	66.67	42.85	Potentiation
	72	53.33	76.67	43.77	Potentiation
	96	56.67	80.00	41.17	Potentiation
Indoxacarb + Stearic acid	48	56.67	40.00	-29.42	Antagonism
	72	63.33	46.67	-26.31	Antagonism
	96	66.67	50.00	-25.00	Antagonism

Table (4): Joint action of selected fatty acids at LC_{10} with lufenuron at LC_{50} against the 4th instar larvae of *S. littoralis* after different times of exposure:

Combinations	Time (hrs)	Expected mortality (%)	Observed mortality (%)	Co-toxicity factor	Action type
Lufenuron + Oleic acid	48	43.33	50.00	15.39	Additive
	72	53.33	60.00	12.50	Additive
	96	56.67	66.67	17.65	Additive
Lufenuron + Linoleic acid	48	46.67	63.33	35.70	Potentiation
	72	53.33	70.00	31.26	Potentiation
	96	60.00	76.67	27.78	Potentiation
Lufenuron + Stearic acid	48	46.67	36.67	-21.43	Antagonism
	72	56.67	43.33	-23.54	Antagonism
	96	73.33	56.67	-22.72	Antagonism

3.3. Sub-lethal effects of the selected insecticides and fatty acids on some biological parameters of *S. littoralis*:

The obtained data of the effects of sub-lethal concentrations of emamectin benzoate, indoxacarb and lufenuron at LC_{10} and LC_{25} and fatty acids (oleic and linoleic acids) at LC_{10} on some biological parameters of the 4th instar larvae of *S. littoralis* were represented in Tables (5 and 6). The data of mean weight of pupae that exposed to all treatments were significantly suppressed compared to the control treatment (Table 5). The mean weight of pupae were 175.4, 188.6 and 181.7 mg/pupa when the 4th instar larvae were exposed to emamectin benzoate, indoxacarb and lufenuron at LC_{25}, respectively, compared to 307.5 mg/pupa in control. Meanwhile, the LC_{10} treatments, mean weight of pupae were 190.7, 202.3, 195.8, 285.2 and 232.4 mg/pupa when larvae were exposed to emamectin benzoate, indoxacarb, lufenuron, oleic acid and linoleic acid, respectively. Emamectin benzoate, indoxacarb and lufenuron at concentrations equivalent to LC_{25} had the highest effect on the pupation percentages with values of 42.3, 52.5 and 46.8 %, respectively, compared to control treatment (93.8 %). Furthermore, significant decreases in pupation percentages were achieved with the concentrations of LC_{10} for each of emamectin benzoate (56.2 %), indoxacarb (66.4 %), lufenuron (61.3 %), oleic acid (76.2 %) and linoleic acid

(68.7 %). Also, the LC_{25} of each treatment of emamectin benzoate, indoxacarb and lufenuron prompted the highest reduction in the adult emergence with percentages of 51.4, 61.5 and 57.6 %, respectively. In addition, the reduction percentages in the adult emergence was significantly prompted with the LC_{10} treatment of emamectin benzoate, indoxacarb, lufenuron, oleic acid and linoleic acid with percentages of 64.7, 70.2, 68.9, 82.3 and 77.4 % respectively, compared to 94.3 % in the control treatment (Table 5). On the other hand, pupal duration did not significantly differ in all insecticides treatments compared to control treatment (Table 5).

Table (5): Sublethal effects of the selected insecticides and fatty acids on the pupal mean weight, pupal duration, pupation and adult emergence percentages of the treated 4th instar larvae of *S. littoralis*:

Treatments	Conc. (mgL^{-1})	Pupal mean weight (mg/pupa) ± SE	Pupal duration (days) ± SE	Pupation (%) ± SE	Adult emergence (%) ± SE
Control	-	$307.5^{a} \pm 2.8$	$9.2^{a} \pm 0.4$	$93.8^{a} \pm 2.4$	$94.3^{a} \pm 2.1$
Emamectin benzoate	0.02×10^{-3}	$190.7^{ef} \pm 3.6$	$9.4^{a} \pm 0.6$	$56.2^{d} \pm 2.3$	$64.7^{ef} \pm 2.8$
	0.097×10^{-3}	$175.4^{g} \pm 3.2$	$9.5^{a} \pm 0.8$	$42.3^{e} \pm 1.8$	$51.4^{h} \pm 1.6$
Indoxacarb	0.002	$202.3^{d} \pm 1.9$	$9.7^{a} \pm 0.3$	$66.4^{c} \pm 2.1$	$70.2^{d} \pm 1.4$
	0.01	$188.6^{ef} \pm 2.5$	$9.8^{a} \pm 0.5$	$52.5^{d} \pm 1.3$	$61.5^{fg} \pm 2.3$
Lufenuron	0.05	$195.8^{de} \pm 2.1$	$9.3^{a} \pm 0.2$	$61.3^{cd} \pm 1.2$	$68.9^{de} \pm 1.2$
	0.24	$181.7^{fg} \pm 2.3$	$9.6^{a} \pm 0.3$	$46.8^{e} \pm 1.6$	$57.6^{g} \pm 1.7$
Oleic acid	228.5	$285.2^{b} \pm 3.4$	$9.2^{a} \pm 0.4$	$76.2^{b} \pm 1.5$	$82.3^{b} \pm 1.9$
Linoleic acid	152.4	$232.4^{c} \pm 2.6$	$9.8^{a} \pm 0.7$	$68.7^{c} \pm 1.9$	$77.4^{c} \pm 2.4$

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

The obtained results of sub-lethal effects of selected insecticides as well as fatty acids on the fecundity, fertility and longevity of the adult stage of *S. littoralis* developed from the treated 4th instar larvae were demonstrated in Table (6). Emamectin benzoate, indoxacarb and lufenuron at concentrations equivalent to LC_{25} have the highest effect on the fecundity with mean numbers of 568.3, 732.8 and 605.9 eggs / female, respectively. While, emamectin benzoate, indoxacarb, lufenuron, oleic acid and linoleic acid at the LC_{10} significantly decreased the fecundity with mean numbers of 833.2, 956.4, 944.6, 993.5 and 978.2 eggs / female, respectively, compared to 1184.7 eggs / female in control treatment. The data of fertility percentages showed significantly decreases for the larvae received all insecticide treatments. Emamectin benzoate, indoxacarb and lufenuron at LC_{25} achieved the highest effects on the fertility with mean percentages of hatched eggs of 49.5, 61.4 and 56.3 %, respectively, compared to 96.0 % in control treatment. Moreover, reductions in the fertility percentages were significantly occurred with the LC_{10} treatments of each of emamectin benzoate, indoxacarb, lufenuron, oleic acid and linoleic acid with mean percentages of 68.2, 77.3, 72.8, 84.5 and 81.7 %, respectively. The data of adult longevity from treated 4th instar larvae with all insecticides and fatty acids treatments were significantly decreased compared in control treatment. These durations were 6.2 and 5.4 days for emamectin benzoate, 6.7 and 5.9 days for indoxacarb, 6.4 and 5.6 days for lufenuron at their LC_{10} and LC_{25}, respectively. Meanwhile, these durations were 7.8 and 7.2 days for oleic and linoleic acids at their LC_{10}, respectively, compared to 8.3 days in control (Table 6).

Table (6): Sublethal effects of the selected insecticides and fatty acids on the fecundity, fertility percentage and longevity of the adult stage of *S. littoralis* developed from the treated 4th instar larvae:

Treatments	Conc. (mgL^{-1})	Fecundity (no. eggs laid / female) ± SE	Fertility (% egg hatch) ± SE	Adult longevity (days) ± SE
Control	-	$1184.7^{a} \pm 42.6$	$96.0^{a} \pm 3.8$	$8.3^{a} \pm 0.6$
Emamectin benzoate	0.02×10^{-3}	$833.2^{c} \pm 36.2$	$68.2^{ef} \pm 3.2$	$6.2^{ef} \pm 0.7$
	0.097×10^{-3}	$568.3^{e} \pm 21.7$	$49.5^{h} \pm 2.4$	$5.4^{g} \pm 0.2$
Indoxacarb	0.002	$956.4^{b} \pm 32.4$	$77.3^{cd} \pm 3.6$	$6.7^{d} \pm 0.3$
	0.01	$732.8^{d} \pm 24.5$	$61.4^{fg} \pm 4.2$	$5.9^{fg} \pm 0.8$
Lufenuron	0.05	$944.6^{b} \pm 38.2$	$72.8^{de} \pm 2.6$	$6.4^{de} \pm 0.9$
	0.24	$605.9^{e} \pm 34.6$	$56.3^{g} \pm 1.8$	$5.6^{g} \pm 0.3$
Oleic acid	228.5	$993.5^{b} \pm 26.8$	$84.5^{b} \pm 3.4$	$7.8^{b} \pm 0.5$
Linoleic acid	152.4	$978.2^{b} \pm 29.3$	$81.7^{bc} \pm 2.2$	$7.2^{c} \pm 0.4$

Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

3.4. Efficacy of oleic and linoleic acids on the residual toxicity of emamectin benzoate, indoxacarb and lufenuron against the 4th instar larvae of *S. littoralis* (semi-field trials):

The obtained data of efficacies of the residual toxicity of emamectin benzoate, indoxacarb and lufenuron alone at their half-field (0.5FR) and field rates (FR) compared to their mixtures at the (0.5FR) with each of oleic and linoleic acids at rates equivalent to their LC_{10} (R_{10}) against the 4th instar larvae of *S. littoralis*. Results revealed that, both oleic and linoleic acids increased the residual toxicity of emamectin benzoate, indoxacarb and lufenuron against the 4th instar larvae of *S. littoralis* during two successive seasons of 2017 and 2018 (Tables 7 and 8). Mortality percentages of the 4th instar larvae of *S. littoralis* after 96 hrs from exposure to cotton leaves treated with emamectin benzoate alone (FR) at 0, 4, 8, 12 and 16 days of spraying were 90.00, 63.33, 26.67, 23.33 and 13.33 %, respectively, during the season of 2017, and 83.33, 60.00, 30.00, 26.67 and 10.00 %, respectively, during the season of 2018. Both oleic and linoleic acids significantly increased and extended the residual toxicity of emamectin benzoate at the 0.5 FR. During season of 2017, the mortality percentages of the 4th instar larvae of S. *littoralis* after 96 hrs from exposure to cotton leaves treated with emamectin benzoate 0.5 FR / oleic acid R_{10} mixture at 0, 4, 8, 12 and 16 days of spraying were 73.33, 43.33, 23.33, 26.67 and 13.33 %, respectively. Meanwhile, the mortality percentages of the 4th instar larvae after 96 hrs from treatment with emamectin benzoate 0.5 FR / linoleic acid R_{10} mixture at 0, 4, 8, 12 and 16 days of spraying were 73.33, 53.33, 50.00, 23.33 and 10.00 %, respectively, compared to emamectin benzoate 0.5 FR alone were 80.00, 30.00, 16.67, 16.67 and 6.67%, respectively.

Regarding to season of 2018, the mortality percentages of 4th instar larvae of *S. littoralis* treated with emamectin benzoate 0.5 FR / oleic acid R_{10} mixture were 76.67, 40.00, 23.33, 16.67 and 13.33 %, respectively. While, mixture of emamectin benzoate 0.5 FR / linoleic acid R_{10} were 70.00, 46.67, 53.33, 26.67 and 6.67 %, respectively, compared to emamectin benzoate 0.5FR alone were 83.33, 26.67, 20.00, 20.00 and 3.33%, respectively, at intervals of 0, 4, 8, 12 and 16 days of spraying (Tables 7 and 8). Referring to indoxacarb (FR) alone, the mortality percentages of 4th instar larvae of *S. littoralis* after 96 post-treatment were 66.67, 100.00, 80.00, 73.33 and 30.00 %, relevant to intervals of 0, 4, 8, 12 and 16 days of spraying, respectively, during the season of 2017 as well as the mortality percentages were 60.00, 100.00, 73.33, 70.00 and 23.33, respectively, in season of 2018. Concerning the treatment of indoxacarb 0.5 FR / oleic acid R_{10} mixture, mortality percentages of 4th instar larvae of *S. littoralis* were 70.00, 100.00, 83.33, 76.67 and 30.00 %. While, mixture of indoxacarb 0.5 FR / linoleic acid R_{10} the mortality percentages were 73.33, 100.00, 100.00, 86.67 and 46.67 % compared to indoxacarb alone 0.5 FR were 53.33, 100.00, 63.33, 56.67 and 26.67 % relevant to intervals of 0, 4, 8, 12 and 16 days of spraying, respectively, at season 2017. The same trend was recorded in season of 2018, the mortality percentages of 4th instar larvae of *S. littoralis* after 96 hrs post-treatment with indoxacarb 0.5 FR / oleic acid R_{10} mixture were 66.67, 96.67, 76.67, 66.67 and 30.00 %, respectively. Whereas, indoxacarb 0.5 FR / linoleic acid R_{10} mixture led to mortality percentages of 70.00, 93.33, 100.00, 80.00 and 43.33 % compared to indoxacarb 0.5 FR alone had mortality percentage of 46.67, 96.67, 70.00, 53.33 and 23.33% relevant to intervals of 0, 4, 8, 12 and 16 days, respectively (Tables 7 and 8). In the respect of treatment of lufenuron (FR) alone, mortality percentages of the 4th instar larvae of S. *littoralis* after 96 hrs post-treatment were 100.00, 76.67, 60.00, 53.33 and 26.67 % relevant to intervals of 0, 4, 8, 12 and 16 days of spraying, respectively, during the season of 2017. Likewise, mortality percentages after 96 hrs post-treatment were 96.67, 73.33, 63.33, 53.33 and 30.00 % at the same days of spraying, respectively, during the season of 2018. Mortality percentages of the 4th instar larvae exposed to lufenuron 0.5 FR / oleic acid R_{10} mixture were 83.33, 50.00, 53.33, 43.33 and 20.00 % at the same days of spraying, respectively. While, mixture of lufenuron 0.5 FR / linoleic acid R_{10} the mortality percentages were 66.67, 53.33, 46.67, 40.00 and 23.33 %, respectively, compared to lufenuron alone 0.5 FR at percentages of 50.00, 40.00, 36.67, 23.33 and 10.00 %, respectively, during season of 2017. In season of 2018, the mortality percentages of 4th instar larvae of *S. littoralis* after 96 hrs post-treatment caused by lufenuron 0.5 FR / oleic acid R_{10} mixture were 80.00, 53.33, 46.67, 50.00 and 16.67 %, respectively. While, mixture of lufenuron 0.5 FR / linoleic acid R_{10} the mortality percentages were 76.67, 50.00, 43.33, 46.67 and 13.33 %, respectively, compared to lufenuron alone 0.5 FR at percentages of 43.33, 33.33, 30.00, 26.67 and 10.00 % relevant to intervals of 0, 4, 8, 12 and 16 days of spraying, respectively (Tables 7 and 8). Eventually, the residual toxicity of indoxacarb at 0.5 FR / oleic acid and 0.5 FR / linoleic acid mixtures were higher than emamectin benzoate and lufenuron compared to each of them alone at 0.5 FR in the two growing seasons.

Table (7): Efficacy of oleic and linoleic acids on the residual toxicity of emamectin benzoate, indoxacarb and lufenuron against the 4th instar larvae of *S. littoralis*, season 2017:

Insecticides	Rates	% Mortality***				
		0-day**	4-days	8-days	12-days	16-days
Emamectin benzoate	0.5 FR* alone	80.00^{bcd}	30.00^{g}	16.67^{f}	16.67^{e}	6.67^{e}
	FR alone	90.00^{ab}	63.33^{c}	26.67^{f}	23.33^{e}	13.33^{cde}
	0.5 FR + Oleic acid	73.33^{cd}	43.33^{ef}	23.33^{f}	26.67^{e}	13.33^{cde}
	0.5 FR + Linoleic acid	73.33^{cd}	53.33^{d}	50.00^{de}	23.33^{e}	10.00^{de}
Indoxacarb	0.5 FR alone	53.33^{ef}	100.00^{a}	63.33^{c}	56.67^{c}	26.67^{b}
	FR alone	66.67^{de}	100.00^{a}	80.00^{b}	73.33^{b}	30.00^{b}
	0.5 FR + Oleic acid	70.00^{cd}	100.00^{a}	83.33^{b}	76.67^{ab}	30.00^{b}
	0.5 FR+ Linoleic acid	73.33^{cd}	100.00^{a}	100.00^{a}	86.67^{a}	46.67^{a}
Lufenuron	0.5 FR alone	50.00^{f}	40.00^{f}	36.67^{e}	23.33^{e}	10.00^{de}
	FR alone	100.00^{a}	76.67^{b}	60.00^{cd}	53.33^{c}	26.67^{b}
	0.5 FR + Oleic acid	83.33^{bc}	50.00^{de}	53.33^{cde}	43.33^{d}	20.00^{bcd}
	0.5 FR + Linoleic acid	66.67^{de}	53.33^{d}	46.67^{e}	40.00^{d}	23.33^{bc}

*FR means field rate.** The leaves samples were collected from the field after 0, 4, 8, 12 and 16 days of spraying. ***The mortality percent was recorded after 96 hrs of exposure. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

Table (8): Efficacy of oleic and linoleic acids on the residual toxicity of emamectin benzoate, indoxacarb and lufenuron against the 4th instar larvae of *S. littoralis*, season 2018:

Insecticides	Rates	% Mortality***				
		0-day**	4-days	8-days	12-days	16-days
Emamectin benzoate	0.5 FR*alone	83.33^{ab}	26.67^{f}	20.00^{g}	20.00^{f}	3.33^{e}
	FR alone	83.33^{ab}	60.00^{bc}	30.00^{fg}	26.67^{ef}	10.00^{cde}
	0.5 FR + Oleic acid	76.67^{bc}	40.00^{def}	23.33^{g}	16.67^{f}	13.33^{cde}
	0.5 FR + Linoleic acid	70.00^{bcd}	46.67^{cde}	53.33^{cde}	26.67^{ef}	6.67^{de}
Indoxacarb	0.5 FR alone	46.67^{ef}	96.67^{a}	70.00^{bc}	53.33^{bc}	23.33^{bc}
	FR alone	60.00^{de}	100.00^{a}	73.33^{b}	70.00^{a}	23.33^{bc}
	0.5 FR+ Oleic acid	66.67^{cd}	96.67^{a}	76.67^{b}	66.67^{ab}	30.00^{ab}
	0.5 FR+ Linoleic acid	70.00^{bcd}	93.33^{a}	100.00^{a}	80.00^{a}	43.33^{a}
Lufenuron	0.5 FR alone	43.33^{f}	33.33^{ef}	30.00^{fg}	26.67^{ef}	10.00^{cde}
	FR alone	96.67^{a}	73.33^{b}	63.33^{bcd}	53.33^{bc}	30.00^{ab}
	0.5 FR + Oleic acid	80.00^{bc}	53.33^{cd}	46.67^{def}	50.00^{cd}	16.67^{bcd}
	0.5 FR + Linoleic acid	76.67^{bc}	50.00^{cd}	43.33^{ef}	46.67^{cd}	13.33^{cde}

*FR means field rate.**The leaves samples were collected from the field after 0, 4, 8, 12 and 16 days of spraying. ***The mortality percent was recorded after 96 hrs of exposure. Within a column, means possessing the same letter do not differ significantly at $P = 0.05$.

4. Discussion

Fatty acids comprised of defensive constituents that featured by biocompatible activity besides the considerations of their safety applications without residual effects on ecosystem. Therefore, fatty acids may involve agricultural usages in controlling insect pests [23, 24]. Accordingly, the outlook of this research was motivated to investigate the usages of certain fatty acids in binary mixtures with selected insecticides that increase the persistence of these insecticides against *S. littoralis* in field.

The laboratory and field experiments were carried out to investigate the effects of sub-lethal concentrations of certain long-chain of free carboxylic fatty acids associated with different numbers of double bonds on the toxicity of emamectin benzoate, indoxacarb and lufenuron. Several binary mixtures of certain fatty acids at LC_{10} with the tested insecticides at LC_{50} in laboratory were established against the 4th instar larvae of *S. littoralis*. The three selected fatty acids had chains of the same length but possessed variance numbers of unsaturated bonds. The mixture of linolenic acid ($C_{18:2}$) with all tested insecticides brought out potentiation effects. Meantime, the mixtures of oleic acid ($C_{18:1}$) with all tested insecticides led to additive effects. In the contrary, stearic acid ($C_{18:0}$) had antagonistic effects in its binary mixtures with the same tested insecticides. The previous data of binary mixtures tests showed that the more number of double bonds in the chain of fatty acids, the highest toxic effect had been occurred against the 4th instar larvae of *S. littoralis*. Thus, the absent of double pond in along chain structure of stearic acids caused nontoxic effects. These findings were in accordance with the results of polyunsaturated fatty acids toxicity that possessed prevalence effects more than the effects of the saturated fatty acids on the growth of ruminal bacteria of *Butyrivibrio fibrisolvens* and *Clostridium proteoclasticum* as well as rat caecal bacterium [25-27].

Selections of tested insecticides of emamectin benzoate, indoxacarb and lufenuron were contributed to their common characteristics of safeness to environment, short dissipations on plant matrix and photo-degradation in agriculture environment. Relative rapid dissipations of these selected insecticides might affect the efficacies of their residual toxicity in controlling cotton leafworm. Therefore, the guidance was to prolong the efficiency of these insecticides as well as mitigate their environmental impact. These assumptions were supported by the short half-life of emamectin benzoate that ranged from 1.72 to 8.66 days in paddy of different crops like cabbage as well as apple [28-30]. Single and double recommended dosages of lufenuron in cauliflower showed shorter half-life values of 1.32 and 1.47 days, respectively, besides the half-life values of indoxacarb single and double dosages at 2.88 and 1.92 days, respectively, in cabbage and reached to 3.19 day in cowpea [31-33].

Referring to the data of tested insecticides effectiveness on some biological parameters of *S. littoralis*, the sub-lethal concentrations of the tested insecticides at LC_{25} were more effective on biological parameters compared to LC_{10}. Emamectin benzoate was the most potent insecticides on pupal weight, pupation and adult emergence percentages followed by lufenuron then ended with indoxacarb. Emamectin benzoate and lufenuron had the highest effects followed by indoxacarb on fecundity, fertility percentage, and adult longevity of the 4^{th} instar larvae of *S. littoralis*. These data came in agree with the results of biological aspects of treated *Helicoverpa zea* with sub-lethal dosages emamectin benzoate. Adults females *H. zea* had significant reduction in both of duration of larvae survived to the pupal stage and percentages of eggs hatchability. Meanwhile, the treated 3^{rd} instar larvae of *Helicoverpa armigera* had declines in pupal weight, fecundity, egg hatching and prolongations in duration of larval and pupal development as well as adult longevity [34-36]. On the other hand, studied of the sub-lethal effects of lufenuron on larvae, pupae and adults of the diamondback moth, *Plutella xylostella* (Linnaeus) resulted in significant reductions of pupal weights, fecundity and oviposition periods [37]. In addition, relevant studies of the sub-lethal dosages of chlorfluazuron that the same action of lufenuron (inhibitors of chitin biosynthesis, Benzoylureas) showed adversely effects on the development of *Spodoptera litura* instars, molting to pupae, emergence of adults, besides larval and pupal weights [38]. Furthermore, indoxacarb at sub-lethal concentration caused feeding deterrent activity of *Spodoptera litura* larvae. Therefore, feeding impairment of treated larvae could led to prolongation in larval instars and subsequently leading to a reduction in the percentage of pupation and adult emergence [39, 40]. On the other hand, oleic and linoleic acids had significant lower effects on the biological parameters compared to the tested insecticides. These present results were compatible with the many studies carried out on linolenic acid and linoleic acid at low concentrations that had insectistatic and insecticidal activities compared to the insecticidal activity of palmitic acid and stearic acid against *Spodoptera frugiperda* [9, 11]. These insecticidal activities might be due to the ability of various fatty acids to involve either the site of acetyl cholinesterase or octopaminergic receptors [41, 42].

Regarding to the data of the binary mixtures tests and biological aspect, the surpassed fatty acids including linoleic and oleic acids at LC_{10} and adequate sub-lethal concentrations of the tested insecticides were picked out to submit the semi-field experiments for residual toxicity test. The residual toxicity of the tested insecticides alone at their (0.5FR) and (FR) were compared to their (0.5FR) mixtures with each of the tested fatty acids at (R_{10}) against the 4^{th} instar larvae within period of 16 days post-treatment during the seasons of 2017 and 2018. The results of the residual toxicity during the two successive seasons revealed that the most potent binary mixture was indoxacarb (0.5 FR) with linoleic acid (R_{10}). In the second rank, the mixture of indoxacarb (0.5 FR) with oleic acid (R_{10}) appeared and followed by lufenuron (0.5 FR) with each of oleic acid (R_{10}) and linoleic acid (R_{10}) and finally ended in mixtures of emamectin benzoate (0.5 FR) with oleic acid (R_{10}). All these binary mixtures at most interval times overpassed the efficacies of the corresponding tested insecticides alone at their (0.5FR) and (FR). These data were supported with the activity of binary mixtures of some inorganic acids and organic acids with certain synthetic insecticides against the 4^{th} instar larvae of *Spodoptera littoralis* in laboratory. They found that combinations of methomyl and organic acids, both acetic and linolenic acids exhibited high synergistic activity followed by hexanoic and oleic acids [43]. Therefore, the outlook should be oriented to new approaches to motivate the uses of certain fatty acids in pests control and within some insecticides formulations to increase their persistency against insect pests besides keeping on environment safety.

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