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**Toxicological and Biological Studies on Using Lufenuron, Chlorpyrifos, Spinosad and Emamectin Benzoate Insecticides for Controlling Cotton Leafworm, *Spodoptera littoralis* (Boisd.)**

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**ABSTRACT**

This study was evaluated the toxicity and latent effect of four insecticides; chlorpyrifos (Dursban H 48% EC) as organophosphates, emamectin benzoate (Proclaim 5% SG) as bioinsecticide, lufenuron (Match 5% E.C) as IGR and spinosad (Spintor 24% SC) as bioinsecticide against 2nd and 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* lab and field strain. The present data proved that values of LC<sub>50</sub> for second instar larval were 1.04, 1.16, 2.73 and 3.12 ppm for 2nd instar larvae for lab strain, while values of LC<sub>50</sub> increased to 2.94, 2.86, 3.26 and 4.96 ppm for field strain when treated larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively. Moreover, values of LC<sub>50</sub> were 3.82, 3.01, 4.54 and 6.28 ppm for 4th instar larvae lab strain, and increased to reach 5.48, 4.95, 6.67 and 9.97 ppm for 4th instar larvae field strain when treated larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively. As for the late effects, the results indicated that in case of second instar larvae the lowest percentage of pupation were 18.55 and 28.56% when treated larvae with lufenuron whereas, the highest 39.55 and 44.23% with spinosad for larvae of lab and field strains, respectively and adult emergence was the lowest when treated larvae with chlorpyrifos, lufenuron and spinosad for lab strains, while larvae of field strains gave the lowest percentage with treated by lufenuron (14.32%) and the mean of eggs /female were the lowest (155.45 egg/female) when treated larvae with lufenuron for lab and field strains and was the lowest (138.0 egg /female) when treated larvae with spinosad for field strain. In case of fourth instar larvae, larvae of lab strain showed the lowest percentage of pupation (31.23%) when treated with spinosad while the lowest percentage of pupation (40.42%) when treated larvae with chlorpyrifos and hatchability percent increase in field strain compare to lab strain.

**INTRODUCTION**

The attack of pests to cotton leads to low yield which is related to damage in bolls by attacking cotton leafworm to the crop and the control of cotton leafworm is done by using different chemical insecticides. Among the most economically and environmentally suitable insecticides are chlorpyrifos, spinosad and lufenuron, these compounds are used in a wide variety against agricultural crops. Spinosad causes muscles to flex uncontrollably this leads

to paralysis and death (Kirst 2010) emamectin benzoate, which belongs to the avermectin family of 16-membered macrocyclic lactones generated by the soil-dwelling microorganism, *Streptomyces avermitilis* (Crouch *et al.*, 1997 and Jansson *et al.*, 1997), is a promising insecticide for lepidopteran insect control. Emamectin benzoate is used against several species of lepidopteran such as; *Heliothis virescens*, *Plutella xylostella*, *Pseudoplusia includes*, *Spodoptera frugiperda*, *Trichoplusia ni*, *S. littoralis*, *Spodoptera exigua* and *Mamestra Brassicae* (Trumble *et al.*, 1987, Argentine *et al.*, 2002, Firake & Pande 2009 and Moustafa *et al.*, 2016), with less toxic to non-Lepidopteran and most beneficial insects (Jansson *et al.*, 1997). Emamectin benzoate is composed of ~90% avermectin B1a and ~10% of avermectin B1b. The insect growth regulator is interfered with insect growth and development by inhibiting chitin synthesis in insects, lowest toxic effect to mammals, birds, and fish (Flint and Smith, 1977) and (Mokbel *et al.* 2017) cited that, emamectin benzoate is a new and effective insecticide used against lepidopteran insect pests. Also, the mixing of these compounds with traditional insecticides increases the efficiency of these insecticides (Abdel-Sattar and EL-Guindy 1988); (Ahmed 2020) and (Abdel Aziz 2019). Abdel-Mageed *et al.* (2006) reported that spinosad gave moderately initial and residual effects when tested alone. (Elhadek, Hafez, and Ali 2020) indicates the high efficacy of some IGRs and Bio-insecticides to be used as an effective alternative method of *S. littoralis* control while insecticide resistance has developed for other traditional insecticides.

Based on these studies, the present work was conducted to evaluate the toxicity and latent effects of chlorpyrifos, as organophosphorus insecticides, emamectin benzoate, as bioinsecticide lufenuron as IGR and spinosad a bioinsecticide against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of the cotton leafworm, *Spodoptera litraltois* lab and field strain.

## MATERIALS AND METHODS

### Rearing Technique:

The Laboratory strain of *S. littoralis* (Boisd.) of this study was obtained from Plant Protection Institute, Agriculture Research Center, Dokki, Giza, Egypt. This strain was not exposed to any insecticides. The colony was reared under constant conditions at  $25 \pm 2$  °C,  $65 \pm 5$  % R.H. and photoperiod 12:12 L:D. The field strain started as egg masses collected from Beni-Suef Governorate, Egypt and reared as described by El-Dafrawy *et al.* (1964) under laboratory conditions at  $25 \pm 2$  °C and  $65 \pm 5$  % relative humidity. Eggs masses were kept separately in a 400 ml glass jar covered with muslin. Larvae were transferred three days after hatching to clean larger jars. The jars were provided with castor bean leaves for larval feeding. The prepupae were allowed to pupate in clean jars pupation. The resulting pupae were transferred to glass jars containing filter papers and were kept in suitable cages (35 × 35 × 35 cm) for mating the emerged moths. Emerged moths fed on a piece of cotton dipped in 10% sugar solution cages were supplied with leaves of *Nerium oleander* (L.) that served as an oviposition site.

### Insecticides Used:

The following insecticides were employed:

- 1) Dursban H 48% EC (Chlorpyrifos) as an organophosphate, obtained from Daw Agrosciences.
- 2) Match 5% EC (Lufenuron) as an insect growth regulator, obtained from Syngenta Argo Egypt company.
- 3) Spintor 24% SC (Spinosad): is an extract of the fermentation broth of soil actinomycete bacterium, *Saccharopolyspora spinosa*, obtained from Daw Agrosciences.
- 4) Proclaim 5% SG (Emamectin benzoate) is a bio-insecticide produced by the soil microorganism, *Streptomyces avermitilis*, obtained from Syngenta Argo Egypt.

### Toxicity Tests:

To determine LC<sub>50</sub> values for each tested compound used serial concentrations from each compound were prepared by diluting the formulation product with distilled water 30, 15, 7.5, 3.75, 1.87, 0.93 and 0.46 ppm for chlorpyrifos, emamectin benzoate, lufenuron and spinosad. Castor bean leaves were dipped for 15 seconds in each concentration then left to dry at room temperature and offered to the newly moulted 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* lab and field strain. Three replicates were carried out for each concentration, ten larvae for each replicate placed inside each jar, three treated leaves of each concentration were transferred to these glasses. Treated larvae were allowed to feed on the treated leaves for 24 hr, in case chlorpyrifos but in cases lufenuron, emamectin benzoate and spinosad, larvae were fed for 72 hr, then in all cases of feeding periods, the larvae were transferred to untreated leaves until pupation. Three replicates were dipped in distilled water for the same periods as a check treatment.

Corrected mortality percent was obtained using check treatments and Abbott formula (1925). The corrected percentage of mortality of each insecticide was statically calculated according to Finney (1952) to determine the LC<sub>50</sub>.

Pupae resulting from treated larvae with deferent LC<sub>50</sub>, were kept in plastic tubes and the pupal was recorded until an adult emergency. Emerged moths resulting from treated and untreated larvae were sexed (male & female) and put in a cage and provided with a sugar solution of 10 % till egg-mass depositions throughout their life span. For each egg mass, the eggs were calculated and put in a clean jar with untreated castor bean leaves until hatching. Newly hatched larvae were recorded to calculate the hatchability %.

## RESULTS AND DISCUSSION

### Toxicity Effects:

Results in Table (1) state the toxicity of chlorpyrifos, emamectin benzoate, lufenuron and spinosad at different concentrations to 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* lab and field strain. The present data proved that values of LC<sub>50</sub> for second instar larval were 1.04, 1.16, 2.73 and 3.12 ppm for lab strain, while values of LC<sub>50</sub> increased to 2.94, 2.86, 3.26 and 4.96 ppm for field strain when treated larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively. Moreover, values of LC<sub>50</sub> were 3.82, 3.01, 4.54 and 6.28 ppm for 4<sup>th</sup> instar larvae lab strain, and increased to reach 5.48, 4.95, 6.67 and 9.97 ppm for 4<sup>th</sup> instar larvae field strain when treated larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively.

**Table 1:** Toxicity of chlorpyrifos, emamectin benzoate, lufenuron and spinosad against 2<sup>nd</sup> and 4<sup>th</sup> inster larvae for lab and field strains of *Spodoptera littoralis*.

Compounds	Concentration (ppm)	2 <sup>nd</sup> instar larvae		4 <sup>th</sup> instar larvae	
		Lab strain	Field strain	Lab strain	Field strain
Chlorpyrifos	LC <sub>50</sub>	1.04	2.94	3.82	5.48
	Slope	0.301 ± .037	0.49± 0.056	0.34 ±0.080	1.2± 0.937
Emamectin benzoate	LC <sub>50</sub>	1.16	2.86	3.01	4.95
	Slope	1.218±0.123	0.67 ±0.044	0.21±0.056	0.59±0.064
Lufenuron	LC <sub>50</sub>	2.73	3.26	4.54	6.67
	Slope	0.40 ± 0.085	0.51±0.040	0.59 0.148	0.67±0.218
Spinosad	LC <sub>50</sub>	3.12	4.96	6.28	9.97
	Slope	0.39 ±0.155	0.546±0.045	0.34±0.090	0.78±0.088

These results agree with the previous studies El-Helaly and El-bendary (2015) cited that LC<sub>50</sub> values of spinosad to 2<sup>nd</sup> of *S. littoralis* tested at 1, 2, 3, 4,5, 6 and 7days after

treatment were 37.580, 19.050, 9.028, 7.019, 5.0182, 4.0181 and 2.0109 mg x kg(-1), respectively. Spinosad at a sublethal dose significantly extended the developmental period of survivor larvae, and reduced larval weight. Mery *et al.*, (2019) reported that lufenuron was more effective than chlorpyrifos and spinosad on the 2nd instar larvae. Where, the LC<sub>25</sub> values for lufenuron, chlorpyrifos and spinosad were 0.0005, 2.21 and 8.1 ppm, respectively. Hendawi *et al.*, (2017) cited that emamectin benzoate followed by chlorfluazuron proved to be the most effective, on the 4th instar larvae of a laboratory strain of cotton leafworm, *S. littoralis*. (Lotfy and Embaby 2020) reported that the highest reduction in infestation percentages was recorded with Jasper followed by Hamer then Dipel 2x. It recorded 96.30%, 90.06%, 80.32%, 78.70% and 68.52%, 67.31% with Jasper, Hamer and Dipel 2x during 2018 and 2019, respectively. Moataz *et al.*, (2018) studied the effect of four emamectin benzoate formulations on second instar larvae of *S. littoralis*. They found that Emi-Mainar was more toxic (LC<sub>50</sub>= 0.007 µg/ml) than Absoluota and Proclaim, Camaro (0.015 and 0.019 µg/ml). Also, Korrat *et al.*, (2012) cited that, emamectin benzoate was a very effective insecticide (LC<sub>50</sub> = 0.017 ppm) then by chlorfluazuron (LC<sub>50</sub>= 0.42 ppm) and profenofos (LC<sub>50</sub>= 10.9 ppm) against 2nd instar larvae of *S. littoralis* under laboratory conditions and finally spinosad which showed the less toxic effect (LC<sub>50</sub>= 19.9 ppm). After 12 days of the treatment, and at the LC<sub>25</sub> level, spinosad recorded the longest residual effect then by chlorfluazuron, profenofos and emamectin benzoate. Also, Ismail (2020) cited that emamectin benzoate showed the most toxic compound followed by lufenuron, cyfluthrin, spinetoram, and profenofos with the corresponding LC<sub>50</sub> values of 0.05, 49.18, 70.99, 130.26, and 156.78 ppm on cotton leafworm, respectively.

#### **Latent Effect of The Tested Compounds on *S. littoralis*:**

Data in Tables (2 and 3) showed that the latent effects of the tested compounds (chlorpyrifos, emamectin benzoate, lufenuron and spinosad) when treated with LC<sub>50</sub> on pupation percent, adult emergence, mean of eggs/female and percentage hatchability of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of *S. littoralis* lab and field strain.

The present data in Table (2) proved that the lowest % pupation was 18.55 and 28.56% when treated larvae with lufenuron whereas, the highest 39.55 and 44.23% with spinosad for larvae of lab and field strains, respectively. Pupation percent of these compounds were 25.55, 32.21, 18.55 and 39.55 % for larvae of lab strain and increased to 38.22, 38.22, 28.56 and 44.23% for larvae of field strain when treated second instar larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively compared to 87.89% in untreated. Percentage adult emergence was the lowest when treated larvae with chlorpyrifos, lufenuron and spinosad for lab strains, while larvae of field strains gave the lowest percentage with treated by lufenuron (14.32%), where percentage adult emergence was 10.54, 35.21, 10.55 and 10.55% for larvae of lab strain and increased to 28.31, 42.24, 14.32 and 18.67% for larvae of field strain when treated second instar larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively compared to 88.44 and 84.43% in untreated for lab and field strain, respectively.

The mean of eggs /female was the lowest (155.45 egg/female) when treated larvae with lufenuron for lab and field strains and was the lowest (138.0 egg /female) when treated larvae with spinosad for field strain. In case of lab strain, values of the mean of eggs /female were 225.23, 245.43, 155.45 and 285.34 egg /female, while were 182.0, 385.0, 182.0 and 138.0 egg /female for field strain when treated second instar larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively compared to 899.6 and 821.5 in untreated for lab and field strain, respectively. Concerning hatchability percentages were 42.21 and 22.13% when treated with emamectin benzoate and Spinosad for lab strain, respectively and in case of field strain, hatchability percentages were 42.26, 52.21, 8.32 and

29.23% for chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively compared to 94.18 and 92.18% in untreated for lab and field strain, respectively.

**Table 2:** Latent effects of the tested compounds on 2nd instar larvae of *S. littoralis* lab and field strain.

Treatments	2nd instar larvae lab strain				2nd instar larvae field strain			
	% Pupation	%Adult emergence	Mean of eggs /female	% Hatchability	% Pupation	%Adult emergence	Mean of eggs /female	% Hatchability
Chlorpyrifos	25.55	10.54	225.23±2.1	-	38.22	28.31	182.0±2.8	42.26
Emamectin benzoate	32.21	35.21	245.43±3.1	42.21	38.22	42.24	385.0±3.5	52.21
Lufenuron	18.55	10.55	155.45±2.32	-	28.56	14.32	182.0±2.8	8.32
Spinosad	39.55	10.55	285.34±1.01	22.13	44.23	18.67	138.0±1.41	29.23
Control	87.89	88.44	899.5±2.1	94.18	87.89	84.43	821.5±2.1	92.18
L.S.D at 5 %	-	-	1.911	-	-	-	2.911	-

Data in Table (3) showed that the latent effects of the tested compounds at LC<sub>50</sub> on fourth instar larval for lab and field strains, results indicate that, in the general percentage of pupation decrease in a lab strain compare to field strain.

In case of lab strain, the lowest percentage of pupation was 31.23 % when treated 4th instar larvae with spinosad and the highest was 35.65% with chlorpyrifos. Whereas in case of field strain, the lowest percentage of pupation was 40.42 % when treated 4th instar larvae with chlorpyrifos and the highest were 62.24% with lufenuron. Pupation percent of these compounds were 35.65, 32.21, 35.34 and 31.23% for larvae of lab strain and increased 45.42, 45.34, 54.32 and 42.01% for larvae of field strain when treated larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively compared to 87.19 and 85.43% in untreated for lab and field strain, respectively. Percentage of adult emergence was 35.55, 35.21, 52.32 and 38.46% for lab strain and increased to 40.67, 52.32, 62.24 and 49.12% for field strain when treated larvae with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively.

Also, the mean of eggs /female and hatchability percent increase in case field strain compare to lab strain. Where, mean of eggs /female was 194.5, 245.43, 489.55 and 150.75 egg /female for lab strain and were 213.5, 489.55, 496 and 213.5 egg /female for field strain and concerning of percentage of hatchability were 56.24, 42.21, 62.02 and 10.69% for lab strain and were 68.56, 62.02, 68.34 and 16.34% for field strain when treated fourth larval instar with chlorpyrifos, emamectin benzoate, lufenuron and spinosad, respectively.

**Table 3:** Latent effects of the tested compounds on 4th instar larvae of *S. littoralis* lab and field strain.

Treatments	4th instar larvae lab strain				4th instar larvae field strain			
	% Pupation	%Adult emergence	Mean of eggs /female	% Hatchability	% Pupation	%Adult emergence	Mean of eggs /female	% Hatchability
Chlorpyrifos	35.65	35.55	194.5±17.67	56.24	45.42	40.67	213.5±6.36	68.56
Emamectin benzoate	32.21	35.21	245.43±3.1	42.21	45.34	52.32	489.55±2.12	62.02
Lufenuron	35.34	52.32	489.55±2.12	62.02	54.32	62.24	496±4.9	68.34
Spinosad	31.23	38.46	150.75±4.3	10.69	42.01	49.12	213.5±6.36	16.34
Control	87.19	81.33	822.5±2.1	94.18	85.43	87.53	881.5±2.1	95.28
L.S.D at 5 %	-	-	2.42	-	-	-	5.45	-

These results agree with results recorded by many authors Saleh *et al.*, (2021) reported that Owner showed the highest decrease in pupation. Where the pupation percentages were 20.0, 28.89, 43.33 and 56.67% at lufenuron (Owner 5%), diflubenzuron (Dimilin 48%), emamectin benzoate (Emafel 4%) and indoxacarb (Strong 30%),

respectively and weight of pupae in all treatments was insignificant between treatments and control. However, larval mortality percentage was 80% in owner 5%EC while, was 10 % in control (Korrat *et al.*, 2012) found that, all concentration, of spinosad and chlorfluazuron was very effects on pupation, moth emergence, hatchability and sterility against larvae of cotton leafworm under laboratory conditions. Thus, it could be mentioned that using low doses of insecticide gave excellent control of the insects compared to their high doses at the same time also minimize the environmental pollution. Hussein and Eldesouky (2019) reported that the sublethal-concentrations of chlorfluazuron on 4th larval instars of *S. littoralis* was significant in reducing of pupae and larvae weight; longevity of adults; pupation percent; the emergence of adults and fecundity of females, while increased larvae and pupae durations. Abdel-Rahim *et al.*, (2008) found that fecundity and fertility were inhibited when treating the second larvae and the 4th instar larvae on *A. ipselon* with spinosad. Also found that when treated 4th instar larvae *A. ipselon* with spinosad the adult emergence percentage was decreased.

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### ARABIC SUMMARY

دراسات سمية وبيولوجية لمركبات كلوروبيريغوس وامامكتين بنزوات وليوفينورون وسبينوساد ضد حشرة دودة ورق القطن

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تم في هذه الدراسة تقييم السمية والتأثير المتأخر لأربعة مبيدات حشرية وهي: كلوربيريفوس (دروسبان H 5% EC) من مجموعة الفسفور العضوية، إيمامكتين بنزوات (بروكليم 5% SG) كمبيد حيوي، ليفينورون (ماتش 5% EC) كمبيد منظم نمو حشري ومركب سبينوساد (سبينتور 24% SC) كمبيد حيوي ضد يرقات العمر الثاني والرابع لدودة ورق القطن للسلاطين المعملية والحقلية. أوضحت البيانات أن قيم  $LC_{50}$  ليرقات العمر الثاني كانت 1.04 و 1.16 و 2.73 و 3.12 جزء في المليون ليرقات العمر الثاني للسلالة المعملية، بينما زادت قيم  $LC_{50}$  إلى 2.94 و 2.86 و 3.26 و 4.96 جزء في المليون للسلالة الحقلية عند معاملة اليرقات بالمركبات كلوربيريفوس وإيمامكتين بنزوات وليوفينورون وسبينوساد على التوالي. علاوة على ذلك، كانت قيم  $LC_{50}$  ليرقات العمر الرابع للسلالة المعملية 3.82 و 3.01 و 4.54 و 6.28 جزء في المليون، وزادت لتصل إلى 5.48 و 4.95 و 6.67 و 9.97 جزء في المليون للسلالة الحقلية عند معاملة اليرقات بالمركبات كلوربيريفوس ، إيمامكتين بنزوات ، ليفينورون و سبينوساد ، على التوالي. أما بالنسبة للتأثيرات المتأخرة فقد أشارت النتائج إلى أنه في حالة يرقات العمر الثاني كانت أقل نسبة تعذير 18.55 و 28.56% عند معاملة اليرقات باستخدام مركب ليفينورون بينما كانت أعلى نسبة 39.55 و 44.23% عند المعاملة بمركب سبينوساد ليرقات السلالات المعملية والحقلية على التوالي. و نسبة خروج الحشرات الكاملة كان الأقل عند معاملة اليرقات بمركب كلوربيريفوس ، ليفينورون وسبينوساد للسلالات المعملية ، بينما أعطت يرقات السلالات الحقلية أقل نسبة بالمعاملة بمركب ليفينورون (14.32%) وكان متوسط عدد البيض/أنثى هو الأقل (155.45 بيضة/أنثى) عند معاملة اليرقات بمركب ليفينورون في حالة السلالة المعملية بينما كان الأقل عند معاملة اليرقات بمركب سبينوساد (138.0 بيضة/ أنثى) في حالة السلالة الحقلية. في حالة يرقات العمر الرابع، أظهرت يرقات السلالة المعملية أقل نسبة تعذير (31.23%) عند معاملة اليرقات بمركب سبينوساد بينما أقل نسبة تعذير (40.42%) عند معاملة اليرقات بمركب كلوربيريفوس كما ان نسبة الفقس زادت في سلالة الحقل مقارنة بالسلالة المعملية.