

**COMPARATIVE TOXICITY OF THREE NOVEL BIOTIC COMPOUNDS, SPINOSAD PYRIDALYL AND RADICAL IN RELATIVE TO A CONVENTIONAL INSECTICIDE, LANNATE AGAINST THE FIELD AND LABORATORY STRAIN OF THE SECOND AND FOURTH INSTARS LARVAE OF COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.)**

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**ABSTRACT**

The toxic activity of three novel compounds (Pyridalyl, Radical, Spinosad) and conventional insecticide (Lannate) against the second and fourth instars of the lab and field strains of *Spodoptera littoralis* were evaluated under laboratory conditions. The obtained 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of the field and lab strains were fed for 48h on castor leaves, *Ricinus communis* were dipped for 15 seconds in series of concentrations of each tested compounds to determine the LC<sub>50</sub> values. Radical was the most toxic one against both of 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of the susceptible and resistance strains. The LC<sub>50</sub> values were 1.1, 2.7 and 1.95, 4.4 ppm for both second and fourth instar larvae of the two susceptible and resistance strains, respectively. While, Pyridalyl was the second one, the LC<sub>50</sub> values were 1.8, 5 and 6.2, 9.4 ppm for the two instars of both strains, respectively. Whereas, Lannate was the third one, its LC<sub>50</sub> values were 3.9, 6 and 11, 19 ppm of both instars for of both strains, respectively. While Spinosad was the fourth one, its LC<sub>50</sub> values were 21, 62.5 and 31.3 and 130 ppm of both instars for both strains, respectively. The biological activities of larvae were affected with the treatment of the second and fourth instars of both lab and field strains with the four tested compounds. The effect varied according to the strain, larval instar and tested compound, therefore, the larval treatment for both instars of the both strains with the four tested compounds caused highly significantly effect led to pupation and adult emergence percentages decrease at the tested four treatments. While, Pyridalyl treatment had the highest effect in larval duration, pupal and adult malformations increase; adult fecundity, fertility and longevity decrease in case of larval treatment of the two instars of both strains with this compound and it had the highest effect in pupal duration increase and weight decrease in case of treatment of the second instar of the field strain with this compound and it was effective against the sex ratios, the males increase and females decrease, as respect to control, with the treatment of fourth instar of lab strain with it. Whereas, Radical had the greatest effect on adult fecundity and fertility with fourth instar treatment of field strain with it. Also, it was effective against the pupal weight with the treated second instar of field strain and it had the highest effect on larval duration and adult malformations in case of the treatment of the second and fourth instars of lab strain with it and it had an adversely effect on the sex ratio (it caused males decrease and females increase) with the treatment of fourth instar of field strain with it. However, Spinosad had the highest effect on both adult fecundity and fertility with the treated fourth instar of field strain and it was the effect on adult malformations with the treatment of both instars of lab strain with it and affect the sex ratio, lead to males increase and females decrease with the treatment of fourth instar of lab strain with it. While, Lannate, had the highest effect on pupal malformations with the larval treatment of both instars of both strains with it and it was effective on pupal weight

and adult malformations with the treatment of either second or second and fourth instar together of field strain with it also, it was effective on adult fecundity, fertility and longevity with the treated fourth instar of field strain and it had the highest effect on larval duration with the treatment of the fourth instar of the lab strain with it.

## **INTRODUCTION**

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is one of the major pests that cause a considerable damage to many of the important vegetables and field crops in Egypt. The rising consumption of currently used insecticides in developing countries has led to a number of problems such as insect resistance, environmental pollution and the health hazards associated with pesticide residues. It is therefore necessary to complement our reliance on synthetic pesticides with less hazardous, safe and biodegradable substitutes. Among these compounds, biotic compounds such, Spinosad played an important role in pest control, gets its name from the microbe that produces it, a soil-dwelling bacterium called *Saccharo-polyspora spinosa*. Spinosad represents a new class of insecticides acting by a novel mode of action (Thompson *et al.*, 2000) possess less risk than most insecticides to mammals, birds, fish and beneficial insects. It was used for control of lepidopterous insects (Temarak, 2003a). Also, Pyridalyl is an insecticide of a novel chemical class (unclassified insecticides) with an unknown mode of action that causes loss of vigour and death within 2-3 hours in lepidopterous larvae and is effective in the control of lepidopterous pests and thrips in cotton and vegetables. Toxicity of Pyridalyl against *S. littoralis* was evaluated in the laboratory (Shigeru *et al.*, 2004 and Isayama *et al.*, 2005). It active against the resistant strain of diamondback, *Plutella xylostella* (L) and *Heliothis virescens* (F) that are resistant to various insecticides. It also produces unique insecticidal symptoms, so it may have a different mode of action from other existing insecticides. Also, Radical is one of the novel compounds, it can be obtained from *Streptomyces avermitilis*, It's avermectin derivatives from combination of methyl amine and avermectin, its efficacy was estimated as insecticide by Grove and Bovington (2008). A conventional insecticide, Lannate was used for the lepidopterous pest's control (Kassem *et al.*, 1986).

The aim of the present study is to compare the insecticidal efficacy of three novel compounds (Radical, Pyridalyl and Spinosad) in relative to a conventional insecticide (Lannate) against the field and laboratory strains of second and fourth instar larvae of *S. littoralis*.

## **MATERIALS AND METHOD**

### **1. The Field strains.**

Field strain egg masses were collected from cotton fields at Sides Station Research, Ben-Sueif Governorate during 2006-2007 cotton growing seasons at which CLW larvae have been exposed to field routine selection pressure of certain conventional insecticides that are usually applied every

year from June to September. These insecticides were insect growth regulators, organophosphates (OPs) as Dursban and Tilton insecticides, pyrethroids (PYs) as Sumi-alpha, biotic compounds as Spintor and Agerin. The egg-masses were collected during June and reared on castor bean leaves *Ricinus communis* (L.) under temperature ranged between 25-28°C and 60-65% relative humidity until egg hatching. The obtained second and fourth instar larvae were used for bioassay tests.

## 2. The laboratory strains:

The cotton leaf worm, *S. littoralis* was reared in the laboratory for several generations at room temperature ranged between 25-28°C and 60-65% R.H. Larvae were fed on castor bean leaves, *Ricinus communis* (L.) in a wide glass jars until pupation period and adults emergence. The newly emerged adults were mated inside glass jars supplied with a piece of cotton wetted 10% sugar solution as feeding source for the emerged moths and branches of Tafia (*Nerium oleander* L.) or castor bean leaves as an oviposition site (El-Defrawi *et al.*, 1964). Egg masses were kept in plastic jars until hatching. The obtained second and fourth instar larvae were used for bioassay tests.

## 2- Material used:

### 2.1. Spinosaci, the used spinosad (24% SC):

**Trade name:** The insecticide was introduced by Dow Agro Sciences for control lepidopterous pests in cotton under the trade name Tracer (Thompson *et al.*, 1997).

**Chemical name:** The name Spinosad is derived from combining the characters Spinosyn A and D. The rate of application was 50 cm<sup>3</sup>/fed.

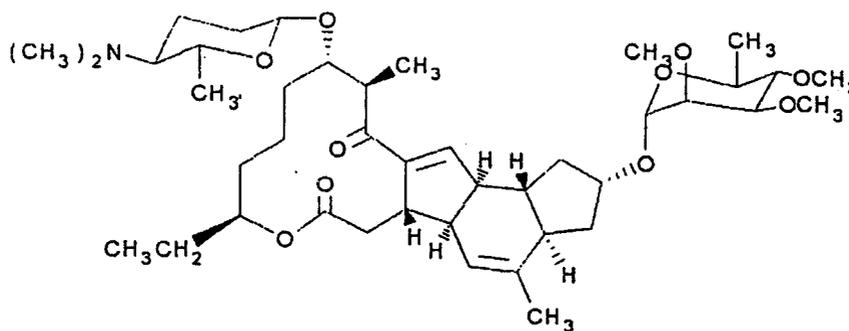
**Empirical formula:** Spinosyn A: C<sub>41</sub>H<sub>65</sub>NO<sub>10</sub>.

Spinosyn D: C<sub>42</sub>H<sub>67</sub>NO<sub>10</sub>.

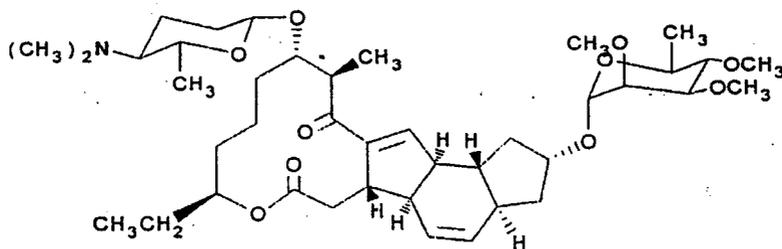
**Molecular weight:** Spinosyn A: 731.98.

Spinosyn D: 745.

### Structure:



**Spinosyn D**



Spinosyn A

**2.2.Common name (ISO name):** Pyridalyl

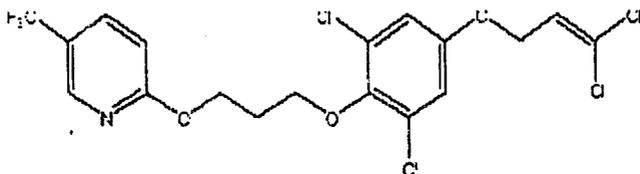
**Trade name:** The insecticide was introduced by Valent USA for control lepidopterous pests in cotton under the trade name Pyridalyl (S-1812): The rate of application was 50-200g ai/ha.

**Chemical name:** 2,6- Dichloro -4- (3,3-dichloroallyloxy) phenyl 3 [5(trifluoromethyl)2-pyridyloxy] propyl ether

**Molecular Formula:** C<sub>18</sub>H<sub>14</sub>C<sub>14</sub>F<sub>3</sub>NO<sub>3</sub>.

**Molecular Weight:** 491.12.

**Structure:**



**2.3.Common Name:** Lannate, Lanox 216, NuBait II, Nudrin, SD 14999.

**Molecular formula:** C<sub>5</sub>H<sub>10</sub>N<sub>2</sub>O<sub>2</sub>S.

**Chemical Name:** S-Methyl-N-[(methylcarbonyl)oxy]-thioacetimidate

**Molecular weight:** 162.20.

**2.4. Radical (0.5% ES):**

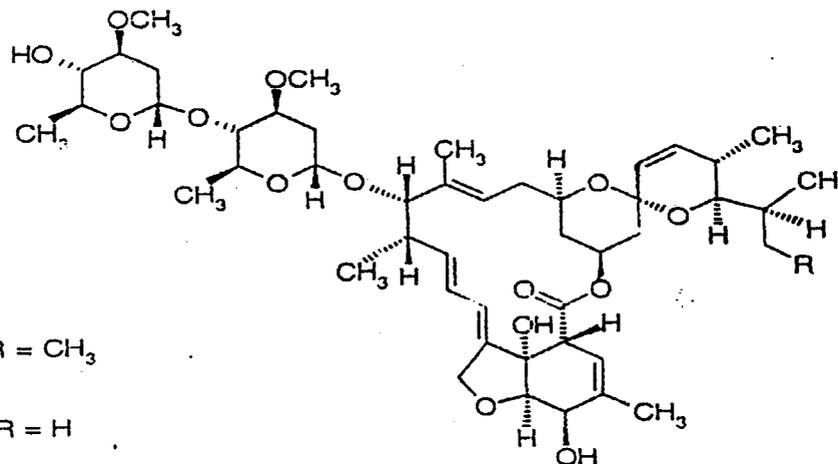
**Source:** its avermectin derivatives consist of combination of methylamine and avermectin, El-Aserai Company.

**Avermectin:** which can be obtained from *Streptomyces avermitilis* are referred to as A1a, Alb, A2a, A2b, B1a, B1b, B2a and B2b. The compounds referred to as "A" and "B" have a methoxy radical and an OH group, respectively, in the 5-position. The "a" series and the "b" series are compounds in which the substituent R1 (in position 25) is a sec-butyl radical and an isopropyl radical, respectively.

**Molecular formula:** C<sub>48</sub> H<sub>72</sub>O<sub>14</sub>.

**Molecular weight:** 873.1

**Structure:**



### 3. Test procedures:

A series of different concentrations of each of the four tested compounds, Pyridalyl, Radical, Spinosad and Lannate were prepared on the active ingredient basis (ppm) by diluting the material of the compounds in the water as solvent. Both Pyridalyl and Radical were tested at 31.3, 15.6, 7.8, 3.9, 1.95 and 0.975 ppm Spinosad was tested at 500, 250, 125, 62.5, 31.3 and 15.6 ppm; Lannate was tested at 62.5, 31.3, 15.6, 7.8, 3.9 and 1.95 ppm. The leaves of castor were dipped for 15 seconds in each concentration, then left to dry in air current for about 1hr. Also, castor leaves were dipped in only distilled water and used as control. About forty larvae in two replicates of each second and fourth instar larvae of both susceptible (laboratory) and resistance (field) strains of each concentration of the tested compound and of the control were used. After 48h., the treated leaves were replaced by another untreated one and the larvae fed on it until the pupation. The jars were examined daily to determine the larval mortality. The different biological effects such larval and pupal duration, pupation and adults emergence percentage, pupal weight, adult fecundity, fertility, longevity, sex ratio were studied at the LC<sub>50</sub> values of each of the four corn pounds. Also, the observed malformations were recorded and photographed.

### 4. Statistical analysis:

The total percent of the larval mortality after 48h of the larval feeding of both second and fourth instars of both susceptible and resistance strains of the four tested compounds were recorded and corrected according to Abbott formula (Abbott, 1925). The data were then analyzed using the probit analysis (Finney, 1971) and the LC<sub>50</sub> values were estimated for each of the four tested compounds of both susceptible and resistance strains. The different biological effects such larval and pupal duration pupation and adult emergence percentage, adult fecundity, fertility, longevity, sex ratio were estimated at the LC<sub>50</sub> values. The data of the biology were statically

calculated through Excel for windows computer program to determine the F-value, P value and L.S.D) (least significant difference at 0.05 or 0.01 freedom degrees).

## RESULTS AND DISCUSSION

### 1. Toxic effect:

Data illustrated in Table (1) showed the toxic effect of the four tested compounds, Pyridalyl, Radical, Spinosad and Lannate against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of both susceptible and resistance strains of *S. littoralis*. Radical was the most toxic one against both 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of both susceptible and resistance strains. The LC<sub>50</sub> values were 1.1, 2.7 and 1.95, and 4.4 ppm for both second and fourth instar larvae of both susceptible and resistance strains, respectively. While, Pyridalyl was the second one, the LC<sub>50</sub> values were 1.8, 5 and 6.2, 9.4 ppm for both instar larvae of both strains, respectively. Whereas, Lannate was the third one; its LC<sub>50</sub> values were 3.9, 6 and 11, 19 ppm for both instar larvae of both strains, respectively. While, Spinosad was the fourth one, its LC<sub>50</sub> values were 21, 62.5 and 31.3 and 130 ppm for both instar larvae of both strains, respectively.

**Table (1): Insecticidal activity of Pyridalyl, Radical, Spinosad and Lannate against 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of lab and field strains of *Spodoptera littoralis*.**

Treatment	Strain	2 <sup>nd</sup> instar				4 <sup>th</sup> instar			
		LC <sub>50</sub> values ppm	Slope function	95% confidence limit		LC <sub>50</sub> values ppm	Slope function	95% confidence limit	
				Upper	Lower			Upper	Lower
Pyridalyl,	Lab	1.8	5.625	2.4	1.3	5.0	2.96	8.9	2.8
	Field	6.2	2.95	10.5	3.7	9.4	2.63	10.3	8.6
Radical,	Lab	1.1	2.19	1.7	0.7	2.7	2.934	2.97	2.46
	Field	1.95	2.639	2.34	1.625	4.4	2.944	5.3	3.7
Spinosad	Lab	21.0	3.8	29.4	15	62.5	4.398	112.5	34.7
	Field	31.3	4.63	62.4	15.7	130	4.565	195.0	86.7
Lannate	Lab	3.9	5.145	7.7	2.0	6	3.792	8.4	4.3
	Field	11	3.365	18.7	6.5	19	3.9	41.8	8.6

These results are agreement with those obtained by Grove and Bovington (2008) who proved the toxic activity of thiocyno radical through a ketomethylene group due to a lipid soluble hydrocarbon residue gives rise to knock-down activity. They mentioned that the most active  $\alpha$ -thiocyanolcetones R.CO.CH<sub>2</sub>.SCN and thiocynoacetates R.O.CO.CH<sub>2</sub>.SCN are too irritant to the eyes and nose for inclusion in domestic fly-sprays. Also, Temarak (2007) showed that a radiant 12 SC (new generation) of Spinosad was 7 times stronger than Spintor 24 SC (old generation) to control of egg masses of *S. littoralis* in laboratory tests based on the LC<sub>50</sub> values. He found that the radiant 12 SC was 5 times stronger (it was active at 5.76 ppm) than the Spintor 24 SC (it was active at 28.8) in the field. This is similar to the results obtained by Hilal and Oktay (2006) tested the susceptibility of the field strain of third instar larvae of the cotton leaf worm, *S. littoralis* as compared to

the susceptible strain (S) at the lethal dose using the leaf dip method. They recorded the LC<sub>50</sub> values for field and susceptible strains were 43.691 and 10.037 ppm, respectively, thus, he mentioned that the field strain was approximately 4.4-fold less sensitive than the susceptible strain and suggests that Spinosad is potentially important in the control of *S. littoralis*. Isayama *et al.* (2005) mentioned that the potency of Pyridalyl was highly effective against all development stages (2<sup>nd</sup> to 6<sup>th</sup> instar larvae) of *S. littoralis*. Also, Shigeru *et al.* (2004) observed the insecticidal action of Pyridalyl at various dosages against *S. littoralis* larvae. They found that larvae treated with 100 mg/larva and higher dosages were killed within 6 hr without any conspicuous symptoms, while the larvae treated with 25 mg/larva and lower dosages showed unique symptoms similar to scar burns at the site treated with Pyridalyl after molting. They reported that such symptoms caused interference with metamorphosis, would suppress populations of *S. littoralis* at lower dose rates). Cook *et al.* (2004) mentioned that the LC<sub>50</sub> values of indoxacarb and Pyridalyl for beet armyworm and fall armyworm exceeded the highest concentrations tested (100-200 µg/vial) in the adult vial test. They found that the dose-mortality values of indoxacarb and Pyridalyl were higher than dis-criminating concentrations of cypermethrin, methomyl, profenofos and endosulfan used in the adult vial test for monitoring tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Heliothis zea* (Boddic). Also, Temarak (2003a) found that the field strain of the cotton leaf worm *S. littoralis* (known to be tolerant or resistant to most of the conventional insecticides) was to be more susceptible to Spinosad (Spintor 24 SC) than the laboratory strain (known as susceptible to conventional insecticides). Moulton *et al.* (1999) recorded the LC<sub>50</sub> values of field populations ranged from 0.6 to 14 µg Spinosad/ml. They mentioned that field populations were 3.0 to 70-fold less susceptible to Spinosad than was a susceptible reference population. David *et al.* (1996) reported that the two formulations of Spinosad, NAF-85 and NAF-127 were effective for control of black cutworm, *Agrotis ipsilon* and Sod webworms, *Agrotis palustris*, the NAF-85 was active at 15 ppm, while NAF-127 was active at 8 ppm. Kassem *et al.* (1986) found that Methomyl (Nudrin 24.1%L and Lannate 90% SP) was the most effective among the tested insecticides (Fenvalerate 20%, Fenitrothion 50%, Carbaryl 85%, Profenofos 72% and Dimilin 25%) against *S. littoralis*, *E. insulana* and *P. gossypella*. They mentioned that the mixtures of methomyl with Fenitrothion increased the initial mortality of *S. littoralis* and reduced infestation by *E. insulana* and *P. gossypella* compared with treatments with either compound alone. While the methomyl mixtures with Carbaryl, diflubezuron, Profenofos or Fenitrothion did not increase their efficacy compared with that of each insecticide alone.

## **2. Latent effect:**

### **2.1. Larval and pupal periods:**

Data in Tables (2 and 3) indicated that the larval treatment of both second and fourth instars of the field (resistance strain) and laboratory strains (susceptible one) with Pyridalyl at LC<sub>50</sub> values had the strongest effect on the larval duration, it highly significantly ( $p < 0.01$ ) increased the larval duration to average 25, 21 and 23, 18 days, of the two instars of both strains, respectively, as compared with 19, 9.5 and 16.3, 8.8 days, respectively, of

control. Also, the treatment of the lab strain of the fourth instar with Radical and Lannate induced highly significantly ( $p < 0.01$ ) increase in the larval duration to average 21 and 22d, respectively, as compared with 6.3 d of control. Whereas, the treatment of the second instar larvae of lab and field strains with Radical, Spinosad, Lannate caused significant ( $p < 0.05$ ) increase in the larval duration to average 24.3, 14.3; 23.3, 14 and 24, 14 days of both strains, respectively, as compared with 19 and 9.5 d of control, respectively. While, the treatment of the field of the fourth instar with Radical and the lab strain of the same instar with Spinosad gave none significant increase in the larval duration, it averaged 12.3 and 19.3 d, as compared to control (8, 8 and 16.3 d, respectively).

Tables (2 and 3) showed that the treatment of the second instar of field strain with Pyridalyl had highest effect on the pupal duration, it highly significantly ( $p < 0.01$ ) increased the pupal duration to average 13.8 d, as compared with 8.8 d of the check. While the larval treatment of the fourth instar of same strain with the same compound induced significantly ( $p < 0.05$ ) increase in the pupal duration to average 12.3 d, as compared with 7.5 d of control. However, the treatment of second instar of lab and field strains with Radical and of the second and fourth instar of field strain with Spinosad; while the second and fourth instars of lab strain with Lannate significantly ( $p < 0.05$ ) increased the pupal duration to average 15.3, 11.5; 12.5, 11.8 and 14, 12.5 d compared with 10.5, 8.8 and 10.3, 7.5 d of the second and fourth instars of the lab and field strains, respectively of control. Whereas, both second and fourth instars of the lab strain with Pyridalyl; whereas the fourth instar of the lab and field strains with Radical and both second and fourth of lab strain with Spinosad with the second and fourth instar of the field strain with Lannate gave none significant increase in the pupal duration to average 12.3, 11; 11.5, 9.8; 13.3, 10.8 and 10.5, 9.3, respectively as compared with control (10.5, 8.8 and 10.3, 7.5d of both instars of the two strains, respectively).

These results are agreement with those obtained by Ahmed (2004) who mentioned that the larval period was elongated and the pupal period shorted for the new hatched larvae of pink and spiny bollworms (laboratory and field strains) treated with the higher concentrations of Spinosad when compared with untreated larvae. Also, Ivan and Jesus (2000) demonstrated that cotton treated with Spinosad in Texas had fewer damaging bollworm and budworm larvae than plots treated with the other pesticides and they suggested that Spinosad prevented small larvae from becoming larger and more damaging.

## **2.2. Pupation and adult emergence:**

Data in Tables (2 and 3) demonstrated that the treatment of the second instar larvae of both lab and field strains with the four tested compounds, Pyridalyl, Radical, Spinosad and Lannate and also of the fourth instar of the two strains of the with both Pyridalyl and Radical at the  $LC_{50}$  values, caused highly significantly ( $p < 0.01$ ) reduction of the pupation percentages as compared control. The pupation ranged from 51.7-57.7 and 53-60% of the second instar for the lab and field strains, respectively, treated with the four tested compounds as compared to that of the check (100% pupation of both strains) and also, the treatment of the fourth instar of the lab

and field strains with both Pyridalyl and Radical caused highly significantly ( $p < 0.01$ ) decrease in the pupation to average 58, 60.7 and 59.7, 62% of the second and fourth instars of both strains treated with the two compounds, respectively compared with control (100%). However, the larval treatment of the fourth instar of lab and field strain with Spinosad and of the field strain with Lannate induced significant ( $p < 0.05$ ) decrease in the pupation to average 63.3, 68.3 and 64.7%, respectively when compared with control (100%).

Data in Tables (2 & 3) showed that the treatment of the second and fourth instars larvae of both lab and field strains with the four tested compounds, Pyridalyl, Radical, Spinosad and Lannate at  $LC_{50}$  values, highly significantly ( $p < 0.01$ ) reduced the adult emergence percentages when compared that of the check, it ranged from 52.8 to 62.7 and 60 to 66.3% of the second instar of the lab and field strains, respectively treated with the four tested compounds as compared to 100% of control and it ranged from 57-67 and 72-75% of the fourth instar of the lab and field strains, respectively, treated with the four tested compound when compared with control (100%).

These results are in agreement with those obtained by Ahmed (2004) who found that the average percentage of pupations and adult emergence for pink and spiny bollworms gradually decreased with increasing concentrations of the tested compounds (Agerin, Dipl 2X, Naturalis L, Spinosad) in laboratory and field strains, respectively. Also, results obtained by Abdel-Rahim (2002) who recorded that the larval treatment of *A. ipsilon* with *A. maritima* extract induced the highest reduction in the adult emergence by a contact method. Also, Abo-El-Ghar *et al.* (1994) demonstrated a decrease in the adult emergence of *A. ipsilon*, 4<sup>th</sup> instar larvae treated with petroleum ether extracts of *L. cylindrica*, *A. najus*, *C. elegans* and *V. rosea* compared with control

### **2.3. The pupal weight:**

The treatment of the second instar larvae of the field strain with Pyridalyl, Radical and Lannate highly significantly ( $p < 0.01$ ) reduced the weight of the resulting pupae to average 160, 182 and 184 mg, as compared with 377 mg pupal weight of control. While the treatment of second instar of the lab strain with Pyridalyl, Radical and the lab and field strains with Spinosad significantly ( $p < 0.05$ ) decreased the pupal weight to 258; 262 and 267, 264 mg, respectively compared with 377 and 390 mg pupal weight of the second instar of the lab and field strains of control. However, the larval treatment of fourth instar of both strains did not give any significant decrease in the pupal weight, as compared to control (Tables 2 and 3).

These results are similar with that obtained by Ahmed (2004) who recorded that the Spinosad, Agerin and Cascade treatments caused a significant gradual reduction in pupal weight of pink and spiny bollworms in the laboratory and field strains, while Tagetes oil was the least effective one. Abdel-Rahim (2002) reported that the larval treatment of *A. ipsilon* with *C. fistula*, *A. maritima* and *T. tipu* extracts decreased the pupal weight of the resulting pupae.

Table (2): Biological activity of Pyridalyi, Radical, Spinosad and Lannate against the 2<sup>nd</sup> instar larvae of lab and field strains of *Spodoptera littoralis* at the LC<sub>50</sub> values.

Treatment	Strain	Larval duration (days)±SD	Pupation%		Pupal duration (days)±SD	Pupal weight (mg)±SD	%Adult emergence ±SD	
			Normal Mean±SD	Malformation %			Normal	Malformation%
Pyridalyi,	Lab	25±3.3**	57.7±5**	16.7**	12.3±3ns	258±39*	61±1.1**	27.3**
	Field	21± 6.3**	60±8.2**	15.4**	13.8±1**	160±35**	63±0.3**	26.2**
Radical,	Lab	24.3±2.5*	57.0±5**	8.1*	15.3±3*	262±62*	52.7±3**	20**
	Field	14.3±1.3*	58.3±4**	6.7ns	11.5±0.9*	182±51**	60±11**	8.1*
Spinosad	Lab	23.3±1.3*	51.7±9**	6.3ns	13.3±1ns	267±59*	62.7±13**	25.6**
	Field	14.0±2.1*	53.0±5**	2.2ns	12.5±3*	264±5.1*	66.3±4**	10*
Lannate	Lab	24.0±2.8*	56.1±3**	30**	14.0±1.7*	291±32ns	58.4±12**	10*
	Field	14.0±1.0*	59.0±4.8**	20**	10.5±1ns	184±116**	64±8**	20**
Control	Lab	19.0±2	100	0	10.5±0.5	390±46	100	0
	Field	9.5±1.5	100	0	8.8±0.4	377±44	100	0
F value	Lab	20.573	183.3	240.5	15.40	19.130	317.9	78.22
	Field	73.9	139.6	70.56	46.837	32.624	126.2	186.6
P value	Lab	0.0297	0.00793	0.0193	0.0425	0.0231	0.00072	0.0073
	Field	0.0227	0.007973	0.0026	0.00379	0.0288	0.00658	0.0053
LSD at 0.05	Lab	3.6	16.5	10.9	3.35	93.1	17.7	17.3
	Field	4.4	17.0	0.725	2.88	130.9	14.6	5.4
LSD at 0.01	Lab	6.6	38.1	25.1	6.125	170.9	40.5	39.98
	Field	8.1	39.2	1.675	5.28	240.5	33.6	12.5

\*\* Highly Significant (p&lt;0.01)

\* Significant (p&lt;0.05)

SD = Standard deviation

LSD = Least significant difference

Lab = Laboratory strain

Table (3): Biological activity of Pyridalyl, Radical, Spinosad and Lannate against the 4<sup>th</sup> instar larvae of lab and field strains of *Spodoptera littoralis* at the LC<sub>50</sub> values.

Treatment	Strain	Larval duration (days)±SD	Pupation%		Pupal duration (days)±SD	Pupal weight (mg)±SD	%Adult emergence ±SD	
			Normal Mean±SD	Malformation%			Normal	Malformation%
Pyridalyl,	Lab	23±1.5**	54±4.6**	13.2**	11±0.7ns	316±63ns	63±5**	25**
	Field	18± 5.6**	60.7±4.2**	10.8**	12.3±1.8*	181±50ns	74±1.4**	22**
Radical,	Lab	21±0.9**	59.7±6.9**	6.7ns	11.5±2ns	362±91ns	57±1**	8.3*
	Field	12.3±2ns	62±5**	5.9ns	9.8±1.8ns	229±41ns	75±25**	4.8ns
Spinosad	Lab	19.3±3ns	63.3±10*	3.1ns	10.8±0.4ns	333±28ns	67±1.5**	23.1**
	Field	12.5±1.5*	68.3±8.5*	2.2ns	11.8±3*	284±34ns	72±**	3.3ns
Lannate	Lab	22±2**	57.3±5.3*	18.7**	12.5±1.7*	355±34ns	61±7**	6.7ns
	Field	13.3±1.3*	64.7±6.9*	16**	9.3±1.3ns	280±34ns	75±1**	24.5**
Control	Lab	16.3±1.3	100	0	10.3±0.4	373±56	100	0
	Field	8.8±1.3	100	0	7.5±0.9	285±35	100	0
F value	Lab	123.3	127.09	44.81	3.6793	3.04629	1936.05	280.5
	Field	17.4	91.067	9.4205	84.08	3.3361	1816.59	130.4
P value	Lab	0.01573	0.0154	0.00156	0.05767	0.0487	0.00433	0.00648
	Field	0.0384	0.01656	0.02048	0.01931	0.5217	0.00133	0.00421
LSD at 0.05	Lab	2.87	19.5	1.95	3.6	106.6	8.75	4.7
	Field	5.06	18.55	2.6	2.45	136.9	2.457	4.9
LSD at 0.01	Lab	4.97	44.975	4.5	6.6	195.8	20.17	10.8
	Field	9.0275	42.775	5.97	4.5	251.32	5.63	11.2

\*\* Highly Significant (p<0.01)

LSD = Least significant difference

\* Significant (p<0.05)

Lab = Laboratory strain

SD = Standard deviation

#### **2.4. Morphogenetic effects:**

Data obtained in (Tables 2&3) showed that the treatment of the second and fourth instars larvae of both lab and field strains of *S. littoralis* with PyridalyI and Lannate induced highly significant ( $p<0.0$ ) increase in the pupal malformations to average 16.7, 15.4 and 30, 20% of the second instar of both strains, respectively treated with the two compounds, respectively as compared to 0% of control and it reached to 13.2, 10.8 and 18.7 and 16% of the fourth instar of both strains, respectively treated with the same two compounds, respectively, as compared to control (0%). Whereas, the larval treatment of second instar of lab strain with Radical induced significant ( $p<0.05$ ) increase in the pupal malformations was 8.1%. While, the larval treatment of the fourth instar of both lab and field strains with Radical and of the second instar of the field strain with the same compound and also, of the second and fourth instars of lab and field strains with Spinosad gave non significant increase in the pupal malformations as respect to control.

With regarded to the adult malformations (Tables 2 & 3), it was found that the treatment of the second and fourth instar larvae of both lab and field strains of *S. littoralis* with PyridalyI and of the second instar of lab strain with Radical of the second and fourth instars of lab strain of with Spinosad and second and fourth instar of field strain with Lannate induced highly significant ( $p<0.01$ ) increase in the adult malformations to reach and 27.3, 26.2 and 25, 22; 20; 25.6 and 23.1 and 20, 24.5%, respectively, when compared with control (0%). However, the treatment of the second instar of field strain and of the fourth instars of lab strains of with Radical and of the second instar of field strain with Spinosad and of the second instar of the lab strain of with Lannate caused significant ( $p<0.05$ ) increase in the adult malformations reached to 8.1 and 8.3; 10 and 10, respectively when compared with control (0%). While, the treatment of the fourth instar of the field strain with Radical and Spinosad and of lab strain with Lannate gave non significant increase in the adult malformations compared with control.

These results are similar to that obtained by Ahmed (2004) reported that Spinosad gave malformed pupal and adults in both laboratory and field strains of both Pink and Spiny bollworms. Abdel-Rahim (2002) indicated that *A. maritima* extract was the most potent extract in inducing noticeable malformations in both pupae and adult stages of *A. ipsilon* that treated as 4<sup>th</sup> instar with this extract by a contact method. Also, Abo-El-Ghar *et al.* (1994) obtained the same results on the *S. littoralis*.

Malformations of *S. littoralis* pupae resulting from the larval treatment of 2<sup>nd</sup> and 4<sup>th</sup> instars of both field and lab strains with both PyridalyI and Radical in the present work mostly appeared a malformed pre-pupa failed to cast the old cuticle with complete blackening of the body leading to death (Figs 1, 2) or larval-pupal monstrosity with larval cuticle patches, head capsule and thoracic legs; posterior half of the body has the pupal properties (Figs 3, 4, 5) or pupa with vestiture of larval skin undersized pupa (Fig. 6 ), while, the moth malformations showing body with poorly developed and twisted wings (Figs 7, 8, 9, 10 and 11). However, the treatment of both of 2<sup>nd</sup> and 4<sup>th</sup> instars of field and lab strains with Spinosad, appeared as abnormal pupae showing body shrinkage (Fig. 12) or larval- pupal monstrosity with

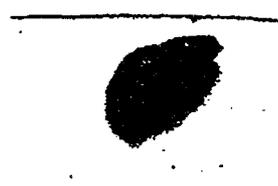
larval cuticle patches, head capsule and thoracic legs; posterior half of the body has the pupal properties (Fig. 13) and the moth malformations appeared with body bear malformed twisted wings (Fig. 14, 15, 16). Also, the treatment of both 2<sup>nd</sup> and 4<sup>th</sup> instars of field and lab strains Figs (12 to 16): Pupae and adults Malformations of *S. littoralis*, resulting from the larval treatment of the field and lab strains of the 2<sup>nd</sup> and 4<sup>th</sup> instars with the Spinosad.



**Figs (1, 2):** mostly appeared as a malformation pre-pupa failed cast the old cuticle with complete blackening of the body leading to death.



**Figs (3, 4, 5):** larval-pupal monstrosity with larval cuticle patches, head capsule and thoracic legs; posterior half of the body has the pupal properties.



**Fig. (6):** Pupa with vestiture of larval skin undersized pupa.



**Figs (7, 8, 9, 10, 11):** Moth malformations showing body with poorly developed and twisted wings.

**Figs (1 to 11):** Pupae and adults Malformations of *S. littoralis*, resulting from the larval treatment of both the field and lab strains of the 2<sup>nd</sup> and 4<sup>th</sup> instars with the both Pyridalyl and Radical.



**Fig. (12):** Abnormal pupae showing body shrinkage.



**Fig. (13):** Larval-pupal monstrosity with larval patches, head capsule and thoracic legs; posterior half of the body has the pupal properties.



**Figs (14, 15, 16):** Moth malformations appeared with body bear malformed twisted wings.



(Fig. 17,18) : mostly appeared as a malformed pre-pupa with complete blackening of the body leading to death

(Fig .19 ) or larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs; posterior half of the body has the pupal properties

(Fig. 20,21,22 ) malformed adults had abnormal body and wings.

**Figs. (17 to 22): Pupae and adults Malformations of *S .littoralis*, resulting from the larval treatment of both the field and lab strains of the 2<sup>nd</sup> and 4<sup>th</sup> instars with the Lannate.**



**Figs. (23, 24 ) :Normal pupae and adults**

With Lannate showed as a malformed pre-pupae with complete blackening of the body leading to death (Figs 17, 18) or larval-pupal intermediates with larval cuticle patches, head capsule and thoracic legs; posterior half of the body has the pupal properties (Fig. 19) while, the malformed adults had abnormal body and wings (Figs 20, 21, 22) as compared to normal pupae and adults (Figs 23, 24).

#### **2.5. Adult fecundity and fertility:**

Data presented in Table (4) indicated that the treatment of the fourth instar of lab and field strains of *S. littoralis* with PyridalyI and field strains of the same instar with Radical, Spinosad and Lannate, highly significantly ( $p<0.01$ ) reduced the adult fecundity to average 15, 62.3; 66; 30 and 80 eggs/f, respectively, compared with 572.3 and 294.3 eggs/f of control. However, the treatment of lab strain of the same instar with Spinosad and Lannate, significant ( $p<0.05$ ) decreased the adult fecundity to average 105 and 140 eggs/f, respectively, as compared to control, while the larval treatment of the fourth instar of lab strain with Radical gave non significant reduction in the adult fecundity as compared to control.

Likewise, the treatment of the fourth instar of both lab and field strains of *S. littoralis* with PyridalyI and field strains of the same instar with Radical, Spinosad and Lannate were highly significantly ( $p<0.01$ ) reduced the adult futility to average 4 and 43: 45.7; 21.3; and 52.3 eggs/f, respectively

when compared with 536.3 and 283.3 eggs/f, for control, respectively. However, the treatment of lab strain of the same instar with Spinosad and Lannate, significant ( $p < 0.05$ ) decreased the adult fertility to average 53 and 102 eggs/f, respectively, as compared to control (536.3 and 283.3 eggs/f, respectively), while the larval treatment of the fourth instar of lab strain with

**Table (4): Biological activity of Pyridalyl, Radical, Spinosad and Lannate against the adults of *Spodoptera littoralis* treated as 4<sup>th</sup> instar larvae of lab and field strains the LC<sub>50</sub> values.**

Treatment	Strain	Fecundity	Fertility	Longevity	Adult ratio (%)	
		Mean±SD (eggs/f)	Mean±SD (eggs/f)	Mean±SD (days)	Male	Female
Pyridalyl,	Lab	15±5**	4±2.2**	3.3±0.8**	58.0	42.0
	Field	62.3±2.1**	43±1.6**	4.4±1.6**	51.8	48.2
Radical,	Lab	2.35±12.2ns	197±2.1ns	5.8±1.3*	55	45
	Field	66±3.7**	45.7±3.3**	5.3±4.3*	46.7	53.3
Spinosad	Lab	105±7.3*	53±5*	7.3±0.4ns	58.3	41.7
	Field	30±5**	21.3±2.1**	6±2.1ns	43.9	56.1
Lannate	Lab	140±8.2*	102±4.9*	5.3±1.1*	50	50
	Field	80±5**	52.3±2.1**	5±1.2**	50.6	49.4
Control	Lab	572.3±129	536.3±113	9.8±2.3	50	50
	Field	294.3±28	283.3±27	8.8±2.2	50	50
F value	Lab	26.701	30.842	15.5985		
	Field	163.586	174.3	35.526		
P value	Lab	0.0375	0.0342	0.02956		
	Field	0.006717	0.00581	0.00945		
LSD at 0.05	Lab	408.8	350.8	4.175		
	Field	81.625	79.655	2		
LSD at 0.01	Lab	942.97	808.98	7.7		
	Field	188.3	183.71	3.7		

\*\* Highly Significant ( $p < 0.01$ )

\* Significant ( $p < 0.05$ )

SD = Standard deviation

LSD = Least significant difference

Lab = Laboratory strain

Radical gave non significant reduction in the adult fecundity, as compared to control.

These results are agreement with those obtained by Pineda *et al.* (2007) who reported that Spinosad and methoxyfenozide reduced in a dose-dependent manner the fecundity and fertility of *S. littoralis* adult when treated oral and residually. Also, Ahmed (2004) reported that the number of eggs produced by spiny bollworm females resulting from the treated larvae with the Spinosad for laboratory and field strains larvae was decreased per female as compared with the control. He added that the average% hatchability for the eggs of treated females in both strains was decreased in both of the pink and spiny bollworms as compared with control. Whereas, Hashem *et al.* (1994) recorded a reduction in both fecundity and fertility as a result of abnormalities in the ovaries of *S. littoralis* adults fed as 4<sup>th</sup> instar larvae on artificial diet mixed with 2% of fruit extract of *M. azedarach* for 72h.

### **2.6. Adult longevity:**

Data obtained in Table (4) showed that the treatment of the fourth instar of both field and lab strains of *S. littoralis* with Pyridalyl and of the field strain of the same instar with Lannate, highly significantly ( $p < 0.01$ ) reduced the adult longevity to average (3.3 & 4.4) and (5.3 & 5) days, respectively, as compared with 9.8 and 8.8 days, for control, respectively, adult longevity of control. While, the larval treatment of the fourth instar of both lab and field strains with Radical and of the lab strains of the same instar with Lannate, significant ( $p < 0.05$ ) decreased the adult longevity lasted (5.8 & 5.3) and (5.3 & 5.0) days, respectively, as compared with control. Whereas, the treatment of the fourth instar of both lab and field strains with Spinosad gave none significant decrease in the adult longevity to average 7.3 and 6.2 days, respectively

These results are in agreement with that obtained by Abdel-Rahim (2002) who demonstrated a significant decrease in the adult longevity of *A. ipsilon* by the larval treatment of 4<sup>th</sup> instar with *A. maritima* and *T. tipu* extracts by a contact method.

### **2.7. Adult sex ratio:**

Data obtained in Table (4) demonstrated that the larval treatment of the fourth instar of lab strain with both Pyridalyl and Spinosad had the highest effect in the sex ratio shifting of adult males and females, it induced males increase and females decrease, as respect to that of control, it reached 58:42 and 58.3:41.7% of both adult males: females, respectively, as compared with 50:50 of control, while the treatment of the instar of the same strain with Lannate had the least effect on sex ratio, it recorded the same ratios of control (50:50%). However, the treatment the fourth instar of field strain with Spinosad had the contrast effect in adult males decrease and female increase to reach 43.9:56.1% of both adult males: females, respectively, as compared to 50:50 of control, while the treatment of the fourth instar of the same strain of the with Radical had the next effect on the sex ratio it reached 46.7:53.3% of both adult males: females, respectively, as compared with control (50:50%), while the treatment of the instar the same strain with both Pyridalyl and Lannate had the least effect, it recorded approximately ratios of that of control.

### **Conclusion:**

The results of the present work demonstrated that the four tested novel compounds were effective against the survival of the 2<sup>nd</sup> and 4<sup>th</sup> instar larvae of both susceptible and resistance strains of *S. littoralis*. Radical had the highest efficacy against the survival of the insect, while Pyridalyl had the most potent against the studied insect biology. Other investigations proved that Pyridalyl was less harmful than existing insecticides to various beneficial arthropods, so it should provide an important tool in IPM and insecticidal management programmes for control lepidopterous pests on cotton and vegetables, without phytotoxicity (Sakamoto *et al.*, 2004). Also, Spinosad had a unique mode of action coupled with a high degree of activity on targeted pests and low toxicity to non-target organisms (including many beneficial arthropods). It possess rapid efficacy competitive with the best synthetic standards and consider an excellent new tool for management of insect pests

(Gary *et al.*, 1999). Thus, these compounds were effective if applied at the obtained lethal concentrations within the integrated control program of this pest for reduction of classic synthetic insecticides use for serious effects on the environment.

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**تأثيرات مقارنة لسمية ثلاث مركبات إبادية حديثة البيردال، الرديكال، الأسبينوساد ومبيد تقليدي اللانثيت ضد يرقات العمر الثاني والرابع للسلالة المعملية والحقلية لدودة ورق القطن الكبرى**

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أجريت هذه الدراسة بغرض مقارنة التأثير السمي لثلاث مركبات جديدة وهي البيردال، الرديكال، الأسبينوساد ومبيد تقليدي اللانثيت تحت الظروف المعملية. غذيت يرقات العمر الثاني والرابع للسلالة المعملية والحقلية المتحصل عليها في صورة لقطع للفقس من الحقل لمدة 48 ساعة علي ورق خروع غطس لمدة 15 ثانية في سلسلة تركيزات لكل مركب من المركبات الأربعة المختبرة لتحديد قيم التركيز النصفى لكل مركب. أوضحت النتائج أن مركب الرديكال له التأثير الأقوي والغالب ضد كل من العمر الثاني والرابع للسلالتين المعملية والحقلية حيث بلغت قيمة التركيز النصفى القاتل له 1.95، 4.4، 1.1 و 2.7 للسلالتين الحساسة والمقاومة للعمرين الثاني والرابع بالتتالي وكان مركب البيردال التأثير الثاني حيث بلغت قيمة التركيز النصفى له 6.2، 9.4، 1.8 و 5 للسلالتين للعمرين علي الترتيب. بينما كان لمركب اللانثيت التأثير الثالث حيث بلغت قيمة التركيز النصفى له 11، 19، 3.9، 6 وبلغت قيمة التركيز النصفى لمركب الأسبينوساد 31.3، 130، 21، 62.5 لكل من العمرين للسلالتين بالتتالي. تأثرت المعايير البيولوجية لليرقات مع المعاملة لكل من العمرين الثاني والرابع للسلالتين المعملية والحقلية بالمركبات الأربعة. التأثير تنوع مع إختلاف السلالة والعمر اليرقي ومع المركب المختبر وبناء علي ذلك كان لمعاملة العمرين لكل من السلالتين بالمركبات الأربعة المختبرة التأثير الأقوي في خفض نسب التعذير والإختراق للحشرة الكاملة في كل من المعاملات الأربعة المختبرة بينما كان لمركب البيردال التأثير الأقوي في زيادة العمر اليرقي والتشوهات العذرية والحشرية ونقص في عدد البيض وخصوبته والعمر للحشرة الكاملة وذلك في حالة المعاملة اليرقية لكل من العمرين للسلالتين المعملية والحقلية بهذا المركب. كما كان له التأثير الأقوي في زيادة البقاء العذري ونقص الوزن العذري وذلك في حالة معاملة العمر الثاني للسلالة الحقلية بهذا المركب كما ثبتت فعاليته في زيادة نسب الذكور ونقص نسب الإناث بالمقارنة بالكنترول وذلك في حالة معاملة العمر الرابع للسلالة المعملية بهذا المركب في حين كان لمركب الرديكال التأثير الفعال في خفض عدد البيض وخصوبته وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب كما كان له التأثير الأعلي علي وزن العذاري في حالة معاملة العمر الثاني للسلالة الحقلية بهذا المركب. كما انه ادي الي زيادة العمر اليرقي والتشوهات الحشرية في حالة معاملة العمر الثاني والرابع للسلالة المعملية بهذا المركب وكما انه أثر في النسب الجنسية فهو ادي الي نقص نسب الذكور وزيادة في عدد الإناث بالنسبة للكنترول وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب. كما كان لمركب الأسبينوساد التأثير الاقوي للنظر في نقص عدد البيض وخصوبته وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب وكما انه أعطي زيادة في نسب التشوهات الحشرية وذلك في حالة معاملة العمر الثاني والرابع لهذا المركب كما أنه نود نسب الذكور ونقص نسب الإناث الحشرية وذلك في حالة معاملة العمر الرابع للسلالة المعملية بهذا المركب. في حين وجد لمركب اللانثيت التأثير الأكبر في زيادة التشوهات العذرية وذلك في حالة معاملة العمرين الثاني لكل من السلالتين بهذا المركب وكان له التأثير الأقوي في نقص الوزن العذري وزيادة التشوهات الحشرية وذلك في حالة معاملة العمر الثاني أو العمر الثاني والرابع للسلالة الحقلية بهذا المركب كما كان له أثر علي عدد البيض وخصوبته وعمر الفراشات وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب كما أنه نود البقاء اليرقي وذلك في حالة معاملة العمر الرابع للسلالة المعملية بهذا المركب.