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 INSTAR LARVAE OF COTTON LEAFWORM, *Spodoptera Littoralis* (BOISD.)

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Abstract

The toxic activity of three novel compounds(Pyridayl, Radical, Spinosad) and conventional insecticide(Lannate)against the second and fourth instars of the Lab. and Field strains of *Spodoptera littoralis.* was evaluated under laboratory conditions through determination their LC50 values.` Radical was the most toxic one against both of 2nd and 4th instar larvae of the lab. and field strains . The LC_{50}values were 1.1 , 2.7 and 1.95 , 4.4 ppm for both second and fourth instar larvae of the two lab. and field strains , respectively . Pyridalyl was the second one with the LC_{50}values of 1.8 , 5 and 6.2 , 9.4 ppm for the two instars of both strains, respectively. Lannate was the third one ,its LC_{50}values were 3.9, 6 and 11,19ppm for both instars of the strains , respectively. While , Spinosad was the fourth one, its LC_{50}values were 21, 62.5 and 31.3 and 130 ppm for both instars of the strains, respectively. All the treated larvae were biologically affected by the four tested compounds . The effect varied according to the strain , larval instar and tested compound. Therefore ,the treated larvae were significantly affected and resulted in decreased pupation and adult emergence percentages. While , Pyridalyl treatment had the strongest 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 , 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, it induced the males increase and females decrease, as respect to control, with the treatment of fourth instar of lab. strain . Whereas, Radical ,had the greatest effect on adult fecundity and fertility with the of fourth instar treatment of field strain .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 .However, 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. Spinosad had the highest effect on both adult fecundity and fertility with the treated fourth instar of field strain and it induced the strongest effect of adult malformations with the treatment of both instars of lab. strain , and it affect the sex ratio, lead to males increase and females decrease with the treatment of fourth instar of lab .strain. Lannate , had the highest effect on

pupal malformations with the larval treatment of both instars of both strains ,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 ,also, it was effective on adult fecundity, fertility and longevity with the treated fourth instar of field strain and it had the strongest effect on larval duration with the treatment of the fourth instar of the lab. strain .

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd) is one of the major pests that cause a considerable damage to many of the important vegetable 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 such as the biotic novel compounds: Spinosad, Pyridalyl and Radical. Spinosad played an important role in pests control and gets its name from the microbe that produces it, a soil-dwelling bacterium called *Saccharopolyspora spinosa.* Spinosad which represents a new class of insecticides that acting by a novel mode of action (Thompson *et al.* 2000)and possesses less risk than most insecticides to mammals, birds, fish, and beneficial insects, was used for control of Lepidoptera 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 to insect pest specie, *Spodoptera litura*, 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, as a novel compound may be obtained from *Streptomyces avermitilis* was estimated as insecticide by Grove and Bovington(2008).The conventional insecticide, Lannate was used for the lepidopterous pests 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 the conventional insecticide (Lannate)against the second and fourth instar larvae of the field and laboratory strains of *S. littoralis*..

MATERIALS AND METHODS

1. The Field strains

Field strain egg masses of cotton leafworm (CLW) were collected from cotton fields at Sides Station Research , Beni-Suef 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 temp. ranged between 25– 28C 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 temp. ranged between 25 - 28 C^o 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 and supplied with a piece of cotton wetted with 10% sugar solution as feeding source for the emerged moths and branches of Tafla (*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-Materials used

2.1–Spinosad, the used spinosad (24 %SC)

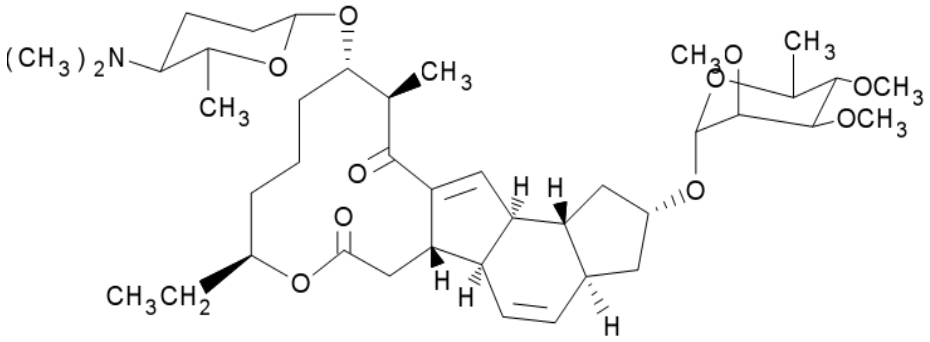
Trade name: The insecticide was introduced by Dow Agro Sciences for control Lepidopterous pests in cotton under the trade name Tracer (Thompson *et al*., 1997).

Chemical name : The name spinosad is derived from combining the characters Spinosyn A and D. The rate of application was 50 cm^3 / fed

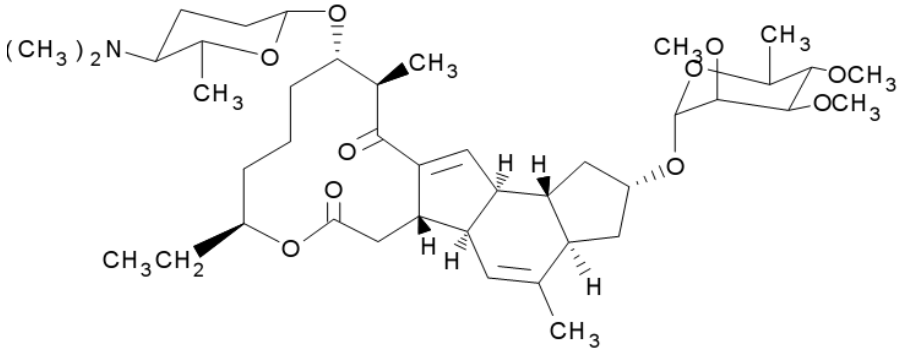
Empirical formula: Spinosyn A:C41H65NO10 , Spinosyn D:C42H67NO10

Molecular weight , Spinosyn A:731.98, Spinosyn D:745

Structure :



spinosyn A



spinosyn D

2.2-Common name (ISO name) :Pyridalyl

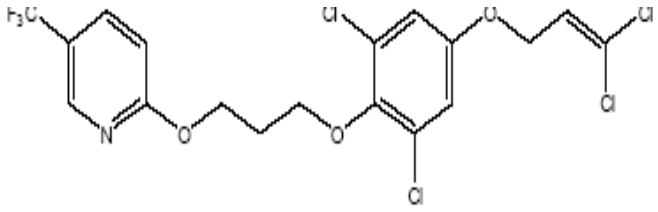
Trade name: The insecticide was introduced by Valent USA for control Lepidopterous pests in cotton under the trade name Pyridalyl (S-1812): The rate of application was 50-200g ai/ha.

Chemical name: 2,6-Dichloro-4-(3,3-dichloroallyloxy)phenyl3[5(trifluoromethy) 2-pyridyloxy]propyl ether

Molecular Formula: C18 H14 Cl4 F3 NO3

Molecular Weight: 491.12

Structure:



2.3-Common Name: Methomyl

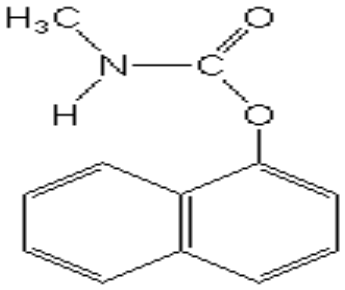
Trade names: Lannate, Lanox 216, NuBait II, Nudrin, SD 14999

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

Molecular formula: C5 H10 N2 O2 S

Molecular weight:162.20

Structure:



2.4-Radical (0.5%E.S)

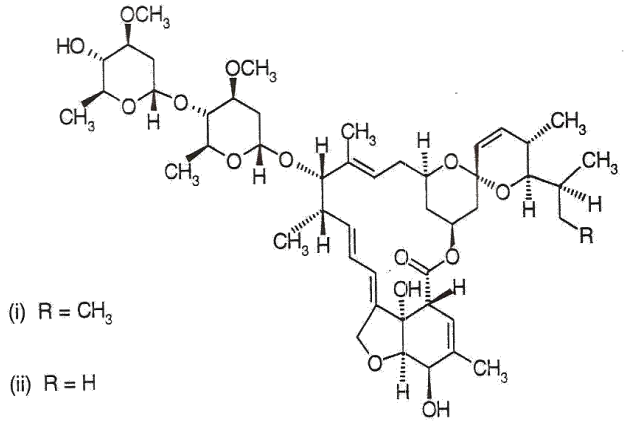
Source: It 's avermectin derivatives consist of combination of Methylamine and averment **,** El- Aserah company.

Averment: which can be obtained from Streptomyces avermitilis are referred to as A1a, A1b, A2a, A2b, B1a, B1 b, 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:C48 H72 O14

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 (p.p.m)using water as a solvent for dilution . 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 15.6ppm, Lannate was tested at 62.5, 31.3 , 15.6 ,7.8 ,3.9 and 1.95ppm. 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 resistant(field) strains for all treatments including the control were used . After 48h.,the treated leaves was replaced by another untreated ones 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 determined at the LC50 values of the four compounds. Also ,the observed malformations were recorded and photographed.

4-Statistical analysis

The total percent of the larval mortality of the four tested compounds were recorded after 48h of the larval feeding of both second and fourth instars of both susceptible and resistant strains of the four tested and corrected according to Abbott formula (Abbott, 1925). The data were then analyzed using the probit analysis (Finney, 1971) and the LC_{50} values of the four tested compounds were estimated for both susceptible and resistant 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_{50} values. The obtained 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 effects of the four tested compounds, Pyridalyl , Radical , Spinosad and Lannate against the 2nd and the 4th instar larvae of both lab. and field strains of *S. littoralis*. Radical was the most toxic one against both 2nd and 4th instar larvae of both lab. and field strains. The LC_{50}values were 1.1 , 2.7 and 1.95 , 4.4 ppm for both second and fourth instar larvae of both lab. and field strains, respectively .While, Pyridalyl was the second one, the LC_{50}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_{50}values were 3.9, 6 and 11,19ppm for both instar larvae of both strains, respectively. While , Spinosad was the fourth one, its LC_{50}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 the 2nd and 4th instar larvae of Lab. and Field strains of *Spodoptera littoralis.*

Treatment	Strain	2nd instar				4th instar			
		LC50 values P.p.m	Slope function	95% confidence limit		LC50 values P.p.m.	Slope function	95% confidence limit	
				Upper	Lower			Upper	Lower
Pyridalyl	Lab.	1.8	5.625	2.4	1.3	5	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	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	86.7
Lannate	Lab.	3.9	5.145	7.7	2	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 in agreement with those obtained by Grove and Bovington (2008)who proved that the toxic activity of thiocyano radical through a ketomethylene group due to a lipoid soluble hydrocarbon residue gives rise to knock-down activity . They mentioned that the most active α-thiocyanoketones $R.CO.CH_2.SCN$ and thiocyanoacetates R. O. CO. CH_2. 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 *Spodoptera littoralis* in laboratory tests based on the LC50

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 (2006)who 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. He recorded that the LC50 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 lab. 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 (2nd to 6th instar larvae) of *S. litura.* Also, Shigeru *et. al*(2004)observed the insecticidal action of pyridalyl at various dosages against *S. litura* larva .They found that larvae treated with 100 mg or more/larva were killed within 6 hr without any conspicuous symptoms, while the larvae treated with 25 mg or less/larva and lower 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. litura* even at lower dose rates. Cook *et al*. (2004)mentioned that the LC50 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 discriminating concentrations of cypermethrin, methomyl, profenofos, and endosulfan used in the adult vial test for monitoring tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie). 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 LC50 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, *Agrostis palustris* ,the NAF-85 was active at 15ppm ,while NAF-127 was active at 8 ppm. Kassem *et al*.(1986)found that Methomyl (Nudrin 24.1%L and Lannate90%sp)was the most effective among the tested insecticides (Fenvalerate20%, Fenitrothion 50% , Carbaryl 85%,Profenofos72% and Dimilin 25%) against *S .littoralis , E.insulana* and *P. gossyipella* .They mentioned that the mixtures of methomyl with Fenitrothion

increased the initial mortality of *S .littoralis* and reduced infestation by *E .insulana* and *P. gossyipella* 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 and laboratory strains with Pyridalyl at LC_{50} values had the strongest effect on the larval duration. The larval duration was highly significantly ($p<0.01$)increased to average 25 , 21 and 23 ,18 days, of the two instars of both strains , respectively, as compared to 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 significant($p<0.01$)increase in the larval duration to average 21 and 22 days, respectively , as compared to 16.3 days 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 to 19 and 9.5 days of control, respectively. The treatment of the fourth instar of the field and the lab. strains with Radical and Spinosad ,respectively, gave none significant increase in the larval duration, it averaged 12.3 and 19.3 days, as compared to control (8.8 and 16.3 days, respectively).

Tables (2 and 3)showed that the treatment of the second instar of field strain with Pyridalyl had the highest effect on the pupal duration, it highly significantly ($p<0.01$)increased the pupal duration to average 13.8 days,as compared to 8.8 days of the check. Also, the larval treatment of the fourth instar of same strain with the same compound induced significant ($p<0.05$) increase in the pupal duration to average 12.3d, as compared to 7.5d of control. However, the treatment of second instar of lab. and field strains with Radical significantly ($p<0.05$)increased the pupal duration to average15.3 and 11.5 , respectively, as compared to10.5 and 8.8 of control. And the second and fourth instar of field strain treated with Spinosad induced significant($p<0.05$) increase in the pupal duration to average12.5 and 11.8 days, respectively, as compared to 8.8 and 7.5 days of control. Also, the treatment of the second and fourth instars of lab. strain with Lannate significantly ($p<0.05$) increased the pupal duration to average 14 and 12.5 days, respectively, as compared to10.5 and 10.3 days of control . Whereas the treatment of both second and fourth instars of the lab. strain with Pyridalyl gave none significant increase in the pupal duration to average12.3 and 11 days, respectively, as compared to 10.5 and 10.3 days of control.

Also, the fourth instar of the lab. and field strains treated with Radical did not significantly increased the pupal duration, it averaged 11.5 and 9.8, respectively, as compared to 10.3 and 7.5 days of control. And the second and fourth instar of the lab. strain treated with Spinosad recorded none significant increase in the pupal duration to average13.3 and 10.8 days , respectively, as compared to 10.5 and 10.3of control. Also, the second and fourth instar of the field strain treated with Lannate gave none significant ($p>0.05$) increase in the pupal duration, it averaged 10.5 and 9.3 respectively ,as compared to that of the control (8.8 and 7.5 days).

Table 2. Biological activity of Pyridalyl ,Radical, Spinosad and Lannate at their LC_{50} values against the 2nd instar larvae of Lab. and Field strains of *Spodoptera littoralis*.

Treatment	Strain	Larval duration (days) ± SD	Pupation %		Pupal duration (days) ± SD	Pupal weight (mg) ±S.D	% Adult emergence ±S.D	
			Normal Mean± SD	Malfo. %			Normal	Malfo %.
Pyridalyl	Lab.	25±3.3**	57.7±5**	16.7**	12.3±3n.s	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±5**	8.1*	15.3±3*	262±62*	52.7±3**	20**
	Field	14.3±1.3*	58.3±4**	6.7n.s.	11.5±0.9*	182±51**	60±11**	8.1*
Spinosad	Lab.	23.3±1.3*	51.7±9**	6.3n.s	13.3±1n.s	267±59*	62.7±13**	25.6**
	Field	14± 2.1*	53.± 5**	2.2n.s	12.5±3 *	264±5.1*	66.3±4**	10*
Lannate	Lab.	24±2.8*	56.1±3**	30**	14±1.7*	291±32n.s	58.4±12**	10*
	Field	14± 1*	59±4.8**	20**	10.5±1n.s	184±116**	64±8**	20**
Control	Lab.	19±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	0.00973	0.0026	0.00379	0.0288	0.00658	0.0053
L.S.D.at.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
L.S.D.at.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 ($p<0.01$) * Significant ($p<0.05$)

S.D.=Standard deviation Malfo.= Malformation%

L.S.D.= Least significant difference Lab.=Laboratory strain

n. s=none Significant ($p>0.05$)

These results are in 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.

Table 3 . Biological activity of Pyridalyl ,Radical, Spinosad and Lannate at their LC_{50} values against the 4th instar larvae of Lab. and Field strains of *Spodoptera littoralis*

Treatment	Strain	Larval duration (days) ± SD	Pupation %		Pupal duration (days) ± SD	Pupal weight (mg) ±S.D	% Adult emergence ±S.D	
			Normal Mean± SD	Malfo. %			Normal	Malfo. %
Pyridalyl	Lab.	23±1.5**	58± 4.6**	13.2**	11±0.7n.s	316±63n.s	63±5**	25**
	Field	18±5.6**	60.7±4.2**	10.8**	12.3±1.8*	181±50n.s	74±1.4**	22**
Radical	Lab.	21±0.9**	59.7±6.9**	6.7n.s	11.5±2n.s	362±91n.s	57±1**	8.3*
	Field	12.3±2n.s	62±5**	5.9n.s	9.8±1.8n.s	229±41n.s	75±25**	4.8n.s
Spinosad	Lab.	19.3±3n.s	63.3±10*	3.1n.s	10.8±0.4n.s	333±28n.s	67± 1.5**	23.1**
	Field	12.5±1.5*	68.3±8.5*	2.2n.s	11.8 ±3*	284±53n.s	72±**	3.3n.s
Lannate	Lab.	22±2**	57.3±5.3**	18.7**	12.5±1.7*	355±34n.s	61±7**	6.7n.s
	Field	13.3±1.3*	64.7±6.9*	16**	9.3±1.3n.s	280±34n.s	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
L.S.D.at.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
L.S.D.at.01	Lab.	4.97	44.975	4.5	6.6	195.8	20.17	10.8
	Field	9.275	42.775	5.97	4.5	251.32	5.63	11.2

** = Highly Significant (p<0.01) * Significant (p<0.05)
S.D.=Standard deviation Malfo.= Malformation%
L.S.D.= Least significant difference Lab.=Laboratory strain
n. s=none Significant (p>0.05)

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 the treatment of the fourth instar larvae of the two strains with both Pyridalyl and Radical at their LC_{50} values, caused highly significant (p<0.01) reduction of the pupation percentages ,as compared to control . The pupation ranged from 51.7-57.7and 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). Also the treatment of the fourth instar of the lab. and field strains with both Pyridalyl and Radical caused highly significant(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 , as compared to control (100%). However, the larval treatment of the fourth instar of lab. and field strain with Spinosad and the treatment 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, as compared to control (100%).

Data in Tables (2 &3) showed that the treatment of the second and fourth instar larvae of both lab. and field strains with the four tested compounds, at their LC_{50} values, highly significantly ($p<0.01$) reduced the adult emergence percentages as compared to that of the check. The adult emergence rates ranged from 52.8 to 62.7 and 60 to 66.3% for the second instar larvae of the lab. and field strains, respectively, as compared to 100% of control . In case of the fourth instar larvae, these rates ranged from 57 - 67 and 72 -75%, as compared to that of the 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 , Diple 2x Naturalis L , Spinosad) in laboratory and field strains. Similar results were 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, and Abo -El - Ghar *et al*. (1994) who demonstrated a decrease in the adult emergence of *A .ipsilon* treated as 4th instar larvae with petroleum ether extracts of *L. cylindrica*, *A. majus*, *C. elegans* and *V. rosea* , as compared to 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, respectively, as compared to that of control (377mg) . 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 and 264mg, respectively ,as compared to 390 and 377 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 those 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. Adel-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.

2.4. Morphogenetic effects

Data obtained (Tables2&3) showed that the treatment of the second and fourth instars larvae of both lab. and field strains of *S .liitoralis* with Pyridalyl and Lannate induced highly significant ($p<0.01$) increase in the pupal malformations. The average of the pupal malformation rates in the case of the second instar larvae treated with Pyridalyl were 16.7 and 15.4 % of both strains, respectively , while those of the fourth instar larvae treated with the same compound were 13.2 and 10.8 %, respectively. While, The average of the pupal malformation rates in the case of the second instar larvae treated with Lannate were 30 and 20% of both strains, respectively ,while those of the fourth instar larvae treated with the same compound were 18.7 and 16 %, respectively, comparing to (0%) for the control treatment . The larval treatment of second instar of lab. strain with Radical induced significant ($p<0.05$) increase in the pupal malformations of 8.1% . While, the larval treatment of the fourth instar of both lab. and field strains and of the second instar of the field strain with Radical, as well as the treatment of the second and fourth instar larvae of lab. and field strains with Spinosad gave none 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 instars larvae of both lab. and field strains of *S . liitoralis* with Pyridalyl, and of the second instar of lab. strain with Radical, and of the second and fourth instars of lab. strain of with Spinosad , and of second and fourth instar of field strain with Lannate induced highly significant ($p<0.01$) increase in the adult malformations to reach 27.3, 26.2 and 25, 22 , 20, 25.6 and 23.1, and 20,24.5%,respectively,as compared to 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 8.1 and 8.3, 10, and 10, respectively, as compared to control (0%). While, the treatment of the fourth instar of the field strain with Radical and Spinosad and of lab. strain with Lannate gave none significant increase in the adult malformations, as compared to control.

These results are similar to those obtained by Ahmed (2004) who reported that Spinosad gave malformed pupal and adults in both laboratory and field strains of both Pink and Spiny bollworms and , Abdel- Rahim (2002) who 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 4th instar with this extract by a contact method. Also, Abo- El - Ghar *et al.*(1994) obtained similar results on the *S.littoralis* .

Malformations of *S .littoralis* pupae resulting from the larval treatment of 2nd and 4th instars of both field and lab. strains with both Pyridalyl 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 (Fig. 1,2),or larval-pupal monstrosity with larval cuticle patches , head capsule and thoracic legs, posterior half of the body has the pupal properties(Fig. 3,4 ,5) or pupa with vestiture of larval skin undersized pupa(Fig. 6) .Moreover, moth malformations showing body with poorly developed and twisted wings(Fig. 7,8,9,10 and 11).However, the treatment of both of 2nd and 4th 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 2nd and 4th instars of field and lab. strains with Lannate showed as a malformed pre-pupae with complete blackening of the body leading to death(Fig.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 (Fig. 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 Pyridalyl , and of 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 ,as compared to 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 none 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 Pyridalyl , and of field strains of the same instar with Radical ,Spinosad and Lannate highly significantly ($p<0.01$) reduced the adult fertility to average 4 and 43, 45.7, 21.3, and 52.3 eggs/f, respectively, ,as compared to 536.3 and 283.3 eggs/f, 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 Radical gave none significant reduction in the adult fecundity ,as compared to control .

These results are in 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 mentioned that the average % hatchability for the eggs of treated females in both strains were decreased in both of the pink and spiny bollworms as compared with control. 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 4th instar larvae on artifical diet mixed with 2% of fruit extract of *M. azedarach* for 72h.

Table 4. Biological activity of Pyridalyl ,Radical, Spinosad and Lannate against the adults of *Spodoptera littoralis* treated as 4th instar larvae of Lab. and field strains with the LC_{50} values.

Treatments	Strain	Fecundity	Fertility	Longevity	Adult sex ratio (%)	
		Mean±S.D. (eggs/f)	Mean±S.D (eggs/f)	Mean±S.D (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± 1.6**	51.8	48.2
Radical	Lab.	235±12.2n.s	197± 2.1n.s	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±0.4 n.s	58.3	41.7
	Field	30± 5**	21.3±2.1**	6± 2.1n.s	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		
L.S.D.at.05	Lab.	408.8	350.8	4.175		
	Field	81.625	79.655	2		
L.S.D.at.01	Lab.	942.97	808.98	7.7		
	Field	188.3	183.71	3.7		

** = Highly Significant (p<0.01) * Significant (p<0.05)
S.D.=Standard deviation Malfo.= Malformation%
L.S.D.= Least significant difference Lab.=Laboratory strain
n. s=none Significant (p>0.05)

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 and 4.4 , and 5 days, respectively , as compared to 9.8 and 8.8 days,

respectively, adult longevity of control. The larval treatment of the fourth instar of both lab. and field strains with Radical, and of the lab. strain of the same instar with Lannate , significantly($p<0.05$) decreased the adult longevity to 5.8 and 5.3,and 5.3 days, respectively, as compared to 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 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 4th 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 to 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 contract effect in adult males decrease and female increase to reach 43.9:56.1% of both adult males: females, respectively , as compared to50:50 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 to 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.

2.8.Conclusion

The results of the present work demonstrated that the three tested novel compounds were effective against the survival of the 2nd and 4th instar larvae of both susceptible and resistant 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 the control of lepidopterous pests on cotton and vegetables, without phytotoxicity(Sakamoto *et al.*,2004).Also, Spinosad had an 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 possesses 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 be effective if applied at the obtained lethal concentrations within the integrate control program of this pest for reduction of classic synthetic insecticides use of serious effects on the environment.

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مقارنة لسمية ثلاث مركبات إبادية حديثة،البيريداليل،الراد يكال،الأسبينوساد والمبيد التقليدي،لانيت ضد يرقات العمر الثاني والرابع للسلالة المعملية والحقلية لدودة ورق القطن الكبرى

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معهد بحوث وقاية النباتات – مركز البحوث الزراعية – الجيزة _ الدقي .

أجريت هذه الدراسة بغرض مقارنة التأثير السأم لثلاث مركبات جديدة وهى البيريداليل، الراد يكال،الأسبينوساد ومبيد تقليدي ،لانيت تحت الظروف المعملية. غذيت يرقات العمر الثاني والرابع للسلالة المعملية والحقلية المتحصل عليها في صورة لطع للفقس لمدة ٤٨ ساعة على ورق خروع تم غمره لمدة ١٥ثانية في سلسلة تركيزات لكل مركب من المركبات الأربعة المختبرة لتحديد قيم التركيز النصفى لكل مركب. أوضحت النتائج أن مركب الراد يكال كان له التأثير الأقوى والغالب ضد كل من العمر الثاني والرابع للسلالتين المعملية والحقلية حيث بلغت قيمة التركيز النصفي القاتل له ١.٩٥ و٤.٤، ١.١و٢.٧ للسلالتين ألمعمليه والحقلية العمرين الثاني والرابع على التوالي وكان لمركب البيريداليل التأثير الثاني حيث بلغت قيمة التركيز النصفي له ٦.٢و٩.٤ ، ١.٨و٥ للسلالتين للعمرين على الترتيب.ينما جاء مركب اللأنيت في المرتبة الثالثة حيث بلغت قيمة التركيز النصفي له ١١و ١٩،٣.٩و٦ وبلغت قيمة التركيز النصفي لمركب الأسبينوساد ٣١.٣و١٣٠،٢١ و٦٢.٥ لكل من العمرين للسلالتين على التوالي. تأثرت المعايير البيولوجية لليرقات بعد المعاملة لكل من للعمرين الثاني والرابع للسلالتين المعملية والحقلية بالمركبات الأربعة. التأثير تنوع مع اختلاف السلالة والعمر اليرقى و مع المركب المختبر وبناء على ذلك كان لمعاملة العمرين لكل من السلالتين بالمركبات الأربعة المختبرة التأثير الأقوى في خفض نسب التعذير والخروج للحشرة الكاملة في كل من المعاملات الأربعة المختبرة.بينما كان لمركب البيريداليل التأثير الأقوى في زيادة العمر اليرقى والتشوهات للعذراء والحشرة الكاملة ونقص في عدد البيض وخصوبته والعمر للحشرة الكاملة وذلك في حالة المعاملة اليرقيه لكل من العمرين للسلالتين المعملية والحقلية بهذا المركب. كما كان له التأثير الأقوى في زيادة مدة طور العذراء ونقص وزنها وذلك في حالة معاملة السلالة الحقلية للعمر الثاني 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 الراد يكال التأثير الفعال في خفض عدد البيض وخصوبته وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب .كما كان له التأثير الأعلى على وزن العذراء في حالة معاملة العمر الثاني للسلالة الحقلية بهذا المركب، كما انه أدى إلى زيادة العمر اليرقى والتشوهات الحشرة الكاملة في حالة معاملة العمر الثاني والرابع السلالة المعملية بهذا المركب وكما انه اثر في النسب الجنسية حيث أدى إلى نقص نسب الذكور وزيادة في عدد الإناث

بالنسبة للكنترول وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب . كما كان لمركب الأسبينوساد التأثير ألافت للنظر في نقص عدد البيض وخصوبته وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب حيث أعطى زيادة في نسب التشوهات للحشرة الكاملة وذلك في حالة معاملة العمر الثاني و الرابع بهذا المركب كما انه رفع نسب الذكور ونقص نسب الإناث وذلك في حالة معاملة العمر الرابع للسلالة المعملية بهذا المركب. في حين وجد لمركب ألللانيت التأثير الأكبر في زيادة التشوهات العذرية وذلك في حالة معاملة العمرين الثاني والرابع لكل من السلالتين بهذا المركب.وكان له التأثير الأقوى في نقص الوزن العذري وزيادة للتشوهات للحشرة الكاملة وذلك في حالة معاملة العمر الثاني والرابع للسلالة الحقلية بهذا المركب.كما انه أثر على عدد البيض وخصوبته وعمر الفراش وذلك في حالة معاملة العمر الرابع للسلالة الحقلية بهذا المركب .كما انه أطال فتره البقاء اليرقى وذلك في حالة معاملة العمر الرابع للسلالة المعملية بهذا المركب.