

## Toxicological evaluation and biochemical impacts for radiant as a new generation of spinosyn on *Spodoptera littoralis* (Boisd.) larvae

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### ABSTRACT

Radiant SC12% (Spinetoram) is a new generation of spinosyn group. Effect of this bio-insecticide against larvae of *Spodoptera littoralis* (Boisd) was studied to evaluate the susceptibility of 2<sup>nd</sup> and 4<sup>th</sup> larval instars in laboratory. The LC<sub>50</sub> of radiant was 0.05 and 0.03 ppm. After 24 and 48 hours, respectively for the 2<sup>nd</sup> larval instars. Where the LC<sub>50</sub> were 6.67 and 2.86 ppm after 24 and 48 hours, respectively for the 4<sup>th</sup> larval instars. In the semi-field experiment, recommended doses of radiant exhibited high mortality 100 & 95.7 % after 0 and 1 days, respectively then decreased gradually to reach 58.1 % after 7 days. Also the field experiment showed high mortality 91.4% after 2 days then reduced gradually to reach 83.1% after 8 days. The effect of different concentrations in laboratory and recommended doses in field showed 100% mortality of entire hatched egg masses. Effect of recommended doses of radiant against predators inhabiting cotton field demonstrated that radiant was safe to natural enemies. The effect of LC<sub>50</sub> of radiant on the major biochemical component of 4<sup>th</sup> larval instars after 24 hours showed that, the amount of total carbohydrates, total proteins, carbohydrate hydrolyzing enzymes (invertase, trehalase and amylase), and acid & alkaline phosphates were significantly decreased. Where the acetylcholinesterase activity was significantly increased.

**Key words:** Radiant SC12%, Spinetoram, New generation of Spinosyn, *Spodoptera littoralis* ( Boisd ), Bio-chemical study.

### INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd) is one of the most notorious and destructive phytophagous insect pests in Egypt, not only to cotton, but also to other field crops and vegetables (**Kandil et al., 2003**). These caterpillars are very polyphagous, causing important economic losses in both greenhouses and open field on a broad range of ornamental, industrial and vegetable crops. Besides many populations have acquired resistance towards most insecticide groups (**Alford, 2000**). During the last two decades research has been made for new and non-traditional control agents effective against this pest since resistance has been recorded for most conventional insecticides (**Rashwan et al., 1991-1992**). Spinosad is a more recent commercial insecticide derived from metabolites of actinomycete bacterium, *Saccharopolyspora spinosa* (**Mertz and Yao, 1990**). The active ingredient is composed of Spinosyn A and Spinocyn D, have strong insecticidal activity (**Thompson et al., 1997**) with low level of mammalian toxicity and little toxicity to non-target insects (**Bret et al., 1997 and Sparks et al., 1998**). Spinosad has been classified as a bioinsecticide (**Copping and Menn, 2000**). It was selected as a candidate natural product insecticide since is active as ingestion and contact (**Spark et al., 1998**). The mode of action of spinosad is completely novel, making it a useful resistance management tool, it has unique mode of action on the insect nervous

system at the nicotinic acetylcholine receptors and it has additional effects on gamma aminobutyric acid or GABA receptor sites, leading to continuous activation of motor neurons and causing cessation of feeding, tremors of most muscles in the body and later, paralysis and death (Salgado 1997, 1998 and Semiz *et al.*, 2006). It has broad spectrum nematocidal, acaricidal and insecticidal properties (Putter *et al.*, 1981). It acts on various Lepidoptera pests of economic importance (Strong and Brown, 1987). It was highly toxic to house flies (Jeffery 1998); effective against stored product insects like *Sitophilus oryzae*, *Tribolium castaneum* and larvae of Indian meal moth *Plodia interpunctella* in stored wheat (Liang Fang & Frank 2002). Conventional toxicity tests indicate that spinosad has virtually no toxicity to birds and mammals. Spinosad has also been reported to be practically nontoxic to insect natural enemies such as *Orius* spp., *Chrysopa* spp. Coccinelids and the predaceous mite *Phytoseiulus persimilis* (Bret *et al.*, 1997). Additional studies in which spinosad treated aphids were fed by coccinelids and chrysopids larvae reported no predator mortality (Schoonover and Larson, 1995).

In Egypt, Temerak (2007) used the spinosyn products, spinosad and spinetoram to combat egg masses of cotton leaf worm, he indicated that Radiant SC12% was 5 and 7 times stronger than spintor SC24% in the field and laboratory respectively. On the other hand Ministry of agriculture (MOA) cancelled all conventional insecticides from spraying on egg masses to conserve their enemies and uses IGR<sub>s</sub> mainly for the newly hatched larvae during this period (Temerak 2002). For the time being, Spinosad is the recommended rapid product by the MOA to face egg masses and conserve the natural enemies.

The present study was carried out to evaluate the susceptibility of various larval instars of field, semi field and laboratory strains of *Spodoptera littoralis* (Boisd) to Radiant SC12% and to study the biochemical activities of some enzymes in fourth larval instars which treated with LC<sub>50</sub> of radiant in laboratory. It is also determined total carbohydrates, total protein, hydrolyzing enzyme groups, acid phosphatase, alkaline phosphatase and acetylcholine enzymes.

## MATERIALS AND METHODS

### Test insects:-

The culture of the cotton leaf worm, *Spodoptera littoralis* (Boisd) was initiated from freshly collected eggs masses supplied from the division of cotton leaf worm of plant protection research Institute, Dokki, Egypt, formed the basis of the culture designed to provide insects used in the present work. All stages of *Spodoptera littoralis* were cultured and tested at 27±2° C and 70±5% R.H. Larval stages were reared on castor bean leaves which were provided daily. The formed pupae were collected and placed in clean Jars with moist saw dust placed at the base to provide the pupation site. Adults were provided with 10% sugar solution.

### Insecticide:

Common name:- Radiant SC 12% (Spinetoram) is the second generation of the spinosyn group. It is a trademark of Dow AgroSciences.

Different concentrations of the insecticide were prepared. For each concentration, leaves of castor bean were dipped for 20 seconds, the treated leaves were then allowed to dry under laboratory conditions. The treated leaves were placed into a Petri dish with its cover. Three replicates with 20 larvae of second or fourth instars were used for each concentration. Control larvae were fed on untreated leaves (dipped in water). For each concentration larvae were allowed to feed on treated leaves

for 48 hours. The dead larvae were recorded after 24 and 48 hours Post-treatment. Leaf dipping was used to assess the susceptibility of different larval instars of cotton leaf worm.

#### **Treatment of egg masses of lab. Strain:-**

Different concentrations of the insecticide were prepared. For each concentration, piece of paper with egg masses on it dipped for 20 seconds, then the treated egg masses allowed to dry and put in Petri dish until hatching. The control one was dipped in water and left to dry. Microscopic examination was made after 3&4 days.

#### **Biochemical studies:-**

The biochemical studies of 4<sup>th</sup> larval instars were measured after 24 hours of treatment. Total carbohydrates content was measured according to the methods described by **Singh and Sinh (1977)**. The total protein content of the total body was determined according to **Bradford (1976)**. The principle based on the digestion of trehalose, sucrose and starch by trehalase, invertase and amylase, respectively according to the method described by **Ishaaya and Swiriski (1976)**. Acetylcholine esterase were measured according to method described by **Simpson et al., (1964)**. Acid and alkaline phosphatase activities were determined by the method described by **Laufer and Schin (1971)**.

#### **Field Experiments:-**

The experiments were conducted at Kaha research station, Toukh district, Qalyobia Governorate to evaluate the field efficiency of one novel biocide (Radiant Sc 12%) against cotton leaf worm, *Spodoptera littoralis* (Boisd). The field area was cultivated with Giza86 cotton variety on March27, 2008 and the normal agricultural practices were applied. The experimental area was divided into plates of 1/16 feddan (262.5 m<sup>2</sup>). The treatment was arranged in randomized complete blocks design (RCBD) with four replicates each. Application of insecticide was on July11. A motor sprayer was used. The volume of spray solution was 40 liters/feddan. The number of larvae were recorded on one meter lengthwise for five times (four at corners and the last one on plot center), before the spray and on 2,4,6 and 8 days after the spray. Reduction percent in the *Spodoptera littoralis* population was estimated using **Henderson and Tilton (1955)**.

#### **Semi field Experiment:-**

From the same experiment area of the treated cotton leaves were collected after zero time, 1,2,3,4,5,6 and 7 days and transfer directly to the laboratory for feeding the second larval instars of cotton leaf worm to estimate the mortality percent.

#### **Treatment of egg masses of field strain:-**

The egg masses produced from the second generation of field strain of *S. littoralis* field strain were treatment by the recommended dose of Radiant (25ml/feddan). After 3 and 4 days, the egg masses were examined under microscope.

#### **Statistical analysis:-**

The statistical analysis of data on mortality was subjected to the Abbott formula (**Abbott, 1925**) for correction wherever required. Probit analysis was determined to calculate LC<sub>50</sub> (**Finney, 1971**), through software computer program. Statistical significant difference between individual means were determined by one way analysis of variance (**ANOVA**).

## RESULTS AND DISCUSSION

### Susceptibility of 2<sup>nd</sup> and 4<sup>th</sup> larval instars of *Spodoptera littoralis* (Boisd) to different concentrations of Radiant SC12% (spinetoram).

Susceptibility test on a laboratory strain of the 2<sup>nd</sup> and 4<sup>th</sup> larval instars of the cotton leaf worm, *Spodoptera littoralis* was carried out at different concentrations after 24 and 48 hours post treatment. The data in **Tables 1&2** indicate that the percentage mortality of larvae indicate positive correlations with the radiant concentrations. The response of larvae to different concentrations was represented by straight regression lines, indicating homogeneity of the population to the tested concentrations and the LC<sub>50</sub>'s of radiant in 2<sup>nd</sup> larval instars after 24 and 48 hrs were 0.05 and 0.03 ppm respectively where in 4<sup>th</sup> larval instars were 6.7 and 2.9 ppm respectively. The present data consistent with results reported by (Temerak 2007).

Table (1): Susceptibility of Second instar larvae of *Spodoptera littoralis* (Boisd.) to different concentrations of Radiant SC 12%.

Radiant Concentrations (ppm)	After 24 hours of treatment		After 48 hours of treatment	
	Mean of dead larvae± SE	Mortality%	Mean of dead larvae± SE	Mortality %
0.01	2.67±1.2	13.3	4 ± 2	20
0.02	9.67±2.03*	48.3	10.7±2.34*	53.3
0.08	11±0.58*	55.8	14±1*	70
0.2	14±1*	70	15 ± 1.16*	76.7
0.4	14.3±1.73*	71.7	17.3±1.67*	87.5
0.6	19.33±0.33*	96.7	19.7±0.33*	98.3
0.8	19.67±0.3*	98.3	20±0*	100
Untreated	0	0	0	0
LC <sub>50</sub>	0.05		0.03	

Sixty larvae were used for each concentration

Table (2): Susceptibility of fourth instar larvae of *Spodoptera littoralis* (Boisd) to different concentrations of Radiant SC 12%.

Radiant Concentrations (ppm)	After 24 hours of treatment		After 48 hours of treatment	
	Mean of dead larvae± SE	Mortality %	Mean of dead larvae± SE	Mortality %
1	2±0.58	10	3±0.58	15
5	2.7±0.66	13.3	14.7±0.88	73.7
8	12±2.11	60	16.3±1.77	81.7
15	17.0±1.53	85	18.7±0.66	93.3
20	17.3±1.46	86.7	19.7±0.33	98.3
25	18.0±0.0	90	20.0±0.0	100
30	20.0±0.0	100	20.0±0.0	100
Untreated	0	0	0	0
LC <sub>50</sub>	6.67		2.86	

Sixty larvae were used for each concentration

**Efficiency of radiant on 2<sup>nd</sup> larval instars of *S. littoralis* (Boisd) in the semifield.**

Data in Table (3) showed the efficiency of recommended dose 35ml/ feddan of radiant against 2<sup>nd</sup> larval instars of *S.littoralis* under semifield condition .Data revealed that the insecticide exhibits high mortality (100 % & 95.7%) after zero and 1 days respectively then decreased gradually to reach (58.1%) after 7days of treatment. This results indicate that there is a short residual time of radiant.

Table (3) Percentage Corrected larval mortality of cotton leaf worm after treatment with Radiant during 2008 cotton season.

Rate of application	Corrected mortality %								
	Zero	1	2	3	4	5	6	7	Mean
35ml/Fedan	100	95.7	90.3	83.7	78.9	72.6	66.0	58.1	80.7

**Efficiency of radiant on larvae of *S. littoralis* (Boisd) in the field**

Data in **Table (4)** showed the efficiency of recommended dose 35ml/feddan of radiant against larvae of *S. ittoralis* under field condition during 2008 cotton season .The insecticide exhibited high mortality ( 91.4%) after 2 days then reduced gradually to reach (83.1%) after 8 days of treatment. These results indicate that the residual activity may be decreased with the time.

Table (4): Reduction % in Population of *Spodoptera littoralis* (Boisd) after treatment by recommended dose of Radiant during 2008 cotton season.

Rate of application	Reduction %				
35 ml/ Fedan	2	4	6	8	General mean
		91.4	89.4	88.8	83.1

**Ovicidal activity of Radiant on egg masses of *Spodoptera littoralis* (Boisd).**

**Fig (1,a-c)** Showed that the used of spinetoram induces (100%) ovicidal activity on egg masses at different concentrations in lab. Strain. Also in **Fig. (1, d-f)** there is evident of 100% ovicidal activity against entire egg masses of field strain Where

**Fig. (1,g)** showed total hatchability of larvae from egg masses in control one. This results are consist with results by **Temerak (2007) and Nolting et. al. (1997)** they indicating that mortality of treated eggs of *Heliothis* was from larvae ingested spinosad as they fed on chorine of the eggs during hatching.

**Effect of LC<sub>50</sub> concentration of Radiant on total carbohydrates and protein contents of Homogenate of 4<sup>th</sup> larval instars of *Spodoptera littoralis* (Boisd.).**

Total carbohydrates and total proteins are major biochemical components necessary for an organism to develop, grow and perform its vital activities, thus the mean values of homogenate contents of carbohydrates and protein were estimated in the 4<sup>th</sup> larval instars treated with the LC<sub>50</sub> of radiant after 24hours . Data in **Table (5)** Showed that the total carbohydrates was significant reduced after treatment by LC<sub>50</sub> of radiant by – 65.06 % as compared to untreated group.

Table (5) Total carbohydrates and protein contents of homogenate 4<sup>th</sup> larval instars of *Spodoptera littoralis* (Boisd.) after 48 hours of treatment with LC<sub>50</sub> of Radiant.

Applied concentration (ppm)	Total carbohydrates contents (mg/ml) Mean ± SE	Total protein contents (mg/ml) Mean ± SE
Control	2.029 ±0.11	26.98 ± 1.05
LC <sub>50</sub>	0.709 ±0.04 *	8.13 ± 0.32*
Activity %	-65.06	-69.87

\*= Highly significant (P<0.01)

The same results found by, **Bennett and Shotwell, (1973)** reported a rapid reduction in the haemolymph carbohydrates was observed following injection of bacteria into some insect species the Japanese beetle larvae, *popillia japonica*. (**El-Kattan, 1995**), in the Indian meal moth larvae, *Plodia interpunctella* and the lesser cotton leaf worm larvae, *S. exigua* (**Younes *et al.*, 2002**).

While the total protein was significant decreases by - 69.87 % compared to control group, this reduction in the protein content may be due to inhibition of DNA and RNA synthesis. The decrease of the total protein in treated 4<sup>th</sup> larval instar may reflect the decrease in the enzymatic activities of various enzymes. These results accordance with the demonstrated by **Abd El-Aziz *el at.*, (2007)**. However, our results disagree with results obtained by **Raja *et al.*, (1986)** and **Lohar and Wright (1990)**.

#### **Effect of Radiant at LC<sub>50</sub> on Carbohydrates hydrolyzing enzymes of homogenate 4<sup>th</sup> larval instars of *Spodoptera littoralis* ( Boisd).**

Carbohydrates are very efficiency utilized by insects and most species drive the main part of their nourishment depend on the digestive enzymes (invertase, trehalase and amylase). The present study evaluated the effect of radiant at LC<sub>50</sub> on this biochemical parameter of treated larvae (**Table 6**).

Table (6): Carbohydrates hydrolyzing enzymes of homogenate 4<sup>th</sup> larval instaes of *Spodoptera littoralis* ( Boisd) after 48 hours of treatment with LC<sub>50</sub> of Radiant.

Applied concentration (ppm)	Amylase (µg Glu/min) Mean ± SE	Trehalase (µg Glu/min) Mean ± SE	Invertase(µg Glu/min) Mean ± SE
Control	105.52 ±3.11	480.74± 9.24	494.11 ± 8.40
LC <sub>50</sub>	50.93±1.69 *	262.23 ± 6.22*	304.13 ± 6.12*
Activity %	-51.73	-45.45	-38.45

\*= Highly significant (P<0.01)

The activities of the carbohydrate hydrolyzing enzymes (Amylase, trehalase and invertase) were decreased after 24 hours post treatment -51.73, -45.45 and -38.45 % respectively as compared to control group. It is quite clear from the present results larval treatment with radiant at LC<sub>50</sub> significantly decreased the activity of the carbohydrate hydrolyzing enzymes (Amylase, trehalase and invertase) as compared to control group. The general disturbance in carbohydrate metabolism as expressed by reduction of carbohydrate hydrolyzing enzymes activities could be result from a chain effect originating primarily from inhibition of chitin synthesis (**Salem *et al.*, 1995**). The disturbance of trehalase activity might hamper the supply of glucose needed for chitin build up (**Kandy and Killy, 1962**). The present data are consist with results reported by **Shakoori *et al.* (1998)**; **Kheder (2002)** and **Younes *et al.* (2008 )**.

On the other hand our results disagree with those obtained by **Abdel-Fattah *et al.* (1986)** who reported that the activities of the carbohydrate hydrolyzing enzymes were much higher at the initial intervals (96 hrs.) of the applied concentration (LC<sub>15</sub>- LC<sub>30</sub> and LC<sub>50</sub>) of triflumeron and diflubenzuron on *Spodoptera littoralis*.

#### **Effect of Radiant at LC<sub>50</sub> on Acid , Alkaline phosphatase and acetylcholinestrace activity of Homogenate of 4<sup>th</sup> larval instars of *Spodoptera littoralis* ( Boisd)**

The results illustrated in Table (7) indicated that the activity of AcP significantly decreased ( p < 0.01) in 4<sup>th</sup> larval instars after 24 hours at the LC<sub>50</sub> of radiant , the percentage decrease in acid phosphatase was -21.80 % and -52.79 % in alkaline phosphatase relative to control. Our results are in conformity with the

finding of Youns *et al.*, 2008, , Shaaban and Sobeiha (1997) and Ayyanger and Rao (1990).

Bassel and Ismail (1985) and Reda *et al.* (2007). suggested that the most probable action of the IGRs is possibly via strong inhibition of the ecdyson that followed by subsequent decrease in number of lysosomes which reflect a decrease in lysosomal ACP activity. On the other hand, as shown in Table (7) the activity of acetylcholinestrace was significantly increased by 43.67 % compared to control .Since Radiant Sc 12 % at LC<sub>50</sub> increase the level of ACh.E in 2<sup>nd</sup> larval instars of *S. littoralis*. This results are in agreement with Martin *et al.* (2000) found that pyrethroids increase the level of ACh.E in the cotton boll worm, *Helicoverpa armigera* and El-Nemaky *et. al.* (2005) found that the ACh.E activity was much higher in the pink and the spiny boll worm larvae which exposed to synthetic pyrethroids than the exposed to organophosphorus compounds. Also Raslan (1994) indicated that the tested compounds synthetic pyrethroids, organophosphates and carbamate compounds caused high level of A.Ch.E, in the treated larvae of PBW compared with the untreated one. Increasing the activity of A.Ch esterase resulted by treatment with the teste compound reflects gaining of the tested pests of resistance phenomena.

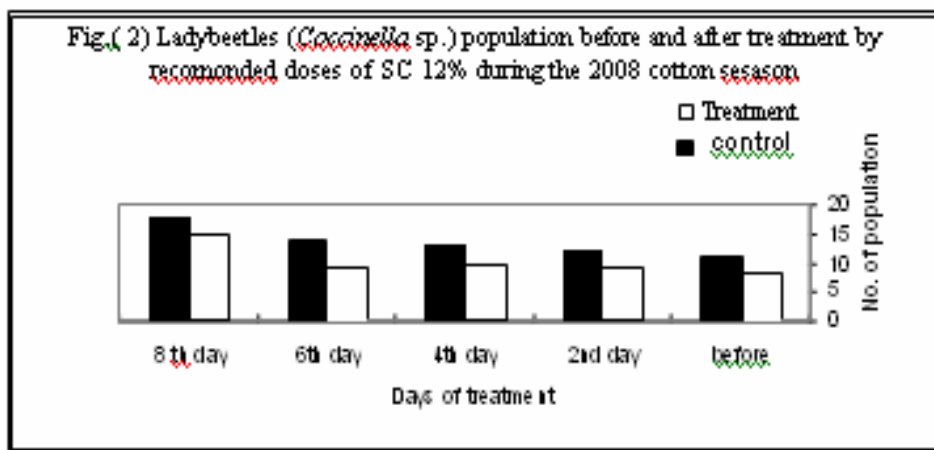
Table (7): Acid , Alkaline phosphatase and acetylcholinestrace activity of homogenate 4<sup>th</sup> larval instars of *Spodoptera littoralis* (Boisd.) after 48 hours of treatment with LC<sub>50</sub> of Radiant

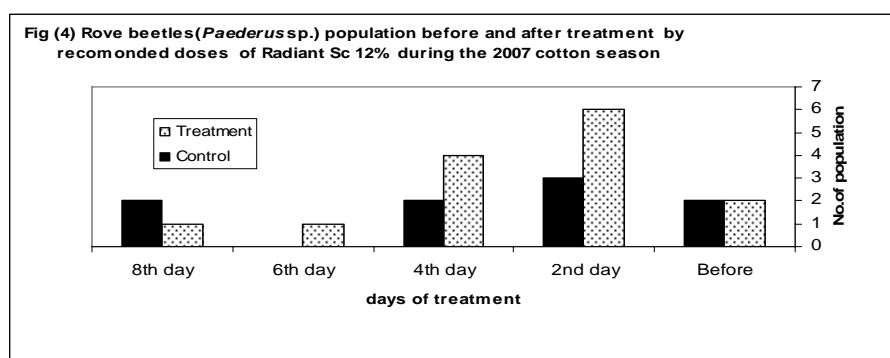
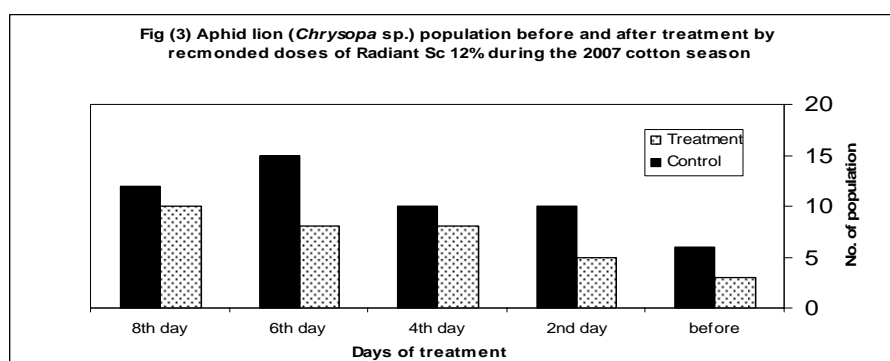
Applied concentration (ppm)	Acid phosphatase (µg phenol/min) Mean ± SE	Alkaline phosphatase (µg phenol/min ) Mean ± SE	Acetylcholinestrace (µg Ach Br/min) Mean ± SE
Control	5.23 ± 0.04	198.42 ± 4.48	30.89 ± 1
LC <sub>50</sub>	4.09 ± 0.14 *	93.68 ± 1.84*	44.38 ± 1.81*
Activity %	-21.80	-52.79	43.67

\*= Highly significant (P<0.01)

#### The field efficiency of radiant on natural enemies.

Data concerning the effect of the radiant against predators inhabiting cotton field during 2008 cotton season are illustrated in Figs. (2,3 and 4) It is clear that the recommended doses of radiant doesn't have harmful effect on population of lady beetles *Coccinella* spp., aphid lion, *Chrysops* spp. and Rove beetle *Paederus* spp. This indicate that radiant as spinosad have little impact on predators this agrees with results founded by Williams *et al.* (2003), Sparks *et al.* (1998), Schoonover and Larson (1995) and Bret *et al.* (1997) on spinosad.





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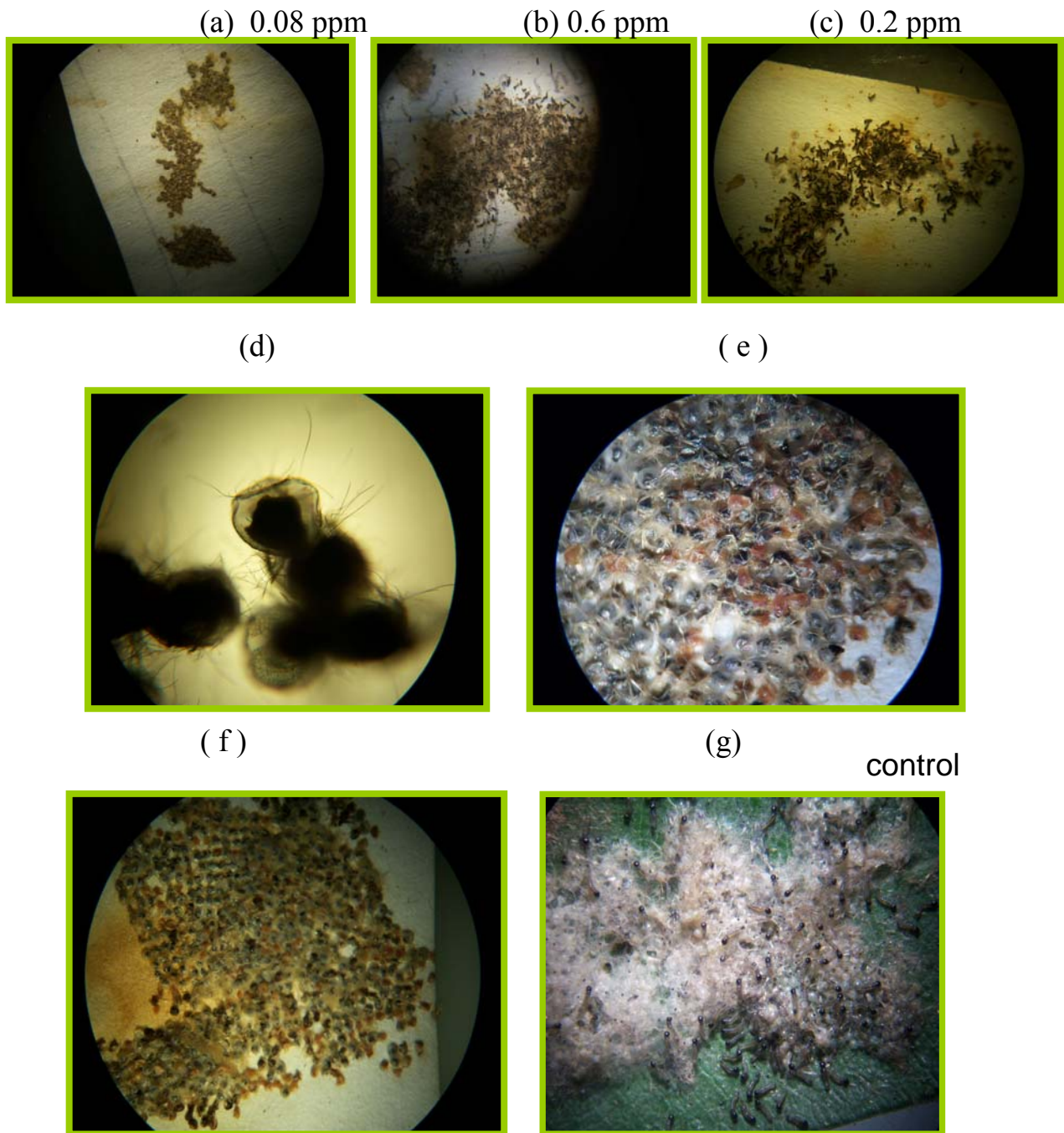


Fig. 1. Effect of Radiant SC 12 % on egg masses of lab. strains ( using different concentrations), and field strain (using recommended dose).  
Lab. (a – c). Field (d-f) and Control (g).

## ARABIC SUMMERY

## تقييم السمية و التأثيرات الكيموحيوية للجيل الجديد من السبينوسين علي يرقات دودة ورق القطن.

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يعتبر الرادينت اس سي 12% الجيل الجديد من مجموعة السبينوسين. وقد تم دراسة حساسية العمرين الثاني و الرابع لدودة ورق القطن لهذا المبيد الحيوي في المعمل. وأسفرت النتائج عن تحديد الجرعة نصف المميّنة بعد 24 و 48 ساعة ، حيث وجدت أنها 0.05 جزء في المليون بعد 24 ساعة و 0.03 جزء في المليون بعد 48 ساعة للعمر اليرقي الثاني بينما كان 6.67 جزء في المليون بعد 24 ساعة 2.86 جزء في المليون بعد 48 ساعة للعمر اليرقي الرابع. تم تطبيق الجرعة الموصي بها في تجربة شبه الحقل ووجد أنها تعطي نتيجة 100% بعد التطبيق مباشرة و7 و95% بعد مرور يوم واحد وبعد ذلك بدأت في التناقص التدريجي حتى وصلت إلي 58.1% في اليوم السابع. وكانت النتيجة متشابهة في تجربة الحقل حيث وصلت نسبة الخفض في التعداد إلي 91.4% بعد يومين وبدأت في التناقص التدريجي حي وصلت إلي 83.1% في اليوم الثامن. وفي دراسة لتأثير التركيزات المختلفة من هذا المبيد الحيوي علي لطم البيض و المفترسات في المعمل أو التركيزات الموصي بها في الحقل وجد أن نسبة الوفيات لليرقات 100% بعد الفقس في المعمل وفي الحقل. ولم يكن هناك تأثير معنوي للجرعات الموصي بها علي المفترسات الموجودة في حقل القطن المعامل بهذا المبيد الحيوي. وعلي الجانب الآخر تم دراسة التأثيرات الكيموحيوية لهذا المبيد علي الطور اليرقي الرابع لدودة ورق القطن ، حيث أظهرت النتائج تناقص معنوي في المحتوى الكلي للكربوهيدرات و البروتين و الانزيمات المحللة للكربوهيدرات ( الأنفرتيز-التريهاليز و الاميلز ) وإنزيم الفوسفات الحمضي و القلوي بينما وجد تزايد معنوي في محتوى إنزيم الأسيثيل كولين.