Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 55(3) (2017),651 – 656 **http://aasj.bu.edu.eg/index.php**

Effectiveness of spinosad on adults of the cowpea beetle, *Callosobruchus maculatus*(F.) and their control costs

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Abstract

Laboratory bioassays were carried out to evaluate the effectiveness of spinosad by using five concentrations (20, 15, 10, 5 and 1 mg/kg) on cowpea seeds against the cowpea beetle, *C. maculatus* (F.) (Coleoptera: Bruchidae). The results indicated that the adults of cowpea beetle were more susceptible to spinosad at 30ºC (LC_{50}: 0.925 mg/kg, LC_{90}: 28.95 mg/kg) than at 20ºC (LC_{50}: 1.403 mg/kg, LC_{90}: 86.7 mg/kg) after 72 hrs from treatment. The shortest lethal time values (LT_{50s} and LT_{90s}) were obtained at spinosad concentration of 20 mg/kg when the tested insect treated at 30ºC, LT_{50s} and LT_{90s} were 12.68 and 37.95 hrs, respectively, and 16.51 and 65.35 hrs at 20ºC, respectively. Results also showed the greatest reductions in the insect progeny were 100.00 and 99.78% at the highest concentration tested (20 mg/kg) under 20 and 30ºC, respectively. Furthermore, control coasts of the tested insect were estimated about 50.5 EL/1000 kg of cowpea seeds. It was concluded that spinosad had considerable toxicity against adults of cowpea beetle and it may be an effective alternative bio-pesticide for control adults of this pest in storage.

Key words: spinosad**,** bioassays, *C. maculatus***,** toxicity**,** progeny

Introduction

The cowpea beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae), has attracted great attention because it is widely distributed throughout the tropical and sub-tropical regions. It is recorded that 55.00 to 60.00% loss in seed weight and 45.50 to 66.30% loss in protein content of pulses is due to infestation caused by this beetle **(Islam *et al.*, 2007)**. Adult females act as host on various beans and chickpea plants and it lays single fertilized eggs on the external surface of seeds. The larva that hatched from the egg, burrows from the egg through the seed coat into the endosperm of the bean and then undergoes a series of moults and burrows to a position just underneath the seed coat prior to pupation. After the pupation period the adult chews through the seed coat and emerges from seeds **(Christopher *et al.*, 2009)**. Seed protectants have an important role in enhancing the storability of the legume seed by protecting them for a long time against stored product insects during storage **(Singh *et al.*, 2016)**.

Spinosad is the first active ingredient proposed for a new class of insect control products **(Sparks *et al.*, 1995**, **Thompson *et al.*, 1996, Sadat and Asghar 2006, Sanon *et al.*, 2010** and **Vidyashree *et al.*, 2015)**. The active ingredient of spinosad is derived from the metabolites of the naturally occurring bacteria, *Saccharopolyspora spinosa,* it is a mixture of two macrocyclic lactones, spinosad A and spinosad D and it has been shown to be active on insects including species from the orders; Lepidoptera, Diptera, Hymenoptera, Thysanoptera and a few Coleoptera **(Thompson *et al.*, 1997)**. The mode of action of spinosad is associated with excitation of the insect nervous system and acts at the nicotinic acetylcholine receptor (nACHRs) and exhibits activity on the gamma-aminobutyric acid receptor GABA **(Salgado, 1998)**. There are a number of factors that effect on the toxicity of insecticide such as, temperature, exposure time, humidity and formulation. Temperature is one of the most important factor affecting biological processes in all living organisms **(Amarasekare and Edelson, 2004)** and it is also a major factor affecting insecticide toxicity **(DeVrise and Georghiou, 1979)**. The effect of temperature on efficacy can be either positive or negative. The relationship between temperature and efficacy has been found to vary depending on the mode of an insecticide, target species, method of application and quantity of insecticide ingested or contacted (**Johnson 1990**). **Musser and Shelton (2005)** recorded negative temperature coefficients for spinosad against *Ostrinia nubilalis* but other research showed that toxicity of spinosad increased with increasing temperature at 15, 25 and 35°C **(Amarasekare and Edelson, 2004)**.

The present work aimed to study the effect of spionsad on adults of the cowpea beetle, *C. maculatus* under laboratory conditions and the control cost of *C. maculatus* treated using spionsad in the grain store.

Materials and Methods

1. Insect

Laboratory strain of the cowpea beetle, *C. maculatus* (F.) was used as an adult stage in these

experiments. The insect was reared in glass jars (approx. 250 ml), each jar contained (about 200 g) cowpea seeds and covered with muslin cloth and fixed with a rubber band. Insect culture was kept under controlled conditions of 28±1°C and 65±5% RH in the rearing room of the laboratory. Cowpea seeds were treated by freezing at -18°C for 2 weeks before application to eliminate any possible infestation by any insect species **(El-lakwah *et al.*, 2004)**. The moisture content of the grains was around 14%. About 300 adults of *C. maculatus* (1-2 weeks old) were introduced into the jars for laying eggs then kept at 28±1°C and 65±5% RH in the laboratory for many generations according to **El-Sawaf (1956)**. One week later, all insects were separated from the food, and the jars were kept again at the controlled conditions in the rearing room. This procedure was repeated several times in order to obtain a large number of the adults needed to carry out the experiments during this study. The emerging adults were collected daily and stored in jars of cowpea seeds until used **(Southgate *et al.*, 1957 and Halstead, 1963).**

2. Bio-insecticide

Spinosad (a mixture of 50-95% of spinosyn A and 50-5% spinosyn D)

Spinosyn A: (2R, 3aS, 5aR, 5bS, 9S, 13S, 14R, 16aS, 16bR) - 2- (6-deoxy-2,3,4-tri-O-methyl- α- Lmannop-yranosyloxy) – 13 - (4-dimethylamino - 2, 3, 4, 6- tetradeoxy- β-Derythrop-yranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16bhexadecahydro- 14- methyl - 1H-8 oxacyclododeca [b] as-indacene-7,15-dione

Spinosyn D: (2S, 3aR, 5aS, 5bS, 9S, 13S, 14R, 16aS, 16bR) - 2- (6-deoxy-2, 3, 4- tri- O-methyl- α-Lmannop-yranosyloxy) -13- (4-dimethylamino - 2, 3, 4, 6- tetradeoxy- β-Derythrop-yranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16b hexadecahydro-4,14-dimethyl- 1H-8-oxacyclododeca [b] as-indacene-7,15-dione

The insecticide formulation was Spintor (24% SC) produced by Dow Agro- Sciences.

2. Culture of *C. maculatus* F.:

The cowpea beetle, *C. maculatus* (F.) was obtained from the Department of Stored product pests, Plant Protection Research Institute in Cairo. Culture of the cowpea beetle, *C. maculatus* was reared on sterilized cowpea seeds.

3. Bioassay tests

Spinosad was applied as solutions against *C. maculatus* (F.) adults in cowpea seeds at five concentrations(20, 15, 10, 5 and 1 mg/kg). Water solution (1ml of each concentration) was added to 10 g cowpea seeds (in glass jars of approx. 250 ml). In addition to 10 g cowpea seeds, which served as controls, were treated with (1 ml) distilled water. The glass jars of treated cowpea seeds were manually shaken for 10 mins to achieve an equal distribution of the insecticide in the entire grain mass. Batches (30 insect adults) of *C. maculatus* (2-3 days post-emergence) were introduced to different treatments. Every treatment was conducted in three replicates. Glass jars were covered with muslin cloth, fixed with rubber band, and kept at 20 and 30±1ºC and 65±5% RH. Mortality was recorded after 6, 12, 24, 48, 72, 120, 168 and 240 hours of the initial treatment.

4. Statistical analysis:

Mortality percentages were corrected by **Abbott's formula (1925).** A probit computer program of **Noack and Reichmuth (1978)** and **Finney (1971)** was used to determine the lethal concentrations and lethal times for the insecticide. The obtained data were statistically analyzed according to the method of **CoStat (2005)** statistical program analysis, computer program software.

5. Control cost of *C. maculates* adults by spinosad:

The control cost of *C. maculatus* adults was done according the equation of **Mangoud (2000)**:

Cost = [(amount of compound/ton seeds) x price of compound)] + Labour for treatment + Machinery for treatment.

Results and Discussion

In this study, spinosad compound was evaluated against *C. maculatus* adults under two constant temperatures (20 and 30 ±1ºC and 65±5 % RH). The adults of cowpea beetles, *C. maculatus* were more susceptible to spinosad when treated at 30ºC (LC_{50}: 0.93 mg/kg) than at 20ºC (LC_{50}: 1.40 mg/kg) after 72 hrs from treatment. In addition, LC_{90s} were 28.95 and 86.7 mg/kg at 30 and 20ºC, respectively at the same period of treatment. Data showed that, the reduction in progeny of cowpea beetle ranged between 70.92 and 100.00% at 20ºC, while at 30ºC, it ranged between 69.64 and 99.78% **(Tables 1, 2 and 3)**.

Table 1. Accumulative mortality of *C. maculatus* adults treated by certain concentrations of spinosad and the progeny reduction at 20±1°C and 65±5% RH

Concentration (mg/kg)	Accumulative mortality (%) after indicated hours								Progeny after 60 days (mean)	Reduction in progeny (%)
	6	12	24	48	72	120	168	240		
20	25.55^{a}	36.66^{a}	52.87^{a}	78.15^{a}	87.34^{a}	100.00^{a}	100.00	100.00	0.00^{c}	100.00^{a}
15	14.44^{b}	24.44^{b}	49.42^{a}	67.80^{ab}	82.75^{ab}	100.00^{a}	100.00	100.00	0.22^{c}	99.81^{a}
10	0.00^{c}	0.00^{c}	32.18^{b}	63.21^{b}	68.96^{bc}	83.90^{ab}	100.00	100.00	3.65^{c}	96.98^{a}
5	0.00^{c}	0.00^{c}	24.13^{c}	50.57^{c}	60.91^{cd}	73.55^{bc}	100.00	100.00	9.99^{b}	91.75^{a}
1	0.00^{c}	0.00^{c}	16.08^{d}	35.62^{d}	48.27^{d}	66.65^{c}	100.00	100.00	35.21^{a}	70.92^{b}
$LSD_{0.05}$	4.98	3.64	7.42	12.42	15.71	16.77	-	-	5.02	18.38

Different lowercase letters within each column of each tested temperature indicate significant differences ($p < 0.05$)

Table 2. Accumulative mortality of *C. maculatus* adults treated by certain concentrations of spinosad and the progeny reduction at 30±1°C and 65±5% RH

Concentration (mg/kg)	Accumulative mortality (%) after indicated hours								Progeny after 60 days	Reduction in progeny
	6	12	24	48	72	120	168	240		
20	28.88^{a}	42.22^{a}	58.88^{a}	94.44^{a}	100.00^{a}	100.00	100.00	100.00	0.33^{c}	99.78^{a}
15	16.66^{b}	27.77^{b}	47.77^{ab}	85.55^{ab}	93.02^{ab}	100.00	100.00	100.00	3.66^{c}	97.63^{a}
10	0.0.00^{c}	0.00^{c}	38.88^{bc}	74.44^{bc}	83.72^{bc}	100.00	100.00	100.00	5.66^{c}	96.33^{a}
5	0.00^{c}	0.00^{c}	33.33^{c}	62.22^{c}	69.78^{c}	96.66	100.00	100.00	17.66^{b}	88.56^{a}
1	0.00^{c}	0.00^{c}	23.33^{d}	41.11^{d}	52.34^{d}	85.55	100.00	100.00	46.88^{a}	69.64^{b}
$LSD_{0.05}$	11.51	11.51	18.19	18.19	14.87	-	-	-	11.87	18.19

Different lowercase letters within each column of each tested temperature indicate significant differences ($p < 0.05$)

Results in **Table (4)** revealed that the shortest time required to obtain 50% mortality of the cowpea beetle adults was recorded at concentration of 20 mg/kg, also the adults were more susceptible when treated with spinosad under 30°C (LT_{50s}: 12.68 hrs) than treated under 20°C (LT_{50s}: 16.51 hrs). This time increased by the reduction in the concentration to reach 49.94 and 67.57 hrs at concentration of 1 mg/kg at 30 and 20°C, respectively.

Data in **Table (5)** indicated that the rate of application of spinosad reached 100 mg (AI)/kg which found in about 500 ml of spinosad formulation/1000 Kg of seeds and the price of litre of compound was 80 Egyptian pound (LE), the cost of spinosad 40 LE/1000 Kg of seeds. In the second item (Labours) two labours was sufficient to treat the seeds with spinosad, every labour needed 5 LE/1000 Kg of seeds, with total, 10 LE. The third item is sprayer (machinery of treatment), it costs 0.5 LE/1000 Kg of seeds.

The total costs were as following: cost of spinosad (40 LE), labours (10 LE) and sprayer (0.5 LE) with a total 50.5 LE/1000 Kg of seeds.

Total cost = A + B + C = 40 + 10 + 0.5 = 50.5 LE

A 500 ml/1000 Kg seeds X 80 LE/L. = 40 LE

B = Labour for treatment = 2X5=10 LE

C = Machinery for treatment = 0.5 LE

Table 3. Lethal concentration values of spinosad against *C. maculates* adults under laboratory conditions at 20 and 30 ± 1°C and 65 ± 5% RH

Temp. (°C)	Exposure period (hrs)	LC_{50} mg/kg	LC_{90} mg/kg	95% Confidence limits				Slope±SD	R
				LC_{50}		LC_{90}			
				Lower	Upper	Lower	Upper		
20	48	3.46	132.43	2.16	4.86	62.33	519.19	0.81±0.13	0.969
	72	1.40	86.79	0.53	2.33	35.66	618.14	0.72±0.15	0.926
30	48	1.89	36.21	1.13	0.74	21.29	89.49	1.00±0.15	0.975
	72	0.93	28.95	0.32	1.55	13.97	150.71	0.86±0.18	0.974

SD: Standard deviation of mortality regression line R: Correlation coefficient of regression line

Table 4. Lethal time values of spinosad on adults of *C. maculatus* under laboratory conditions at 20 and 30 ± 1°C and 65 ± 5% RH

Temp. (°C)	Concentrations mg/kg	LT_{50} hrs	LT_{90} hrs	95% Confidence limits				Slope±SD	R
				LT_{50}		LT_{90}			
				Lower	Upper	Lower	Upper		
20	20	16.51	65.35	13.69	19.92	49.15	86.88	2.15±0.49	0.958
	15	24.88	72.55	20.64	30.003	55.22	95.32	2.76±0.44	0.956
	10	38.95	107.72	32.10	47.26	83.86	138.37	2.90±0.52	0.936
	5	47.76	124.73	40.60	56.19	97.13	160.17	3.07± 0.90	0.907
	1	67.57	130.64	57.13	79.93	99.86	170.91	4.48±0.90	0.819
30	20	12.68	37.95	10.46	15.36	28.15	51.17	2.69±0.48	0.959
	15	21.37	58.01	17.66	25.87	44.92	74.90	2.96±8.45	0.992
	10	30.99	70.88	23.96	40.08	52.28	96.10	3.57±0.17	0.975
	5	37.19	90.35	31.25	44.27	72.01	113.35	3.33± 0.28	0.972
	1	49.94	118.61	43.17	57.77	54.02	149.58	3.41±0.73	0.936

SD: Standard deviation of mortality regression line R: Correlation coefficient of regression line

The obtained results are agree with those obtained by **Sanon *et al.* (2010)** they found spinosad caused high mortality of adult *C. maculatus* and decreased the number of eggs laid by females. In addition, **Vidyashree *et al.* (2015)** evaluated of newer molecule insecticides for the management of *C. maculatus* on storability of chickpea variety JG-11 under ambient storage condition. The treatment of spinosad at 2 ppm was more effect followed by emamectin benzoate at 2 ppm and it protects the chickpea seeds from *C. maculatus* infestation. **Sadat and Asghar (2006)** determined the effect of post-treatment temperature on the toxicity of a commercial formulation of spinosad (Tracer24®) against adults of *C. maculatus*. A direct relationship between spinosad dosages and post-treatment temperature was detected. Mortality of the adults of *C. maculatus* after 72 hours post-treatment was more effective than 24 and 48 hours.

Table 5. Control costs of the cowpea beetle, *C. maculatus* using spinosad at 30±1°C and 65±5% RH

Item	LE*
Spinosad**	40
Labour	10
Machinery	0.5
Total costs	50.5
Efficacy***	100%

* Egyptian pound (LE) **Average price of spinosad

*** Mortality of cowpea beetle after 168 hrs treatment

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كفاءة مبيد سبينوساد على الطور الكامل لخنفساء اللوبيا وتكاليف مكافحتها

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*** الادارة المركزية للحجر الزراعى– القاهرة

أجريت الاختبارات المعملية لتقييم فعالية خمسة تركيزات من مبيد إسبينوساد باستخدام ضد خنفساء اللوبيا.

و قد أظهرت النتائج عند درجة الحرارة 30 درجة مئوية كانت الحشرة أكثر تأثراً بالمبيد حيث كانت قيمة LC_{50} و LC_{90} بعد 72 ساعة من المعاملة هى 0.925 و 28.95 ملجم/كجم على التوالى ، بينما كانت هذه القيم عند 20 درجة مئوية 1.403 و 86.7 ملجم/كجم على التوالي بعد 72 ساعة. من ناحية أخرى كانت قيم LT_{50s} لخنفساء اللوبيا المعاملة بمبيد سبينوساد بتركيز 2 ملجم/كجم عند 30 ، 20 درجة مؤية 12.68 و 16.51 ساعة على التوالى و LT_{90s} عند 30 ، 20 درجة مؤية هى 37.95 و 65.35 ساعة على التوالى. كما أظهرت النتائج أن أكبر خفض فى النسل الناتج من الحشرة (100 و 99.78%) تم الحصول عليه عند المعاملة بتركيز 2 ملجم/كجم عند 30 ، 20 درجة مؤية على التوالى. وقد قدرت تكاليف مكافحة هذه الحشرة فى المخزن بحوالى 50.5 جنيه مصرى لكل طن من بذور اللوبيا. ويستنتج من ذلك أن مبيد إسبينوساد يمكن أن يكون بديل فعال كمبيد حيوى فى مكافحة خنفساء اللوبيا فى المخزن.