J. Plant Prot. and Path., Mansoura Univ., Vol. 5 (12): 1073 - 1080, 2014

FIELD AND LABORATORY EVALUATION OF SPINOSAD AND SPINETORAM AGAINST HOUSEFLY, *Musca domestica* L. (DIPTERA: MUSCIDAE)

Abo-El-Maged, T. M.
Plant Protection Dept., Faculty of Agric., Assiut Univ., Assiut 71516, Egypt
E-mail: tnmn203@yahoo.com

ABSTRACT

Two spinosyn compounds, spinosad 12% SC and spinetoram 12% SC were evaluated against housefly under laboratory and field conditions. In field study, the two compounds showed high reduction in fly population especially at the highest rate used (2.4 ml/m^2). According to LT_{50} value spinosad showed relatively higher activity than spinetoram. This indicated that females were more tolerant two spinosyn products than males.
In laboratory study the toxicity of spinosad against 1st and 3rd instars was higher than spinetoram. The third instar larvae were more tolerant to the two compounds, than the first instar larvae. Against pupae, spinosad showed slightly high toxicity as compared with spinetoram.
Keywords: Housefly, *Musca domestica,* Spinosyn products, Animal husbandry.

INTRODUCTION

The housefly, *Musca domestica* L. (Diptera: Muscidae) represents a pest of great economic importance in animals husbandry, and transmits a variety of pathogens to animals, as well as causes problems by invading homes surrounding animal farms, affecting the quality of life of these populations (Farkas *et al*., 1998 and Patricia and Claudio, 2008). In many countries, house flies are the probable carriers of more than 65 human and animal intestinal diseases (Greenberg, 1965), including bacterial infections (salmonellosis, shigellosis and cholera); protozoan infection (amebic dysentery); helminthic infections (roundworms, tapeworms and hookworms) and rickettsial infections. Flies also transmit eye diseases and infect wounds or skin with diseases (Keiding, 1986, Shono and Scott, 2003 and Geden, 2012). High level of insecticide resistance in housefly and public demands for reducing pesticide use around animal food have promoted interest in the development of other control strategies of this pest (Geden *et al*., 1995). In view of the severity of the problem, it is imperative that control of housefly must be improved through the application of occupationally and environmentally safe natural pesticides (Kaufman *et al*., 2001 and Shono and Scott, 2003). The spinosyns are a large family of unprecedented compounds produced from fermentation of two species of saccharophyspora. It was first identified in a soil sample by Eli Lilly and Co in 1982 and commercially introduced by Dow Agro Sciences during 1997 (Tomlin, 2000 and Saleem *et al*.2009).

Spinosyns show potent insecticidal activities against many commercially significant species that cause extensive damage to plants and activity against important external parasites of animals and humans (Herbert,

2010). They have potent activity and lower environmental effect (Thompsom *et al*., 2000; Racke, 2007 and Huang *et al*., 2009).
Several recent studies of spinosyn products have shown their efficacy against flies and fleas when administered to animals (White, 2007, Deken, 2009 and Franc and Bouhsiro, 2009). The spinosyns are also useful in integrated pest management and insecticide resistance management programs (Racke, 2007).

The aim of the present study was to evaluate two spinosyn products, spinosad 12% and spinetoram 12% against housefly, *M. domestica* in the animal husbandry at Assiut area.

MATERIALS AND METHODS

Housefly culture:

Adult of housefly were collected from the animal husbandry in Lilian Tracher orphanage (5 km north of Assiut city) during May 2014 by sweeping net, the flies were transferred to the laboratory where they were reared at 27C, 50±5% RH and photoperiod of 14:10 (L:D). Adult were maintained in cages (50x50x50 cm^3) covered by gauze. Water and food in the form of sugar and powdered milk were provided and replenished every 24-48 hr. larval medium comprised 55 g wheat bran, 3 g powdered milk suspended in 150 mL water. One cup of 250 mL of this medium was put in each cage for adult ovipositor and subsequent development of larvae. The food media in each cage were replaced by new one each 24-48 hr according to the method of Sharififard *et al*., 2011.

The percentage of reduction:

Field evaluation of the toxicity effect of spinosad and spinetoram against *M. domestica* adults was conducted in eight animal-sheds located in Lilian Tracher orphanage, Assiut Governorate.

The spinosad 12% and spinetoram 12% were sprayed at rates of 0.6, 1.2 and 2.4 mL/m^2 (0.072, 0.144 and 0.288 gm/m^2), while one untreated animal-shed was used as control.

Samples were taken three times from each treatment in addition to control by using the sweeping net. The numbers of housefly males and females were recorded before spray and after 1, 3, 5, 7, 10, 15 and 21 days of treatment. The reduction percentages in housefly adult population were calculated by using Henderson & Tilton (1955) formula:

$$\% \text{ Reduction} = (1 - \frac{\text{Ta x Cb}}{\text{Tb x Ca}}) \text{ x } 100$$

Where,
Ta= Number of males or females after treatment.
Tb= Number of males or females before treatment.
Ca= Number of males or females in control after treatment.
Cb= Number of males or females in control before treatment.

Laboratory study: In order to evaluate the toxicity of spinosad 12% or spinetoram 12% against *M. domestica* immature stages, four concentrations of both compounds (0.3, 0.6, 1.2 and 2.4 ppm) were used. The housefly

immature stages were treated using feeding or dipping bioassay methods. Twenty larvae of both 1st and 3rd instars, in addition to twenty pupae were used per replicate, and each treatment was replicated three times. Larvae and pupae in the control groups were treated with distilled water. Dead larvae or pupae were counted daily for all treatments. The predicted effects of spinosad and spinetoram treatments were compared with the observed mortality of the control ones. Mortality percentages were corrected using Abbott's formula (Abott, 1925) and LC_{50}, slope values of LCP lines were determined by using the computerized probit analysis program significance among results obtained underwent to the applying linear model test (F-test) using MSTATE statistical Package Software (Anonymous, 1986) . The least significant difference test (LSD) of 5% probability level (Steel and Torrie, 1984) and the probit analysis were done using SPSS software (Anonymous, 1998).

RESULTS AND DISCUSSION

Field study:

Data Table 1 represent the percentages of reduction in housefly female population after application of spinosad and spinetoram at 3 rates for 21 days. Spinosad at 0.6 ml/m^2 caused 62% reduction after one day of application, the activity of the compound increased with increase in rate to attain 94.5% at 2.4 ml/m^2. The percentage of reduction at all rates decreased gradually to attain 22.4% at a rate of 2.4 ml/m^2 after 21 of application. Result of spinetoram showed the same trend of spinosad, at a rate of 0.6 ml/m^2, the percentage of reduction was 50%, and at 2.4 ml/m^2 it was 83.8%. After 21 days, only 9.5% reduction was recorded at 2.4 ml/m^2.

Table (1): Reduction (%) of *M. domestica* (female) after being treated with spinosad 12% SC and spinetoram 12% SC in the animal husbandry.

Days after field spray	% Reduction					
	Spinosad 12% SC			Spinetoram 12% SC		
	0.6 ml/m^2	1.2 ml/m^2	2.4 ml/m^2	0.6 ml/m^2	1.2 ml/m^2	2.4 ml/m^2
1	62.0 cd	80.2 b	94.5 a	50.0 d	68.3 c	83.8 b
3	47.4 d	63.0 c	86.5 a	42.4 de	49.5 d	72.2 b
5	43.3 de	60.0 c	84.1 a	33.3 e	48.2 d	66.1 b
7	35.0 d	49.1 b	61.2 a	12.5 e	44.7 c	50.9 b
10	27.8 d	48.0 b	56.9 a	7.9 e	33.3 d	39.7 c
15	17.3 d	25.0 bc	35.3 a	0.0 e	12.5 d	32.7 b
21	0.0 e	6.8 c	22.4 a	0.0 e	1.8 d	9.5 b

In each row, means followed by the same letter are not significant different (Duncan's test a= 0.05)

Data of Table 2 showed the same trend of female for the two compounds. However, males were more susceptible to two compounds as compared with females. In general, spinosad showed great activity than spinetoram.

According to LT_{50}, and LT_{90} values (Table 3), spinosad showed high effect of both male and female of housefly as compared with spinetoram. Females were more tolerant to the two compounds than males. Comparing

slope value housefly showed high homogeneity response to the two compounds, except at the higher rate of spinosad.

Table (2): Reduction (%) of *M. domestica* (male) after being treated with spinosad 12% SC and spinetoram 12% SC in the animal husbandry.

Days after field spray	% Reduction					
	Spinosad 12% SC			Spinetoram 12% SC		
	0.6 ml/m^2	1.2 ml/m^2	2.4 ml/m^2	0.6 ml/m^2	1.2 ml/m^2	2.4 ml/m^2
1	73.9 d	95.0 b	100 a	72.1 d	81.6 c	92.3 b
3	64.3 d	86.2 b	90.3 a	62.4 d	79.4 c	87.7 b
5	57.7 cd	77.8 b	85.2 a	57.7 cd	64.3 c	77.5 b
7	53.2 c	75.6 b	77.8 a	47.3 c	62.3 d	69.6 cd
10	35.5 d	47.9 c	64.6 a	28.9 d	45.5 c	55.7 b
15	26.6 c	34.5 b	44.5 a	17.5 d	28.9 c	36.5 b
21	9.4 d	21.7 b	29.8 a	1.7 e	12.2 d	15.4 c

In each row, means followed by the same letter are not significant different (Duncan's test a= 0.05).

Table (3):Probit analysis parameters of spinosad 12%SC and spinetoram 12%SC tested against females and males of *M. domestica*. in the animal husbandry.

Compound	Rate (ml/m^2)	Sex	LT$_{50}$ days	Confidence limits		Slope	LT$_{90}$ days	Confidence limits	
				Lower	Upper			Lower	Upper
Spinosad 12%SC	0.6	F	2.48	1.698	3.240	0.984	0.12	0.029	0.283
		M	5.03	2.790	8.047	1.316	0.53	0.045	1.298
	1.2	F	5.56	3.147	9.054	1.457	0.73	0.072	1.655
		M	10.34	7.966	14.409	2.053	2.46	1.143	3.611
	2.4	F	9.39	6.282	15.978	2.312	2.62	0.655	4.328
		M	13.37	11.909	15.333	2.368	3.85	3.095	4.532
Spinetoram 12%SC	0.6	F	1.48	0.102	2.868	1.512	0.21	0.001	0.778
		M	4.16	1.821	7.0170	1.547	0.62	0.029	1.531
	1.2	F	3.26	1.085	5.592	1.387	0.39	0.008	1.139
		M	7.30	4.420	12.934	1.539	1.07	0.129	2.232
	2.4	F	6.49	4.390	9.554	1.570	0.99	0.247	1.868
		M	10.15	7.137	16.969	1.969	2.26	0.722	3.699

Laboratory study:

Data presented in Tables 4 and 5 show the LC_{50}, LC_{90} and slope of LCP lines of spinosad and spinetoram tested against 1st and 3rd instar larvae of *M. domestica* for 48 and 72 hrs.

After 48 h exposure, the LC_{50} and LC_{90} values of spinosad were 0.49 and 4.45 ppm for 1st instar and 0.95 and 7.57 ppm for 3rd instar. As for Spinetoram the corresponding values were 0.65 and 5.62 ppm for 1st instar, and 1.20 and 12.45 ppm for 3rd instar. According to LC_{50} and LC_{90} values, spinosad showed relatively higher toxicity against both instars than

spinetoram. On the other hand, the third instar significantly tolerated the two compounds than first instar larvae.

Results after 72 h exposure showed the same trend of 48 h results. However, values of LC_{50} and LC_{90} after 72 were much lower than that after 48 h exposure.

Regardless of period of exposure or insect instars, values of slope showed high homogeneity response of housefly to the two compounds.

Data of pupae Table 6 show that the LC_{50} and LC_{90} value of spinosad was 0.73 and 3.25 ppm. The corresponding values for spinetoram were 0.81 and 4.63 ppm, respectively. As for larvae, spinosad showed slightly high toxic effect against pupae, as compared with spinetoram.

Table(4):Probit analysis parameters of spinosad 12%SC and spinetoram 12%SC tested against *M. domestica* 1st and 3rd instar larvae in the laboratory after 48 hr.

Compound	Instar	LC_{50} ppm	Confidence limits		Slope	LC_{90} ppm	Confidence limits	
			Lower	Upper			Lower	Upper
Spinosad 12%SC	1st	0.49	0.357	0.621	1.339	4.45	2.857	9.971
	3rd	0.95	0.773	1.193	1.423	7.57	4.580	17.880
Spinetoram 12%SC	1st	0.65	0.508	0.811	1.371	5.62	5.62	12.664
	3rd	1.20	1.033	1.762	1.306	12.45	6.580	39.658

Table(5):Probit analysis parameters of spinosad 12%SC and spinetoram 12%SC tested against *M. domestica* 1st and 3rd instar larvae in the laboratory after 72 hr.

Compound	Instar	LC_{50} ppm	Confidence limits		Slope	LC_{90} ppm	Confidence limits	
			Lower	Upper			Lower	Upper
Spinosad 12%SC	1st	0.24	0.158	0.327	1.670	1.44	1.122	2.148
	3rd	0.35	0.192	0.402	1.410	2.43	1.727	4.364
Spinetoram 12%SC	1st	0.29	0.190	0.382	1.533	1.99	1.478	3.248
	3rd	0.38	0.252	0.498	1.302	3.67	2.407	7.822

Table (6):Probit analysis parameters of spinosad 12% and spinetoram 12% tested against *M. domestica* pupae in the laboratory (recorded till the adults emergence).

Compound	LC_{50} ppm	Confidence limits		Slope	LC_{90} ppm	Confidence limits	
		Lower	Upper			Lower	Upper
Spinosad 12%SC	0.73	0.619	0.854	1.977	3.25	2.459	4.890
Spinetoram12%SC	0.81	0.674	0.971	1.69	4.63	3.221	8.149

The activity of spinosyn products against houseflies has previously been reported by many authors (Bret *et al*., 1997; Scott, 1998; Shono and Scott, 2003; Salgado and Sparks, 2005; Dencutis *et al*., 2006 and Sharififard *et al*., 2011). Spinosyn products were toxic to houseflies by feeding, exposure to a residue, in addition to the topical application.

Results indicated variation in the susceptibility to both products in flies collected from animal-sheds. Whereas selections of field collected flies have produced a highly resistant strain of housefly after 8 generations of selection (Shono and Scott, 2003). Spinosad works at a novel target site (Salgado and Sparks, 2005) and resistance in the housefly is highly evaluated in field populations (Georghiou, 1983 and Kaufman *et al*., 2010). In general the two spinosyn products in the present study showed high toxicity on housefly adult and immature stages population. However, spinosad and its congeners must be used judiciously and periodic monitoring of resistance should continue. This limitation will be important to identify the gene responsible for spinosad resistance, so that a more sensitive detection method can be developed.

CONCLUSION

The spinosyn products showed great effect against housefly, *M. domestica* in the animal husbandry. The spinosad12%SC and spinetoram 12%SC tested gave a successful biocontrol to adult (female and male) and immature stages of housefly. According to percentage of mortality recorded after application and LT_{50} and LT_{90}values, females were more tolerant than males.

Acknowledgment

Sincere thanks are due to Dr. Youssef Omar, Associate Professor in Plant Protection Department, and Faculty of Agriculture Assiut University for his advice in the statistical analysis and Prof. Dr. Sayed A. El- Eraky, Professor in Plant Protection Department, Faculty of Agriculture Assiut University for useful discussions.

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التقييم الحقلي والمعملي للإسبينوساد والإسبينوترام ضد الذبابة المنزلية ، مسكا دومستيكا (ذات الجناحين:مسكيدى)

طارق محمد أبو المجد

قسم وقاية النبات-كلية الزراعة-جامعة أسيوط-مصر

أجريت الدراسة لتقييم سمية مركبى الإسبينوساد والإسبينوترام على الذبابة المنزلية تحت ظروف المعمل والحقل.وأظهرت الدراسة الحقلية لمركبى (الإسبينوساد والإسبينوترام) نسبة خفض عالية للذبابة المنزلية خصوصا فى المعاملة بتركيز 2.4 مليلتر/مترمربع . وأوضحت الدراسة طبقا لقيم LT_{50} أن الإسبينوساد أكثر فعالية نسبيا عن الإسبينوترام .وأظهرت الدراسة أن إناث الذبابة المنزلية أكثر تحملا من الذكور.وفى الدراسة المعملية كانت سمية الإسبينوساد ضد يرقات العمر الأول والثالث أعلى سمية عن الإسبينوترام. وأوضحت أن يرقات العمر الثالث أكثر تحملا للمركبين عن يرقات العمر الأول. كما أظهرت الدراسة أن مركب الإسبينوساد أعلى سمية نسبيا عن مركب الإسبينوترام ضد عذارى الذبابة المنزلية.