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EFFECTIVENESS OF SPINOSAD AS A LARVICIDAL AND PUPACIDAL AGAINST CULEX SPECIES IN BENI SUEF GOVERNORATE, EGYPT

SUMMARY

The present study was conducted to evaluate the larvicidal and pupacidal efficacy of Spinosad on *Culex* species in their breeding habitats in village of Saft, Beni Suef Governorate, Egypt. The results showed that Spinosad caused a pronounced toxicity against *C. pipiens*. The total mortality of second and fourth larval instars was achieved by lower concentrations of Spinosad. While the highest pupal morality of *C. pipiens* was obtained by concentration of 5000 ppm (93.3%), after 168 h under laboratory conditions. Spinosad was applied with concentration of 5000 ppm at semi-field experiments and resulted total mortality of larval instars of *C. pipiens*, *C. theileri* and *C. perexiguus* after 24 and 48 h of exposure. The same concentration induced strong pupal mortalities of *C. pipiens*, *C. theileri* and *C. perexiguus* (90, 60 and 70%), respectively after 48 h. The field experiments, Spinosad (5000 ppm) induced total mortality of larval instars of *Culex* species and the pupal mortality was higher in breeding habitat, cement irrigation tank (77.8 %) and decreased to 36.4% in agriculture canal. The present study concluded that Spinosad is a promising larvicide that can be used in treatment of mosquito breeding habitat sites with low efficacy on pupae at some habitat sites such as agricultural canals.

Keywords: *Culex* species; Cement irrigation tank; Breeding habitats; Mosquitos, Agricultural canal

INTRODUCTION

Mosquitoes are a source of nuisance and transmission of diseases to humans and animals. Their risk to transmit diseases through the bite of female mosquitoes increases during their blood meal search before oviposition. The diseases transmitted by female mosquitoes include chikungunya, yellow fever,

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malaria, filariasis, Zika virus disease and dengue fever. These diseases cause risk to millions of people in Egypt and around the world (Southgate, 1979; Meegan *et al*., 1980; Darwish and Hoogstraal, 1981; Warrell, 1993; Elgendy *et al.*, 2018).

Management of mosquito species is applied by using the chemical insecticides (Cetin *et al*. 2006; Aney *et al.* 2018; Eissa *et al*. 2020). However, the frequent use of high application rates of these substances is associated with serious problems, such as increase resistance of insects and detrimental effects on environment, animals and humans (Liu *et al*. 2019; Talipouo *et al*. 2021). Thus, alternative strategies have been examined and used for management of mosquito species, such as Spinosad, essential oils and entomopathogenic fungi (Bond *et al.* 2004; Howard *et al.* 2010; Williams *et al.* 2019; Baz *et al*. 2022).

Spinosad is a bioinsecticide and member of the spinosyn family of insecticides, and one of the most promising alternative methods for controlling mosquito species. It consists of a mixture of two active components, spinosyn A and D derived from a rare, naturally occurring soil dwelling actinomycete bacterium called *Saccharopolyspora spinosa* (Hertlein *et al.* 2010). Several reports were evaluated Spinosad as a larvicidal against mosquito species under laboratory conditions during the last years (Bahgat *et al*. 2007; Perez *et al.* 2007; Benhissen *et al*. 2014; Moselhy *et al*. 2015; Sisodiya *et al*. 2021). However, insufficient knowledge is available on the assessment of Spinosad on mosquito species in their breeding habitats under field experiments (Bond *et al*. 2004; Williams *et al.* 2019). Therefore, the aim of the present study was to determine the efficacy of Spinosad on larvae and pupae of *Culex* species in their field breeding habitats of Beni Suef Governorate, Egypt.

MATERIAL AND METHODS

Insects and bioinsecticide

The field strain of *Culex* species was collected as larvae and pupa from various breeding habitats of tested sites area at village of Saft, Beba, Beni Suef Governorate, Egypt and identified in Research Institute of Medical Entomology, Ministry of Public Health and Population, Dokki, Giza, Egypt. The colony was maintained under the laboratory conditions of 26±2°C and 70±5% R.H. The 2^{nd} and 4^{th} instars larvae and pupae of *Culex* species were collected for the bioassay tests.

Spinosad (Spintor 48% SC) obtained from Dow AgroSciences Co., Cairo, Egypt.

Laboratory experiments

The susceptibility of *C. pipiens* to Spinosad was tested in the laboratory using a methodology adapted from the Elliot larval test (WHO, 1975). Groups of 20 second instar larvae, 20 fourth instar larvae and 20 pupae were separately placed in 150 mL plastic cups containing a solution of Spinosad at one of the following concentrations: (0.125, 0.25, 0.50, 1.0, 2.0, 5.0, 10.0, 15.0 and 20.0 ppm) for 2^{nd} and 4^{th} instars larvae and concentrations of Spinosad were increased to 50, 100, 150, 200, 500, 1000, 1250, 2500 and 5000 ppm for pupae. Each concentration was replicated four times. An additional cup contained clean water as an untreated control. A mixture of ground dried bread and Brewer's yeast pellets (3:1) were added daily larval food. Dead second and fourth instar larvae were recorded after 1 and 24 h while in case of pupae, the pupal mortality was counted after 24, 48, 72 and 168 h.

Semi-field experiments

Semi-field experiments were conducted in village of Saft, Beba distract, Beni Suef Governorate, Egypt. The village of Saft contains several breeding habitats of *Culex* species such as *C. pipiens*, *C. theileri* and *C. perexiguus*. The semi field experiments were performed in plastic tanks (27.0 cm height; 26.5 cm diameter) locality in the mosquitoes breeding habitats sites were filled with 2000 ml water. Then, fifty of larva and pupae were introduced in plastic tank contained water treated with Spinosad (5000 ppm). A small amount (15 mg) of mixture of ground dried bread and Brewer's yeast pellets was provided daily to the larvae. Three plastic tanks were applied for second and fourth instar larvae and pupae of *Culex* species. Dead larvae and pupae were recorded after 1, 24 and 48 h.

Field experiments

The field experiments were performed in two breeding habitats include, cement irrigation tank and agricultural canals in village of Saft, Beba, Beni Suef Governorate, Egypt between March and May 2022. These breeding habitats contained almost *Culex* species mainly *C. pipiens, C. theileri, C. perexiguus* and *C. antennatus*. For field experiments breeding sites were selected which were unlikely to be used for drinking water by animals (Mohamed *et al*. 2022). Spinosad with 5000 ppm were assessed at two breeding habitats. Application doses were achieved by spraying of precalculated amount of oxymatrine with the help of a hand compression sprayer. Prior to spraying, density of immatures was estimated by dipper sampling method using a standard dipper. Density of immatures per dip (five dip x three replicates) was monitored in control and treated habitats at 1 and 24 h. The data obtained at two different times of observation were pooled together to get means of reduction. The percent of reduction in larval and pupal density of *Culex* species was calculated by Mulla *et al*. (1971).

Data analysis

The mortality was corrected according to Abbott's formula (1925) and mortality data transformed by arcine and submitted to a one-way analysis of variance (ANOVA) by using SPSS 21.0 Software (SPSS, Chicago, IL, USA). Tukey's HSD test was used to detect differences between mean mortality values at the 0.05 significance level.

RESULTS AND DISCUSSION

Laboratory experiments

The results demonstrated that concentrations of Spinosad caused higher mortality of larval instars of *C. pipiens* compared to the untreated treatment. The highest larval mortality of second instar (60.0 %) and fourth instar (46.7 %) was achieved by concentration of Spinosad (20 ppm) after 1h of exposure and most concentrations of Spinosad were caused total mortality of two larval instars after 24 h of exposure (Table 1). On the other hand, Spinosad up to 1000 ppm not affected on treated pupae during 72 h of exposure, then the pupal mortality gradually increased at the higher concentrations and the highest pupal morality of *C. pipiens* was obtained at concentration of 5000 ppm (93.3%), after 168 h under laboratory conditions (Table 2).

Table 1. Mortality percentage of larval instars of *Culex pipiens* after 1 and 24 h after exposure to different concentrations of Spinosad under laboratory conditions

Treatment (Concentratio, ppm)	Mortality percentage of *Culex pipiens* larvae			
	Second larval instar		Fourth larval instar	
	1 h	24 h	1 h	24 h
0.0	0.0g	0.0b	0.0g	0.0c
0.125	3.3fg	100.0a	3.3fg	90.0b
0.25	13.3ef	100.0a	13.3ef	90.0b
0.5	13.3ef	100.0a	16.7de	96.7a
1.0	20.0de	100.0a	13.3ef	100.0a
2.0	26.7cd	100.0a	26.7cd	100.0a
5.0	33.3c	100.0a	33.3bc	100.0a
10.0	46.7b	100.0a	40.0ab	100.0a
15.0	56.7a	100.0a	43.3ab	100.0a
20.0	60.0a	100.0a	46.7a	100.0a

Mean values bearing the same letters within a column are not significantly different (P<0.05).

Table 2. Mortality percentage of pupal stage of *Culex pipiens* after 24, 48, 72 and 168 h after exposure to different concentrations of Spinosad under laboratory conditions

Treatment (Concentration, ppm)	Mortality percentage of *Culex pipiens* pupae after (h)			
	24	48	72	168
0.0	0.0d	0.0c	0.0d	0.0e
50.0	0.0d	0.0c	0.0d	0.0e
100.0	0.0d	0.0c	0.0d	0.0e
150.0	0.0d	0.0c	0.0d	20.0d
200.0	0.0d	0.0c	0.0d	30.0c
500.0	0.0d	0.0c	0.0d	33.3c
1000.0	0.0d	0.0c	0.0d	33.3c
1250.0	60.0c	73.3b	73.3c	73.3b
2500.0	80.0b	86.6a	86.6b	86.6a
5000.0	86.6a	90.0a	93.3a	93.3a

Mean values bearing the same letters within a column are not significantly different (P<0.05).

Semi-field experiments

The data presented in Table (3) indicated that Spinosad (5000 ppm) caused total mortality of larval instars of three *Culex* species (*C. pipiens, C. theileri* and *C. perexiguus*) in treated plastic tanks compared to the control treatment after 24 and 48 h. While the highest pupal mortality percentages of three *Culex* species (*C. pipiens, C. theileri* and *C. perexiguus*) were achieved after 48 h of exposure to 5000 ppm of Spinosad (90, 60 and 70%), respectively.

Table 3. Mortality percentages in immature stages of *Culex* species after 1, 24 and 48 h exposures to 5000 ppm of Spinosad in plastic tank under semi-field conditions

Concentration, ppm	Mortality percentage of								
	Second larval instar			Fourth larval instar			Pupal stage		
	Cx. pipiens	*Cx. theileri*	*Cx. perexiguus*	*Cx. pipiens*	*Cx. theileri*	*Cx. Perexiguus*	*Cx. Pipiens*	*Cx. theileri*	*Cx. perexiguus*
0.0	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 c	0.0 b	0.0 b	0.0 b
5000.0 after (1 h)	40.0 b	50.0 b	40.0 b	30.0 b	40.0 b	40.0 b	0.0 b	0.0 b	0.0 b
5000.0 after (24 h)	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	90.0 a	60.0 a	70.0 a
5000.0 after (48 h)	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	100.0 a	90.0 a	60.0 a	70.0 a

Mean values bearing the same letters within a column are not significantly different (P<0.05).

Field experiments

The percent of reduction in density per five dip of larval instars of *Culex species, C. pipiens, C. thelerei, C. perexiguus* and *C. antennatus* in treated cement irrigation tanks and agriculture canals with Spinosad is presented in Tables (4 and 5). The results were demonstrated the complete reduction (100%) of larval density of *Culex* species were achieved at 5000 ppm of Spinosad at both breeding habitats, cement irrigation tank and agriculture canal after 24 h.

Table 4. Percent of reduction in density of *Culex* species larvae per five dips after exposure for 1 and 24 h with Spinosad at 5000 ppm in cement irrigation tank under field conditions

Concentration, ppm	Percent of reduction of second larval instar							
	C. pipiens		*C. theileri*		*C. Perexiguus*		*C. antennatus*	
	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)
0.0	5.0	0.0 c	2.0	0.0 c	1.0	0.0	3.0	0.0
5000.0 after (1 h)	3.0	40.0 b	1.0	50.0 b	1.0	0.0	0.0	100.0
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0	0.0	100.0
Percent of reduction of fourth larval instar								
0.0	8.0	0.0 c	5.0	0.0 c	3.0	0.0 c	3.0	0.0 c
5000.0 after (1 h)	6.0	25.0 b	3.0	40.0 b	1.0	66.7 b	2.0	33.3 b
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a

Mean values with the same letter within a column are not significantly different (P<0.05), PR% = Percent of reduction.

Table 5. Percent of reduction in density of *Culex* species larvae per five dips after exposure for 1 and 24 h with Spinosad at 5000 ppm in agriculture canal under field conditions

Concentration, ppm	Percent of reduction of second larval instar							
	Cx. pipiens		*Cx. theileri*		*Cx. perexiguus*		*Cx. Antennatus*	
	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)	No. density	PR (%)
0.0	6.0	0.0 c	6.0	0.0 c	1.0	0.0	1.0	0.0
5000.0 after (1 h)	4.0	33.3 b	3.0	50.0 b	0.0	100.0	0.0	100.0
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0	0.0	100.0
Percent of reduction of fourth larval instar								
0.0	11.0	0.0 c	7.0	0.0 c	4.0	0.0 c	3.0	0.0 c
5000.0 after (1 h)	8.0	27.3 b	6.0	14.3 b	2.0	50.0 b	2.0	33.3 b
5000.0 after (24 h)	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a	0.0	100.0 a

Mean values with the same letter within a column are not significantly different (P<0.05), PR% = Percent of reduction.

On the other hand, the percent of reduction in pupal density of *Culex* species in two breeding habitats was decreased to 77.8 and 36.4% in cement irrigation tank and agriculture canals, respectively after 24 h (Table 6).

Table 6. Percent of reduction in density of *Culex* species pupae per five dips after exposure for 1 and 24 h with Spinosad at 5000 ppm in two breeding habitats (cement tank and agriculture canal) under field conditions

Concentration, ppm	Percent of reduction in density of pupae			
	Cement tank		Agriculture canal	
	No. density	PR (%)	No. density	PR (%)
0.0	9.0	0.0 c	11.0	0.0 c
5000.0 after (1 h)	7.0	22.2 b	8.0	27.8 b
5000.0 after (24 h)	2.0	77.8 a	7.0	36.4 a

Mean values with the same letter within a column are not significantly different (P<0.05), PR% = Percent of reduction.

Efficacy of Spinosad on mosquito species has been reported by several studies (Bond *et al*. 2004; Williams *et al*. 2019; El Sayed *et al.* 2020). These previous reports indicated that Spinosad had the ability to manage mosquito larvae as biocide. Our results indicated that Spinosad caused higher toxicity against *C. pipiens* and the total mortality of second and fourth larval instars was achieved at lower concentrations. While the highest pupal morality of *C. pipiens* was obtained at concentration of 5000 ppm (93.3%), after 168 h under laboratory conditions. These findings were matched with previous studies demonstrated efficacy of Spinosad on *Culex* species such as Bahgat *et al.* (2007) showed that Spinosad was tested in laboratory and field against some *Culex* species in El-Ismailia Governorate and found the LC_{50} value of the liquid formula of Spinosad for *C. pipiens* was 0.002 ppm after 24 h. This higher toxicity of Spinosad was observed on fourth instar larvae of *C. pipiens* (Benhissen *et al.* 2014). Moselhy *et* *al*. (2015) revealed that Spinosad was the most effecting tested insecticide on *C. pipiens* larvae. El Sayed *et al.* (2020) showed that efficacy of Spinosad was studied to control *C. pipiens* larvae by different concentrations. The mortality percentage of larvae was increased by increasing concentrations and time. While 100% mortality was obtained at the concentration 1000 µl/10 ml of Spinosad after 48 h. This higher insecticidal efficacy of Spinosad on *Culex* species may be due to their mode of action involving the postsynaptic nicotinic acetylcholine and GABA receptors (Salgado, 1998; Watson, 2001).

The results of current study revealed that Spinosad (5000 ppm) showed pronounced toxicity against larval stages of *Culex* species with total mortality of larval instars, while the pupal mortality was more than 60% in semi-field experiments. Similar results were obtained by Bond *et al.* (2004) found that Spinosad 1 ppm compared with the standard temephos (Abate1) 1% granules 100 g/m3 water prevented Aedes aegypti breeding in plastic containers of water for 8 weeks, at 10 ppm Spinosad prevented breeding for > 22 weeks. Gharib (2021) found that Spinosad showed the highest larvicidal effect against *C. pipiens* with mortality percentage (69.5%) in semi field experiments. The same finding was obtained previously by other insecticides on mosquito species (Thavara *et al.* 2004; Marcombe *et al.* 2011; Mohamed *et al*. 2022).

The results of current study demonstrated that Spinosad (5000 ppm) caused total mortality of larval instars of *Culex* species and the pupal mortality was higher in breeding habitat, cement irrigation tank (77.8 %) and decreased to 36.4% in agriculture canal. Similar results were obtained by Bond *et al*. (2004) showed that the field experiment of Spinosad (5 ppm) caused completely eliminated reproduction of *A. aegypti* for 13 weeks. They also predict that Spinosad is likely to be an effective larvicide for treatment of mosquito breeding sites. Williams *et al.* (2019) examined the efficacy of Spinosad for the control of mosquito larvae present in experimental tires in Veracruz State, Mexico in the period 2015–2016 and found that Spinosad granules provided control of larvae and pupae of *A. aegypti*, *A. albopictus* and *Culex* spp. in used tires in Veracruz State, Mexico.

CONCLUSIONS

It could be concluded that Spinosad is highly effective for the management of mosquitos. It's able to achieve total larval mortality of *Culex* species in their field breeding habitats such as cement irrigation tank and agricultural canal as strong larvicide alternative to synthetic insecticides. The results demonstrated also, the low efficiency of Spinosad on pupal stage of *Culex* species in field experiments. Further studies should be done to assess Spinosad effectiveness as pupalcide against mosquito species in various ecological regions, different mosquito populations, and species in their field breeding habitats.

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