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Effects of Sublethal Concentrations of Vectobac 12 AS on Some Biological Parameters of the Malaria Vector *Anopheles superpictus*

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Abstract: The effects of sublethal concentrations of *B.t.i.* (Vectobac 12 AS, LC_{20} = 0.15 and LC_{70} = 0.76 mL m^{-2}) on life parameters of *Anopheles superpictus* were assessed in the laboratory for 3 generations. According to the data, sex ratios were affected by exposure to sublethal doses of *B.t.i.* Developmental time was prolonged significantly in groups treated with LC_{70}, while, LC_{20} had no effect. Pre-oviposition periods were not affected by sublethal concentrations. Oviposition period in the LC_{70} group was prolonged, whereas, in the control and LC_{20} groups it was nearly identical. Longevity was affected by sublethal exposure and increased from F_1-F_3 in both groups. Gross and net reproductive rates were adversely affected in both exposure groups and these effects increased with increasing *B.t.i.* concentration. Generation time was affected by exposure to sublethal concentrations and decreased from generation to generation. Main daily fecundity did not differ significantly between treatment groups and the control group but was slightly higher in the latter. Significant differences ($p<0.05$) between generations in terms of survival rate were found in the LC_{20} group but not in the LC_{70} group. Life parameters were affected adversely and this effect was more pronounced in lines exposed to higher concentrations. Lower concentration effects were not clear and parameters fluctuated between generations when compared to the control group.

Key words: *Bacillus thuringiensis* var. *israilensis*, vectobac, *Anopheles superpictus*, sublethal dose effects, gross reproductive rate, net reproductive rate, generation time

INTRODUCTION

Thirteen *Anopheles* sp. have been recorded in Turkey (Ramsdale *et al*., 2001). Of these, *Anopheles* (*Cellia*) *superpictus* Grassi is one of the most important and widely distributed species, especially in malarious regions of Turkey (Parrish, 1959; Postiglione *et al*., 1973; Ozer *et al*., 2001, Simsek, 2006). The efficiency of this species as a vector of *Plasmodium vivax* (Kasap *et al*., 1987) and *Plasmodium falciparum* (Luty *et al*., 2006) has been demonstrated under laboratory conditions. It is also, considered to be an important malaria vector in the Middle East, Middle Asia and Mediterranean countries and a secondary vector in other regions (Barkai and Saliternik, 1968; Zahar, 1974; Romi *et al*., 1997). For these reasons, *An. superpictus* is always taken into consideration in malaria control programs conducted in Turkey. The microbial insecticide *Bacillus thuriensis* var. *isralensis* (*B.t.i.*) was integrated into mosquito control programs in the last decade of the 20th century. Recently, *B.t.i.* has been commonly used in mosquito larvae control programs in Turkey (Matur and Ceber, 1988; Simsek *et al*., 2005) and *B.t.i* applications have increased in *Anopheles* larvae control programs. Larvicidal agents, if administrated at high enough concentrations, will yield complete or almost complete mortality in exposed populations. A number of chemical larvicides and mosquito control agents have been shown to manifest delayed effects at sublethal doses in the survivors. Adugelo-Silva and Spielman (1984) have shown that in the laboratory inefficient larviciding reduces larval competition among the survivors and increases the density and average body size of the resulting adult population. Hare and Nasci (1986) noted delayed mortality in surviving larvae of *Aedes aegypti* exposed to a median lethal concentration of *B.t.i.* Mulla and Singh (1991) examined some biological parameters and morphogenetic aberrations of *Culex quinquefasciatus* larvae, pupae and adults after larvae

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were treated with sublethal concentrations (LC_{25} and LC_{80}) of *B.t.i.* Flores *et al*. (2004) have indicated that inefficient larviciding with *B.t.i.* reduces the developmental time and fecundity of *Ae. aegypti*. They examined the effects of sublethal concentrations (LC_{30}, LC_{50} and LC_{70}) of *B.t.i.* on survival, longevity, fecundity and sex ratio of adults for surviving larvae and their F_1 progeny. However, it is well known that the effects of insecticides and pesticides vary from generation to generation. Therefore, in order to have a more complete understanding of the effects of sublethal concentrations of *B.t.i.* on mosquito populations it is necessary to monitor populations for several generations. The present study aims to fill such a gap by assessing the effects of sublethal concentrations of *B.t.i.* (Vectobac® 12 AS) on different biological parameters of *An. superpictus* for three generations.

MATERIALS AND METHODS

Mosquitoes: The *An. superpictus* specimens were from a colony established in the insectary of Hacettepe University Ecological Sciences Research Laboratory (ESRL). This colony originally was obtained from the village of Magarali, 10 km southeast of Birecik (37°, 01'N and 37°57'E) district in Sanliurfa province, in the malarious region of Turkey (Simsek *et al*., 2005).

B.t.i.: A commercial *B.t.i.* product, Vectobac® 12 AS (1200 ITU mg^{-1}, Valent Biosciences), was used to determine the effects of sublethal concentrations of *B.t.i.* on biological parameters of *An. superpictus*. The sublethal concentrations examined in this study were LC_{20} (0.15 mL m^{-2}) and LC_{70} (0.76 mL m^{-2}), one under and one above the LC_{50} concentration. The LC_{20} value was chosen because this was the minimum concentration available that would not violate the 10% error rate. The LC_{70} value corresponds to the same concentration above the LC_{50} value. These concentrations were determined in ESRL in 2004 and repeated again in 2005.

Experimental procedure: The laboratory was maintained at 26±2°C, 65%, ±5 relative humidity with a 12:12 h light:dark cycle photoperiod. Cohorts of 3000 eggs were used in the establishment of the *An. superpictus* colony. After hatching, 1st stage larvae were transferred into rearing pans filled with 2 L of distilled water (25±2°C). The larvae were fed twice daily with 0.01-0.04 g of powdered larval food (TetraMin® fish food), which was spread evenly onto the water surface (Bangs *et al*., 2002; Kuhn, 2002). Late 3rd instars larvae were exposed to sublethal concentrations (LC_{20} and LC_{70}) of *B.t.i.* Treatments were conducted on a total of 500 larvae within plastic rearing cups containing 200 mL of deionized water and 25 larvae each (total 20 cups). The 1st cohort exposed to sublethal concentrations was named the F_1 generation. After 24 h of exposure the surviving larvae were transferred to fresh containers including 200 mL of distilled water. Every 24 h the larvae cups were checked and surviving pupae were collected and transferred to adult cages. After adult emergence 40 females and 40 males were picked at random and transferred to new cages. Adult mosquitoes were fed with 10% sugar water. A live rabbit was used for blood feeding of female mosquitoes (1 h every day). Surviving females and their eggs were recorded every 24 h until the death of the last female. Survival, longevity and fecundity of the females were calculated using the data obtained by methods outlined in Krebs (1985). This procedure was carried out for 3 generations. Offspring from the F_1 generation were used in establishment of the F_2 generation and similarly F_2 offspring were used in establishment of the F_3 generation. Differences in main daily fecundity and developmental cycle were compared using the Tukey test. Survival curves were compared by means of the log rank test for the survivors of the exposed generations and the control group.

RESULTS

Sex ratio and developmental cycle: Sex ratio results (Table 1) indicated that in the treatment group female ratios were slightly higher than male ratios. The sex ratios in the LC_{20} treatment group fluctuated but the last generation sex ratios were same as the 1st generation ratios. Sex ratio in the F_1 generation of the LC_{70} group was 1:1.13 but in all other generations for both treatment concentrations sex ratios were close to 1:1. Sex ratio of the control group was 1:1.40.

Developmental time of *An. superpictus* from F_1-F_3 generation exposed to LC_{20} did not differ significantly ($p>0.05$) between the groups. LC_{20} and control group development time were nearly identical. LC_{70} group results were significantly higher ($p<0.05$) than LC_{20} group and control group results.

Oviposition: According to our results, both oviposition and pre- and postoviposition periods in females showed differences between the control and treatment groups (Table 2). The preoviposition period in the treatment groups was in general longer than that in the control group but the difference was not statistically significant. Oviposition period in the LC_{20} group varied between 38 and 56 days and fluctuated from F_1-F_3. Oviposition period for the two treatment groups showed a significant increase in the F_2 generation (LC_{20}: 56, LC_{70}: 54).

Table 1: Mean developmental time in days and male:female ratio of *Anopheles superpictus* exposed to sublethal concentrations of *B.t.i.* for 3 generations

Concentration/	LC_{20}				LC_{70}			
generation	Mean	SE	Male	Female	Mean	SE	Male	Female
F1	15	1.71	1	1.04	20.5	2.44	1	1.13
F2	16	1.71	1	1.02	19.5	2.44	1	1.02
F3	14	1.71	1	1.04	21.0	2.73	1	1.04
Control	16	1.20	1	1.40	-	-	-	-

Table 2: Periods of the pre-oviposition, oviposition, postoviposition and longevity of female *Anopheles superpictus* that emerged from surviving larvae after sublethal concentrations of *B.t.i.* for 3 treatment periods

Concentration/	LC_{20}			LC_{70}			
generation	F1	F2	F3	F1	F2	F3	Control
Preoviposition	7	6	6	6	6	5	4
Oviposition	40	56	38	44	54	52	39
Postoviposition	8	2	18	11	3	6	7
Longevity	55	64	62	61	63	63	50

Table 3: Population parameters and rate of hatching, pupae and emergence of *Anopheles superpictus* surviving from larvae exposed to sublethal concentrations of *B.t.i.* for 3 generations

Parameters	Control	LC_{20} F1	LC_{20} F2	LC_{20} F3	LC_{70} F1	LC_{70} F2	LC_{70} F3
Net Reproductive rate (Ro)	2480	1779	3006	1474	1369	2311	1044
Gross Reproductive Rate (GRR)	10145	10114	14376	8531	9634	9791	6314
Mean generation Time (Tg)	40.3	40.7	40.01	34.01	39.29	36.31	34.56
Intrinsic growth rate (rm)	0.19	0.18	0.2	0.21	0.18	0.21	0.2
Finite growth rate (ë)	1.2	1.19	1.22	1.23	1.19	1.23	1.22
Instantaneous birth rate (b)	0.8	0.9	0.86	0.99	0.86	1.045	1.08
Instantaneous mortality rate (d)	0.61	0.71	0.66	0.78	0.68	0.83	0.88
Hatching (%)	85	73	73	72	75	62	78
Pupae rate (%)	52	51	43	41	58	43	51
Emergence of adults (%)	34	40	33	27	46	33	42

Postoviposition period varied between the 2 treatment groups and was very variable and no directional change could be determined. In general, longevity increased in all treatment groups for all generations.

Growth parameters, fecundity, hatching, pupation and emergence rate: The results showed a decrease in Gross Reproductive Rate (GRR) with increasing concentrations.

In all generations individuals showed greater GRR values at LC_{20} when compared with LC_{70}. In both treatment groups, there was an initial drop in GRR in the F_1 generation (LC_{20}: 10114, LC_{70}: 9634), followed by an increase in the F_2 generation, reaching values above or similar (LC_{20}: 14376, LC_{70}: 9791) to those of the control group (10145). However, in the F_3 generation GRR values again dropped below those of the control group for both treatment lines (Table 3).

Mean generation time showed a decrease in both treatment groups, while this drop occurred in the F_3 generation in the LC_{20} group (34.01). It occurred in the F_2 generation in the LC_{70} group (36.31).

The Net Reproductive Rate (NRR) substantially fell from F_1-F_3 for both treatment lines. However, F generation values were higher than or similar to those of the control group.

Although, GRR and NRR values changed with treatment and showed a decreasing trend, the finite and intrinsic growth rate did not show any differences between generations or treatment lines.

Hatching rates were substantially reduced in all generations for both treatment groups, when compared to the control group. Although, no trend in hatching rate was observed in the LC_{20} group, in the LC_{70} group there was 1st a decrease (F_2: 62%) and then an increase (78%) in hatching rate from F_1-F_3. Pupation rates were not correlated with hatching rates. Pupation rates decreased from F_1-F_3 in the LC_{20} group and decreased from F_1-F_2 and increased from F_2-F_3 in the LC_{70} group. The change in direction of emergence rates for both treatment lines was similar to that seen for pupation rates.

Fecundity: Mean daily fecundity did not show any significant ($p<0.05$) differences between generations (F_1-F_3) or treatment groups (LC_{20}, LC_{70}) or between generations and controls. Although, we did not determine any significant differences between the control and treatment groups, mean daily fecundities observed in both lines for all generations were lower than those in the control group. In addition, mean daily fecundity of the LC_{70} line was slightly lower than that of the LC_{20} line and control line (Table 4).

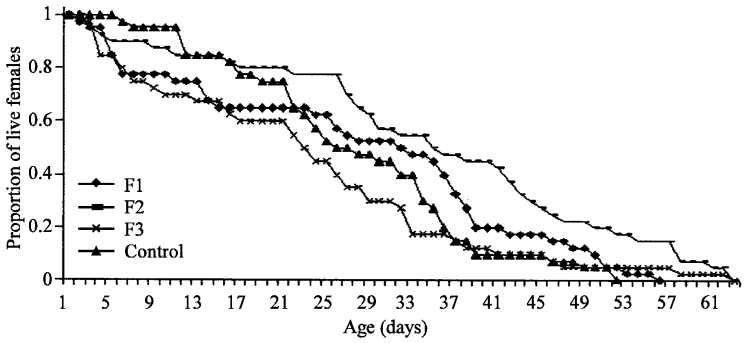

Fig. 1: Survivorship curves of *Anopheles superpictus* that emerged from larvae surviving LC_{20} concentrations of Vectobac 12 AS for 3 treatment generations and the control group

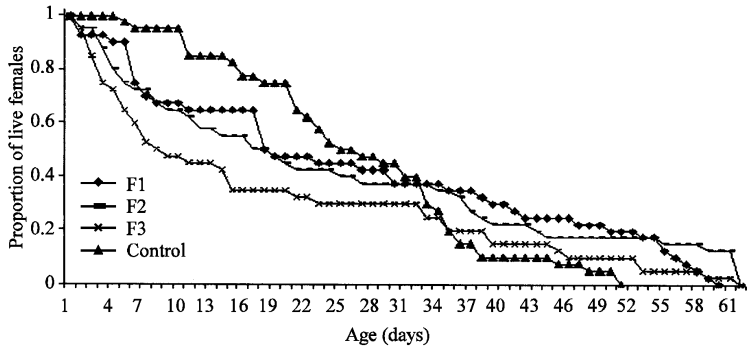

Fig. 2: Survivorship curves of *Anopheles superpictus* that emerged from larvae surviving LC_{70} concentrations of Vectobac 12 AS for 3 treatment generations and the control group

Table 4: Mean daily fecundity of female *Anopheles superpictus* that emerged from larvae surviving treatment with (three generation) 2 different sublethal concentrations of Vectobac 12 AS and the control group

	LC20		LC70		Control	
Generation	Mean	SD	Mean	SD	Mean	SD
F1	11.1	5.82	10.41	5.89	15.7	8.79
F2	13.3	4.67	11.97	4.79	-	-
F3	13.8	1.52	9.02	4.9	-	-

Survival: The survival curves of the treatment groups (all generations) and control group (Fig. 1 and 2) were compared by means of the log-rank method. The only significant differences ($p<0.05$) in the LC_{20} group were between F_2 and F_1 and between the F_3 generation and the control group. In the LC_{70} group there were no significant differences between the F_1, F_2 and F_3 generations.

DISCUSSION

According to the results, some life parameters were adversely affected more than others. Development cycle was influenced by increasing *B.t.i.* concentrations. The effects of a low sublethal concentration (LC_{20}) were not significant ($p>0.05$) but a high concentration (LC_{70}) had serious effects on developmental time, prolonging the maturation period. In other words, exposure to a low concentration of *B.t.i.* may shorten the duration of the development cycle (results were statistically not significant), whereas, a high concentration (LC_{70}) may prolong the duration of the development cycle. However, Flores *et al.* (2004) found somewhat different results showing that exposure to low concentrations of *B.t.i.* significantly shortened the duration of the developmental cycle and exposure to high concentrations caused no apparent significant differences.

Sex ratios were influenced by *B.t.i.* application but there was no clear difference between the generations and treatment groups except for in the LC_{70} F_1 generation. The effects are biased towards a reduction in the proportion of females compared to the control group. Based on the results obtained here, it can be concluded that the sex ratio did not differ between generations or among individuals exposed to different sublethal concentrations of *B.t.i.* However, sex ratios in all exposure groups differed from those of the control group. Flores *et al.* (2004) obtained a reduction in female ratios after treatment with *B.t.i.* This indicates that treatment of populations with

B.t.i. could lead to a reduction in reproductive population size. At the same time, studies on the effects of chemical insecticides on sex ratio indicate a distortion towards males (Priyalakshmi *et al*., 1999). All preliminary observations and results shown here indicate that the effects of chemical and biological insecticides on sex ratios are nearly the same.

Results also revealed that there was an extension in total ovipositional period compared to the control group. This effect increased with increasing *B.t.i.* concentration, but was only observed for generations in the LC_{70} line. Postovipositional results were not correlated with the results obtained during the ovipositional period. This period varied widely. As a result, Vectobac causes an increase in female longevity but this is not related to *B.t.i.* concentration. Flores *et al*. (2004) showed an extension of the preovipositional period but the control group preoviposition period was shorter than that of the treatment groups in that study. At the same time, they showed an extension of longevity.

Net and gross reproductive rates decreased with *B.t.i.* exposure. The reduction was greater with increasing *B.t.i.* concentrations but this trend was not observed in the 2nd generation. We cannot fully explain this result but it may be a response by the population to continuous insecticide pressure resulting from *B.t.i.* exposure. The reduction in the GRR and R_0 with increasing *B.t.i.* concentration indicates lower reproductive potential in females. In this study, this was reflected by a decline in total fecundity. While, results showed a decline in total fecundity, Foo and Yap (1982) could not show any significant differences between control group and *B.t.i.* H-14 treated groups for fecundity, but Zahiri and Mulla (2006) reported a reduction in oviposition values by *B.t.i.* and *B.s.* in tests with a range of concentrations from 0.1-2.0 mg L^{-1}. Flores *et al*. (2004) showed a decline in GRR with increasing *B.t.i.* concentrations and reported lower reproductive potential in females with increasing *B.t.i.* concentrations. They also, indicated that this was reflected by a decline in total fecundity resulting from an exposure to concentrations higher than LC_{50}. According to Prilayakshmi *et al*. (1999), chemical insecticides (fenitrothion, deltamethrin and cypermethrin) have the same effects.

The generation time determined with sublethal concentrations is shorter than that of the control group except for the LC_{20} F_1 generation but is not significantly higher than the control group. Results for generation time indicate a reduction in values from generation to generation. Results obtained from the 1st generation were nearly identical to those from the control group, but generation time decreased substantially in the F_2 and F_3 generations for both treatment lines. This resulted in a daily reduction in population size although finite growth rates did not show any significant differences between generations. Flores *et al*. (2004) found an increase in generation time in *Ae. aegypti* lines that were treated with a sublethal concentration.

Although, GRR and R_0 values varied between generations, the intrinsic growth rate showed no significant differences between generations, concentrations, or the control group.

LC_{70} group survival curves showed no significant difference from those of the control group and no significant difference between generations. However, the LC_{20} group showed significant differences from the control group and also between the F_2 and F_1 and F_2 and F_3 generations. Zahiri and Mulla (2006) found that survival rates of larvae decreased with increasing *B.t.i.* exposure. Their study demonstrated that egg raft deposits are adversely affected by an increase in *B.t.i.* concentrations and also demonstrated that females die before they can deposit their whole compliment of eggs. Flores *et al*. (2004) found significant differences among the exposed individuals in all treatment groups, but they did not show any differences between the control group and groups treated with LC_{50} or LC_{70} concentrations.

Mean daily fecundity was adversely affected by sublethal concentrations. These effects increased with increasing *B.t.i.* concentrations but were not statistically significant.

Concentrations of *B.t.i.* used in this study were not higher than the normal application rates used in mosquito control studies. We noted some adverse effects of *B.t.i.* concentrations on some biological parameters. These results suggest additional advantages of *B.t.i.* for use in control programs. These findings along with the findings of Flores *et al*. (2004) and Zahiri and Mulla (2006) have revealed the extra potential of this agent as a larvicide.

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