Turk J Zool
31 (2007) 317-323

The Efficacy and Longevity of VectoBac® 12 AS and VectoBac® G (both based on *Bacillus thuringiensis* subsp. *israelensis*) for the Control of Mosquitoes in Turkey*

Adnan ALDEMİR

Kafkas University, Faculty of Science and Letters, Department of Biology, 36100 Kars - TURKEY

Received: 16.01.2006

Abstract: Under the field conditions of the Gölbaşı district of Ankara, commercial preparations of VectoBac® 12 AS (aqueous suspension) and VectoBac® G (corn cob formulation), both based on *Bacillus thuringiensis* subsp. *israelensis* (H-14), were tested against *Anopheles sacharovi*, *An. maculipennis*, *Culex pipiens*, and *Cx. theileri* larvae at the edge of a lake, a flood plain, and a swamp. Applying VectoBac® 12 AS at the rate of 1.0-1.25 l/ha and VectoBac® G at 7.5-10.0 kg/ha produced excellent initial control (90%-100%) of all mosquito larvae under Central Anatolian conditions. An increase in larval mortality was detected in all treatments as dose rates increased, but there was no significant difference between dose rates and residual activity on post-treatment day 20 ($P > 0.05$).

Key Words: Mosquito, biocontrol, *Bacillus thuringiensis israelensis*, field application, Turkey

Türkiye'de Sivrisinek Kontrolünde *Bacillus thuringiensis* subsp. *israelensis*'in VectoBac® 12 AS ve VectoBac® G Preparatlarının Etkinlik ve Kalıcılığı

Özet: Ankara ilinin Gölbaşı ilçesinde, alan şartlarında, *B. thuringiensis* (H-14)'in ticari preparatlarından VectoBac® 12 AS (aqueous suspension) and VectoBac® G (corn cob formulation) göl kenarı, su birikintisi ve bataklık habitatında *Anopheles sacharovi*, *An. maculipennis*, *Culex pipiens* ve *Cx. theileri* larvalarına karşı denendi. Orta Anadolu şartlarında, VectoBac® 12 AS'nin 1-1,25 l/ha, VectoBac G'nin 7,5-10 kg/ha oranları ile, bütün sivrisinek larvaları için mükemmel bir başlangıç kontrolü (% 90-100) sağlandı. Uygulamaların tümünde, doz oranlarına bağlı olarak larva ölümlerinde artış belirlendi, fakat; uygulama sonrası 20. günde doz oranı ile kalıcı etkinlik arasında anlamlı bir ilişki belirlenemedi ($P > 0,05$).

Anahtar Sözcükler: Sivrisinek, biyokontrol, *Bacillus thuringiensis israelensis*, alan uygulaması, Türkiye

Introduction

For almost 2 decades, *Bacillus thuringiensis israelensis* (Bti) and *Bacillus sphaericus* have been successfully used as biological control agents against mosquitoes, black flies, and some other nematoceran flies, without any evidence of harmful environmental effects (Becker, 2000). Both agents are spore-forming bacteria that produce parasporal proteinaceous crystal toxins demonstrating high activity against larvae of certain dipterans (Mulla et al., 1999). Presently Bti is used on all continents and several commercial formulations are available, namely liquid concentrates, wettable powders, granules, pellets, dunks and briquettes, tablets, polymer matrixes, and ice granules (Margalith et al., 2000).

Continental semi-arid climate conditions prevail in the present study area in which mosquitoes are a problematic nuisance. Additionally, a public survey conducted in this area found that the local population were very sensitive to environmental issues and wanted mosquito control programs that did not negatively impact the environment (Aldemir and Boşgelmez, 2005).

*This study is a part of the doctoral dissertation of A. Aldemir.

The purpose of this study was to determine the effective dose and longevity of VectoBac® 12 AS and VectoBac® G for the control of mosquito larvae under Central Anatolian conditions.

Materials and Methods

Study area

The study was performed during July 2002 in the Lake Mogan vicinity near Ankara, the capital city of Turkey, which is a good indicator of the climatic conditions typical of Central Anatolia. This site is an important recreation area for the inhabitants of Ankara and the region has been declared a protected area because of its biological diversity. The lake area is 6.35 km^2 and its drainage area is 925 km^2. Since a major part of this drainage area is used for agricultural purposes, there is significant sediment flow into the lake. Lake Mogan is a fresh water lake with a eutrophic structure; this lake has gradually been turning into a swamp area and hosts massive macrophyte growth (Yerli, 2002). The diversity of larval habitats and the suitability of the climatic conditions are the major reasons behind the high mosquito population in the lake vicinity (Aldemir et al., 2002).

Three major mosquito breeding sites were selected for field trials: the lake edge, flood plain, and swamp. For each habitat type, each dose was applied in a 100 m^2 area and between these areas 3 m^2 were left untreated. In addition, for each habitat type 100 m^2 of breeding sites were left untreated as control areas.

Application

Using 4 different doses of each biocontrol agent, field applications were performed in each of the 3 different habitats (2 agents × 4 application rates × 3 larval breeding areas). The methods and procedures described by Karch et al. (1991) and Mulla et al. (1997, 1999) were used for applications and assessments, with some modifications according to field conditions.

The required amounts of the VectoBac® 12 AS formulation were placed in a compression spray tank and the required amount of tap water was added. The mixture was stirred with a stick and then shaken in the tank. The tank was then sealed and pumped to pressurize the spray mixture. The required amounts of VectoBac® G granules were applied by hand as evenly as possible.

Sampling

Larval and pupal sampling was performed by dipping with a standard 400-ml dipper. During sampling, 5 dip samples were taken from every treated 100-m^2 area each time. Similarly, 5 dip samples were taken each time in every control site. The dip samples were taken on post-treatment days 1, 3, 6, 12, and 20, depending on the larval growth duration of mosquito species in each trial area.

During pretreatment and post-treatment larval sampling, the contents of each dipper were transferred into different pots and transported to the laboratory. Immatures were counted and categorized as 1st and 2nd instars, and 3rd and 4th instars. The 3rd and 4th instars were identified immediately with the use of keys (Harbach, 1985, 1988; Snow, 1990), while the 1st and 2nd instars were reared to 3rd and 4th instars. Pupae were not included in the immature population sampling, since *B. thuringiensis* does not kill them.

Efficacy Assessment

In order to determine the magnitude of reduction of immatures, the sampling counts of different post-treatment days were compared to those of the pretreatment. Efficacy rates, in terms of percentage larval mortality, of the different doses of applied formulations on the species in different larval breeding areas are shown in Tables 1-3.

Larvae were counted on post-treatment day 20. The relationship between larval number and treatment dose was statistically analyzed using one-way analysis of variance (ANOVA).

Physical characteristics of the larval habitats

Physical parameters, such as water temperature, dissolved oxygen, conductivity, pH, and water depth, were measured 3 times during the field trials and mean values were calculated. During this study the mean air temperature was 24.8 °C and there was no precipitation.

Predominant macrophytes in the study areas were *Phragmites australis* (Cav.), *Typha angustifolia* L., *Bolboschoenus maritimus* (L.), *Potamogeton pectinatus* L., *Chara* sp., and *Najas marina* L.

Results

The results obtained from the 3 different habitats are shown in the relevant tables (Tables 1-3). There are essential similarities in the results obtained from different habitats. Low doses of each formulation (0.5 and 0.75 l/ha VectoBac® AS; 2.5 and 5.0 kg/ha VectoBac® G) were generally more effective on *Culex* species than *Anopheles* species. With high doses of each formulation (1 and 1.25 l/ha VectoBac® AS and 7.5-10 kg/ha VectoBac® G) in all treatments, larval mortality obtained in all species was about 90%-100% on post-treatment day 1.

Larval mortality rates of *An. maculipennis* and *Cx. pipiens* on post-treatment day 1 were 55%-60% and 97%-98% respectively, following the application of 0.75 l/ha liquid formulation and 5 kg/ha granule formulation, both of which were carried out at the lake edge (Table 1). In addition, while the larval mortality rates of *Cx. pipiens* and *Cx. theileri* were more than 90% on post-treatment day 1 following application of the same doses in the flood plain, the larval mortality rate was 50%-57% for *An. sacharovi* (Table 2). Similar results were determined in the swamp area (Table 3).

Generally, a decrease in the larval mortality rate was detected after post-treatment day 6. No larval reduction was observed on post-treatment day 12 or 20 in some areas in which high doses were used; that is, pretreatment larval numbers recovered (*An. maculipennis*, Table 1; *An. sacharovi*, Table 2; *Cx. pipiens*, Table 3).

On post-treatment day 20, the number of larvae in the treatment areas and the rates of applied doses in these areas were compared. There was no relationship observed between larval number and dose rate ($P > 0.05$); high dose rates did not increase the residual effect.

Discussion

Low doses of both tested formulations were more effective on *Culex* species than *Anopheles* species. In previous studies, it was reported that culicinae larvae are usually more susceptible than anophelines (Goldberg and Margalit, 1977; Lacey and Singer, 1982). Decreased anopheline susceptibility may be related more to surface feeding behavior and settling of the toxin than to innate susceptibility (Reuben et al., 1990).

Under Central Anatolian conditions it was established that the effective control rates of *Anopheles* and *Culex* species were 1.0-1.25 l/ha VectoBac® 12 AS and 7.5-10.0 kg/ha of VectoBac® G, whereas larval mortality was approximately 90%-100%. At the end of the field trial it was found that the residual activity of Bti preparations was not durable. On post-treatment day 3 larvae were found in many treatment areas and pretreatment larvae population numbers recovered in some treatment areas on post-treatment day 20, with a mean reduction value of 0% (Tables 1-3). These results show that mosquitoes lay eggs in areas treated with Bti because Bti spores in the substratum do not affect newly hatched larvae on the water surface. The duration of larvicidal activity of VectoBac® 12 AS is relatively short, usually lasting less than a week (Mulla et al., 1993).

In a study carried out in Zaire by Karch et al. (1991), polluted gutter water, the breeding site of *Cx. quinquefasciatus*, was treated with 2, 4, and 6 l/ha of VectoBac® 12 AS. At all doses larval mortality was more than 95% on post-treatment day 1, but larval mortality was less than 40% on post-treatment day 2 and the larval population began to recover 7 days after treatment. In the same research, irrigation ponds, the breeding sites of *An. gambiae*, were treated with 10, 15, and 20 kg/ha of the VectoBac® G granule formulation of Bti, and larval mortality was 90%-100% on post-treatment day 1, but the population began to recover 5 and 7 days after each treatment.

No significant relationship between dose rate and residual activity was found in our study ($P > 0.05$). This phenomenon can be explained by Bti's efficiency in nutrition zones of mosquito larvae. Bti is effective against larvae only while subsiding and after subsiding it does not show any effect against larvae. Mulla et al. (1993) carried out a study in field ponds in southern California in which 0.2, 1.0, and 2.0 kg/ha of VectoBac® 12 AS doses were evaluated against mixed populations of *Cx. quinquefasciatus*, *Culex stigmatosoma*, and *Cx. tarsalis*. According to the results of that study, excessive VectoBac® 12 AS doses did not significantly extend the duration of its effectiveness for controlling mosquito larvae.

In some application areas new larvae were found on post-treatment day 3 in the present study. The short-term residual activity of VectoBac® 12 AS and VectoBac® G may have been affected by some factors, such as the eutrophic structure of Mogan Lake (a freshwater lake), high conductivity values, and exposure to sunlight in the tested habitats. According to Margalith et al. (2000),

Table 1. Evaluation of VectoBac® 12 AS (1200 ITU) and VectoBac® G (200 ITU) formulations of Bti against *Anopheles maculipennis* and *Culex pipiens* larvae at the edge of Mogan Lake, Gölbaşı District, Ankara Province.

Rate		Mosquito species	Mean no. larvae/dip, pretreatment		Mean (%) reduction after treatment (days)									
Vec. AS (l/ha)	**Vec. G (kg/ha)**				1		3		6		12		20	
0.5	**2.5**	*An. maculipennis*	15	**13**	53	**69**	7	**15**	0	**0**	0	**7**	20	**0**
0.75	**5**	_	20	**15**	55	**60**	50	**53**	35	**0**	45	**6**	10	**33**
1	**7.5**	_	15	**18**	100	**89**	100	**100**	53	**44**	53	**22**	20	**5**
1.25	**10**	_	9	**22**	100	**100**	100	**100**	41	**64**	29	**59**	0	**18**
Control			17		18		0		29		35		12	
0.5	**2.5**	*Cx. pipiens*	42	**47**	79	**88**	67	**68**	50	**41**	14	**24**	0	**17**
0.75	**5**	_	64	**60**	97	**98**	91	**69**	94	**64**	67	**11**	55	**0**
1	**7.5**	_	61	**73**	98	**100**	69	**91**	84	**88**	44	**61**	20	**10**
1.25	**10**	_	34	**54**	100	**100**	87	**88**	89	**86**	43	**70**	9	**38**
Control			53		6		11		0		0		9	

Water temperature 25.5 °C; dissolved oxygen 8 mg/l; conductivity 2220 µmhos/cm; pH 8.9; water depth 35-40 cm.
Bold figures show doses and application results of VectoBac® G.

Table 2. Evaluation of VectoBac® 12 AS (1200 ITU) and VectoBac® G (200 ITU) formulations of Bti against *Anopheles sacharovi*, *Culex pipiens*, and *Cx. theileri* larvae in the flood plain at Gölbaşı District, Ankara Province.

Rate		Mosquito species	Mean no. larvae/dip, pretreatment	Mean (%) reduction after treatment (days)									
Vec. AS (l/ha)	**Vec. G (kg/ha)**			1		3		6		12		20	
0.5	**2.5**	*An. sacharovi*	12	25	**26**	17	**33**	20	**0**	17	**13**	8	**0**
0.75	**5**	_	8	50	**57**	25	**18**	0	**0**	30	**0**	0	**0**
1	**7.5**	_	12	100	**100**	33	**100**	66	**30**	0	**40**	25	**10**
1.25	**10**	_	13	100	**100**	62	**100**	38	**18**	23	**18**	0	**0**
	Control		14	7		29		14		0		14	
0.5	**2.5**	*Cx. pipiens*	43	74	**70**	56	**79**	51	**59**	42	**9**	21	**7**
0.75	**5**	_	72	94	**93**	89	**90**	81	**78**	74	**70**	61	**57**
1	**7.5**	_	55	100	**100**	93	**97**	62	**86**	46	**82**	32	**49**
1.25	**10**	_	38	100	**100**	78	**100**	76	**91**	61	**71**	53	**55**
	Control		60	0		10		13		18		32	
0.5	**2.5**	*Cx. theileri*	88	90	**63**	89	**78**	83	**65**	75	**41**	60	**46**
0.75	**5**	_	65	97	**95**	95	**90**	85	**68**	60	**47**	43	**41**
1	**7.5**	_	76	97	**99**	94	**97**	88	**72**	55	**49**	39	**58**
1.25	**10**	_	68	100	**100**	97	**96**	93	**84**	58	**60**	45	**55**
	Control		81	0		0		9		38		42	

Water temperature 28.5 °C; dissolved oxygen 8.7 mg/l; conductivity 3970 µmhos/cm; pH 8.9; water depth 15-35 cm.
Bold figures show doses and application results of VectoBac® G.

Table 3. Evaluation of VectoBac® 12 AS (1200 ITU) and VectoBac® G (200 ITU) formulations of Bti against *Anopheles sacharovi*, *Culex pipiens*, and *Cx. theileri* larvae in the swamp at Gölbaşı District, Ankara Province.

Rate		Mosquito species	Mean no. larvae/dip, pretreatment	Mean (%) reduction after treatment (days)									
				1		3		6		12		20	
Vec. AS (l/ha)	**Vec. G (kg/ha)**												
0.5	**2.5**	*An. sacharovi*	24	50	**42**	50	**42**	38	**8**	21	**0**	33	**0**
0.75	**5**	_	16	75	**47**	50	**53**	31	**63**	19	**21**	25	**26**
1	**7.5**	_	13	92	**89**	100	**100**	77	**72**	62	**44**	31	**39**
1.25	**10**	_	10	100	**100**	100	**100**	40	**79**	30	**67**	40	**29**
		Control	21	0		29		0		43		19	
0.5	**2.5**	*Cx. pipiens*	34	65	**88**	44	**75**	15	**53**	0	**50**	0	**20**
0.75	**5**	_	41	93	**91**	63	**81**	46	**58**	27	**40**	22	**0**
1	**7.5**	_	36	97	**96**	42	**80**	11	**41**	0	**37**	0	**33**
1.25	**10**	_	30	100	**100**	57	**85**	3	**42**	0	**42**	0	**10**
		Control	44	0		0		0		0		5	
0.5	**2.5**	*Cx. theileri*	10	50	**67**	20	**25**	0	**0**	0	**0**	0	**0**
0.75	**5**	_	21	95	**90**	57	**40**	57	**50**	0	**0**	0	**0**
1	**7.5**	_	15	93	**100**	53	**89**	0	**39**	0	**39**	20	**50**
1.25	**10**	_	16	100	**100**	69	**57**	13	**48**	31	**43**	31	**26**
		Control	14	29		0		0		0		0	

Water temperature 23 °C; dissolved oxygen 9.4 mg/l; conductivity 3750 µmhos/cm; pH 8.5; water depth 15-25 cm.
Bold figures show doses and application results of VectoBac® G.

many environmental factors affect control performance of Bti, such as water quality, solar radiation, high organic content, suspended material, water currents, and weather conditions, as well as larval and mosquito species. They reported that Bti has a short residual activity, often only 1-2 days, due to adsorption by particles and sinking to the bottom, beyond the feeding zone of larval mosquitoes and black flies.

The most important point that should be considered in the application interval of VectoBac® 12 AS and VectoBac® G is the larval growth duration of target species in the field. Larval growth duration was 16-18 days for *Anopheles* species and 11-12 days for *Culex* species in the July-August period under the semi-field conditions of the Gölbaşı area (Şimşek, 1997; Aldemir, 2003). Considering the climatic conditions, the application interval for these preparations in the Central Anatolia region should be 15-20 days for *Culex* and *Anopheles* species. Additionally, since high dose applications do not increase residual effect, VectoBac® 12 AS at 1 l/ha and VectoBac® G at 7.5 kg/ha should be applied for larval control in Central Anatolia. Due to climatic conditions, this application interval can be shorter in the warmer Aegean, the Mediterranean, and the southeastern Anatolian regions of Turkey.

Acknowledgments

The research was financially supported by Kafkas University, Hacettepe University, and Gölbaşı Municipality. We thank biologist Mustafa Ege for his valuable contributions to the field studies. We also thank Dr. Birol Mutlu, who identified the plants in the application areas.

References

Aldemir, A. 2003. Ankara-Gölbaşı'nda sivrisineklere karşı entegre mücadele. PhD thesis. Hacettepe University, Ankara, 188 pp.

Aldemir, A. and Boşgelmez, A. 2005. Ankara-Gölbaşı'nda sivrisinek (Diptera: Culicidae) kontrol çalışmaları hakkında yöre halkının düşünce, beklenti ve önerileri, Türkiye Parazitoloji Dergisi (Acta Parasitologica Turcica) 29: 50-55.

Aldemir, A., Boşgelmez, A. and Çıngı, H. 2002. Gölbaşı Sivrisinekleri. Bizim Büro Basımevi, Ankara.

Becker, N. 2000. Bacterial control of Dipterans – general strategy and further development. In: Proceedings of the 13th European SOVE Meeting, (Eds., S.S. Çağlar., B. Alten., N. Özer), Belek-Antalya, pp. 63-70.

Goldberg, LJ. and Margalit, J. 1977. A bacterial spore demonstrating rapid larvicidal activity against *Anopheles sergentii*, *Uranotaenia unguiculata*, *Culex univittatus*, *Aedes aegypti* and *Culex pipiens*. Mosq. News. 37: 355-358.

Harbach, R.E. 1985. Pictorial keys to the genera of mosquitoes, subgenera of *Culex* and the species of *Culex* (*Culex*) occurring in southwestern Asia and Egypt, with a note on the subgeneric placement of *Culex deserticola* (Diptera:Culicidae). Mosquito Systematics 17: 83-107.

Harbach, R.E. 1988. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera:Culicidae). Contributions of the American Entomological Institute. 24: 240 pp.

Karch, S., Manzambi, Z.A. and Salaun, J.J. 1991. Field trials with Vectolex® (*Bacillus sphaericus*) and Vectobac® (*Bacillus thuringiensis* (H-14)) against *Anopheles gambiae* and *Culex quinquefasciatus* breeding in Zaire. J. Am. Mosq. Control Assoc. 7: 176-179.

Lacey, L.A. and Singer, S. 1982. Larvicidal activity of new isolates of *Bacillus sphaericus* and *Bacillus thuringiensis* (H-14) against anopheline and culicine mosquitoes. Mosq. News. 42: 537-543.

Margalith, Y., Zaritsky, A., Barak, Z., Manasherob, R., Schnieder, B. and Ben-Dov, E. 2000. *Bacillus thuringiensis israelensis* (*Bti*) in integrated biological control (IBC) of mosquitoes and black flies – A global view. In: Proceedings of the 13th European SOVE Meeting, (Eds., S.S. Çağlar., B. Alten., N. Özer), Belek-Antalya, pp. 84-98.

Mulla, M.S., Chaney, J.D. and Rodcharoen, J. 1993. Elevated dosages of *Bacillus thuringiensis* var. *israelensis* fail to extend control of *Culex* larvae. Bull. Soc. Vector Ecol. 18: 125-132.

Mulla, M.S., Rodcharoen, J., Ngamsuk, W., Tawatsin, A., Pan-Urai, P. and Thavara, U. 1997. Field trials with *Bacillus sphaericus* formulations against polluted water mosquitoes in a suburban area of Bangkok, Thailand. J. Am. Mosq. Control Assoc. 13: 297-304.

Mulla, M.S., Su, T., Thavara, U., Tawatsin, A., Ngamsuk, W. and Pan-Urai, P. 1999. Efficacy of new formulations of the microbial larvicide *Bacillus sphaericus* against polluted water mosquitoes in Thailand. J. Vector Ecol. 24: 99-110.

Reuben, R., Rao, D.R., Sebastian, A., Corbet, P.S., Neng, W. and Guohou, L. 1990. Biological control methods suitable for community use. In: Proceedings of Appropriate Technology in Vector Control, (Eds., C.F. Curtis), Florida, pp. 139-158.

Snow, K.R. 1990. Mosquitoes, Naturalist Handbook 14. Richmond Publ. Co. Ltd. England

Şimşek, F.M. 1997. Gölbaşı İlçesi-Mogan-Eymir Gölleri'nde ve İmrahor Vadisi'nde *Culex* ve *Culiseta* (Diptera: Culicidae) türlerinin biyo-ekolojisi üzerine araştırmalar. MSc thesis, Hacettepe University, Ankara, 155 pp.

Yerli, S.V. 2002. Mogan gölü havzası biyolojik zenginlikleri ve ekolojik yönetim planı. Çevre Bakanlığı, Özel Çevre Koruma Kurumu, 167 p. Balgat, Ankara.