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Determination of Mortality Effect of some Biological Larvicites on the Mosquito *Culex* sp.

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HIGHLIGHTS

- *Bacillus thrungiensis* var. *israilensis*.causes mortality of *Culex* sp. larvae.
- Spinosad causes mortality of *Culex* sp. larvae.
- Bio-larvicides were an effective controlling tool for mosquitoes in Kahramanmars.

Abstract: The use of biocidal larvicides in the control against mosquitoes is important in reducing the adverse effects on non-target organisms and the environment. In this study, it was aimed to determine the most effective concentrations and durations of *Bacillus thrungiensis* var. *israilensis* and spinosad larvicides for controlling of the *Culex* sp. larvae collected from Kahramanmaraş region. For this pupose, *Culex* sp. larvae were exposed to *Bacillus thrungiensis* var. *israilensis* at the concentrations of 0, 0.025, 0.05, 0.1, 0.2, 0.5, 1.0, ve 2.0 µL/200 cm^2 and exposed to spinosad at the concentrations of 0, 0.0075, 0.015, 0.03, 0.06, 0.15, 0.3, ve 0.6 µL /200 cm^2 along 5 different times (3, 6, 12, 18 and 24 hours). According to the results of the variance and probit analysis, over 90% of mosquito larvae were observed to have died in almost all of the trial periods, but more than 90% of the larvae were found to have died considerably even1/10(For Bti:1 µL/200cm^2, For Spinosad:0.3 µL/ 200cm^2) concentration after 24 hours which is recommended concentration. The results of the study showed that both bio-larvicides used gave similar results and were an effective controlling tool for mosquitoes. For this reason, it is important to use any of the two larvicides in consideration of local conditions and non-target organisms. For the observation of environmental health and mosquito resistance, making such studies at certain intervals is extremely important in terms of effective control with mosquitoes.

Keywords: Biological larvicide; *Culex* sp.; Mortality.

INTRODUCTION

Mosquitoes generally cause direct and indirect damage to public health worldwide. In addition to disturbing and irritating humans and other animals, they vectorize many living pathogenic diseases that lead to a drop in work productivity and livestock production. Diseases vectored by mosquitoes can be listed as malaria, filariasis, yellow fever, and dengue, and these diseases are highly fatal to humans and animals [1,2]. Microorganisms that cause serious diseases to humans are transmitted during the sucking of these insects [3]. The frequency of mosquito-borne diseases increases day by day due to uncontrolled urbanization. Therefore, mosquito control is an indispensable component for the control of diseases coming through mosquitoes.

Chemical insecticides are used to control pests. In addition to the spread of mosquitoes and the diseases they spread, conventional chemical pesticides are reported to be ineffective over time against insect resistance [4,5]. It is therefore essential to effectively control mosquito populations during their controls. Again, due to some of the chemicals used in the control of the the insects cause neurological diseases and asthma, allergies, hormonal imbalances and cancer on human, biological control methods are developed as an alternative [6]. Therefore, alternative, more effective and environmentally friendly control factors are needed. In recent years, there has been an increasing interest in biological control factors. Many organisms have been investigated as potential factors for vector mosquito control, including viruses, fungi, bacteria, protozoa, nematodes, invertebrate predators and fish, of which efficacy is safe for mammals and the environment [7].

The larvacid effects of *Bacillus* species on various mosquito species are known for more than half a century [8,9]. Among these, *Bacillus sphaericus* and *B. thuringiensis* have very high toxicity to mosquito larvae [10,11]. On the other hand, the negative effect of non-target organisms, namely mammals and other creatures, is negligible compared to chemical insecticides. Spinetoram, a new group of insecticides like Bti in the naturalytes class, is a large-scale semi-biological insecticide against many pests. This insecticide acts against insects via stomach and contact ways [12]. The semi-synthetic Spinosin insecticide has emerged as one of the insecticides that is likely to be used in the fight against pests due to the lower toxicity of spinetoram to both the environment, mammals and birds [13]. All these results may be an important alternative in the controlling mosquitoes with *B. thuringiensis israelensis* (Bti) and Spinosad active ingredient because of the long-term use of broad-spectrum synthetic insecticides, which have very harmful effects on the environment, human health and beneficial organisms and mosquitoes have improved resistance to insecticides that are widely used. Therefore, in the current study, the toxicity of Bti and Spinosad active insecticides against mosquito (*Culex* sp) larvae was investigated under laboratory conditions.

MATERIAL AND METHODS

Insect

In this study, Third instar *Culex* sp. larvae were used. The mosquito larvae were collected from streams and ponds around Kahramanmaras province with plastic containers and identified at the genus level under the microscope. Coordinates of the area where the larvae are collected; K 37 ° 27´ 855´´ and D 036 ° 53´ 764´´ and altitude was measured as 469. Collected *Culex* sp. mosquito larvae were kept in a 5 liter plastic container in a laboratory environment (26 ± 2 °C) until bioassay. The larvae were tested on the day of the collection.

Reagents

Bacillus thuringiensis subsp. *israelensis* (Serotype H-14) (Bti), which is LD_{50} value with low toxicity in birds and mammals; for acute oral rats> 2000 mg /kg.,was used. The Bti Flytech-BTI Suspension Concentrate (SC) that we use in our trials is licensed to mosquito larvae and is produced by Biodalia Microbiological Technologies®. The other larvacide we use is Spinosad active substance and its LD50 value; for acute oral rats> 5000 mg/kg. Mozkill *120 SC is licensed against mosquito larvae under the trade name and is produced by Dow agroScience®.

Bacillus thuringiensis israelensis (Bti) and Spinosad active ingredient insecticidal effect against mosquito larvae

Biological tests were carried out in a laboratory environment (26 ± 2 °C). Different concentrations *of Bacillus thuringiensis israelensis* (Bti) and Spinosad active ingredient larvacides were used in biological tests. The concentration adjustment and dilution were adjusted according to the manufacturer's recommendation of the larvicides. For each larvicide application, the different concentrations of *Bacillus thuringiensis israelensis* (Bti) and Spinosad were prepared in 1 liter plastic containers filled with 500 mL of tap water. No food was provided to the larvae during the experiment. Four replications were used for each test and twenty larvae were used for each replicate. A total of 640 larvae were used for each larvicide, and 640 larvae were used for control since control trials were performed for each application. In trials 8 different concentrations were used for Bti (0, 0.025, 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 µL /200cm2). Mortality was assessed at 3, 6, 12,18 and 24 hours after applications For Spinosad, 8 different concentrations (0, 0.0075, 0.015, 0.03, 0.06, 0.15, 0.3, and 0.6 µL/200 cm2) were also applied to the larvae. Again mortality was assessed at 3, 6, 12,18 and 24 hours after applications. The larvacides used were kept in the refrigerator at 4 ± 1 ºC until the application.

Statistical Analysis

According to the results of the biological tests, mortality rates on mosquito larvae were calculated for each application. In addition, % mortality tables for mosquito larvae were created at each concentration and after application times. These obtained data were subjected to Arcsin transformation [14]. Variance analysis (ANOVA) [15](SAS, 1989) was applied to the data obtained from here and the differences between the averages were compared according to the DUNCAN (p <0.05) test.

RESULTS

Mortality effect of different concentrations of Bacillus thuringiensis israelensis (Bti) on Culex sp. Larvae

Since there is interaction between application concentrations and durations, concentrations were examined separately for each period (p <0.001). Accordingly, all application concentrations and durations are examined in Table 1. According to Table 1., At 2.0 µL application concentration, all larvae died at all application times. At 1.0 µl of application concentration, all of the larvae died during all other application times except for 3 hours of application (94% of the larvae were dead) and 3 hours of application was significantly different from other applications (Duncan <0.05). Almost all of the larvae (99-100%) died at all application times except for 3 hours (81% of the larvae were dead) at 0.5 µl of application concentration, and 3 hours of application was significantly different from other applications (Duncan <0.05). If the application concentration is 0.2 µL, significant differences were found between the application times and the highest mortality rate was achieved within 24 hours (96%), the lowest mortality rate was obtained at the 3rd c (30%) and then at the 6th hour (52%). the same mortality rate (94%) was obtained at 12th and 18th hours. Similar mortality rates (72%, 78% and 92%, respectively) were obtained at the application concentration of 0.1 µL at the 12th, 18th and 24th hours. Significant differences were found at the application concentration of 0.05 µL, and the highest mortality rate (90%) was achieved at the 24th hour, and the lowest mortality rates (2% and 4%) at the 3rd and 6th hours (Duncan <0.05). Similar results were obtained at the application concentration of 0.025 µL, the highest mortality rate (59%), and the lowest mortality rate at 0 hours (0%) at 3 and 6 hours (Duncan <0.05). According to these results, Bti applications have been effective as the time is longer and more than half of the larvae have died within 24 hours even at the lowest concentration (0.025 µL) (Figure 1). At the 24th hour of the application, concentrations of 0.2 µL and above provided an effective control with a mortality rate of over 95% (Figure 1).

Table 1. Mortality rates (%) (± standart error) of *Culex* sp. larvae as a result of the application of different concentrations of *Bacillus thuringiensis israelensis* (Bti).

***Culex* sp. larval mortality ± S. Error**						
Concentrations (µL/200cm²)	**3 hours**	**6 hours**	**12 hour**	**18 hour**	**24 hour**	**F ve P value***
2 µL	100±0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	F= - P= -
1 µL	94±1 Bb	100±0 Aa	100±0 Aa	100±0 Aa	100±0 Aa	$F_{4,15}$=107.6 P<0.0001
0.5 µL	81±3 Cb	99±1 Aa	100±0 Aa	100±0 Aa	99±1 Aa	$F_{4,15}$=36.04 P<0.0001
0.2 µL	30±5.5 Dc	52±10.5 Bb	94±1 Ba	94±2.5 Ba	96±1 ABa	$F_{4,15}$=27.96 P<0.0001
0.1 µL	5±3.5 Ec	24±1 Cb	72±4.5 Ca	78±9.5 Ca	92±1.5 BCa	$F_{4,15}$=32.73 P<0.0001
0.05 µL	2±2.5 Ec	4±2.5 Dc	17±4.5 Db	22±4.5 Db	90±3.5 BCa	$F_{4,15}$=42.50 P<0.0001
0.025 µL	0±0 Ec	0±0 Dc	9±2.5 Eb	16±2.5 Db	59±9.5 Da	$F_{4,15}$=47.45 P<0.0001
Control	0±0	0±0	0±0	0±0	0±0	
F ve P value*	$F_{6,21}$=132.52 P<0.0001	$F_{6,21}$=149.92 P<0.0001	$F_{6,21}$=281.51 P<0.0001	$F_{6,21}$=156.81 P<0.0001	$F_{6,21}$=13.98 P<0.0001	

The values are the mean of three replications, and the different letters in each column indicate a statistical difference between the mortality rates according to Duncan test (P≤0,05).

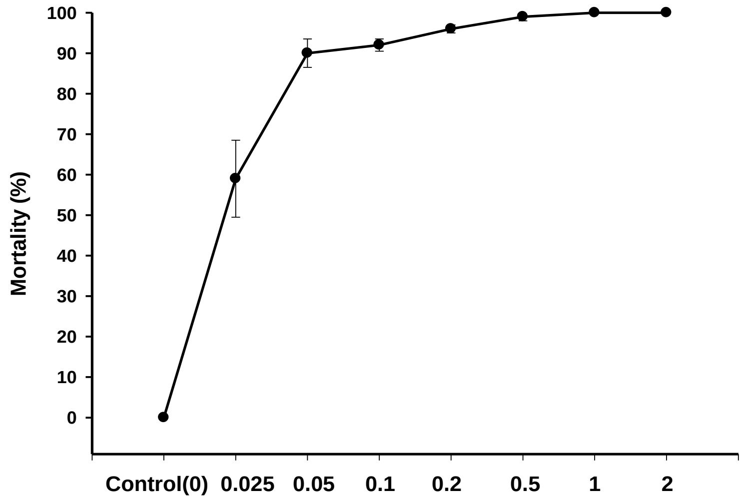

Figure 1. Mortality rates (%) of *Culex* sp. larvae obtained during the 24-hour application period of different concentrations of *Bacillus thuringiensis israelensis* (Bti).

Mortality effect of different concentrations of spinosad on Culex sp. Larvae

Again since there is interaction between application concentrations and durations, concentrations were examined separately for each period (p <0.001). Accordingly, all application concentrations and durations are examined in Table 2. According to Table 2, at the application concentration of 0.6 µL, all the larvae died at all application times. If the application concentration was 0.3 µL 200 cm², all the larvae died during all other application periods except for 3 hours (75% of the larvae were dead) and the 3 hours application was significantly lower than other applications. The majority of the larvae (89-97%) died in all other application periods except for 3 hours (66% of the larvae were dead) at 0.15 µL of the application concentration, and the 3-hour application was significantly different from other applications. If the application concentration was 0.06

µL, significant differences were found between the application times and the highest mortality rate was obtained in the 18th and 24th hours (81% and 90%, respectively), the lowest mortality rate in the 3rd hours (15%), and similar mortality rates (75-81%) were obtained at the 12th and 18th hours. If the application concentration was 0.03 µL, the application times were all statistically different from each other, the mortality rate at 24 hours (99%) was significantly higher than the others, which was 18 hours (86%), 12 hours (70%), 6 hours. (34%) and 3rd hour (6%). At application concentration 0.015 µL, the highest mortality rate (66%) was found at 24 hours, and the lowest mortality rate (2% and 14%) was found at 3rd and 6th hour, with significant differences in application times, except between 12th and 18th hours (30-41%) . Similar results were obtained at the application concentration of 0.0075 µL (74-77%) at 18 and 24 hours, followed by 12 hours (17%); the lowest mortality rate (0-4%) was found at the 3rd and 6th hours. In the control application, there was no difference between the times and no mortality was seen in any of them. When examined Figure 2, at the 24th hour of the application, concentrations of 0.03 µL and above provided an effective control with a mortality rate of more than 90%, and during this period, 50% or more mortality was achieved at all concentrations except the control.

Table 2. Mortality rates (%) (± standart error) of *Culex* sp. larvae as a result of the application of different concentrations of spinosad.

	***Culex* sp. larval mortality ± S. Error**					
Concentrations (µL/200cm²)	**3 hours**	**6 hours**	**12 hours**	**18 hours**	**24 hours**	**F ve P value***
0.6 µL	100±0 ^{Aa}	100±0 ^{Aa}	100±0 ^{Aa}	100±0 ^{Aa}	100±0 ^{Aa}	$F_{4,15}$=- P=-
0.3 µL	75±3.5 ^{Bb}	100±0 ^{Aa}	100±0 ^{Aa}	100±0 ^{Aa}	100±0 ^{Aa}	$F_{4,15}$=170.1 P<0.0001
0.15 µL	66±6 ^{Bb}	89±4 ^{Ba}	91±3 ^{Ba}	94±2,5 ^{Ba}	97±1.5 ^{Aa}	$F_{4,15}$=5.28 P<0.01
0.06 µL	15±2 ^{Cd}	50±6 ^{Cc}	76±4 ^{Cb}	81±2.5 ^{CDab}	90±3.5 ^{Ba}	$F_{4,15}$=56.06 P<0.0001
0.03 µL	6±2.5 ^{De}	34±8.5 ^{Cd}	70±3.5 ^{Cc}	86±4 ^{Cb}	99±1 ^{Aa}	$F_{4,15}$=56.41 P<0.0001
0.015 µL	2±2.5 ^{DEd}	14±2.5 ^{Dc}	30±3 ^{Db}	41±5 ^{Eb}	66±3 ^{Ca}	$F_{4,15}$=43.16 P<0.0001
0.0075 µL	0±0 ^{Ec}	4±2.5 ^{Ec}	17±3 ^{Eb}	74±2.5 ^{DEa}	77±3 ^{Ca}	$F_{4,15}$=115.9 P<0.0001
Control (0 µL)	0±0	0±0	0±0	0±0	0±0	
F ve P value*	$F_{6,21}$=127.42 P<.0.0001	$F_{6,21}$=76.37 P<.0.0001	$F_{6,21}$=97.6 P<.0.0001	$F_{6,21}$=46.76 P<.0.0001	$F_{6,21}$=32.98 P<.0.0001	

The values are the mean of three replications, and the different letters in each column indicate a statistical difference between the mortality rates according to Duncan test (P≤0,05).

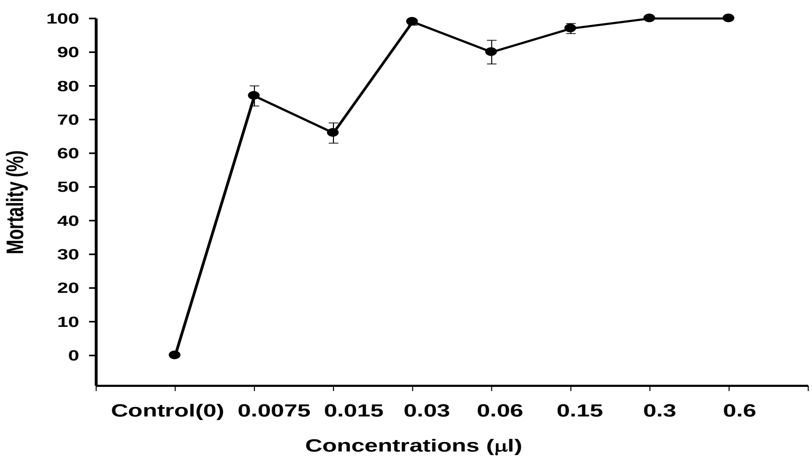

Figure 2. Mortality rates (%) of *Culex* sp. larvae obtained during the 24-hour application period of different concentrations of spinosad.

DISCUSSION

As in many parts of the world, it is known that larvacide application is done intensely in the control of mosquitoes in Kahramanmaraş province. For this reason, it is very important to control mosquitoes both economically and environmentally. The use of chemical insecticides should be minimized by considering the negative effects of synthetic insecticides on the environment in terms of both environmental pollution and disruption of the population balance of non-target organisms. *Culex* sp. which appears intensely in Kahramanmaraş region, is approximately 70% of the mosquito species in the region. There is no study in terms of what kind of method should be followed in the control of this species in this region before. Therefore, in this study, the most effective concentration and duration of Bti and Spinosad biolarvasites were revealed. In summary, using 8 different concentrations for *Bacillus thrungiensis var.israilensis* (Bti) (0; 0.025; 0.05; 0.1; 0.2; 0.5; 1.0; and 2.0 µL / 200 cm2 and 8 different concentrations for Spinosad (0; 0.0075; 0.015; 0.03; 0.06; 0.15; 0.3; and 0.6 µL / 200 cm2) were applied at five different times (3, 6, 12, 18 and 24 hours). Almost 90% of mosquito larvae were observed to die at almost all of the trial periods in the proposed concentrations, but after 24 hours, even at 1/10 of the recommended concentration (for Bti: 1 µL / 200cm^2, for Spinosad: 0.3 µL / 200cm^2) more than 90% of the larvae was observed to die.

According to the results of the study, there are significant variations between Bti and spinosad concentration and duration applications especially in low concentrations. When the mortality rates were examined,100% mortality rate was observed in the two larvacides at the recommended concentrations (for Bti: 1 µL/200cm^2, for Spinosad: 0.3 µL/200cm^2). However, up to 1/5 of the recommended concentration, mortality rates in Bti were higher than in spinosad at 12 and 24 hours of application times. Mortality rates caused by Spionosad were two times higher than mortality rates caused by Bti at 12 hours of application time, especially in concentrations up to 1/20 and 1/40 of the concentration recommended (for Bti: 1 µL/200cm^2, for Spinosad: 0.3 µL/200cm^2). In studies similar to our study, it was reported that mortality rates were lower in low Bti concentrations, but mortality rates increased over time [16,17]. Again in a previous study, different concentrations of Spinosad (20–100 µg L-1) were applied to *C. pipiens* larvae for different periods of time (2–15 days), increases in mortality as concentration and time increased, even at the highest concentration over 80%. mortality rate was obtained in the lowest duration application [18]. In another study conducted in different mosquito species (*C. quinquefasciatus, A. aegypti, A. stephensi*), it was stated that as the concentration of Spinosad substance applied in different concentrations increased, mortality rates increased significantly after 24 hours, especially more than 80% of larvae died in 1 ppm [19]. Similar to these studies, as the concentration and time used in our study increased, the mortality rates increased significantly in *Culex* sp. Larvae at least this region.

CONCLUSION

As a result, considering the results in our study, it has been demonstrated that only 90% of the insecticide costs can be saved. The results of the study showed that both biolarvisides used give similar results and are an effective means of controlling mosquitoes. For this reason, it is important to use any of the two larvacides considering local conditions and non-target organisms. As a result of the study, it was observed that the concentrations of the recommended larvacides are quite high. For this reason, both environmental and material contributions can be made in the review of another study by making concentration experiments considering the environmental and geographical conditions. In addition, it has been reported in the literature that biocidal larvacides are as effective as synthetic insecticides and that mosquitoes are less resistant than synthetic larvacides. Conducting such studies at regular intervals in monitoring environmental health and mosquito resistance is extremely important for an effective control against mosquitoes. It is thought that this first study in the region for *Culex* sp, will lead the future studies.

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Conflicts of Interest: “The authors declare no conflict of interest.”

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