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SUMMARY

In this study, 12 different commercial pesticides, including 5 fungicides, 5 herbicides, 1 aphicide and 1 acaricide, were investigated for their effects on spore viability, toxin stability and larvicidal activity of a mosquito pathogenic *B. sphaericus* 2362 strain. Minimal inhibitory concentrations (MICs) of each pesticide were determined after 48 h incubation of *B. sphaericus* 2362 strain. According to MIC results, all pesticides showed various inhibitory effects on the growth of the test bacterium. Particularly, the pesticides which contain TCA, hymexazol, copper sulphate, hexythiazox, 2,4-D Amine, copper inhibited spore germination significantly and bacterial growth even at the lowest tested concentrations. Our results indicated that various commercial pesticides in different concentrations reduced the spore viability significantly and heat-resistant spore numbers of *B. sphaericus* 2362 strain. In general, the pesticides decreased the spore numbers 10^4 - 10^5 -fold compared to initial ones. Furthermore, it was observed that the larvicidal activity was completely lost when it was grown at the concentration of MIC values or higher. Indeed, SDS-PAGE analysis confirmed that the binary toxin proteins, 41.9 kDa and 51.4 kDa, were lost at the MIC values and higher concentrations although parasporal crystals did not disappear in the much lower pesticide concentration.

KEYWORDS: Pesticides, *B. sphaericus* 2362, toxin stability, larvicidal activity and SDS-PAGE.

INTRODUCTION

Bacillus sphaericus, a gram positive spore-forming bacterium, produced a potent mosquito larvicide at the onset of sporulation [1]. Recently, several pathogenic strains, such as *B. thuringiensis* and *B. sphaericus* strains,

the microbial control agents, were observed to have high levels of insecticidal activity against various mosquito larvae in laboratory and field studies [2]. The effect of biotic and abiotic factors on the viability, toxin stability and larvicidal activity of both biological control agents against many species of mosquito larvae has been summarized by Lacey and Undeen [18] and Mulla [20]. One of the most important environmental factors affecting the larvicidal activity of these bacteria is water pollution rate and depth [5, 12, 23]. *B. thuringiensis* var. *israelensis* serotype H-14 and other H-serotypes have shown great promise as a mosquito larvicide. Also, it is reported that the mosquito pathogenic strains of *B. thuringiensis* have more chance to live in aquatic habitats as they use various kinds of carbohydrates for growth, whereas the strains of *B. sphaericus* differ from them in this regard [3, 24]. However, *B. thuringiensis* var. *israelensis* has lower larvicidal activity and persistence in organically enriched habitats and polluted waters [10, 18]. The findings in the last decade suggested that the larvicidal activity of *B. sphaericus* may persist against many species of mosquito larvae under field conditions including organically enriched habitats [12, 16, 22]. In addition to persistence of toxin, residual larvicidal activity also may be facilitated by recycle ability of the bacterium within larval cadavers under certain conditions [16, 21].

Besides, UV-light, high temperature and pH reduced drastically the viability of *B. sphaericus* 1593 and 2362 spores [6, 27, 28]. Moreover, toxin stability of *B. sphaericus* against different pH levels, high temperature and UV-radiation were enhanced by encapsulation studies with carboxymethylcellulose, alginate and carrageen [3, 14, 17].

So far, detailed studies related to chemical pesticides have not been carried out, despite several studies at present are concerned with the effect of different kinds of chemical compounds on the larvicidal activity and spore viability of mosquito pathogenic strains of *B. sphaericus* [1, 4, 7]. The aim of this study is to determine the inhibitor effects

of 12 different commercial pesticides on the spore viability and larvicidal activity of *B. sphaericus* 2362 strain.

MATERIALS AND METHODS

Bacterium and Spore Suspensions

B. sphaericus 2362 was provided by Prof. Dr. A. A. Yousten (VPI & State University, Blacksburg, VA). Overnight culture of bacterium in NYSM broth was inoculated onto NYSM agar plates (Difco, Detroit, MI) and incubated at 30°C for 5 days for complete sporulation. The sporulation was examined regularly at intervals under phase-contrast microscope. Spores were then collected from the surface of NYSM agar plates with sterile saline (0.85% w/v), and washed three times. Spores were then resuspended in sterile distilled water and the count adjusted to 2×10^{10} spores ml⁻¹ on NYSM agar plate after heating at 80°C for 12 min.

Preparation of Pesticide Stock Solutions and MIC Assays

The commercial pesticides used in research were obtained from Agricultural Research and Control Institute of Turkey. The liquid pesticides were sterilized separately by filtration through cellulose acetate membranes (Sartorius, 0.25 µm diameter). Powder pesticides were sterilized by autoclaving at 121 °C for 15 min. To determine the minimal inhibitor concentrations (MICs) of each pesticide, 2 ml stock solutions were transferred into sterile tubes containing 2 ml NYSM broth [8]. Pure NYSM broth medium alone was used as control. Subsequently, 100 µl of the bacterium spore suspension was inoculated into each tube containing pesticides and control NYSM broth medium. Inoculated serial tubes were then incubated on a rotary shaker (150 rpm) at 30 °C for 48 hours. The minimal inhibitor concentrations of pesticides were determined by checking the growth of the bacterium culture against control group. All of the experiments were carried out in triplicate. MIC values were calculated as mg ml⁻¹ active ingredient in the media.

Preparation of Samples for Spore Counts

2 ml of bacterial samples from incubated culture for 24 and 48 h were transferred into a sterile eppendorf tubes to count heat-resistant spore number and to use for electrophoresis. Then, to remove pesticides' residue from the media, bacterial samples were washed 6-7 times by centrifuging at 15.000 rpm for 5 min. The bacterial samples were kept in deep-freezer at -70°C until use. The heat-resistant spore count of bacterial culture treated with pesticides was performed by heating 1 ml samples at 80°C for 12 min and then plated on NYSM agar using the pour-plate technique. The number of spores was determined from the mean of at least 3 plating.

Phase-Contrast Microscopy

Fresh test strains were prepared from 24 h and 48 h incubated cultures and used to investigate the bacterial

growth, the structure of spore-toxin complex, and bacterial sporulation under phase-contrast microscope.

Electrophoresis

The samples were centrifuged at 15.000 rpm for 10 min and the proteins extracted from the spores by suspending them directly in the sample buffer (pH 6.8, 0.06 M Tris, 2.5 % glycerol, 0.5% SDS, 1.25% β-mercaptoethanol) and boiling for 10 min. Solubilized proteins were subjected to SDS-PAGE in gel slabs of 0.75 mm thickness (1.5 cm, 4.0% stacking and 13.5 cm, 10.0% resolving gels) as described by Laemmli [19], and the gels stained with Coomassie Brilliant Blue R-250.

Bioassays

The larvicidal activity of *B. sphaericus* 2362 strain grown in different pesticide concentrations was tested against 2nd and 3rd instars of *Culex quinquefasciatus* larvae and the LC₅₀ values were calculated [15]. *C. quinquefasciatus* culture was provided by Elisabeth Davidson (Arizona State University, Department of Zoology, Tempe, Arizona, USA). Serial concentrations of pesticides treated as spore samples were added into plastic cups containing 50 mosquito larvae in 30 ml sterile tap water in triplicate, and toxic activity was expressed as LC₅₀, that is, the concentration of bacterial cells that killed 50% of the test insect population in time period under study. Larvae were fed with 0.2 ml of 5% (wt/vol.) debittered brewers' yeast suspensions at the beginning of the assay.

RESULTS AND DISCUSSION

In this study, we investigated the effects of 12 commercial pesticides on spore viability, larvicidal activity and toxin stability of *B. sphaericus* 2362 strain, a mosquito pathogenic bacterium. The commercial name of pesticides, active ingredient, and minimal inhibitor concentration (MIC) values are shown in Table 1. The most effective pesticide was Nata Granule, a herbicide, in all tubes over MIC concentrations, 1.187 mg ml⁻¹. However, fungicides used in this study had more inhibition effect on spore germination compared to other pesticide types. Also Nissorun 5EC, an acaricide, was the most effective after Nata Granule. The other pesticides, Cupper Sulphate, Tachigaren 70 WP, Cupravit fungicides, Agro D-Amine and Cornox Amine herbicides, were active following the order in Table 1. However, 5 pesticides, Brassicol, Anvil, Tefralin EC, Tordon 101 Mixture and Primor 50 WG, decreased the heat-resistant spore numbers, but they did not inhibit the spore germination. Also, parasporal inclusions of the test strains were observed under phase-contrast microscope, when they were grown on media containing different concentrations of all pesticides (data not shown).

The number of heat-resistant spore counted after 24 and 48 h incubation can be seen in Table 1. In all cases, the initial spore numbers was 2×10^7 spores ml⁻¹. Nata Granule

reduced the heat-resistant spore numbers to 2.4×10^2 and 2×10^3 after 24 and 48 h incubation, possibly due to its trichloro acetic acid content that is destructive to proteins. Although the most effective pesticide was Nata Granule, an herbicide, mainly fungicides show high inhibitory effects on spore germination. In addition, these findings showed that Brassicol and Primor 50 WG had a slight inhibitory

effect on the spore viability and spore germination of the bacterium. In general, pesticides reduced approximately the spore numbers 10^4 - 10^5 -fold, compared to initial spore numbers. It was interesting that bacterial spores after 48 h incubation acquired resistance to all pesticides, but they were more sensitive after 24 h incubation. It was also found that spore numbers of test bacterium decreased at different

TABLE 1 - The larvicidal activities (LC₅₀), heat-resistant spore numbers, and MIC values of *B. sphaericus* 2362 grown on media containing serial concentrations of 12 different commercial pesticides after incubation for 24 and 48 h.

Commercial Name	Active Ingredient	Control	Serial Concentrations of the Pesticides (mg ml ⁻¹)							MICs (mg ml ⁻¹)
Agro D amine	2,4-D Amine	0.0	1.562	3.125	6.25	12.50	25	50	100	12.50
		24 h	4.67x10 ⁷	2.56x10 ⁷	7.36x10 ⁵	2.01x10 ⁶	1.1x10 ⁵	4.9x10 ³	1.6x10 ³	0.8x10 ³
		24 h-LC ₅₀	3.1x10 ³	5.2x10 ³	5.3x10 ³	5.4x10 ³	5.5x10 ³	None	None	None
		48 h	2.21x10 ⁸	1.32x10 ⁸	3.41x10 ⁷	1.01x10 ⁷	1.06x10 ⁶	4.8x10 ⁴	5.8x10 ³	4.3x10 ³
		48 h-LC ₅₀	4.1x10 ³	4.6x10 ³	5.1x10 ³	5.1x10 ³	5.2x10 ³	5.4x10 ³	None	None
Anvil	Hexaconazole	0.0	0.39	0.781	1.562	3.125	6.25	12.5	25	*
		24 h	3.34x10 ⁷	2.33x10 ⁷	1.12x10 ⁷	2.43x10 ⁶	1.6x10 ⁵	2.6x10 ⁴	2.4x10 ⁴	2.3x10 ³
		24 h-LC ₅₀	4.8x10 ³	4.9x10 ³	5x10 ³	5.9x10 ³	6.1x10 ³	6.3x10 ³	6.3x10 ³	None
		48 h	2.46x10 ⁸	2.11x10 ⁸	5.83x10 ⁷	1.82x10 ⁷	9.1x10 ⁶	1.25x10 ⁶	9.4x10 ⁵	3.2x10 ⁴
		48 h-LC ₅₀	3x10 ³	4.5x10 ³	4.6x10 ³	4.8x10 ³	5.4x10 ³	5.6x10 ³	5.6x10 ³	5.5x10 ³
Nissorun 5 EC	Hexythiazox	0.0	0.039	0.078	0.156	0.312	0.625	1.25	2.5	1.250
		24 h	3.13x10 ⁷	2.24x10 ⁷	2.36x10 ⁶	4.7x10 ⁴	1x10 ³	0.3x10 ³	2x10 ²	2x10 ²
		24 h-LC ₅₀	3.1x10 ³	5.4x10 ³	6.3x10 ³	7.1x10 ³	None	None	None	None
		48 h	3.64x10 ⁸	1.86x10 ⁸	2.41x10 ⁷	6.14x10 ⁶	1.06x10 ⁵	1.68x10 ⁴	0.4x10 ³	0.3x10 ³
		48 h-LC ₅₀	2.9x10 ³	4.6x10 ³	5.1x10 ³	5.7x10 ³	6.4x10 ³	6.6x10 ³	None	None
Cupravit	Copper	0.0	1.95	3.90	7.81	15.62	31.25	62.5	125	3.90
		24 h	1.5x10 ⁷	1.7x10 ⁴	3.4x10 ³	2.3x10 ²	2x10 ²	1x10 ²	0.9x10 ²	0.1x10 ²
		24 h-LC ₅₀	4.1x10 ³	6x10 ³	None	None	None	None	None	None
		48 h	2.25x10 ⁸	1.8x10 ⁵	0.6x10 ³	2x10 ²	2x10 ²	1.4x10 ²	1x10 ²	3x10 ¹
		48 h-LC ₅₀	3.1x10 ³	5x10 ³	None	None	None	None	None	None
Primor 50 WG	Primicarb	0.0	1.95	3.90	7.81	15.62	31.25	62.5	125	*
		24 h	1.91x10 ⁷	1.41x10 ⁷	9.16x10 ⁶	2.41x10 ⁶	5.11x10 ⁵	1.01x10 ⁴	0.2x10 ³	1.6x10 ²
		24 h-LC ₅₀	3.5x10 ³	5.6x10 ³	5.8x10 ³	6.7x10 ³	7x10 ³	8.1x10 ³	None	None
		48 h	1.63x10 ⁸	1.33x10 ⁸	1.08x10 ⁸	7.02x10 ⁷	6.92x10 ⁶	2.95x10 ⁵	1.22x10 ⁵	9.5x10 ⁴
		48 h-LC ₅₀	2x10 ³	4.4x10 ³	4.6x10 ³	5x10 ³	5.6x10 ³	6.1x10 ³	7.4x10 ³	7.6x10 ³
Cornox amine	2,4-D Amine	0.0	3.90	7.81	15.62	31.25	62.5	125	250	62.50
		24 h	4.13x10 ⁷	2.24x10 ⁷	2.36x10 ⁶	6.71x10 ⁴	0.8x10 ³	1.3x10 ²	1.1x10 ²	1x10 ²
		24 h-LC ₅₀	3.1x10 ³	5.7x10 ³	6.3x10 ³	7.1x10 ³	None	None	None	None
		48 h	2.51x10 ⁸	1.71x10 ⁸	1.61x10 ⁷	6.14x10 ⁶	8.6x10 ⁴	1.4x10 ³	2.4x10 ²	2x10 ²
		48 h-LC ₅₀	3.1x10 ³	4.1x10 ³	5.1x10 ³	5.7x10 ³	6.4x10 ³	None	None	None
Brassicol	Quintozene	0.0	0.703	1.406	2.812	5.625	11.25	22.5	45	*
		24 h	5.1x10 ⁷	2.24x10 ⁷	2.36x10 ⁶	9.67x10 ⁵	4.79x10 ³	1.54x10 ³	7.12x10 ⁴	3.44x10 ⁴
		24 h-LC ₅₀	3.1x10 ³	4.6x10 ³	4.5x10 ³	5x10 ³	5.2x10 ³	5.6x10 ³	5.7x10 ³	5.8x10 ³
		48 h	1.84x10 ⁸	1.11x10 ⁸	1.54x10 ⁷	8.84x10 ⁶	2.06x10 ⁶	1.18x10 ⁶	2.44x10 ⁵	1.16x10 ⁵
		48 h-LC ₅₀	3x10 ³	3.8x10 ³	4x10 ³	4.4x10 ³	5.8x10 ³	5x10 ³	5.4x10 ³	5.4x10 ³
Copper sulphate	Copper sulphate	0.0	0.765	1.53	3.06	6.125	12.25	24.5	49	1.530
		24 h	4.64x10 ⁷	0.6x10 ³	0.3x10 ³	1.9x10 ²	1.6x10 ²	1x10 ²	0.8x10 ²	5x10 ¹
		24 h-LC ₅₀	3.8x10 ³	None	None	None	None	None	None	None
		48 h	4.16x10 ⁸	2.64x10 ⁴	6.5x10 ²	4.4x10 ²	2.6x10 ²	1.4x10 ²	1.2x10 ²	6x10 ¹
		48 h-LC ₅₀	3.1x10 ³	5.7x10 ³	None	None	None	None	None	None
Nata Granule	T.C.A.	0.0	0.074	0.148	0.296	0.593	1.187	2.375	4.75	1.187
		24 h	4.13x10 ⁸	2.24x10 ⁷	2.12x10 ⁶	5.64x10 ⁴	9.8x10 ²	2.4x10 ²	1.6x10 ²	1x10 ²
		24 h-LC ₅₀	3.1x10 ³	5.7x10 ³	6.4x10 ³	7.2x10 ³	None	None	None	None
		48 h	2.12x10 ⁹	1.71x10 ⁸	1.41x10 ⁷	2.14x10 ⁶	8.6x10 ⁴	2x10 ³	2x6x10 ²	2.3x10 ²
		48 h-LC ₅₀	2.8x10 ³	4.1x10 ³	4.6x10 ³	5.7x10 ³	6.2x10 ³	None	None	None
Tachigaren 70 WP	Hymexazol	0.0	1.64	3.281	6.562	13.125	26.25	52.5	105	3.281
		24 h	1.92x10 ⁷	0.5x10 ³	0.1x10 ³	1.1x10 ²	1x10 ²	0.9x10 ²	0.9x10 ²	6x10 ¹
		24 h-LC ₅₀	3x10 ³	None	None	None	None	None	None	None
		48 h	1.54x10 ⁸	0.8x10 ³	0.2x10 ³	0.1x10 ³	1.2x10 ²	1.2x10 ²	1.1x10 ²	1x10 ²
		48 h-LC ₅₀	2.7x10 ³	None	None	None	None	None	None	None
Tefralin EC	Trifluralin	0.0	3.75	7.5	15	30	60	120	240	*
		24 h	4.44x10 ⁷	3.66x10 ⁷	7.44x10 ⁶	8.68x10 ⁵	4.86x10 ⁵	3.44x10 ⁴	1.84x10 ⁴	1x10 ³
		24 h-LC ₅₀	3.1x10 ³	5.1x10 ³	5.4x10 ³	6.1x10 ³	6.1x10 ³	6.4x10 ³	7.1x10 ³	None
		48 h	2.51x10 ⁹	2.11x10 ⁸	5.41x10 ⁷	9.14x10 ⁶	2.3x10 ⁶	2.64x10 ⁵	6.48x10 ⁴	2.42x10 ⁴
		48 h-LC ₅₀	2.9x10 ³	3.4x10 ³	3.5x10 ³	4.2x10 ³	5.1x10 ³	5.4x10 ³	5.6x10 ³	6.6x10 ³
Tordon 101 Mixture	I. P. A. + P.A.	0.0	3.59	7.18	14.37	28.75	57.5	115	230	*
		24 h	4.54x10 ⁷	3.86x10 ⁷	6.44x10 ⁶	8.88x10 ⁵	4.67x10 ⁵	4.44x10 ⁴	1.64x10 ⁴	2.02x10 ³
		24 h-LC ₅₀	3.1x10 ³	4.1x10 ³	4.4x10 ³	5.2x10 ³	5.6x10 ³	6.6x10 ³	7.0x10 ³	None
		48 h	2.51x10 ⁹	2.16x10 ⁸	4.41x10 ⁷	6.14x10 ⁶	2.3x10 ⁶	2.64x10 ⁵	8.48x10 ⁴	4.42x10 ⁴
		48 h-LC ₅₀	2.9x10 ³	3.2x10 ³	3.4x10 ³	4.1x10 ³	4.4x10 ³	5.1x10 ³	5.8x10 ³	6.8x10 ³

* Test strain grew in all concentration of pesticides.

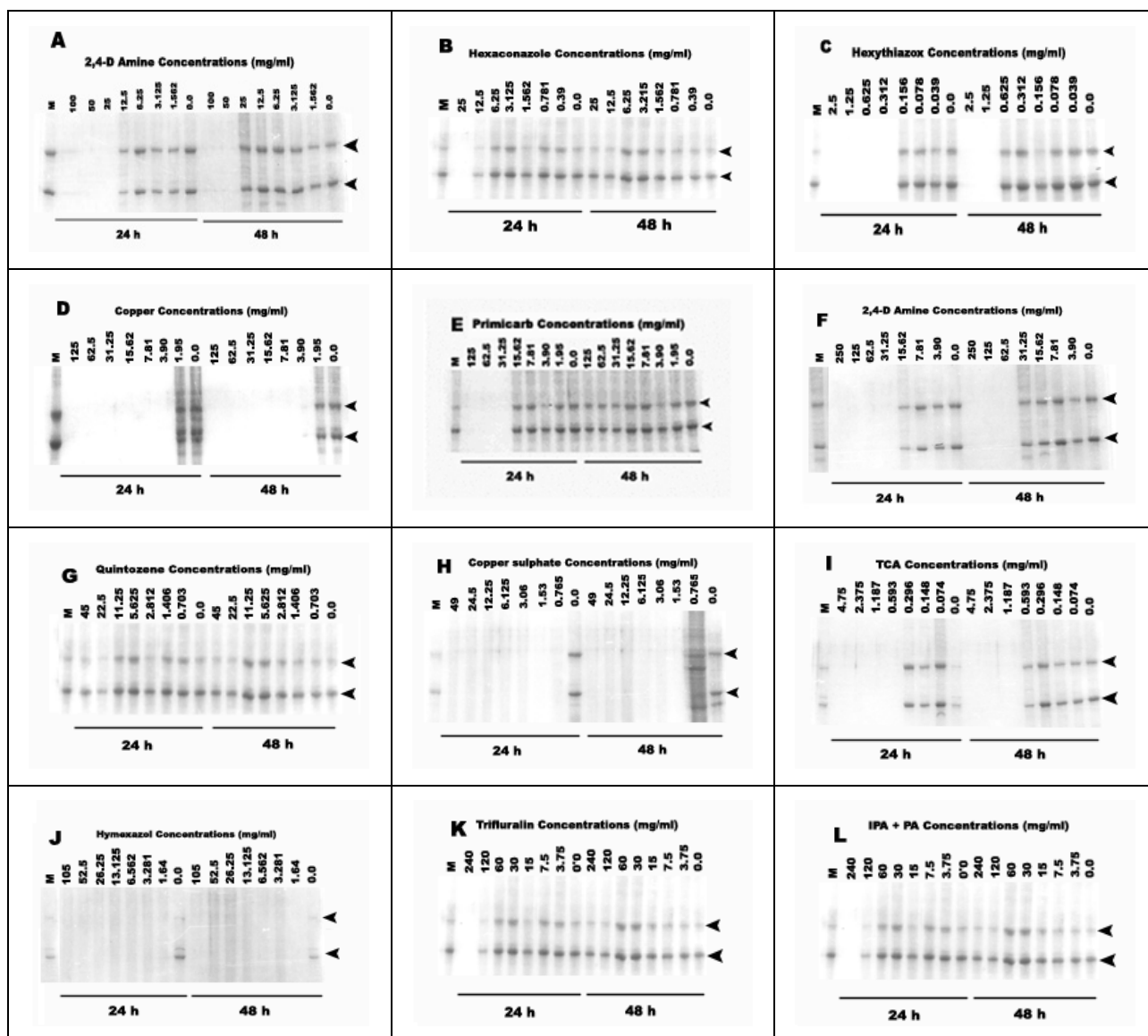


FIGURE 1 - SDS-PAGE analysis of *B. sphaericus* 2362 strain after 24 and 48 hours incubation on media containing 12 different pesticide concentrations. M, Standard toxin bands. Arrows show the binary toxin bands.

rates by the whole group of pesticides studied. These variable effects might be depending on radicals groups consisting of active ingredient including each of pesticide.

The larvicidal activity of *B. sphaericus* 2362 culture grown on media containing different pesticides and incubated for 24 and 48 h were tested against 2nd and 3rd instars *C. quinquefasciatus* larvae separately at the room temperature and their LC₅₀ values were calculated in this study (Table 1). It was found that the larvicidal activity disappeared at MIC values and at higher concentrations, but the larvicidal activity persisted in the case of low concentrations, less than MIC values. In the meantime, it was observed that larvicidal activities decreased relatively (6×10^3 CFU ml⁻¹), compared to the control (2×10^3 CFU ml⁻¹).

It was shown that the use of Primor 50 WG, Tordon 101 Mixture, Tefralin EC, Brassicol and Anvil pesticides in the mosquito larvae habitats for the purpose of chemical control did not effect adversely the larvicidal activities of *B. sphaericus* 2362 strain, but the use of Tachigaren 70 WP, Nissorun 5 EC, Agro D-Amine, Cornox Amine, Nata Granule, Copper sulphate and Cupravit pesticides significantly decreased the larvicidal activity of the bacterium.

The loss of toxin protein bands was also confirmed by SDS-PAGE analysis of spore samples, in which 41.9 kDa and 51.4 kDa binary toxin bands of *B. sphaericus* strain disappeared on the gel when test bacterium was grown at MIC or higher concentrations of pesticides (Figure 1). On the contrary, parasporal inclusions were not affected as

already observed by phase-contrast microscopy, because the parasporal crystals of pesticide-treated spores might not contain toxin proteins and, hence, they did not appear to be damaged. In this case, the destruction of binary toxin proteins, produced by *B. sphaericus* H5a5b serogroup strains, could be attacked by active radicals, such as sulphate, Cl^{-1} , Cu^{+2} , CH_3 , OH^{-1} , NO_2 etc.

Cokmus et al. [9] found that peroxide radicals generated by UV radiation could destroy 41.9 kDa and 51.4 kDa toxic proteins of *B. sphaericus*. Our results are confirmed their findings. A number of studies involving physical and chemical factors were carried out on the larvicidal activity of *B. sphaericus* strains so far. It was observed that different lethal dosages of UV light effectively reduced the spore viability of *B. sphaericus* 1593, but had no effect on larvicidal activity [6, 11, 27]. A few workers [1, 4, 29] investigated the effect of several chemical compounds on the mosquito pathogenic *B. sphaericus* strains [1, 4, 29]. Berber et al. [4] observed that increasing concentrations of paraquat had no adverse effects on the toxin proteins of *B. sphaericus* 1593 and 2362 strains. But, they found that larvicidal activities of *B. sphaericus* 1593 and 2362 strains were maintained, despite the fact that paraquat inhibited the spore germination. We found in this study that various commercial pesticides in different concentrations reduced significantly the spore viability and larvicidal activity of *B. sphaericus* 2362 strain. Nevertheless, it was obvious that the larvicidal activity disappeared, when the bacterium grew at MIC or higher concentrations.

A linear relationship was found between pH and bacterial spore germination, generally enhanced at pH 7. However, complete inhibition of spore germination occurred at and below pH 5 [28]. Also, some chemical agents caused a dramatic reduction in the spore germination [1, 4]. The larvicidal activity of *B. sphaericus* H5a5b serogroup depends on the presence of binary toxin proteins produced during their sporulation [26]. Some researchers have reported that high larvicidal activity occurs at pH 7 by the mosquito pathogenic *B. sphaericus* strains [14, 28]. It was reported that insecticidal activity of 1593 strain was lost in low and high pH values, $\text{pH} < 4$ and $\text{pH} > 10$ [16, 28].

Our findings showed that the reasons for disappearance of larvicidal activity depend on the degradation of binary toxin proteins by free radicals and decrease of pH (< 4) in the media that contain the pesticides. It may be concluded from SDS-PAGE analysis, that the larvicidal activity is dependent on the presence of the binary toxin proteins in the parasporal inclusions. On the other hand, when the bacterial colony exposed to the media containing different pesticide concentrations for 48 h is transferred onto NYSM agar plates, which do not contain pesticides, both spores and larvicidal toxin proteins are produced. This showed that no break occurred in the genes coding the toxin proteins.

In addition to the low water content of the spore, the pH of the core cytoplasm is about one unit lower than

that of the vegetative cell and contains high levels of core-specific proteins called *small acid-soluble spore proteins* (SASPs). These are produced during the sporulation process and have at least two functions. SASPs bind tightly to DNA in the core and protect it from potential damage from ultraviolet radiation, desiccation, and dry heat. In addition, SASPs function as carbon and energy source for the growth of new vegetative cells from the endospores [25]. Since the spores are more resistant to environmental factors than SASP proteins, this germination process might have been prevented due to degradation of these proteins by pesticides examined in this study. Some researchers reported that encapsulated spore-toxin complex of *B. sphaericus* 2362 strain provided more resistance than its free form in the control of mosquito larvae [13, 14, 17]. Actually, it may be useful to use an encapsulated spore-toxin complex of the bacterium to prolong the larvicidal activity in organically and chemically polluted habitats.

One of the most important factors affecting the larvicidal activity is pollution of the water habitat [2]. The area that larvicidal *B. sphaericus* strains are applied to combat the mosquitoes in biological control is generally aquatic, swampy fields that have been exposed to chemical pollution and in which mosquito larvae live. For this reason, it is very important to detect how the spore viability and larvicidal activity of *B. sphaericus* strains will be affected in this kind of habitats that are polluted by various pesticides. In this study, the effects of *B. sphaericus* 2362 strain, an excellent mosquito pathogenic bacterium, on spore viability, toxin stability and larvicidal activity were examined for the first time. It is recommended to apply *B. sphaericus* strains in field conditions against mosquito larvae. It is important, however, to determine the toxin stability and larvicidal activity of the microbiological agent being used.

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