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Effect of *Bacillus sphaericus* and *Bacillus thuringiensis* strains On Larvae of *Ephestia kuehniella* (Lepidoptera)

Nil BAĞRIAÇIK[1]*, Ayten ÖZTÜRK[2]
[1]*Nigde Ömer Halisdemir University, Faculty of Arts and Sciences, Department of Biology, Nigde, Turkey.
[2]Nigde Ömer Halisdemir University, Faculty of Arts and Sciences, Department of Biotechnology, Nigde, Turkey.
*Corresponding author email id: nilbagriacik@hotmail.com

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Abstract **– In this study, new isolates of *Bacillus sphaericus* 51BR strain, *Bacillus thuringiensis* 51Bb and 51BQ strains from soil of Nigde, were tested on larvae of *Ephestia kuehniella*. Spores - crystals mix of the isolates were prepared. Two doses of the mix had been tested on larvae of *Ephestia kuehniella* under laboratory conditions for 18 days until the larvae reached their adult forms and the results were compared with the control groups. The Isolates have been found to cause insecticidal activity of over the average on the larvae and on growth retardation in the survivors. The differences between the groups were determined using the One-Way ANOVA test. As a result, *B. sphaericus* 51BR strain was found to exhibit the most insecticidal activity against larvae even at low dose.**

Keywords **– Biocontrol, bioinsecticide, *Bacillus sphaericus, Bacillus thuringiensis, Ephestia kuehniella.***

I. Introduction

Numerous chemical insecticides are used all around the world for pest control. They were previously effective in controlling the pest. However, their accumulation has adversely affected particular species such as beneficial insects, animals, and people in the environment. Nowadays these insects have gained resistance to such compounds. In order to avoid these problems, biological control agents have been used mostly with host-specific natural enemies that pose low risks and provide great benefits recently. There are many safe bacterial group used in many countries as the biocontrol agents including *Bacillus* species [1], [2].

B. thuringiensis strains, those are toxic against a wide range of insect orders such as Lepidoptera, Diptera and Coleoptera, produces parasporal crystal line inclusions containing Cry and Cyt proteins and its various serotypes of *B. thuringiensis* exhibits insecticidal activity against different insects. However, the most commonly microbial control agent used is *B. thuringiensis* strains, and some of their commercial formulation that have been manufactured against pests [3]-[5].

One of the most important toxins has been found in *B. sphaericus* species, which has insecticidal activity on larvae's of mosquitoes' species. These toxins have been converted into the active form in the digestive systems of the larvae as a result of the enzymatic activity occurring at alkaline condition. *B. thuringiensis* and *B. sphaericus* have been currently used to control the pests that are threatening public health [6], [7].

Ephestia kuehniella (Lepidoptera) is a common pest that grows on stored grain products in Turkey and in the world. Several biological control studies have been used especially *B. thuringiensis* against this pest [8]-[10]. Although *B. sphaericus* was first discovered to have larvicidal properties against the mosquito larvae by Kellen et al [11], up to date the insecticidal activity studies of *B. sphaericus* strains on *Ephestia kuehniella* has not been carried out. In this study, three insecticidal isolates were identified, and their activities on *E. kuehniella* larvaes were investigated. This is the first report on the insecticidal activity of *B. sphaericus* strain on *E. kuehniella*.

II. Material and Methods

A. Ephestia Kuehniella Larvae

The adult male and female individuals of *Ephestia kuehniella* and *B. thuringiensis* strain T03A001 obtained from Ankara University (Ankara, Turkey) were used for control groups.The larvae of *E. kuehniella,* were fed on an artificial medium made of 100 g of wheat flour, corn flour and bran at a ratio of 2: 1: 1 at 60 ° C for 3 hours. Glass containers (9cm diameter x 17cm height) covered with tulle were prepared. This population was maintained for three weeks in the dark at 25 ± 3°C and at 45-60% relative humidity. The mature individuals were transferred to a new container for offspring. The eggs collected were used to maintain the colonies [9], [12] and [13].

Ten early third instars larvae were placed in each glass container and, the container closed with tulle. 16 containers with bacteria-treated nutrient for insecticidal activity test and two containers of untreated nutrient as control group were used with regulated relative humidity and temperature.

B. Isolation and Preparation of Bacteria

In this study, three bacteria were isolated from soil (Nigde, Turkey) and were identified [14], [15]. Five gram of soil was subjected into 100 ml acetate buffered nutrient liquid medium, and then the growing cells and other, non-spore-forming bacteria were eliminated by heat treatment (75-80°C for 10-15 minutes). Morphological and physiological identification tests of isolate bacteria were performed according to Holt [14]. Three isolates which identified as *B. thuringiensis* and *B. sphaericus* species were chosen for insecticidal activity studies. To prepare the sporulated culture, these bacterial strains, *B. thuringiensis* 51Bb, *B. thuringiensis* 51BQ, and *B. sphaericus* 51BR strains were grown in nutrient medium for two weeks at 35°C for sporulation. The cultures were harvested by centrifugation at 5000 rpm for ten minutes. The pellets were washed two times with cold saline and resuspended in saline for microscopy. The crystal and sports forms were examined with light microscope (OLYMPUS) and were photographed in 1000 magnifications. The bacterial suspensions were dried for 48 hours at 37°C. In order to study of insecticidal activity, these dried bacteria were weighed for preparation of larval nutrient.

C. Insecticidal Activity Tests

The bioassay of insecticidal activity was performed in duplicates for each different dose (0.1 and 0.2 doses). Each dose of mixture was added into container containing 10 g larval nutrient (0.1 g/10g and 0.2 g/10g). Some container containing larval nutrient without bacteria were used as the control group. The practice continued until the individuals became mature forms in the control group. During this time, the larvae survived were counted every day.

D. Statistical Studies

The data obtained at the end of the study were analyzed by ANOVA (ONEWAY) test. However, when P is less or equal to 0.05 in the results, it is agreed to be statistically significant.

III. Discussion and Results

A. Isolation and Identification of Bacteria

Three bacterial strains were chosen according to the identification tests and the results of crystal staining (Table 1). Their crystalline and spore forms were examined under light microscope and two bacterial strains were found to belong to a typical *B. thuringiensis* strains with typical parasporal crystal structures (Fig. 1a-b), while the other bacterial strain belonged to the typical *B. sphaericus* bacterial strain (Fig. 1c). However, it is known that *B. sphaericus* species has got a combined exotoxin with sport and these spore forms are fully rounded [14].

Table 1. Identification of *Bacillus sphaericus* 51BR.

Characteristics	Isolated	Standard
Colony morphology	Round,entire margin, white	Round, entire margin, opaque white
Shape of bacteria	Long bacilli	Long bacilli
Gram reaction	+	+
Size; L (µm) xW (µm)	1.5-5.0 x 0.5-1.0	1.5-5 .0 x 0.6-1.0
Endospore	+	+
Endosporepositioninsporangium	Subterminal	Terminal/subterminal
Catalase	+	+
Starch hydrolysis	-	-
Acid production from sugar	-	-
Indol	-	-
Gelatinhydrolysis	+	Variable
Aerobicity	+	+
Sensitivity to streptomisin	Resistant	Resistant
Sensitivity to Chloramphenicol	Sensitive	Variable

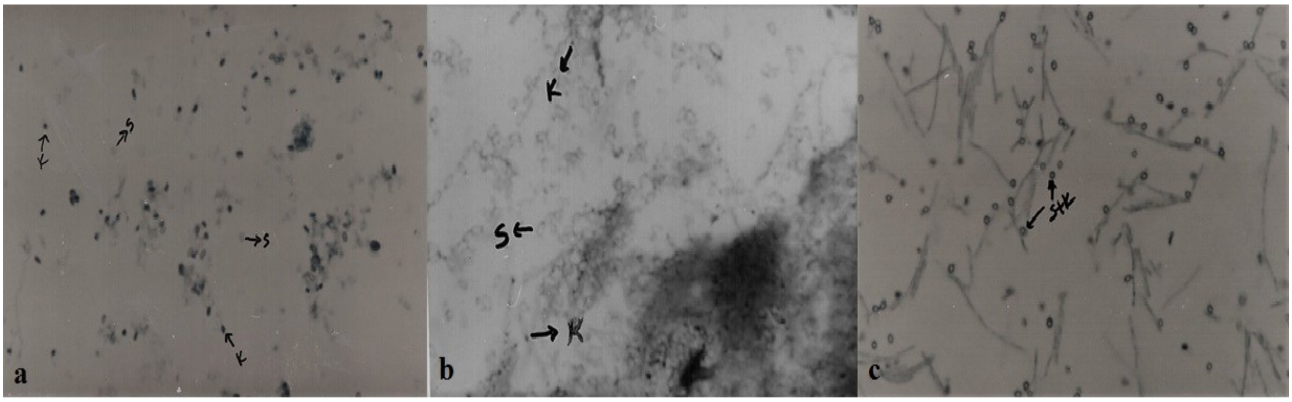

Fig.1. Spore and crystal forms of a) *B. thuringiensis* 51Bb strain, b) *B. thuringiensis* 51BQ strain, c) *B. sphaericus* 51BR strain, S: Spore form; K: Crystal endotoxin

B. Insecticidal Activity

In this study, results from bioassay study were found to show that, insoluble bacterial strains did not only show insecticidal activity on *E. kuehniella* larvae, but also caused growth retardation in survivors when compared with the control group. This has been explained as a tolerance for resistance acquisition in several previous studies [8], [16] and [17]. We have also found that *B. thuringiensis* 51BQ strains were effective when applied in 0.2 doses. Similarly, *B. sphaericus* 51BR strain was effective on the larvae when 0.1 dose was used (Fig. 2a), and that the effect of *B. sphaericus* 51BR strain has increased at this dose (Fig. 2b). However, in the study, the doses used and the presence of larvae have proved to be very effective in their insecticidal activity within the first 150 hours (approximately five days). We have also determined that *B. thuringiensis* var.

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kurstaki has an insecticidal activity reaching 100 % against the larvae (Fig. 3). *B. thuringiensis* var. *kurstaki* were used affectively against some members of the Lepidoptera and Coleoptera orders [18]. It is stated that the larvae are more susceptible to toxin during the periods before the fifth instar period in the study Rahman et al [8] found that maternal activity produced tolerance to the toxin of *B. thuringiensis* strains in subsequent generations. Abdelmalek et al [17] showed that *E. kuehniella* larvae in the fifth instar period showed high tolerance to the Cry1Aa toxin of *B. thuringiensis* var. *kurstaki* HD1 strain, which was used in low doses, whereas it was over 50% of the mortality rates of the first and fifth instar larvae. However, in this study, our isolate *B. sphaericus* 51 BR strain was very effective to larvae of *E. kuehniella* as well.

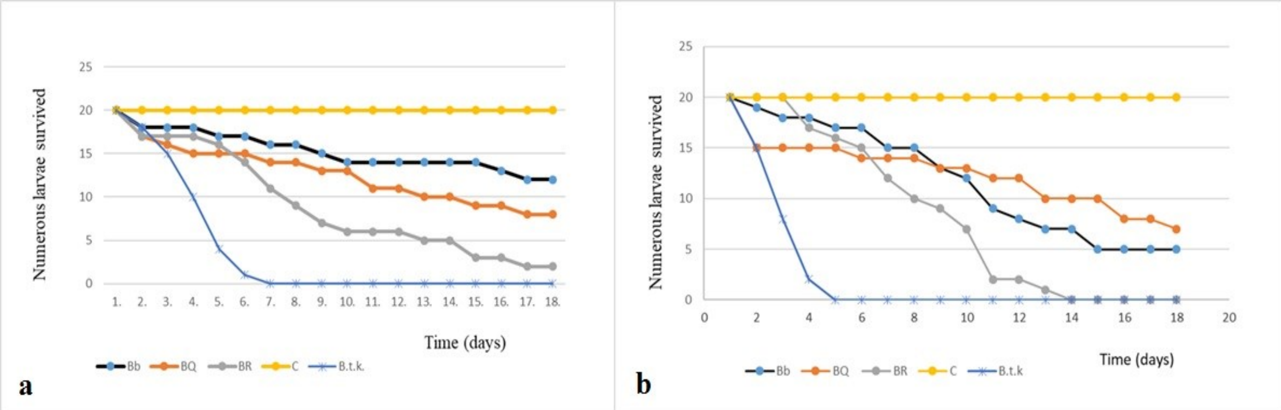

Fig. 2. a) Bioassay studies at 0.1 dose mixture of spore and crystal to *E. kuehniella* larvae; b) Bioassay studies at 0.2 dose mixture of spore and crystal to *E. kuehniella* larvae, Bb: *B. thuringiensis* Bb strain; BQ: *B. thuringiensis* 51BQ strain; BR: *B. sphaericus* 51BR strain C: Negative control (larval nutrient without bacteria); B.t.k: positive control (larval nutrient containing *B. thuringiensis* var. *kurstaki*)

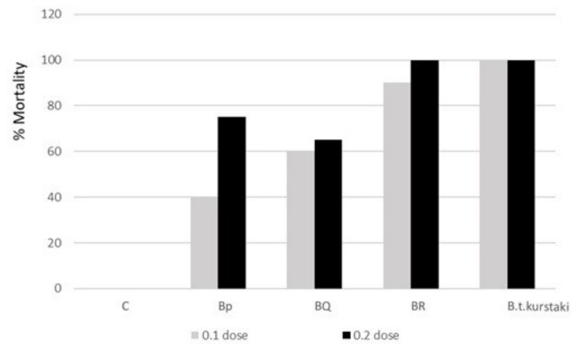

Fig. 3. Mortality rates of isolates.
C: Negative control (larval nutrient without bacteria); Bb: *B. thuringiensis* Bb strain; BQ: *B. thuringiensis* 51BQ strain; BR: *B. sphaericus* 51BR strain; *B.t.kurstaki*: positive control (larval nutrient containing *B. thuringiensis* var. *kurstaki*)

In the literature, Alper and colleagues found that *B. thuringiensis* isolates had the highest mortality rates against to *E. kuehniella*, 42%. They reported that these isolates have high mortality rates and can be used to control *E. kuehniella* as an alternative biopesticides in case of insect resistance against the known *B. thuringiensis* preparations [19]. BenFarhat and colleagues showed that *X. nematophila* and *B. thuringiensis kurstaki* delta-endotoxins act synergistically against *E. kuehniella* and this synergistic effect was proportional to toxins receptor affinity [20].

IV. Conclusions

In this study, the results obtained for B. *thuringiensis* 51 Bb and BQ strains compared to the positive control *B. thuringiensis* var. *kurstaki* strain, seems to be compatible with the literature. *B. spaericus* 51BR strains were found to possess higher insecticidal activity than both *B. thuringiensis* strains. This study is the first report that *B. sphaericus* has insecticidal activity for *E. kuehniella* larvae.

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AUTHORS' PROFILES

Nil BAGRIACIK is Associated Professor of Biology at the University of Nigde Ömer Halisdemir. She received her B.S. from the University of Hacettepe (Ankara/Turkey) in 1991, and M.S. from the University of Gazi (Ankara/Turkey) in 1994 and her Ph.D. in Zoology and Entomology from the University of Ankara in 2004. She has worked at the University since 1994 in Nigde. She has published numerous papers.

Ayten ÖZTÜRK is Professor of Microbiology at the University of Nigde Ömer Halisdemir. She received her B.S. and M.S. from the University of Hacettepe Ankara/Turkey, and her Ph.D. in Microbiology from the University of Ankara in 1996. She has worked at the University since 1996 in Niğde. She is teaching introduction of Molecular Biology and Microbiology. She has published numerous papers. She is head of Biotechnology Department.