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Research Article

Evaluation of Spinosad and Different *Bacillus thuringiensis* (Bacillaceae) Strains against the Larvae of *Hyphantria cunea* (Lepidoptera: Erebidae)

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ABSTRACT

In this study, *Bacillus thuringiensis aizawai* Bonnefoi & de Barjac, 1963 (Florbac), different isolates of *Bacillus thuringiensis* (Bt) *kurstaki* Berliner, 1915 (Biobit, Delfin, Rapax, Rebound) and spinosad (Spintor) were assessed for their efficacy against different instars of *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae) under laboratory and field conditions in 2019 in Duzce Province, Turkey. Spinosad was the most effective bioinsecticide, followed by *Bt aizawai* and *Bt kurstaki* isolates against various instars of *H. cunea*. In the laboratory, at 96 hour post-treatment, spinosad caused 98% mortality in 2nd and 3rd instars, and 83% mortality in 4th and 5th instars; *Bt aizawai* caused 98% mortality in 2nd and 3rd instars and 43% mortality in 4th and 5th instars; and *Bt kurstaki* isolates caused 95% mortality in 2nd and 3rd instars and 51% mortality in 4th and 5th instars. The results from the field study showed similar trends. The older instars were less susceptible to all the tested bioinsecticides than the younger instars, which may be attributable to the phenomenon known as “maturation immunity”. *Bacillus thuringiensis kurstaki* has already been recommended for management of this pest. Whereas the data indicated that spinosad and *Bt aizawai* could also be used as bioinsecticides for control *H. cunea* larvae.

Keywords: *Bacillus thuringiensis*, spinosad, *Hyphantria cunea*

Spinosad ve Farklı *Bacillus thuringiensis* (Bacillaceae) Suşlarının *Hyphantria cunea* (Lepidoptera: Erebidae) Larvaları Üzerindeki Etkinliğinin Değerlendirilmesi

ÖZET

Bu çalışmada, *Bacillus thuringiensis aizawai* Bonnefoi & de Barjac, 1963 (Florbac), *Bacillus thuringiensis* (Bt) *kurstaki* Berliner, 1915 (Biobit, Delfin, Rapax, Rebound)'nin farklı ticari suşları ve spinosad (Spintor)'ın etkinliği *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae)'nin farklı larva dönemlerine karşı laboratuvar ve doğal koşullarda 2019 yılında Düzce ilinde test edilmiştir. *Hyphantria cunea*'nin farklı dönem larvalarına karşı en etkili biyoinsektisidin spinosad olduğu tespit edilmiştir. Spinosad'ı sırasıyla *Bt aizawai* ve *Bt kurstaki* suşları takip etmiştir. Laboratuvar deneylerinde 96 saat sonunda spinosad *H. cunea*'nın 2. ve 3. dönem larvalarında %98, 4. ve 5. dönem larvalarında ise %83 ölüm meydana getirmiştir. *Bt aizawai* 2. ve 3. dönem larvalarının %98'ini, 4. ve 5. dönem larvaların ise %43'ünü, *Bt kurstaki* suşları 2. ve 3. dönem larvaların ortalama %95'ini, 4. ve 5. dönem larvaların ise ortalama %51'ini öldürmüştür. Alan denemelerinde de spinosad en etkili sonuçları sergilemiştir. Çalışmada daha yaşlı larvaların daha az duyarlı oldukları görülmüştür. Bu durumun “maturation immunity” olarak bilinen olguyla bağlantılı olduğu düşünülmektedir. Bu zararlı ile mücadelede *Bacillus thuringiensis* (Bt) *kurstaki*

zaten tavsiye edilmektedir. Bu çalışmada ise spinosad ve *Bt aizawai* etken maddesine sahip biyoinspektisilerin de *H. cunea*'ya karşı kullanılabilceği gösterilmiştir.

Anahtar Kelimeler: *Bacillus thuringiensis*, spinosad, *Hyphantria cunea*

I. INTRODUCTION

The fall webworm, *Hyphantria cunea* (Drury, 1773) (Lepidoptera: Erebidae), a native insect of North America, is now a widespread pest in Europe and Asia [1]. The first reports of *H. cunea* in Turkey were in the Marmara and central Black Sea Regions in 1975 and 1982, respectively [2,3]. It is a gregarious, polyphagous insect that feeds on leaves of more than 400 species of forest, shade and orchard trees [4]. The larvae form a webbed nest and they can defoliate trees, but the defoliation is generally not considered harmful to healthy trees, especially to forest trees because it occurs during late summer and fall. However, the defoliation to shade and orchard trees can reduce their ornamental value [1].

Various control methods have been reported against the adult and larval stages of this pest. For example, a pheromone lure can be used for mass trapping or disrupting the mating of adults. Unfortunately, the lure is not currently available, and light traps may be a means to reduce the population of adults [1]. For controlling the larval populations, a number of insecticidal control options such as the systemic (emamectin benzoate) and non-systemic (cypermethrin, diflubenzuron, carbaryl) synthetic insecticides, and insect growth regulators (IGRs) (methoxyfenozide, etc.) are available in many countries [1]. However, in Turkey, only the chemical insecticides, cypermethrin and diflubenzuron, are currently recommended for the control *H. cunea*. However, these synthetic insecticides can have negative effects on pollinators and non-target organisms, as well as on the environment and humans. Therefore, to avert these negative effects, bioinsecticides such as *Bacillus thuringiensis* (Bt) Berliner, 1915 (Bacillaceae), and toxins from the bacterium, *Saccharopolyspora spinosa* Mertz & Yao, 1990 (Pseudonocardiaceae), can be used [1].

In Turkey, the only bioinsecticide (Bio-T plus) registered against the fall webworm is *Bacillus thuringiensis kurstaki* de Barjac & Lemille, 1970 strain PB-54, but there are at least six commercial bioinsecticides sold under the trade names, Biobit, Delfin, Rapax, Florbac, Rebound and Spintor, which have been registered for used against different insect pests [5]. The active ingredient of Biobit, Delfin, Rapax and Rebound is *Bt kurstaki*, whereas the active ingredient of Florbac is *Bacillus thuringiensis aizawai* Bonnefoi & de Barjac, 1963. The active ingredient(s) of Spintor is spinosad, which is derived from the bacterium, *S. spinosa* [6]. With the availability of these various bioinsecticides in Turkey, the objective of this study was to evaluate the efficacy of different *B. kurstaki* isolates and spinosad against *H. cunea* larvae under laboratory and field conditions.

II. MATERIALS AND METHODS

A. 1. Insect and Bioinsecticides

Hyphantria cunea larvae were collected from hazelnut orchards in 2019 in Duzce Province of Turkey. The branches with the silken webs containing larvae were cut and transferred to the laboratory. The larvae were maintained in an insectarium at room temperature where they were fed fresh hazelnut leaves until the experiments. Before starting the experiments, the larvae were separated into two groups by their body length and color [7]. Group I consisted of 2nd and 3rd instar larvae and group II contained 4th and 5th instar larvae. The susceptibility of both instar groups to the bioinsecticides was determined under laboratory and field conditions.

Six bioinsecticides (Biobit, Delfin, Rapax, Rebound, Florbac, Spintor) registered in Turkey were tested against larvae of the fall webworm. Their active ingredients are 16,000 IU/mg *Bt kurstaki* serotype: H-7, 32,000 IU/mg, *Bt kurstaki* strain SA-11, 24,000 IU/mg *Bt kurstaki* strain EG23-42, 16,000 IU/mg *Bt kurstaki*, 35,000 DBM/mg *Bt aizawai* strain ABTS-1857, 240g/L Spinosad for Biobit, Delfin, Rapax, Rebound, Florbac, Spintor respectively. All the bioinsecticides were prepared at their recommended rates in distilled water just prior to their application. Distilled water was used as a control. During the current experiments, Bio-T plus could not be provided by the distributor. So, Bio-T plus was not included as a control in this study.

Table 1. The active ingredients, product names and recommended rates of the six bioinsecticides used in this study

Active ingredient	Product name	Recommended rate
16,000 IU/mg <i>Bt kurstaki</i> serotype: H-7	Biobit	2g/L
32,000 IU/mg, <i>Bt kurstaki</i> strain SA-11	Delfin	1g/L
24,000 IU/mg <i>Bt kurstaki</i> strain EG23-42	Rapax	1.5ml/L
16,000 IU/mg <i>Bt kurstaki</i>	Rebund	2g/L
35,000 DBM/mg <i>Bt aizawai</i> strain ABTS-1857	Florbac	1.5g/L
240g/L Spinosad	Spintor	0.5ml/L

A. 2. Laboratory Experiments

The method of Saruhan et al. [8] was followed in the laboratory experiments. Fresh hazelnut leaves of equal size and number were dipped in the stock suspension of each bioinsecticide for 30 seconds and placed on a filter paper to air dry for 15 minutes. The air-dried leaves were placed in plastic containers (8 x 14 x 20 cm), and 10 fall webworm larvae were added. Control leaves were dipped in water. Two leaves per container were used. The containers were kept under laboratory conditions at room temperature and moist filter papers were placed in the containers to humidify the air. The number of dead larvae in each container was recorded after 48, 72 and 96 hours. There were 3 containers per group and the experiments were replicated three times on different dates. The experiments were conducted in Biological Control Laboratory at the Department of Biology, Duzce University, Duzce, Turkey in 2019.

A. 3. Field Experiments

Based on the data collected from the laboratory experiments, the three effective bioinsecticides were evaluated in the field. The experiments were conducted in an infested hazelnut orchard with fall webworm on the Düzce University campus, Düzce, Turkey, in 2019. The 2-3rd and 4-5th instar populations of fall webworm on different branches were preferred in the field experiments. Approximately 10 mL suspensions of Florbac, Rebound or Spintor were applied with a sprayer (Baco 2 liter Hand sprayer) per branch. The branches were marked with different colored tapes indicating the different insecticides and covered with a cage made from bridal veil material after spraying each treatment. The branches were cut and brought to the laboratory after 96 hours, and the numbers of dead and alive larvae were recorded per branch. Three branches were used per treatment group (Florbac, Rebound, Spintor), and the Control.

A. 4. Data Analyses

For the laboratory experiments, two-way ANOVA was performed to determine the effects of bioinsecticide, larval stage, larval stage x bioinsecticide and time on the mortality of *H. cunea* [9]. Data were pooled and replicates were considered as a block. Mortality was corrected according to the methodology of Abbott [10]. If there was a significant difference between the mortalities of instars caused by each bioinsecticide, independent sample t-test has been performed [9]. At the field

experiment, Chi-square analysis was performed to determine any significant difference at the $P = 0.01$ level in the mortality rate among the treatments.

III. RESULTS and DISCUSSION

In the first part of study, control potential of *Bt aizawai* (Florbac), spinosad (Spintor) and isolates of *Bt kurstaki* (Biobit, Delfin, Rapax, Rebound) were evaluated against fall webworm in the laboratory. It was observed that, the early instars (2-3rd instar) were more susceptible to all of tested bioinsecticides. than 4-5th instar (Figure 1). Furthermore spinosad (Spintor) caused the highest mortality in both groups of instars under laboratory conditions. And no significant difference was detected only at spinosad (Spintor) ($F=33,977$; $df= 58$; $P=0.88$) in comparison of 2-3rd instar and 4-5th instar larvae.

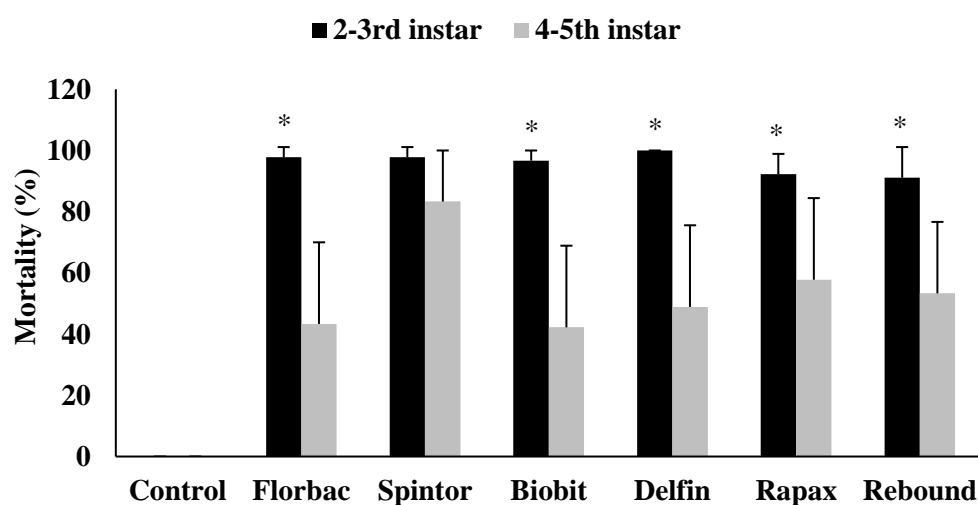


Figure 1. The effects of six bio insecticides on the mortality of different instars of *Hyphantria cunea* after 96 hours. Asterisk (*) indicates a significant difference between the instar groups for the bioinsecticide (Independent Student-t test).

In laboratory experiments, results showed that instar and insecticide had a significant effect on larval mortality. However, the effect of the interaction of instar x insecticide on mortality was not significant (Table 2).

Table 2. Two-way ANOVA results showing the effects of insecticide and instar on *Hyphantria cunea* mortality

Variation	DF	F	P-value
Instar	1	44,601	0,000
Insecticide	6	17,682	0,000
Instar x insecticide	6	2,284	0,064

In time-dependent mortality experiments, the mortality in early instars (2-3rd) occurred faster than in group II. The mean mortality in early instars was 77% after 48 hours but the highest mortality in 4-5th was only 69% after 96 hours. It was observed that there was a significant effect on mortality of instars (2-3rd and 4-5th) but not on “time” and “instar x time” (Table 3).

Table 3. Two-way ANOVA results for the effect of time and instar on *Hyphantria cunea* mortality

Variation	Df	F	P-value
Instar	1	12,849	0,001
Time	2	2,197	0,126
Instar x time	2	1,092	0,346

In the second part, Florbac, Rebound, Spintor were evaluated in the field. Spintor caused the highest larval mortality (99%) of group I instars which was significantly higher than that of Florbac (85%) and Rebound (80%) ($\chi^2=0.00$) (Table 4). In group II, the mortality of larvae caused by Spintor (86%) and Florbac (94%) were significantly higher than for Rebound (52%) ($\chi^2=0.11$)(Table 4).

Table 4. Mortality of early and late instar groups treated with three different biopesticides in the field experiments

Treatments	2-3 rd instar (Group I)			4-5 th instar (Group II)		
	N	Number of dead	Mortality (%)	N	Number of dead	Mortality (%)
Control	274	0 ^a	%0	120	0 ^a	%0
Spintor	543	541 ^b	%99 ^b	58	50 ^b	%86 ^b
Florbac	298	254 ^c	%85 ^c	85	80 ^b	%94 ^b
Rebound	358	288 ^c	%80 ^c	85	45 ^c	%52 ^c

Abbreviations: N: total number of larvae in population. The different letters indicate significant differences (Chi-square test) ($p<0.01$).

The recent study showed that spinosad is effective against fall webworm larvae. Beuzelin et al. [11], Saruhan et al. [8] and Schowalter and Ring [1] mentioned that it is a good option in orchard management. Our results show similarities with Saruhan et al. [8]. Saruhan et al. [8] tested spinosad (Laser, 480 g/L) on 3rd instar of *H. cunea* and recorded the highest mortality rates (92.5 and 95%) after 72 hours. Watson [12] stated that spinosad acts as a neurotoxin that inhibits nicotinic acetylcholine and ionotropic γ -aminobutyric acid (GABA) receptors. When the toxin is taken by larvae, digestion is arrested causing paralysis and death of larvae within 24 hours.

Biobit and Delfin, which include different isolates of *Bt. kurstaki* were tested by Ecevit et al. [13] and Saruhan et al. [8] against *H. cunea*. In the present study, we tested two different isolates of *Bt. kurstaki* (Rapax and Rebound) in addition to Biobit and Delfin. All the *Bt kurstaki* isolates and *Bt aizawai* caused mortality at a slower rate than Spinosad due to differences in the mode of action of *B. thuringiensis*. The Ministry of Agriculture and Forestry in Turkey recommends *Bt aizawai* for other lepidopteran pests that include *Archips rosanus*, *A. xylosteanus*, *Tuta absoluta*, *Lobesia botrana* and *Spodoptera littoralis* but not for *H. cunea* [5]. As far as we are aware, our study is the first to demonstrate the potential of *Bt aizawai* in the management of *H. cunea*.

It was observed that 2-3rd instars of the fall webworm were more susceptible to the bioinsecticides than 4-5th instars both in laboratory and field experiments, a response that may be related to “maturation immunity” [15]. The earlier studies support our data. Ecevit et al. [13] showed that the 1st-3rd instars of *H. cunea* died in 5 days using *Bt kurstaki* in the laboratory. Saruhan et al. [8] observed that two different concentrations of Delfin killed 92.5% and 100% of 3rd instars of *H. cunea* in the laboratory (Figure 2, Table 2).

Tuncer and Kansu [2] reported that the host plant may have an effect on the biology of fall webworm. In our study, we preferred hazelnut as a host plant whereas maple leaves were used by Ecevit et al. [13] and Saruhan et al. [8]. Schowalter and Ring [1] stated that the growth and survival rates of broad generalist insects such as the fall webworm may be reduced by certain host plant traits.

IV. CONCLUSION

The tested bioinsecticides, especially Spinosad, showed promising results in the management of *H. cunea*. In addition to Spinosad, *Bt aizawai* could be used to control *H. cunea*. However, the potential for the development of resistance in populations treated with spinosad and *Bt aizawai* should be monitored in future studies

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