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# Insecticidal Effects of Some Biological Agents on the *Gypsonoma dealbana* (Lepidoptera) and *Hyphantria cunea* (Lepidoptera)

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**Abstract:** *Gypsonoma dealbana* (Corylus bud moth, Lepidoptera) and *Hyphantria cunea* (Fall webworm, Lepidoptera) are common pests of *Coryllus* sp. Up to now, chemical substances have been utilized to control these pests. However, recent concern on the hazardous effect of chemical pesticides in the environment made scientists consider finding more effective and safe control agents. In the present study, in order to find a more effective and safe biological control agent, we tested the insecticidal effects of various biological agents on the larvae of *Gypsonoma dealbana* and *Hyphantria cunea*. The tested agents and their insecticidal effects are respectively *Autographa californica* nuclear polyhedrosis virus (*Ac*NPV, 5 and 25%), *Lymantria dispar* nuclear polyhedrosis virus (*Ld*NPV, 5 and 25%), *Escherichia coli* transformed with plasmid containing *cryIVA*, *cryIVD* and 20-kDa-protein genes (pHE4-ADR, 17 and 17%) of *Bacillus thuringiensis*, and toxins isolated from Tenebrionis (BTS-1, 10 and 5%) and Harry Dumagae (HD-1, 31 and 80%) strains of *B. thuringiensis*. We consider that *Ac*NPV and *Ld*NPV cause the death of larvae through productive infection, however, the rest of the samples cause mortality via cytotoxidity.

**Keywords:** insecticidal effects, biological agents, *Gypsonoma dealbana, Hyphantria cunea*

## Introduction

Hazelnut, one of the highest income sources of the Blacksea zone, is among the important export products of Turkey. Turkey receives 70% of the world hazelnut product and 70-80% of the world export. Even though Turkey places on the top of the list among all countries producing hazelnut in hazelnut production and export, it is way back of many of them in terms of product harvested per unit field. One of the main causes of this situation is that hazelnut has a lot of damagers and they can not be controlled effectively.

*Gypsonoma dealbana* (Corylus bud moth, Lepidoptera) and *Hyphantria cunea* (Fall webworm, Lepidoptera) are among the most common pests of *Coryllus* sp. While *G. dealbana*, prevents growing of hazelnut shoots and leaves by feeding, *H. cunea* causes important damages on hazelnut leaves by feeding, dead shoots without leaves are the typical damage form of these insect (Ural, 1973). *H. cunea* also damages leaves and fruits of mulberry, acer, apple, pear, sour cherry, cherry, plum, etc. (Bulletin of Agricultural Control Agency, 1995). Suprisingly, despite their mass occurrence and wide distribution, very little is known about the pathogens limiting their population. For this reason, these insects are very attractive objects of biological control studies, as well as a target for control by introduction of biological agents. Up to now, chemical substances have been utilized to control these pests. The rapid widespread adoption of inorganic insecticides have been brought the new problems which are the destruction of the biotic agent pressure on pest, wild life and human health, and others. For this reason, scientists consider finding more effective and safe biological control agents (Sezen and Demirbağ, 1999; Yaman *et al.*, 1999).

In this study, in order to find a more effective and safe pesticide, we tested the insecticidal effects of various biological agents on the larvae of *G. dealbana* and *H. cunea*. The tested agents are *Autogropha californica* nuclear polyhedrosis virus (*Ac*NPV), *Lymantria dispar* nuclear polyhedrosis virus (*Ld*NPV, as gypchek), *Escherichia coli* transformed with plasmid containing *cryIVA*, *cryIVD* and 20 kDa-protein genes (pHE4-ADR) of *Bacillus thuringiensis*, and toxins isolated from Tenebrionis (BTS-1) and Harry Dumagae (HD-1) strains of *B. thuringiensis*.

## Materials and Methods

**Virus samples:** Two types of virus samples were used in bioassays: *Autogropha californica* nuclear polyhedrosis virus (*Ac*NPV) was produced in *Spodoptera frugiperda* (SF IPLB-21) cells (Demirbağ *et al.*, 1997). At 3 days post infection (occurrence of polyhedral inclusion bodies), the cells (3 x $10^7$ cell/ml) were centrifuged at 3.000 rpm for one minute. The pellet was resuspended in 5 ml of sterilized phosphate buffer solution (PBS) and used. *Lymantria dispar* nuclear polyhedrosis virus (*Ld*NPV) was obtained from Edward M. Daugherty (USDA, Beltsville, Maryland, 20750 USA) as ready used pesticide (gypchek). Two grams of these samples were suspended in 5 ml of PBS and used.

**Recombinant bacterial strains:** *cryIVA*, *cryIVD* and 20-kDa protein genes (pHE4-ADR, Ben-Dov *et al.*, 1995) of *Bacillus thuringiensis* were expressed in *E. coli* JM101. The recombinant *E. coli* cells were grown at 37 C in nutrient broth for 12 hr. After incubation, the density of cells was set up to 1.89 x $10^9$ cells/ml at $OD_{600}$ and centrifuged at 3.000 rpm for one minute. The pellet was resuspended in 5 ml of PBS and used.

**Bacillus thuringiensis toxins:** Two types of toxins, isolated from Tenebrionis (BTS-1) and Harry Dumage (HD-1) strains of *B. thuringiensis* were (obtained from Plant Genetic Systems J. Plateaustraat 22, 9000 Gent, Belgium) used in bioassays. 0.5 mg of toxin was suspended in 5 ml of PBS (100 µg/ml) and used (Moar *et al.*, 1995).

**Bioassays:** For this study, the larvae of *G. dealbana* and *H. cunea* were collected from coast of Black Sea around Trabzon between 1997-1999, Turkey. The caught insects were taken from the gardens to laboratory with appropriate boxes, larvae were reared in groups of 20 larvae in containers. Containers were punched to permit air flow. Each group was fed for 48 hours with fresh leaves and exiles of host using equal amount from each one. For this purpose, diets were placed into glass containers of 80 mm in diameter for each type of different biological agents. The surface of diet in each container was contaminated individually with all agents prepared in phospate buffer solution (PBS) using sterilized syringe (Dulmage, 1981). Twenty larvae were placed on diet in containers for each assay. After 48 hours, the larvae received fresh diet every 24 hours (Lipa & Wiland, 1972). Twenty control larvae received diet contaminated with PBS, *Spogoptera frugiperda* cells and *E.coli* JM101, respectively, for the first 48 hours and then fresh diet every 24 hours, finally dead larvae were removed.

Hundred larvae of each insect were tested for each agent.
All tested larvae were kept at 26 ± 2 C and 60% RH on a 12:12 hours photoperiod (Lipa and Wiland, 1972; Ben-Dov *et al.*, 1995). Dead larvae were removed immediately, and bioassay checked daily untill 10th day. The data were evaluated by using Abbot's formula.

## Result and Discussion

In this study, in order to find a more effective and safe biological control agent, we tested the insecticidal effects of various biological agents on the larvae of *Gypsonoma dealbana* and *Hyphantria cunea*. The insecticidal effects determined on larvae of *G. dealbana* and *H. cunea* are respectively 5 and 25% with *Autographa californica* nuclear polyherosis virus (*Ac*NPV), 5 and 25% with *Lymantria dispar* nuclear polyhedrosis virus (*Ld*NPV, as gypchek), 17 and 17% with *cryIVA*, *cryIVD* and 20-kDa-protein genes (pHE4-ADR), 10 and 5% with toxins isolated from Tenebrionis strains (BTS-1), and 31 and 80% with Harry Dumagae strain (HD-1). PBS, *Spodoptera frugiperda cells* and *Escherichia coli* were used as control (Fig. 1).

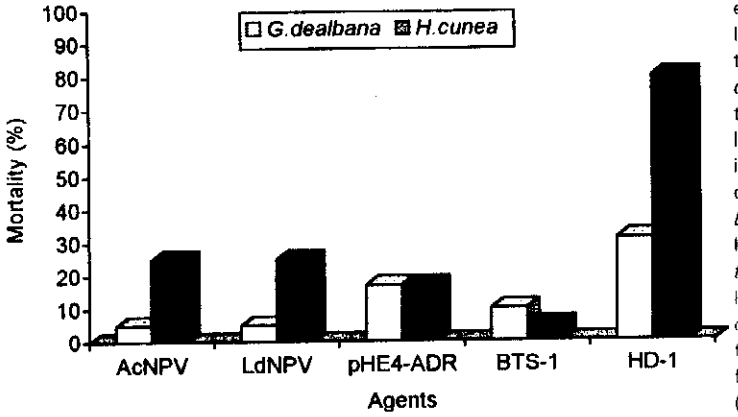


Fig. 1: The insecticidal effects of biological agents on *G. dealbana* and *H. cunea*. *Ac*NPV: *Autographa californica* nuclear polyhedrosis virus; *Ld*NPV: *Lymantria dispar* nuclear polihedrosis virus; pHE4-ADR: *Escherichia coli* transformed with plasmid containing *cryIVA*, *cryIVD* and 20-kDa-protein genes of *Bacillus thuringiensis*; BTS-1: toxin isolated from tenebrionis strain of *B. thuringiensis* and HD1: toxin isolated from Harry Dumagae strain of *B. thuringiensis*.

We observed the larvae during ten days after feeding the biological control agents. Generally, both *G. dealbana* and *H. cunea* displayed sluggish and eppetiteless.
It is possible the NPV's cause the larval death by infection. However the mode of action are not as fast as it is expected. NPV's were taken during feeding. In the midgut of these larvae, protective coat of NPV dissolved and the virus particules present were released into midgut lumen. These particles entered the midgut columnar cells by membrane fusion (Harrap, 1970), so larvae were infected by virus. The major symptom of infection is the decreased larval life of *G. dealbana* and *H. cunea*. Both *Ac*NPV and *Ld*NPV have showed 25% mortality particularly on *H. cunea*. In some studies, Atasanov (1984) and Yaman *et al.* (1999) determined that effect of NPV had high effect on *Euproctis chrysorrhoea*. It is known that some viruses have a remarkable host specificity (Kelly *et al.*, 1988). This comes from host specifity. However, the infectivity of these viruses can be developed by recombinant methods (Maeda, 1993; Lee *et al.*, 1998) While viruses have high effect especially on larvae of *H. cunea*, plasmids which contain *cry* genes of *B. thuringiensis* have 10-20% effect on *H. cunea* larvae. This is understood that the effect of *cryIV* genes are more severe on diptera than on lepidoptera (Ben-Dov *et al.*, 1995). In another study, Martens *et al.* (1990) determined that *S. frugiperda* cells infected with *Ac*NPV, which transformed with plasmid containing *cryIA* (b) gene of *Bacillus thuringiensis*, have insecticidal activity on *Pierris brassicae*.
A variety of insecticidal crystal proteins are known from *B. thuringiensis*, and their proteins are effective on lepidopterans, coleopterans and dipterans (Hofte and Whitely, 1989). Especially, *cryIV* proteins are present in crystals as protoxins which must be proteolytically converted into a toxin peptide after ingestion by the insect (Hofte and Whitely, 1989). According to Ellar *et al.* (1986) and Knowles and Ellar (1987), *B. thuringiensis* toxins function by binding to receptors on midgut epithelium cells, inserting themselves into the plasma membrane and creating pores that result in cell leakage and possibly larval death. It might be possible that because of the high specifity the recombinant product of *cryIVA*, *cryIVD* and 20 kDa protein is not highly effective on the larvae of *G. dealbana* and *H. cunea*.
In the study, we also determined that HD-1 toxin (100 µg/ml) has the highest insecticidal effect on larvae of both *G. dealbana* and *H. cunea*, respectively, 31 and 80%. Moar *et al.* (1995) determined that HD1-toxin (4.83 µg/g of diet) causes 50% mortality against lesser cornstalk borer. Hwang (1998) also determined that insecticidal crystal proteins of *B. thrungiensis* var. *kurstaki* HD1 causes 95% mortality against *Bombyx mori*.
*Bacillus thuringiensis* toxins are generally specific, with activity limited to insects of one order. However, the HD-1 strain of *B. thuringiensis*, the strain used as most commercial products for lepidopteran insect control, also posses low but detectable levels of activity against dipterans. The HD-1 strain has a major protein fraction that is toxic to only, Lepidoptera and a minor protein fraction that has activity against both lepidopterans and dipterans (Yamamoto and McLaughlin, 1981). We think that various species has different resistance againts this toxin, because some insects have a sufficiently high pH in the midgut to solubilize the protein and release the toxin of *B. thuringiensis* (Deacon, 1983).
Biological control is more effective and safe than chemical control, and chemical control effect ecological equilibrium dramatically. Biological agents are used against pests in the world (Phlanzenschutz Nachrinhten Bayer Special Issue). It is very important to use more effective and safe control agents in pest control. In the end of this study we suggest that HD-1 toxin can be used against both *Gypsonoma dealbana* and *Hyphantria cunea*, and *Ac*NPV and *Ld*NPV can also used against *Hyphantria cunea*. However, it is necessary to improve the mode of action of these viruses.

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