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## Insecticidal Effects of Some Biological Agents on the Larvae of *Balaninus nucum* (Coleoptera; Curculionidae) and *Euproctis chrysorrhoea* (Lepidoptera; Lymantriidae)

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### Abstract

In the present study, in order to find a more effective and safe biological control agent against common pests of *Coryllus* sp, *Balaninus nucum* (Hazelnut beetle, Coleoptera) and *Euproctis chrysorrhoea* (Browntail moth, Lepidoptera), we tested the insecticidal effects of various biological agents. The highest insecticidal effects that we determined for each pest within 10 days are 90 percent using *Lymantria dispar* nuclear polyhidrosis virus (LdNPV, as gypchek) and toxin (BTS-1) isolated from tenebrionis strains of *B. thuringiensis* against *B. nucum* and 73.6 percent using LdNPV and 57.8 percent using toxin (HD-1) isolated from Harry Dumage strain of *B. thuringiensis* against *E. chrysorrhoea*. We think that the insecticidal effects of all agents are the results of cytotoxicity.

### Introduction

The main purpose of the agricultural studies is to increase the yield of product per hectare. Even though Turkey places on the top of the list among all the 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.

*Balaninus mucum* (Hazelnut beetle, Coleoptera) and *Euproctis chrysorrhoea* (Browntail moth, Lepidoptera) are common pest of *Coryllus* sp in Turkey while *B. mucum* damages on hazelnut fruit by feeding and laying (Martin, 1949; Ural *et al.*, 1973), *Euproctis chrysorrhoea* has a characteristics of population increases almost in 3 or 4 years, causes important damages on hazelnut leaves by feeding. Surprisingly, despite their mass occurrence and wide distribution, very little is known about the agents limiting their populations. 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 pest. The rapid widespread adoption of organic insecticides have been brought the new problems which are the destruction of the biotic agent pressure on pests, wile fife, human health and to others. For this reason, scientists consider finding more effective and sate control agents. However, a few authors have studied natural enemies of these insects (Demirbag and Yaman, 1999; Sezen and Demirbag, 1999).

In this study, in order to find a more effective and safe pesticide against *B. mucum* and *E. chrysorrhoea*, we tested the insecticidal effects of various biological agents on the larvae of these pests. The tested agents are *Autographa californica* nuclear polyhidrosis virus (AcNPV), *Lymantria dispar* nuclear polyhidrosis virus (LdNPV, as gypchek) and toxins isolated from Harry Dumage (HD-1) and tenebrionis strains (BTS-1) of *B. thuringiensis*.

### Materials and Methods

Virus samples: Two types of virus samples were used in bioassays: (a) *Lymantria dispar* nuclear polyhidrosis virus (LdNPV) was obtained from Edward M. Daugherty (USDA,

Beltsville, Maryland, 20705 USA) as ready used pesticide, gypchek. Two g of this sample was suspended in 5 ml of phosphate buffer solution (PBS) and used; (b) *Autographa californica* nuclear polyhidrosis virus (AcNPV, m.o.i. = 1) was produced in *Spocloptera frugiperda* (Sf IPLB-21) cells (Martens *et al.*, 1990). At 3 days post infection (occurrence of polyhedral inclusion bodies), the cells ( $3 \times 10^7$  cell/ml) were centrifuged at 3.000 rpm for 10 min. The pellet was re-suspended in 5 ml of sterilized PBS and used.

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

**Bioassays:** For this study, the larvae of *B. nucum* and *E. chrysorrhoea* were collected in the vicinity of Trabzon. The caught insects were taken from the gardens to the 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 hr with fresh leaves, exiles or fruits of hazelnut 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 the agent prepared in phosphate buffer solution (PBS) using sterilized syringe (Dulmage, 1981). Twenty larvae were placed on diet in each containers for each assay. After 48 hr, the larvae received fresh diet every 24 hr (Lipa and Wiland, 1972). Twenty control larvae received diet contaminated with PBS for the first 48 hr and then fresh diet every 24 hr. finally dead larvae were removed (Thiery and Frachon, 1997). Hundred larvae of each insect were tested for each agent. All larvae tested were kept at 26 ± 2 C and 60 per cent RH on a 12:12 hr photoperiod (Lipa and Wiland, 1972; Ben-Dov *et al.*, 1995). Dead larvae were removed immediately and bioassay checked daily till 10th day. Data were evaluated by using Abbot's formula.

### Results and Discussion

In this study, in order to find a more effective and safe

pesticide, we tested the insecticidal effects of four different biological agents on the larvae of *B. nuceum* and *E. chrysorrhoea*. The insecticidal effects determined on larvae of *B. nuceum* and *E. chrysorrhoea* respectively, are 90 and 73.6 percent with gypchek, 60 and 36.8 percent with AcNPV, 90 and 26.3 per cent with BTS-1 and 77.7 and 57.8 percent with HD-1 (Fig. 1).

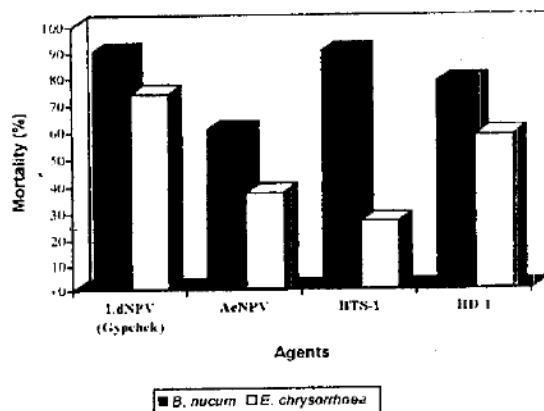


Fig. 1: The insecticidal effects of biological agents on *Balaninus nuceum* and *Euproctis chrysorrhoea*. LdNPV: *Lymantria dispar* nuclear polyhidrosis virus, as gypchek; AcNPV: *Autographa californica* nuclear polyhidrosis virus; BTS-1: toxin isolated from tenebrionis strain of *B. thuringiensis* and HD-1: toxin isolated from Harry Durnagae strain of *B. thuringiensis*

We determined that the larvae which followed during ten days, showed different signs before death. Generally, the larvae of *B. nuceum* displayed sluggish and appetite less. The only major symptom of infection is decreased larval life of both *B. nuceum* and *E. chrysorrhoea*. In all of the assays, the highest insecticidal effect determined on larvae of both *B. nuceum* and *E. chrysorrhoea* respectively are 90 and 73.6 percent with gypchek. The insecticidal effect of AcNPV is 36.8 percent on *E. chrysorrhoea* and 60 percent on *B. nuceum*. While the effect of BTS-1 is 90 per cent on *B. nuceum*, it is only 26.3 percent on *E. chrysorrhoea*. The effect of HD-1 toxin is 77.7 percent on *B. nuceum* and 57.8 percent on *E. chrysorrhoea*. Moar *et al.* (1995) determined that HD-1 toxin (4.83 pg/g of diet) causes 50 percent mortality against lesser cornstalk borer, while the control mortality was <20 percent determined. It is thought that various species has different resistance against this toxin because some insects have a sufficiently high pH in the mid-gut to solubilize the protein and release the toxin of *B. thuringiensis* (Deacon, 1983). According to previous studies, effect of each *Bacillus* strains are

different on various insect species (Esters, 1996). There is some evidence that the enzymes of different insects release different polypeptide from the proteins, so further specificity may arise in this way (Deacon, 1983).

As a result, it was determined that especially BTS-1 and gypchek can be used as biological control agents on *B. nuceum* larvae and that HD-1 gypchek can be used as biological control agents on *E. chrysorrhoea* larvae. The high speed of action and the higher rate of mortality are always desired. Therefore, further researches will be directed to start biological control against *B. nuceum* and *L. chrysorrhoea* using these pesticides or other newly developed pesticides.

## References

- Ben-Dov, E., S. Boussiba and A. Zaritsky, 1995. Mosquito larvicidal activity of *Escherichia coli* with combinations of genes from *Bacillus thuringiensis* subsp. israelensis. *J. Bacteriol.*, 177: 2851-2857.
- Deacon, J.W., 1983. Microbial Control of Plant Pests and Diseases. Van Nostrand Reinhold, Wokingham, Berkshire, England.
- Demirbag, Z. and M. Yaman, 1999. A newly recorded mermithid parasite of *Euproctis chrysorrhoea* L. (Lepidoptera: Lymantriidae). Proceedings of the 7th European Meeting in the IOBC/WPRS Working Group on Insect Pathogens and Insect Parasitic Nematodes, March 22-26, 1999, Vienna.
- Dulmage, H.T., 1981. Insecticidal Activity of Isolates of *Bacillus thuringiensis* and their Potential for Pest Control. In: Microbial Control of Insect Pests and Plant Diseases 1970-80, Burges, H.D. (Ed.). Academic Press, New York, London, pp: 193-222.
- Esters, M., 1996. Genetic engineering in agriculture, pflanzenchutz nachrichten bayer special issue. Crop Protection Business Group Public Affairs/Market Research, D-51368 Leverkusen, Bayerwerk, No. 49, pp: 47-56.
- Lipa, J.J. and E. Wiland, 1972. Bacteria isolated from cutworms and their infectivity to *Agrotis* sp. *Acta Microbiol. Polonica*, 4: 127-140.
- Martens, J.W.M., G. Honee, G. Zuidema, J.W.M. van Lents, B. Visser and J.M. Vlak, 1990. Insecticidal activity of a bacterial crystal protein expressed by a recombinant baculovirus in insect cells. *Applied Environm. Microbiol.*, 6: 2764-2770.
- Martin, H., 1949. Contribution à l'étude des balanin des noisettes (*Balaninus nuceum* L.). *Rev. Path. Veg. Ent. Agric. France*, 28: 3-28.
- Moar, W.J., M. Pusztai-Carey and T.P. Mack, 1995. Toxicity of purified proteins and the HD-1 strain from *Bacillus thuringiensis* against Lesser Cornstalk Borer (Lepidoptera: Pyralidae). *J. Econ. Entomol.*, 88: 606-609.
- Sezen, K. and Z. Demirbag, 1999. Isolation and insecticidal activity of some bacteria from hazelnut beetle (*Balaninus nuceum* L.). *Applied Entomol. Zool.*, 34: 85-89.
- Thiery, I. and E. Frachon, 1997. Identification, Isolation, Culture and Preservation of Entomopathogenic Bacteria. In: Manual of Techniques in Insect Pathology, Lacey, L.A. (Ed.), Academic Press, London.
- Ural, I., M. Isk and A. Kurt, 1973. The some investigation on insects established in hazelnut gardens in east black sea region. *The Bulletin of Plant Protection, Ankara, Turkey*, No. 13, pp: 55-56.