

Efficacy evaluation of *Heterorhabditis bacteriophora* against click beetle (Coleoptera: Elateridae)

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Abstract: The efficacy of the entomopathogenic nematode *Heterorhabditis bacteriophora* was evaluated in the control of click beetles (Coleoptera: Elateridae). The species of harmful insects, responsible for the damage, are *Agriotes lineatus*, *A. obscurus*, *A. sputator* and *A. gurgistanus* in many European regions. The efficiency of *H. bacteriophora* was tested against different stages of click beetle of the species *A. gurgistanus*-wireworms, pupae and adults in the laboratory. *H. bacteriophora* with 100% mortality has been shown to be pathogenic towards wireworms. We also evaluated the effect of nematode infection on wireworm's organism and studied histological changes of the fatty tissues.

Key words: Click beetles, *Agriotes gurgistanus*, Steinernematidae, Heterorhabditidae, wireworm, biological control

Introduction

Click beetles (Coleoptera: Elateridae) are one of the most common harmful agricultural insect pests. Wireworms, the subterranean larval stage of click beetles are major pests of arable crops including potatoes in many parts of the world (Jansson & Seal, 1994). A single larva can successively destroy several plants. Adult wireworms, or click beetles, are hard-shelled and cause no crop damage at this stage of their life cycle. The biological agents, entomopathogenic nematodes of the Steinernematidae and Heterorhabditidae families are pathogenic for a range of pests belonging to the orders Lepidoptera and Coleoptera (Poinnier *et al.*, 2007). These nematodes are symbiotically associated with entomopathogenic bacteria *Photorhabdus* (Boemare *et al.*, 1993) and *Xenorhabdus*.

The aim of the paper is to determine infectivity of entomopathogenic nematode of *H. bacteriophora* against click beetle – *Agriotes gurgistanus* in different stages – wireworms, pupae and adults under laboratory conduction.

Material and methods

All insects were collected between April and May in 2013, in the field, near the village Saguramo (central part of Georgia) using soil cores (10 cm deep, 10 cm wide) and bait traps (Simmons *et al.*, 1998). *H. bacteriophora* were reared at 25 °C in last instar larvae of the wax moth, *Galleria mellonella*, according to the procedures described by Woodring and Kaya (1998). The infective juveniles (IJs) that emerged from cadavers were recovered using modified White traps (Kaya and Stock, 1997) and stored at 7 °C for 7-14 days before use.

Ten insects in the stage of wireworms, pupae and adults were individually placed on a wet filter paper in 10-cm Petri dishes. Suspension of 1000 IJs/ml water (i.e. dose 100 IJs per insect), were sprayed in each Petri dishes. Mortality was assessed after 5 and 7 days and presence of nematodes inside the insects was checked as indicator of nematode infection. Each treatment was replicated four times and included untreated control dishes. To evaluate the effect of EPN infection in wireworm's organism, histological examination was performed.

To study histological changes in the fatty tissues of wireworm's they were fixed 2, 4, 8, 24, 48 hours after treatment. Larvae were cut on 3 parts – front, middle and back, fixed in 10% formalin, then dehydrated in alcohols of increased concentrations and embedded into the paraffin according to the histological methods, sections with the width of 5-6 μm were dyed with hematoxylin-eosin according to Erlich and by Mallory three-colored method (Pease, 1964).

Experiments were carried out under laboratory conditions at temperature 22 ± 2 °C and 80% RH.

Results and discussion

Mortality rates of insects are presented in Figure 1. Within 5 and 7 days exposure, respectively, 92-100%, 82-88% and 52-60% of insects were infected by *H. bacteriophora*. The mortality of wireworms was highest.

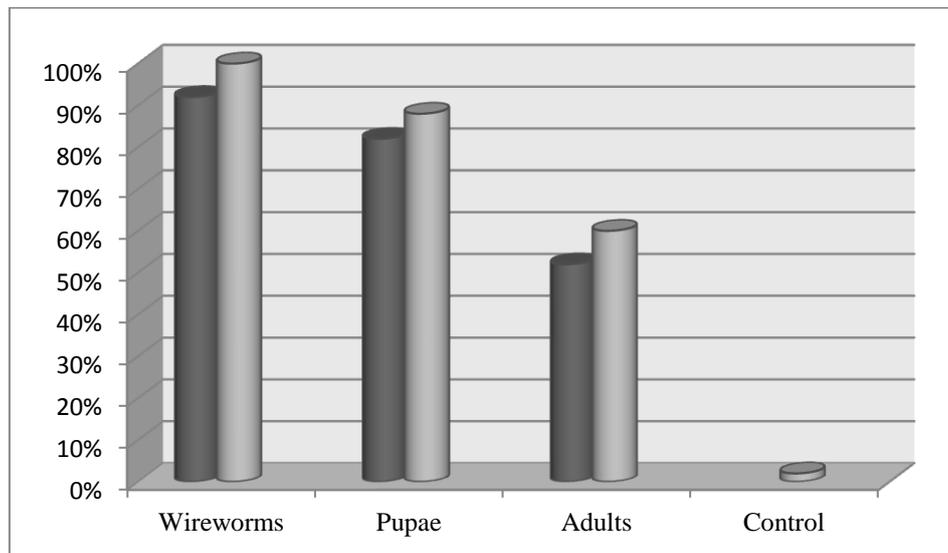


Figure 1. Percentage mortality of different instars of click beetles – wireworms, pupae, and adults – caused by *H. bacteriophora*.

After dissecting of insects was observed destruction of different degree, at the first stage the fat-body preserved its consistence, at the following stage coloring of insects didn't change though under thin cover the nematodes were clearly seen. On the 7th day after the infection, wireworm's intestines were almost destroyed by *H. bacteriophora*, haemocoel was full of different stage of entomopathogenic nematodes. In the fat-body of wireworms IJs were observed as well. In some still alive, but the infected pupae and beetles were observed some

dead IJs of nematodes. All survived insects slightly reacted to irritation, though in their bodies the nematodes weren't seen. In control tests mortality of insects was insignificant (2%).

2 hours after spraying with suspension of *H. bacteriophora*, bacteria are already observed in the cells of the fat-body. The moment of penetration of the bacteria into the fat-body cell through the cell membrane is clearly seen on Figure 2A 4 hours after treatment, appearance of granulation, decrease of fat drops, and abrasion of boundaries between cells, their vacuolization, and dissection of earlier compact fat-body into the separate bands are registered in fat-body cells. Dissection and vacuolization of cells, abrasion of boundaries between cells and also penetration of bacteria into the intestine cells are observed in the cells of the front part of intestinal canal (Figure 2B).

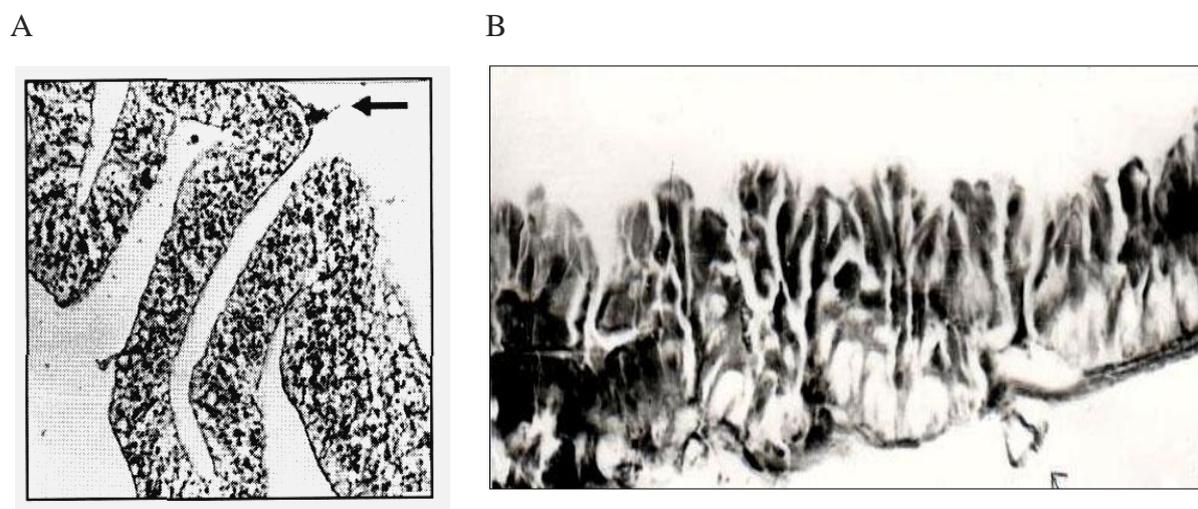


Figure 2. A: The moment of penetration of bacterial cell through the membrane into the fat-body cell of larva (2 hours after spraying with EPN suspension). B: Vacuolization of digestive canal, penetration of bacteria into the intestine (4 hours after spraying with EPN suspension).

Close to the fat-body the increase of the number of formed elements of haemolymph is seen, among which the majority are micro- and macronucleocytes, some of them are cells with pyknosis nucleus and a few dead cells (Figure 3A). Darker cells with oval, dark nucleus are distinguished among cells covering middle part of intestinal canal. Cells by enhanced basal end are attached to the basal membrane, and by apical and also enhanced end directed towards intestine cavity (Figure 3B). Vacuolization of cytoplasm, formation of the nuclear secretion, cell fragmentation are also recorded in the glandular cells. Around the glands aggregation of haemolymph cells arranged as fibers is observed, among which pyknosis and dead cells are revealed. Pathological changes of cells and tissues of wireworm become more significant after 8 hours. As a result of enhancement of bacterial intoxication, nucleus pyknosis and destruction of cellular structure of fat-body during 24 hours were observed. Intestine tissue undergoes destruction: cells are greatly vacuolated, cell borders are nearly revealed. Aggregations formed elements of haemolymph between organs are observed. Numerous pathological changes and dead macronucleocytes and micronucleocytes are revealed as well (Figure 3C). 48 hours post treatment high mortality of wireworms is observed (92%). Utter degradation of fat-body cells and disappearance of fat drops occur. In situ of fat-body only weak granulation is registered.

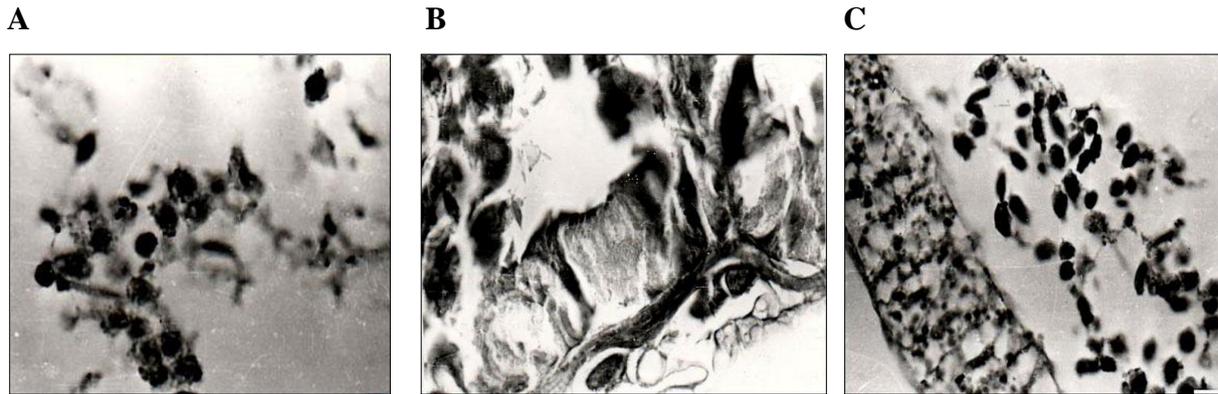


Figure 3. A: Haemolymph cells. B: Intestine cells. C: Destruction of fat-body cells.

The first responding defense system of larva is fat-body, with its nutrient reserve providing normal vital activity of organism. Increase of the number of formed elements of haemolymph 2 hours after treating, argues that insects have well-defined defense reaction.

Similar phenomenon was observed while studying the effect of pathogenic fungi *Beauveria bassiana* on the internals of European spruce bark beetle (*Ips typographus*), and also at many bacterial infections in other insects (Tskhadaia, 1996; Kvinikhidze, 1988; Weiser, 1966).

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