Insect pathogens and entomopathogenic nematodes

IOBC/wprs Bulletin Vol. 66, 2011

pp. 333-335

Biocontrol of leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae) using entomopathogenic nematodes in Iran

Mahbobeh Ashtari[1], Javad Karimi[2], Mohammad Reza Rezapanah[3], and Mahnaz Hassani-kakhki[2]

[1]Azad University of Arak, Arak, Iran; [2]Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran; [3]Iranian Research Institute of Plant Protection, Tehran, Iran

Abstract: Walnuts produced in the Juglans region is among Iran's strategic products and the Leopard moth, *Zeuzera pyrina* L. (Lep.: Cossidae) is the key pest of this tree. Difficulty in chemical application against this caterpillar and special habitats of its larvae encouraged us to address efficiency of some entomopathogenic nematodes (EPNs) against different larval stages. Native isolates of EPNs were isolated from soil habitats of this pest in Arak, Markazi province, Iran. Using ITS and D2/D3 expansions of 28S sequences they were identified as *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *S. carpocapsae*. In laboratory assays,the susceptibility of second, third and fourth larval stages to commercial products of *S. carpocapsae* and *H. bacteriophora*, and native strain of *H. bacteriophora* were studied. For field application, both strains were used via injection of nematode suspensions into the galleries bored in tree stems or branches. This study showed that both tested nematodes at 2,000 IJs/larva proved to be effective on *Z. pyrina*. The results indicated the higher efficiency of *S. carpocapsae* as compared to *H. bacteriophora* on larvae of this moth.

Key word: Insect pathogen, *Heterorhabditis*, *Steinernema*, Iran, microbial control, tiger moth

Introduction

Walnut, Juglans regia L., is believed to have originated from Iran and the production areas it is around 60,000 hectares that allocates about 11.5 percent of the global production (Amirghasemi, 2006). Key pest of this strategic tree is *Zeuzera pyrina* L. (Lep.: Cossidae), a polyphagous caterpillar that infests a large number of shrubs and tree species as well as walnut trees. Neonate larvae bore into the tips of branches and shoots and then attack the larger branches and the trunk, where they form ascending galleries under the bark, later in the wood (Langström et al., 2004). The tree becomes weak, thereby some secondary pests such as bark beetles also can attack the tree (Rajabi, 1987). Chemical applications against this cossid are less suscessful due to the cryptic habitat of the larvae inside the branches and the long oviposition time of adults (Shamseldean et al., 2009). This encouraged us to address the efficiency of entomopathogenic nematodes against different larval stages. Studies on EPNs started during this decade in Iran. Several native isolates were characterized, but information about their biocontrol potential in the field is rare. Currently, *Z. pyrina* is considered a big challenge for walnut production in the country, and several institutes work on the

development of IPM plans for the control of this caterpillar. We assume that native isolates of EPNs may provide better control of this cossid than strains less well adapted to climatic conditions of Iran.

Material and methods

Insect

Larval instars (second, third and fourth) were collected from heavily infested walnut trees in the Arak region, during May until August 2010. EPN strains Three native strains of *Sterinernema* and *Heterorhabditis* were isolated from soil orchards of Walnut tree in the study area during 2010. The isolated EPNs were reared following Kaya and Stock's (1997) methodology and characterized using ITS and D2/D3 expansions of 28S rDNA. The analyisis assigned the isolates to the species *H. bacteriophora*, *S. carpocapsae* and *S. feltiae*. Two additional strains of *H. bacteriophora* and *S. carpocapsae* were provided by Koppert Biological Systems (Berkel en Rodenrijs, NL).

Laboratory experiment

In a laboratory study, virulence of EPNs were evaluated by exposing larval instars to 2,000 IJs/larva with 1.5ml of sterile distilled water in each Petri dish. There were six replicates with five larvae per replicate. To the control treatment, 1.5ml of sterile distilled water without nematodes was added. For five days, the number of infested larva of *Z. pyrina* was determined due to color change and IJs emergence after transferring the cadavers to white trap.

Field experiment

Field experiments were conducted in a walnut orchard in July until November 2010. Infected trees were treated with *S. carpocapsae* and *H. bacteriophora* provided by Koppert and the indigenous strain Arak 2 of *H. bacteriophora*. EPN suspensions were injected into the insect galleries after sunset. The nematode concentrations were 2,000 IJs per active hole. For injection of EPNs, suspensions were applied using a 60 cc plastic syringe. To prevent death of IJs due to UV light and desiccation, half of the treated trunks were covered with a green plastic cover. To assess the effect of the coverage the other tree trunks were left without cover, There were six treatments and each experimental unit was a single trunk. Treatments were replicated five times with six trunk/tree per replication. After 10 days, larvae of *Z. pyrina* were excerpted from the galleries using a wire, and the number of alive and dead larva was determined. Statistical significance was determined by analysis of variance and Dunnet's Multiple Range Test, at $P<0.05$.The results of each experiment were corrected for the control mortality or the number of emerging leopard moth in the control, according to Abbott's formula (Abbott, 1925)

Result and discussion

In laboratory tests, *S. carpocapsae* caused 100% mortality in 2^{nd}, 3^{rd}, and 4^{th} instars larvae 54, 30 and 36 hours after treatment, respectively. *H. bacteriophora* caused 100% mortality in 2nd, 3rd and 4th instars larvae 44, 40 and 52 hours after treatment, respectively. In field experiment, mortality in treatments with *S. carpocapsae* under plastic cover and without cover was 100% and 63%, respectively. Treatments with *H. bacteriophora* caused no significant mortality whether used with or without cover. There was a significant difference between *S. carpocapsae* under plastic cover comparing other treatment (at 5% level). Thus *S.*

carpocapsae was more effective against larvae of *Z. pyrina* than *H. bacteriophora* under conditions in the orchard. Deseö et al. (1984) in Italy achieved similar results and showed low virulence of *Heterorhabditis* species against leopard moth. Abdel-Kawy et al. (1992) found that *S. carpocapsae* was the more virulent pathogen on leopard moth larvae, followed by *H. heliothidis* and *H. bacteriophora* (31 to 88% mortality).
In a field experiment dealing with *Z. pyrina*, injection of nematode suspensions with 3,000 and 5,000 IJs of *H. bacteriophora* and *S. riobrave* caused high larval mortality (>90%) (Shamseldean et al., 2009). However, in another study, Shamseldean (2000) indicated that after injection of 1,000 IJs of *H. bacteriophora* and H. indica, larval mortality reached 98% but a low efficiency was recorded for *S. abbasi* and *S. riobravae*. Results of our work show that *S. carpocapsae* is a suitable candidate for controlling *Z. pyrina* in walnut orchards. This study provides the first characterization of entomopathogenic nematodes and their efficiency from the Arak province of Iran that can be useful in future works.

References

Abbott, W. S. 1925: A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Abdel-Kawy, A. G. M., El-Bishry, M. H. & El-Kifl, T. A. H. 1992: Controlling the leopard moth borer, *Zeuzera pyrina* by three entomopathogenic nematode species in the field. Bulletin of the Faculty of Agriculture, Cairo University 43: 769-780.

Amirghasemi, M. 2006: Identification of Walnut and its properties. Yas publication, Tehran, 100 pp.

Deseö, K. V., Fosch, F. & Rovesti, L. 1984: Results of biological control experiments in apple and pear orchards against the larvae of *Zeuzera pyrina*. SIP XVll Annual Meeting, UC Davis, U.S.A Aug. poster N "17F".

Kaya, H. K. & Stock, S. P. 1997: Techniques in insect nematology In L. A. Lacey (ed.): Manual of Techniques in Insect Pathology. Academic, San Diego, CA.

Langström, B., Heliövaara, K., Moraal, L. G., Turcani, M. Viitasaari, M. & Ylioja, T. 2004: Non-Coleopteran Insects. In: Bark and Wood Boring Insects in Living Trees in Europe, A Synthesis, eds. Lieutier, F., Day, K. R., Battisti, A., Grégoire, J.-C. & H. F. Evans: 501-538.

Rajabi , G. 1987: Destructive insect pests of trees in cold regions of Iran .Publication by the Research Institute of Plant Pest and Diseases , 209 pp.

Shamseldean, M. M., Hasanain, S. A. & Rezk, M. Z. A. 2009: Virulence of entomopathogenic nematodes against lepidopterous pests of horticultural crops in Egypt. Proceedings of the 4th conference on recent technologies in Agriculture "Challenges of Agriculture Modernization" Vol. 1, pp. 74-84.

Shamseldean, M. M. 2000: Laboratory bioassays and field application of entomopathogenic nematode isolates and species belonging to the genera *Heterorhabditis* and *Steinernema* against the leopard moth *Zeuzera pyrina*. J. Agric. Sci. Mansoura Univ. 25: 8151-8161.