control of box tree moth – *Cydalima perspectalis* (Walker, 1859) in laboratory conditions

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Cydalima perspectalis or the box tree moth is a pest of Buxus trees. It originates from South East Asia and recently has been introduced in Europe, and in 2014 in Bosnia and Herzegovina. It has quickly spread over the country and started to make devastating damage to this ornamental plant. Buxus ornamentals are usually grown in private gardens or in public parks. Its damaging potential requires implementation of control measures at regular basis. In this study four species of entomopathogenic nematode *Steinernema feltiae*, *S. carpocapsae S. kraussei* and *Heterorhabditis bacteriophora* local strains were used. Caterpillars of the box tree moths were collected from naturally infested trees during April and May and treated with nematodes in laboratory assay. Four concentrations of nematodes 250, 500, 1000, and 2000 IJ were applied ageist 10 larvae in Petri dishes (diameter 9.5 cm) at room temperature. Mortality was assessed 24 hours and 5 days after the nematode application. At the highest concentration of IJ *S. kraussei* showed 100%mortality after 24 h, while other nematodes this efficacy expressed in observation 5 days after the application.

POSTER SESSION. Wednesday, 16:30 PN-16

Management of black vine weevil (*Otiorhynchus sulcatus*) by entomopathogenic nematodes in Georgia

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The aim of this study was to determine the biological control effect of entomopathogenic nematodes species, *Steinernema carpocapsae* and *Steinernema feltiae* against of *Otiorhynchus sulcatus* Adult weevils can be controlled by using nematodes as biocontrol. Also grubs can be controlled using the fungus *Beauveria bassiana*. Experiments was evaluated under laboratory conditions. Various laboratory bioassays were conducted to determine the effectivity of entomopathogenic nematodes to control *Otiorhynchus sulcatus*. Adults of *O. sulcatus* were screened for susceptibility to two introduced from Israel nematode species. *Otiorhynchus sulcatus* was found to be most susceptible to *S. carpocapsae* and *S. feltiae*, causing mortality 94, 88, 89% and 97,94, 89% on the temperature 25°C and 1000 IJs/ml cm² concentration, respectively. Larvae of *O. sulcatus* was controlled using the fungus *B. bassiana*. Further bioassays illustrated a linear relationship between black vine weevil, mortality and the concentration of nematodes applied, with the highest level of control using a concentration of 1000, 1500 infective juveniles (Ijs)/insect. *Steinernema carpocapsae* proved able to locate and infect black vine weevil, quicker, than *S. feltiae*. For all nematode species, the highest virulence was observed 48,56,88% and 59,45, 78% on the temperature 25°C and 1500 IJs/ml cm² concentration for *S. carpocapsae*, and *S. feltiae*, respectively. Fungal isolate *B. bassiana* imposed more than 50% larval mortality of *O. sulcatus*. In conclusion, it was determined that *O. sulcatus*can be controlled by *S. carpocapsae*, *S. feltiae* and fungus *B. bassiana*, but further studies should be conducted at field conditions.

POSTER SESSION. Wednesday, 16:30 PN-17

Molecular and phenotypic characterization two strains of *Photorhabdus luminescens* associated with Iraqi *Heterorhabditis bacteriophora*

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The bacterial symbionts IRO.1 and IRO.2 were isolated from populations of the insect pathogenic nematode *Heterorhabditis bacteriophora* collected from Iraq. These bacteria were symbiotically associated with entomopathogenic nematodes of genus *Heterorhabditis*, contributing actively to the biological cycle of their host. The Heterorhabditidae family of nematodes involves of obligate insect pathogens. Both the nematode and bacteria work together to overcome the immune response of target insect. The bacteria were isolated from crushed number of infective juveniles. On the indicator NBTA plates, characteristic blue colonies of *Photorhabdus* were developed slowly, then, the colony was picked from isolation plates only after 48 h. This study was based on phylogenetic analysis of sequence data of two genes: 16S rRNA and gyrB. The bacteria were also characterized phenotypically by biochemical and physiological tests. Our results have shown that the *Photorhabdus* strains isolated from *H. bacteriophora* belong to *Photorhabdus luminescens* subsp. *akhurstii*. This is indeed the case for the strain examined in this study which has been isolated from the recently described *H. bacteriophora* from Iraqi soil.

POSTER SESSION. Wednesday, 16:30 PN-18

Comparison of entomopathogenic nematode and insecticide management of western corn rootworm larvae

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The Western Corn Rootworm (WCR), *Diabrotica virgifera virgifera* LeConte, 1868, [Coleoptera, Chrysomelidae], whose larvae cause damage to maize roots, is an important economic insect pest in America and Europe. Its larvae are usually controlled by granular soil insecticides or insecticide-treated seeds. Biological control options, such as entomopathogenic nematodes (EPN), may provide an alternative management option. In a three year field experiment we compared the effectiveness of inundative biological control on the basis of EPN *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae; product Dianem) and the chemical insecticides Force 1.5 g (active substance tefluthrin, pyrethroid) and Sonido (a.s. thiacloprid, neonicotinoid). Additionally, a soil conditioner (a.s. alcohol ethoxylate, product Transformer) was used with the EPN, to check for potential increase of EPN effectiveness. Treatment efficacy was evaluated by counting the emerged beetles in the experimental plots using field cages. Two experiments were performed, one in eastern (Prekmurje) and the other in northern (Gorenjska) Slovenia. The efficacy of the treatments was very similar at both locations, despite the approximately 5-fold lower WCR population in Gorenjska compared to Prekmurje, as well as consistent over time. The highest number of WCR beetles was caught in the negative control, followed by the treatment Sonido (insignificant decrease). Treatments Force, Dianem with and Dianem without Transformer significantly decreased the number of emerged beetles and were statistically indistinguishable. WCR larvae control in maize using entomopathogenic nematode *Heterorhabditis bacteriophora* was comparable to conventionally used chemical control and could thus provide a sustainable WCR biological control management option.

POSTER SESSION. Wednesday, 16:30 PN-19 STU

The earthworm mucus and their feeding activity can decrease the biological control action by entomopathogenic nematodes and entomopathogenic fungi

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Molecular and phenotypic characterizations of *Photorhabdus luminescens* from Iraq

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Background

- *Photorhabdus* species are mutualistic bacteria associated with entomopathogenic nematodes related to genus *Heterorhabditis*.
- They complete their life cycle in intestinal lumen of infective juvenile nematodes (IJs) and hemolymph of an infected insect
- IJs penetrate suitable insect, they realize the bacteria which kill the host within 48 hours (Goodrich and Clarke, 2007).
- Currently, there are three main species that related to genus of Photorhabdus: *P. luminescens*, *P. temperata* and *P. asymbiotica*.

Research Questions

- First, bacterial species were identified by molecular taxonomy; followed by sub-species level identification
- Second, biochemical characterizations for these isolates were considered for Photorhabdus luminescens IRQ.b1,b2 associated with the entomopathogenic nematodes *Heterorhabditis bacteriophora* IRQ.1.

Materials and Methods

Bacterial strains isolation:

- Direct approach:100 -150 of new emerged infective juvenile nematode (IJs) were collected, sterilized and crushed. (NBTA medium used).
- Indirect approach (drop hemolymph):hemolymph of infected larvae (20–35 h), sterilized with 70% (v/v) ethanol, washed by 2% (v/v) sodium hypochlorite and deionized water.

DNA extraction, PCR, and DNA sequencing:

- Single colony from pure culture of bacteria was transferred to 5ml of liquid LB (Luria–Bertani broth) medium.
- The LB tubes with bacteria were placed on shaker with 100± 3rmp and 28±1°C of temperature for 48 hr.
- Two approaches were conducted: Trazol (Sigma) technique and DNA was extracted using the DNeasy Tissue Kit (Qiagen).
- Two genes of bacteria were investigated, 16S rRNA (Fischer et al, 1999) and gyrB (Yamamoto & Harayama, 1995).

Bacteria Phenotypic Characterization:

- Mac-Conkey, NBTA and NA to record the ability of bacterial isolates to absorb dye after 24-48 h.
- Bioluminescence, movement, Phospholipolytic, protease, catalase and other activities were tested

Light and electronic microscope: Bacterial were checked based on shape, size and color using light and scanning microscopes (B,C).

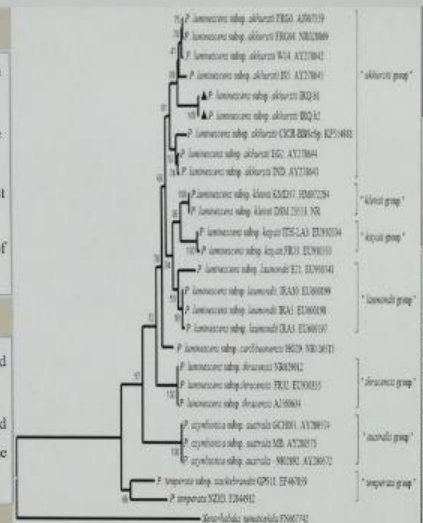


Fig.1: Neighbour-joining tree inferred from 16S rRNA gene sequences showing the phylogenetic relationships of two bacteria isolated from Iraqi *H. bacteriophora* within the genus *Photorhabdus*

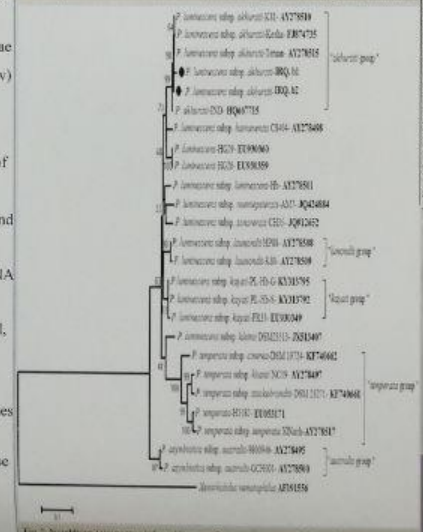
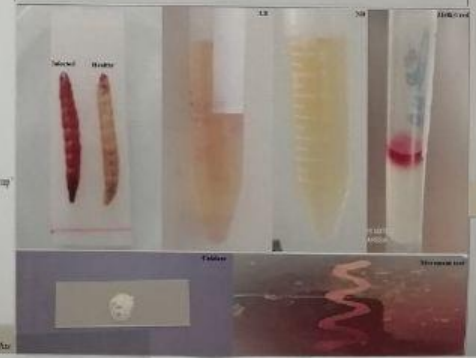


Fig.2: Neighbour-joining tree inferred from *gyrB* gene sequences showing the phylogenetic relationships of two bacteria isolated from Iraqi *H. bacteriophora* within the genus *Photorhabdus*

Biochemical characteristics	IRQ.b1	IRQ.b2	*P. luminescens akhurstii* (Published)
Gram staining	-	-	-
Bromothymol blue from	+ (Ggreen)	+ (Ggreen)	+ (Ggreen)
NBTA [pigmentation]			
Neutral red from Mac	+ (Red)	+ (Red)	+ (Red)
Conkey agar [pigmentation]			
Pigmentation (cultural agar)	+ (yellow)	+ (yellow)	+ (yellow)
SWC medium (sea water complexed)	+	+	+
Motility	+	+	-
Ampicillin resistance	+	+	-
Catalase	+	+	-
Cytochrome oxidase	-	+	+
Citrate utilization	+	+	-
Voges Proskauer test	-	-	-
Methal red test	-	+	+
Hydrolysis of gelatin	-	-	-
Indole production test	+	+	-
Starch Hydrolysis	-	-	-
Amylase production test	-	-	-
Cell shape	rod	rod	rod
Cell length (µm)	2.7±1.2	3.1±1.1	4.0±1.2
Cell width (µm)	1.1±0.2	0.9±0.1	1.3±0.2
Urease	+	+	+
Proteolysis: casein	-	-	-
Gelatin			
Lecithinase	-	-	-
Hemolysis type	β-hemolysis	β-hemolysis	β-hemolysis
Arginine dihydrolase	-	-	-
DNase	+	+	+
Phospholipase	-	-	-
Amylase production test	-	-	-
Maximum temperature for growth (°C) [Luria-Bertani broth]	40	40	37-40



Results and discussion

Phylogenetic analyses:

- The lengths of 16S rDNA gene for the tow Iraqi bacterial isolates (b1 and b2) were 1537 and 1549 bp respectively.
- The BLAST analysis on the basis of the 16S rDNA and *gyrB* sequences that 97% similarity and 98% of query coverage with *P. luminescens* subsp. *akhurstii* (AY278645).
- The main inter-specific distance of 16S rDNA sequences was 0.033% (range 0.01-0.15), which was calculated using Tamura 3-parameter model.

Phenotypic characterization:

- The two isolates of this study had ability to produce pigments and these isolates were green to dark green in color.
- Bacteria have two phases, phase I was round and glossy, while phase II of both colonies was mucoid.
- Bioluminescence was observed visually under dark condition after 30-35 h from inoculation
- Both isolates were gram negative and the colonies showed catalase activity when were examined using hydrogen peroxide, and they were rod shape
- These colonies of bacteria were green to dark green on NBTA plates, light yellow on NA plates and red on McConkey plates

Conclusion

- The molecular and phenotypic characterizations study of mutualistic bacteria of Iraqi EPNs allows us to increase our knowledge about two bacterial isolates associated with H. bacteriophora species
- These bacteria could be used widely to multiply of the mutualistic Iraqi EPNs as a main source for these nematodes and as a part of biological control program against insect pests.

Acknowledgment

The information of this poster is a portion of a Ph.D. thesis by the first author. The authors thank the Ministry of science and technology and University of Ferdowsi Mashhad for financial support this own initiative project.