

**POTENTIAL OF ENTOMOPATHOGENIC NEMATODES APPLICATION AGAINST  
*LIRIOMYZA HUIDOBRENSIS BLANCHARD IN LEBANON***

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**Abstract**

*Liriomyza huidobrensis* Blanchard, the pea leafminer, started to be threaten for vegetable products in Lebanon since the beginning of 1990s. Due to the wide range of insecticide resistance, the control of *L. huidobrensis* by chemicals remains a great challenge and it is important, therefore, to find a friendly environmental control programme against this pest. This study addresses the potential of applying a biological control agent, entomopathogenic nematode against *L. huidobrensis* *in vitro*. Entomopathogenic nematodes (EPNs) are parasites of soil-dwelling insects that occur in natural and agricultural soils around the world. Thanks to their entomotoxicity, EPNs are good tools for biological control in agriculture almost everywhere in the world.

In the current study, one indigenous strain of EPNs, *Heterorhabditis indica*, was sampled on the coastal area in Lebanon and tested against *L. huidobrensis* pupae *in vitro*. Assays consisted of placing Petri dishes containing sterilized soil and entomopathogenic nematode solution in contact with the pupae of the pea leafminer. While previous studies used larval stages, in the current study, pathogenicity of EPNs is tested *in vitro* against *L. huidobrensis* pupae stage for the first time. Out of 150 pupae used during the experiment, 16 ±1.5 % of the pupae emerged into adults of *L. huidobrensis* and 21±2.5% of the pupae were parasited by another *Liriomyza* natural pathogene - *Diglyphus isaea* Walker. Results showed the mortality of 53±1.5% for the *L. huidobrensis* pupae following the application of entomopathogenic nematodes without any emergence of infestive juveniles nematodes, one month following the infestation. The control tests showed that percentage of emergence from pupae were 79±2%. Comparison with the control tests indicates that 53±1.5% of the *L. huidobrensis* pupae are potentially parasited by *H. indica*. The indigenous strain in Lebanon, *H. indica* can therefore be considered as potential agent in biological control regarding its capability to cause pupae mortality *in vitro* and being isolated in favorable environmental conditions to the presence of *L. huidobrensis* pupae which could prevent field trial failures in further studies.

**Keywords:** *Liriomyza huidobrensis*, biological control, Lebanon, entomopathogenic nematodes, *Heterorhabditis indica*

**Introduction**

*Liriomyza huidobrensis* (Blanchard, 1926), a pea leafminer, originated in South America that has spread to other continents with significance damages to agriculture in recent years (Scheffer et al., 2001). Considered as a highly polyphagous leafminer, it is capable of inflicting severe damage to crop and ornamental host plants including field and glasshouse grown vegetables and flowers. Cited for the first time in California in 1945 as a pest of peas and spinach (Lange, 1945), the invasion of *Liriomyza huidobrensis* started at the end of the 80's in England, France, Belgium and the Netherlands (Sunderland et al., 1992). The species reached the Middle East countries in the beginning of 1990s (Weintraub, 1995). Local farmers in Lebanon noticed first outbreak of leafminers' on gebara plants and the attack of a series of crops included leafy vegetables, many greenhouse and field crops such as cucumber and beans. Within the order of Diptera, *Liriomyza*

*huidobrensis* belongs to the family Agromyzidae. The genus *Liriomyza* was discovered in 1894 with more than 300 species recorded (Parrella, 1987). Within this genus, 23 species are considered economically-important vegetables and ornamental plants in both, glasshouses and outdoors due to their leafmining activity. *Liriomyza huidobrensis* adults are small flies (1.3-2.3 mm length) and their flight range is limited. Under laboratory conditions, a female lays about 100 eggs in total (Hincapié *et al.*, 1993). Eggs are deposited within leaf tissue (CABI, 2004; Weintraub and Horowitz, 1995) between the 4th and 10th day of the adult life (Parrella, 1984). Larvae starts feeding on the spongy mesophyll of the leaf immediately after hatching. Three larval instars develop in the leaf. Full grown larvae make an exit hole in the leaf surface through which it emerges to pupate on the soil surface. There is also a fourth larval stage (prepupae) between the puparium formation and actual pupation (Fig. 1). Pupae varies in colour from light brown to almost black, but all instars are combined and referred to “larvae duration” because of the difficulty in separating larval instars.

The duration of the pupal stage varies with temperature but at least 50% of the total development time of a *L. huidobrensis* individuals is spent in this stage. The life cycle can be completed in 16-43 days at 25 and 15 °C, respectively (Lanzoni *et al.*, 2002). Although *L. huidobrensis* is endemic to warm climates, it has demonstrated the ability to survive cold temperatures and extend its range through supercooling (Chen and Kang, 2004).

Fig. 5. Lifecycle of leafminer (*Liriomyza* spp.) (Enkegaard, 1990)

*Liriomyza* spp. can impact crops in at least six ways: (1) by transmission bacterial and fungal diseases (2) by destroying young seedlings, (3) by causing reduction in field crops, (4) by causing leaf drop above developing fruits (‘‘sunburning’’ of the fruit), (5) by reducing the aesthetic value of ornamental plants and (6) by causing some plant series to be quarantined (Parrella, 1987). The most serious damage is caused by larval feeding. The mining activity of the larvae can reduce the photosynthetic capacity of the plant. Heavy infestation causes desiccation and premature fall of leaves. On the other hand, feeding punctures made by the adult females can also be invaded by fungi and bacteria (Price and Hardbaugh, 1981).

*Liriomyza huidobrensis* is a highly polyphagous species and feeds on a large number of flowers, vegetables and weeds (Weintraub and Horowitz, 1995). It attacks different plant families such as Cucurbitaceae (cucumber, melon), Chenopodiaceae (spinach), Solanaceae (pepper, tomato, eggplant), Violaceae (*Viola* spp.).

Due to the wide range of insecticide resistance, the control of *L. huidobrensis* by chemicals remains a great challenge especially moreover because it is difficult to implement biological control for this pest where it is not indigenous (Jayaraj and Rabindra, 1992). With the increasing awareness of society's concern about pesticide residues in food and the desire for a healthy and aesthetic environment, the use of biorational insecticides for the control of the leafminer is a primary factor in its management.

Entomopathogenic nematodes (EPNs) are known for their potential to attach to the insect cuticle and lodge in their intestine a symbiotic bacteria essential for parasitic success. *Steinernema* and *Heterorhabditis* species are the only insect-parasitic nematodes genera that possess biological control attributes. The complex EPNs-bacteria penetrate through natural insect openings to the hemocele, release the symbiotic bacterial cells that multiply and cause the insect death within 48 h. Since these nematode-bacteria complexes are highly virulent to insects, they are considered as one of the best non-chemical insect pest control alternatives. This characteristic is largely exploited for biological control of insect pests in natural and agricultural soils around the world (Hominick, 2002). Many different insect pests are susceptible to infection by these entomopathogenic nematodes, yet no adverse effects have been shown against beneficial insects or other non targets in field studies (Georgis and Gaugler, 1991; Akhurst and Smith, 2002).

In an attempt to find an environmentally safe control measure against *L. huidobrensis*, in Lebanon the main objective of the present research aimed to make use of one indigenous biological control agent from the entomopathogenic nematodes families for the pest control.

## Materials and methods

### Collection of *Liriomyza huidobrensis*

Potential hosts of *Liriomyza huidobrensis* included cultivated host plants and wild host plants such as *Solanum oleracelus*, *Pisum sativum*. In the present study open fields of *Solanum oleracelus*, main host of *L. huidobrensis* in Lebanon, were chosen along the northern coastal area in Lebanon for collecting *L. huidobrensis* pupae during spring season of 2013 (March-April). Additionally, pupae were collected from agricultural fields of fava beans also. Infested leaves are placed in bags and transferred to the laboratory where *L. huidobrensis* Blanchard pupae are directly isolated from the leaves. The pupae are placed in the refrigerator at 4°C. The pupae are then removed and used in the experiments. Pupae of *L. huidobrensis* can be stored for a maximum two weeks after which the mortality starts to increase.

### Nematodes for pathogenicity experiments

An indigenous entomopathogenic nematode, *Heterorhabditis indica*, isolated from a banana field on the coastal area of Lebanon was used for the experiment. The nematode was reproduced on *Galleria mellonella* larvae at 25 °C in laboratory conditions (Poinar, 1979): ten *Galleria* larvae were placed in Petri dish containing autoclaved soil in contact with a solution of *H. indica* for two days. *Galleria* cadavers were then placed on White traps containing Ringer solution (Kaya and Stock, 1997) for a couple of weeks to collect emerging infective juveniles. The larvae solution collected is then washed with Ringer solution, stored at 15 °C and used for the experiments within 2 weeks.

Susceptibility of *Liriomyza huidobrensis* to entomopathogenic nematodes *in vitro*

The aim of this experiment was to determine if the *Liriomyza huidobrensis* pupae are susceptible *in vitro* to the EPNs larvae. For this purpose ten Petri dishes were filled with sterilized soil containing infestive juveniles-IJs of EPNs solution concentrated at 1000IJs/mL. Five pupae were added per Petri dish and placed in obscurity at 22±2°C. In total 50 pupae were used for each test. The experiment was repeated three times. In control Petri dish, *L. huidobrensis* pupae are added to autoclaved soil humidified with Ringer solution only. Due to the small size of *L. huidobrensis* pupae, they were considered as dead if no emergence of adult flies were observed. Pupae mortality was verified every 48hrs during 15 days which represented the duration time for adult flies emergence. Dead pupae were placed on individual white trap (Bedding and Akhurst, 1975) to harvest the emerging of IJs juveniles. Assessment of IJs emergence was done for each pupae cadaver during one month. IJs emergence were considered as an indicator for the success of the EPNs life cycle inside *L. huidobrensis* pupae.

### Results and discussion

Results of this study showed that following the EPNs application and out of the 150 pupae used in the three tests, of 16 ±1.5 % of the pupae emerged into adults of *L. huidobrensis*, 21±2.5% of the pupae were parasited by another natural pathogene for *Liriomyza*, *Diglyphus isaea*) and 53±1.5% of the pupae were found dead, potentially infested by EPNs. The remaining pupae (10±1%) were not found in the Petri dish due to their small size. The control tests showed 79±2% of adult flies eclosion. Out of the 53±1.5% *L. huidobrensis* pupae cadavers potentially infested by EPNs, none of the cadaver showed any emergence of EPNs after one month eventhough the mummification and red color aspects of the cadavers (main signs indicating that an insect is parasited by EPNs).

Previous research on EPNs from the genus of *Steinernema* (*Steinernema carpocapsae* and *Steinernema feltiae*) demonstrated their potential for control of the agromyzid leafminers: *Liriomyza trifolii* Burgess (Hara et al., 1993; LeBeck et al., 1993; Sher et al., 2000; Tomalak et al., 2005) and *Liriomyza huidobrensis* (Williams and Walters, 2000). Results of this experiments showed leafminers larval mortality ranged from 48 to 98%.

Biological control using entomopathogenic nematodes offers an alternative approach for management of *L. huidobrensis*, because of its ability to rapidly develop resistance to chemical insecticides (Mason et al., 1987; Parella et al., 1989). Additionally, chemical insecticides are hazardous to the environment and non-target organisms. Entomopathogenic nematodes, in particular *Steinernema carpocapsae*, *Steinernema feltiae* and *Heterorhabditis bacteriophora* were already tested against different leafminers in the genus *Liriomyza* by several researchers (Harris et al., 1990; Olthof and Broadbent, 1990; 1992); *Liriomyza trifolii* Burgess larval mortality after application of *S. carpocapsae*, were 64% in laboratory (Harris et al., 1990) and 53 to 83% in greenhouse trials (Olthof and Broadbent, 1992), while with *H. bacteriophora* in laboratory trials, mortality were ranged from 76 to 90% (Olthof and Broadbent, 1990). Harris et al. (1990) showed significant reductions in leaf damage after *S. carpocapsae* treatment which encouraged further studies on the application of entomopathogenic nematodes against *Liriomyza*. In addition only few EPNs species were tested against *Liriomyza* and Bedding et al. (1982) showed the importance of selecting the appropriate species and strain of nematodes for each pest species. Accordingly we conducted laboratory experiment using an indigenous species of nematode, not tested previously against *L. huidobrensis* pupae. Our results showed an important mortality rate of *Liriomyza huidobrensis* pupae in laboratory conditions with no validation of EPNs reproductive cycle success inside the pupae (no EPNs infective juveniles emerged from pupae cadavers).

### Conclusion

The main challenge of using entomopathogenic nematodes in biological control programs are adaptation to local conditions such as temperature, predators, soil ph, that might help introducing EPNs in the soil (Klein, 1990). Using indigenous entomopathogenic nematodes may provide more suitable EPNs isolates for biological control uses because of the adaptation to local climate and population regulators of the insect pest (Bedding, 1990). In our study, *H. indica* indigenous strain is isolated along the coastal area of Lebanon, sharing the same environmental conditions as *L. huidobrensis*. Our results shown that this strain could cause a mortality of 53% of *L. huidobrensis* pupae, even without being able to achieve whole reproductive cycle inside of leafminer pupae. Accordingly, *H. indica* can be considered as potential agent in biological control regarding its capability to cause pupae mortality *in vitro* and being isolated in favorable environmental conditions to the presence of its host, *L. huidobrensis* pupae which could prevent field trial failures in further studies. Susceptibility of *L. huidobrensis* pupae against entomopathogenic nematodes under controlled conditions, as reported here, is the first step towards the development of an integrated pest management program. More studies are needed to validate the capacity of EPNs to penetrate *Liriomyza huidobrensis* pupae and to test *H. indica* against larval stages of *L. huidobrensis*. Our results could also have an ecological important component; being unable to reproduce completely inside *L. huidobrensis* pupae, the EPNs will be unable to persist in the environment and therefore the balance of the ecosystem will not be disrupted (Schroeder et al., 1994) and there will be no ecological risk on non target species.

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