Status of entomopathogenic nematodes researches in Iran

Javad Karimi[1*], Tahmineh Safari[1] and Aziz Kharazi-Pakdel[2]

ABSTRACT

Entomopathogenic nematodes (EPNs) in the genera *Steinernema* and *Heterorhabditis* are excellent candidates for biological control of insect pests. Attributes making the nematodes as ideal biological include their broad host range, high virulence, safety for nontarget organisms and high efficacy in favourable habitat. Identification and characterization of EPNs in Iran was started since 2000. Several species of *Steinernema* and *Heterorhabditis* were isolated. Species from *Steinernema* are *Steinernema carpocapsae, Steinernema feltiae, Steinernema glaseri, Steinernema monticolum* and *Steinernema bicornutum*. From *Heterorhabditis* genus, only *Heterorhabditis bacteriophora* has been identified so far. Based on few studies, phylogenetic position of native EPNs species/isolates was investigated. In addition to EPNs, their symbiotic bacteria are identified and characterizied. In laboratory, infectivity of several isolates of EPNs assayed against different soil inhabiting pests. This accompanied by field evaluation of few numbers. Many indigenous EPNs were used in laboratory and field trials to evaluate their potential in control some economically important insect pests of crop, fruit and forest trees. Those insect hosts were *Polyphylla olivieri* (Coleoptera: Scarabaeidae), *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae), *Leptinotarsa decemlineata* (Coleoptera : Chrysomelidae) and *Spodoptera exigua* (Lepidoptera :Pyralidae**)**. Increasing information about EPNs potential will provide suitable biopesticides for using pest management programs.

Key words: Entomopathogenic nematodes, pest management, diversity

INTRODUCTION

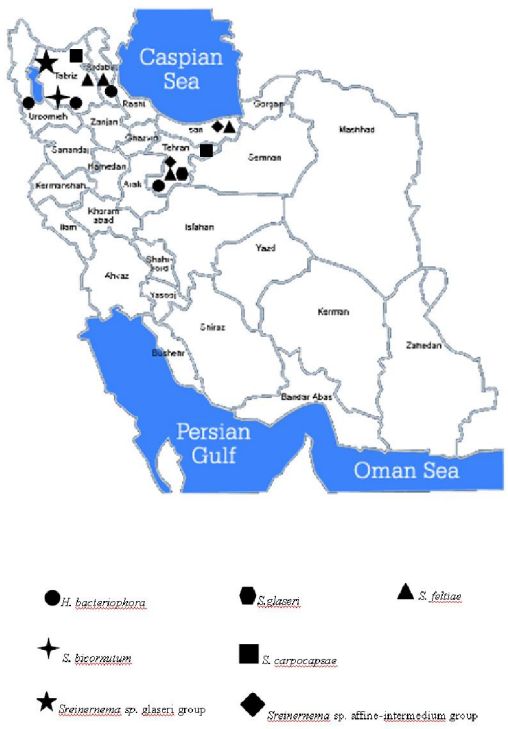
Nematodes are important in biological control of insect pests (Tanada and Kaya, 1993). The entomopathogenic activity of Steinernematid and Heterorhabditid species has been documented against a broad range of insect pests in a variety of habitats. These nematodes are especially efficacious against insect in soil and cryptic habitats (Lacey *et al.*, 2001). Many species of *Steinernema* and *Heterorhabditis* have been commercialized as biopesticides because they have wide host range, ability to kill the host within 48 h, capacity for growth on artificial media, amenable for storage, lack of host resistance and safety to the environment. EPNs invade their hosts through natural openings (mouth, spiracles, anus) or wounds and penetrate into the haemocoel. Bacteria in the genera *Xenorhabdus* or *Photorhabdus* are released and kill the host quickly. Nematodes then develop saprophytically in the cadaver (Ehlers, 1990, 1996; Van Driesche *et al.*, 2008). Iran has ideal suitable climatic conditions for agriculture, resulting in a high diversity of related herbivore insects and their natural enemies. Farmers have problems about the control of these pests and need intensive control methods. In addition to chemical control methods, biocontrol techniques has also an important role in pest management. However, the roles and effects of insect pathogens as microbial agents except *Bacillus thuringiensis* are not well-known in such control strategies. On the other hand, several universities and research institutes working with entomopathogenic nematodes. But all are at first line which needs to be improved. Research with the Iranian fauna of EPNs have only recently been initiated. In this review, a general overview of the studies and the current situation of EPNs research in Iran are discussed**.**

Records and diversity of entomopathogenic nematodes

In Iran , few surveys have been conducted to date. Parvizi (2000 , 2001) recovered unnamed sample of *Steinermema* sp. as well *Heterorhabditis bacteriophora* from the West Azerbaijan province. Tanha Ma'afi *et al*. (2006) found two *Steinernema* species from Mazandaran and Tehran provinces soils which was identified as *Steinernema feltiae* and a member from "*affine-intermedium*" group. Karimi and Kharazi-pakdel (2007) collected and identified eight isolates from three EPNs species from Tehran province as natural pathogens of the white grub, *Polyphylla olivieri* (Coleoptera: Melolonthidae). In this survey, different larval stages of the white grub collected from several sites, and

infected larvae transferred to a modified White trap. This resulted in isolation of *S.glaseri*, *S.carpocapsae* and *H.bacteriophora*. They used morphological and molecular data as well as cross breeding tests to identify them. Molecular analysis of ITS regions were most informative (Adams and Nguyen, 2002). Kary *et al.* (2009) reported occurrence of several EPNs in North Western at natural areas in Iran. They extracted and identified *S. carpocapsae, S.feltiae, S.bicornotum* and *H.bacteriophora*. These species were described by morphological and molecular characters. Nikdel *et al.* (2008) collected and introduced five EPNs from Arasbaran forests in North East Iran.The most commonly found species reported were *S.carpocapsae* and *S.feltiae*, *H. bacteriophora* was also isolated from different regions.

Figure 1. Map of Iran showing distribution of entomopathogenic nematodes complex



Potential of entomopathogenic nematodes

Information on the effectiveness of EPNs on insetcts is limited in Iran. Recently, efficiency of two native EPNs i.e *H. bacteriophora* and *S. bicornotum* was tested against the acorn weevil, *Curculio glandium* . This Curculionid is an important forest pest of oak trees in Iran. In the first experiment, penetration assay was conducted using a suspension of 4000 IJs/ml distilled water in multi-well plates. Penetration rate was 1.6 % for *H. bacteriophora* and 0.55% for *S. bicornutum*. In the second experiment, *H. bacteriophora* and *S .bicornutum* were applied at different concentrations (0, 150, 250, 500, 1000 and 2000 IJs / ml of DW) in the 9 cm Petri plates .The experiments were conducted at two temperature ranges (21-24ºC and 25-28ºC). Maximum mortality caused by *H. bacteriophora* and *S. bicornutum* were 58.3 % , 25% (at 21-24ºC) and 63.5 % , 30.5 % (at 25-28ºC), respectively. *H.bacteriophora* caused higher larval mortality comparing to *S. bicornutum* at both temperature ranges. In this research, it was showed that by increasing of concentration of nematode and temperature, larval mortality was raised. Based on probit analysis, the LC_{50} of *H. bacteriophora* at two temperature ranges of 21-24ºC and 25-28ºC were determined 1331 and 1037 IJs/ml, respectively. *H. bacteriophora* comparing to *S. bicornutum* is more effective and can be suggested for complementary studies toward finding a suitable biocontrol agent of the pest.

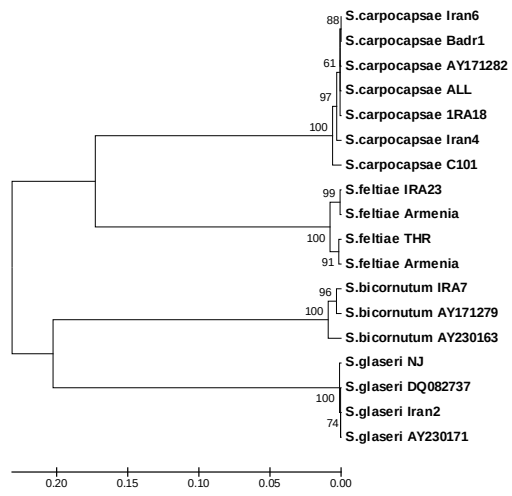


Figure 2 . Phylogenetic analysis of Iranian Steinernematid species /isolates based ITS sequences. The dendrogram was constructed by the maximum parsimony method and Kimura-2 parameter with 1000 resamplings values of bootstrap.

Ebrahimi *et al.* (2008) studied on efficiency of *H.bacteriophora* and *S.feltiae* against *Helicoverpa armigera* at laboratory conditions. This study resulted a mean mortality percentage of 83.33 % with *S.feltiae* and 66.7 % with *H.bacteriophora*. Saghaei *et al.* (2004) examined the effects of indigenous *H.bacteriophora*

Table 1. Record and diversity of entomopathogenic nematodes from Iran

Entomopathogenic nematode	Location	References
Heterorhabditis bacteriophora	West Azerbaijan province, Tehran, north western at natural areas, Arasbaran forests in NW of Iran	Karimi & Kharazi –pakdel(2007) Kary *et al.* (2009), Nikdel *et al.* (2008)Parvizi, (2000 , 2001 and 2002)
S.bicornotum	North Western at natural areas, Arasbaran forests in North West of Iran	Kary *et al.* (2009), Nikdel *et al.* (2008)
S.carpocapsae	Tehran, North Western at natural areas, Arasbaran forests in North West of Iran	Kary *et al.* (2009), Nikdel *et al.* (2008)
S.feltiae	Mazandaran and Tehran, North Western at natural areas, Arasbaran forests in North West of Iran	Tanha Ma'afi *et al.* (2006), Kary *et al.* (2009), Karimi *et al.* (2009) , Nikdel *et al.* (2008)
S.glaseri	Tehran	Karimi *et al.* (2009)
Steinernema sp. *"affine-intermedium"*	Mazandaran and Tehran	Tanha Ma'afi *et al.* (2006)
Sreinernema sp. "glaseri" group	Arasbaran forests North West of Iran	Nikdel *et al.* (2008)

strains (isolates from west Azerbaijan) on *Galleria melonella*.By increasing larval size of wax moth, its susceptibility were increased. In another study, Aramideh *et al*. (2004) evaluated efficiency of some native *Steinernema* strains against *Spodoptera exigua*. In laboratory conditions, pre-pupa is high susceptible stage to EPN. Based on this they recommended using this pathogen at larval and pre-pupa stages in fields. In other studies, Parvizi (2001) conducted a test on infectivity of *H.bacteriophora* and *Steinernema* sp. against white grub ,*P. olivieri*. Both EPNs strains were isolated from soils of West Azerbaian. In this study, IJs with concentration $5 \times 10^{5}/m^{2}$ could cause a mean mortality of 33.8 %, and 45.87% in third larval stage of this scarabaeid. In another test, potential of EPNs strains studied against Colorado potato beetle, *Leptinotrsa decemlineata*. 160 IJs/cm^{2} has the best results and could reduce pest populations about 83.75% (*Steinernema* sp.) and 90% (*H.bacteriophora*). *Synanthedon myopaeformis* (Lepidoptera: Sesiidae) was another test insect used for determing its suscebtibility to EPNs (Parvizi, 2000 , 2001 and 2002).

Distribution of entomopathogenic nematodes

Kary *et al*. (2009) observed low recovery of nematodes (3% of sites). They isolated Four species EPNs from diverse agroecosystems. The reason for low recovery rate could be that only *Galleria mellonella* was used as a trap insect, and it may not be an appropriate host for all EPN species/strains. This was a confirmation of same statement by Spiridonov and Moens (1999) and Griffin *et al.* (1991). However, such low recovery rate is very common in surveys conducted in other regions of the world (Choo *et al.*, 1995; Rosa *et al.*, 2000). The majority of *H. bacteriophora* isolates were found in grasslands and alfalfa fields. Orchards (mainly apple) and vegetable plots yielded the remaining positive samples. *Steinernema feltiae* was isolated mainly from orchards and grasslands in similar proportion (37.5%) followed by alfalfa fields and cereals (12.5%). *Steinernema carpocapsae* and *S.bicornutum* were isolated from an orchard and alfalfa field, respectively (Kary *et al*., 2009).

In bioassays against the white grub, *Polyphylla olivieri*, the LD_{50} of *H. bacteriophora* Iran 1 was 35 IJs/larva, followed by 65 IJs/larva for *S. glaseri* Iran 2. The LD_{50} for *S. carpocapsae* was > 10000 IJs/larva and caused only 16% mortality after 25 days. Tolerance of the three Iranian EPNs (Iran 1 of *H. bacteriophora*, Iran 2 of *S. glaseri* and Iran 3 of *S. carpocapsae*) were compared. Heat tolerance study showed that the *H. bacteriophora* strain was the most tolerant nematode at 32°C, but no nematodes could survive at 36°C after a 4 -5 h exposure. Furthermore, life cycle and natality/ mortality data of the three Iranian

isolates were studied in the wax moth larvae, *G. mellonella*, at the temperatures regims of 5 to 30°C.

While reviewing status of research on symbiotic bacteria associated with EPN species, only *Xenorhabdus poinarii*, *Xenorhabdus nematophila* and *Xenorhabdus bovieni* were isolated. Two subspecies of *Photorhabdus luminescens*, symbiont of the two *H. bacteriophora* isolates were associated with two different *P. luminescens* subspecies. The first strain of *H. bacteriophora* was associated with *P. luminescens* ssp. *laumondii* in all locations. The second isolate of *H. bacteriophora* was associated with *P. luminescens* ssp. *laumondii* in two locations and with *P. luminescens* ssp. *thracensis* in another one.

In biological control programmes, native biocontrol agents is often preferable, since they are adapted to local conditions. Novel species and strains may have superior traits, making them suitable for direct commercial exploitation or as a source of genetic diversity for breeding improved species or strains.

Research on EPNs in Iran have been started recently. Biology, ecology and infectivity studies should be initiated in Iran. Field application of these EPNs should be tried in selected regions. Farmers and growers must be created awareness on the safety of EPNs, their usage, advantages and disadvantages. In addition to scientific studies, regulatory strategies of the government should also aim at supporting the further introduction of EPNs based products as a part of control performance (Susurluk , 2007).

Studies on the nematode taxonomy, biology of the pest insects and symbiotic association with bacteria were as indispensable as the product development involving mass production, formulation and application techniques. The industry requirements for future research includes fundamental research on the characterization most available EPNs strains, screening for virulence strains and mass production.

REFERENCES

Adams B. J. and Nguyen K. B. 2002. Taxonomy and systemtics. in: *Entomopathogenic Nematology*, (R. Gaugler, eds.). CABI Publishing, Oxon, UK. 1–33 **PP**.

Aramideh, Sh., Safaralizadeh, M. H., Pourmirza, A. A. and Parvizi, R. 2004. Studies on the susceptibility of different larval, prepupal and pupal stages of beet armyworm (*Spodoptera exigua* H.) to *Steinernema* sp. On sugar beet under laboratory conditions, *16th. Iranian Plant Protection Congress , Tabriz University, Tabriz*, 29 August-2 September, 77 **P.**

Choo, H.Y., Kaya, H. K. & Stock, S. P. 1995. Isolation of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Korea. *Japanese Journal of Nematology,* **25**: 44-51.

Ebrahimi, L., Niknam, G. R., Nikdel, M. and Hassanpour, M., 2008. Study on the response of cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae) to various concentrations of entomopathogenic nematodes, *Steinernema feltiae* and *Heterorhabditis bacteriophora* (Rhabditida) under laboratory conditions, *18th Iranian Plant Protection Congress, Bu Ali university of Hamedan, Hamedan, Iran*, 25-29 August, 7 **P**.

Ehlers, R. U. 1990. Some contemporary issues in biological control of insects and their relevance to the use of entomopathogenic nematodes. *In*: *Entomopa thogenic Nematodes in Biological Control* (Gaugler, R. and Kaya, H. K. eds.). CRC Boca Raton, FL; 1-19 **PP**.

Ehlers, R. U. 1996. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. *Biocontrol Science and technology,* **6**: 303-316.

Glaser R.W. and Fox. H. 1930. A nematode parasitic of the Japanese beetle (*Popillia japonica* Newman). *Science,* **71** : 16-17.

Griffin C. T., Moore J. F. and Downes., M. J. 1991. Occurence of insect parasitic nematodes (Steinernematidae, Heterorhabditidae) in The Republic of Ireland. *Nematology,* **37**: 92-100.

Karimi J. and Kharazi-pakdel, A. 2007. Incidence of natural infection of the white grub *Polyphylla olivieri* (Coleoptera: Scarabaeidae) with entomopathogenic nematodes in Iran. *Bulletin OILB srop*., **30** (1): 35-39.

Karimi, J. , Kharazi-pakdel A. and Yoshiga, T. 2009. Insect pathogenic nematode, *Steinernema feltiae* from Iran. *IOBC/wprs Bulletin,* **45**: 409-412.

Karimi, J., Kharazi-pakdel, A.,Yoshiga,T. and Koohi-habibi, M. 2009. First Report of *Steinernema glseri* (Rhabditida :Steinernematidae) from Iran *Russian Journal of Nematology* , **17** : 83-85.

Kary , N. E., Niknam ,Gh., Griffin, C. T., Mohammadi, S. A, Mohammadi, M.2009. A survey of entomopathogenic nematodes of the families Steinernematidae and Heterorhabditidae (Nematoda: Rhabditida) in the North-West of Iran. *Nematology*, **11**(1), 107-116.

Lacey, L. A ., Frutos, F., Kaya, H. K. and Vail, V.2001. Insect Pathogens as Biological Control Agents: Do They Have a Future?. *Biological Control*, **21**: 230-248.

Miduturi, J. S. Moens, M., Hominick, W. M., Briscow, B. R. and Reid, A. P. 1996. Naturally occuring entomopathogenic nematodes in the province of West-

Flanders, Belgium. *Journal of Helmintho*logy, **70**: 319-327.

Nikdel, M., Niknam, G., Shojaee, M., Askary, H. and Mohammadi., S. A. 2008. A survey on the response of the last instar larvae of acorn weevil, *Curculio glandium* (Col.: Curculionidae), to entomopathogenic nematodes *Steinernema bicornutum* and *Heteror habditis bacteriophora* in the laboratory . *Journal of Entomological Society of Iran*, **28**: 45-60

Özer, N., Keskin, N. and Kirbas, Z. 1995. Occurrence of entomopathogenic nematodes (Steinernematidae: Heterorhabditidae) in Turkey. *Nematologica*, **41**, 639-640.

Parvizi, R., 2000. Study biocontrol of Colorado beetle by entomopathogenic nematodes, *14th Iranian Plant Protection Congress, Isfahan University of Technology, Isfahan, Iran*, 5-8 September, 66 **P**.

Parvizi, R. 2001.Study infectivity of *H.bacteriophora* and *Steinernema* sp. against white grub ,*Polyphylla olivieri*. *Journal of Entomological Society of Iran*, **21** : 63-72.

Parvizi, R. 2002. Study on effectiveness of entomo pathogenic nematodes, *Steinernema* sp. and *Heterorhabditis bacteriophora* on red-belted clearwing moth (*Synanthedon myopaeformis*), *15th Iranian Plant Protection Congress , Razi University of Kermanshah , Kermanshah, Iran*, 7-11 Sptember , 149 **P.**

Rosa, A. J. S., Boinifassi, E., Ameral, J., Lacey, L. A., Simoes, N. & Laumond, C. 2000. Natural occurrence of entomopathogenic nematodes (Rhabditida: *Steinernema, Heterorhabditis*) in the Azores. *Journal of Nematology,* **32**: 215-222.

Saghaei, N., Pourmirza, A. A., Sadeghi Nasab, F., Hashemi Khabir, Z., Javie, E. and Hossein Zadeh, A. 2004 . Studies on the susceptibility of *Galleria mellonella* L. to *Heterorhabditis bacteriophora* P. under laboratory conditions, *16 th Iranian Plant Protection Congress , Tabriz University, Tabriz, Iran*, 29 August-2 September, 60 **P**.

Spridonov, S. E. and Moens, M. 1999. Two previously unreported species of steinernematids from woodlands in Belgium. *Russian Journal of Nematology,* **7**: 39-42

Susurluk, A. 2007 Review on Entomopathogenic Nematodes in Turkey *Journal of Biological and Environmental Science*, **1**(2): 67-71

Tanada Y. and Kaya, H. K. 1993. *Insect Pathology*. Academic Press. San Diego CA. 666 **PP**.

Tanha Ma'afi, Z., Ebrahimi, N., Abootorabi, E. and Spiridonov, S. E. 2006. Record of two Steinernematid species from Iran, *17 th Iranian Plant Protection Congress , University of Tehran , Karaj, Iran*, 2-5 Sptember, 482 **P.**

Van Driesche, R., Hoddle, M. and Center, T. 2008.*Cotrol of pests and weeds by natural enemies an introduction to Biological Control*,Blackwell Publishing.473 **PP.**.

Javad Karimi[1], Tahmineh Safari[1] and Aziz Kharazi-Pakdel[2]

1. Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran, Phone: +98-911-1700817, E-mail: jkb@um.ac.ir

2.Department of Plant Protection ,Campus of Agriculture and Natural Resources ,University of Tehran, Karaj, Iran.

Received: September 9, 2009; Revised: December 6, 2009; Accepted: February 15, 2010.