



Status of entomopathogenic nematodes researches in Iran

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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 characterized. 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 *Steinernema* 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.bicornutum* 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 insects is limited in Iran. Recently, efficiency of two native EPNs i.e *H. bacteriophora* and *S. bicornutum* 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 (21-24°C and 25-28°C). Maximum mortality caused by *H. bacteriophora* and *S. bicornutum* were 58.3 % , 25% (at 21-24°C) and 63.5 % , 30.5 % (at 25-28°C), 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 21-24°C and 25-28°C 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.

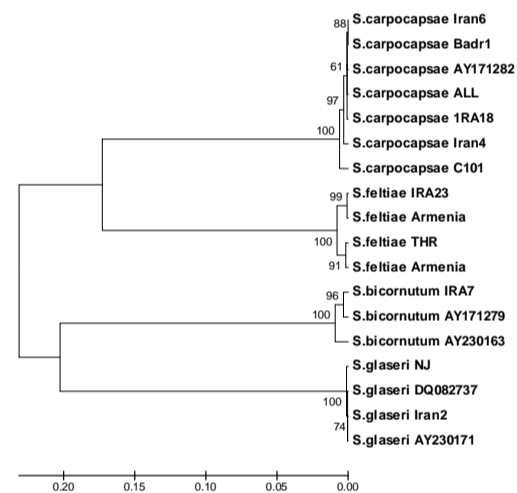


Figure 2. Phylogenetic analysis of Iranian Steinerma species /isolates based ITS sequences. The dendrogram was constructed by the maximum parsimony method and Kimura-2 parameter with 1000 resamplings values of bootstrap.

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

strains (isolates from west Azerbaijan) on *Galleria mellonella*. 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 Azerbaijan. 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/cm² 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 determining its susceptibility 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 LD₅₀ of *H. bacteriophora* Iran 1 was 35 IJs/larva, followed by 65 IJs/larva for *S. glaseri* Iran 2. The LD₅₀ 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 bovienei* 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.

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