DISTRIBUTION OF ENTOMOPATHOGENIC NEMATODES OF THE FAMILIES STEINERNEMATIDAE AND HETERORHABDITIDAE IN POTATO FIELDS IN NORTH-WEST IRAN (NEMATODA: RHABDITIDA)

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ABSTRACT: During 2009 a survey on entomopathogenic nematodes was conducted in potato fields in two provinces in the north-west of Iran. Out of a total of 320 soil samples, 9 were positive for entomopathogenic nematodes (2.81%), with 7 (2.18%) containing *Heterorhabditis* and 2 (0.62%) *Steinernema* isolates. Morphological and molecular studies were undertaken to characterize these isolates. The *Heterorhabditis* isolates were identified as *Heterorhabditis bacteriophora* and one new undescribed species and two species of *Steinernema* isolates were identified as *S. feltiae* and *S. carpocapsae*. *Heterorhabditis bacteriophora* was recovered from 6 sites and *Heterorhabditis* n. sp. Nas7 from only one site like two other species of *Steinernema*.

KEYWORDS: *Heterorhabditis bacteriophora, Heterorhabditis* n. sp. Nas7. Potato fields. *Steinernema carpocapsae*, *Steinernema feltiae*.

Entomopathogenic nematodes (EPNs) of the genus *Steinernema* have been globally used as safe biocontrol agents against soil borne insect pests. These nematodes are symbiotically associated with entomopathogenic bacteria *Photorhabdus* (Boemare et al., 1993) and *Xenorhabdus* (Thomas & Poinar, 1974). The third-stage infective juvenile (IJ) is the only free-living stage that persists in the soil in search of a host. Following entry, IJs release the symbiotic bacteria into the insect hemocoel, multiply and kill the host, usually within 24-48 h (Ciche and Ensign, 2003; Poinar, 1990).

In the first survey in Iran, three species of *Steinernema* and one *Heterorhabditis* species, including *S. bicornutum*, *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* have been reported from north-west regions (Eivazian Kary et al., 2009). Regarding the area wide we conducted second survey in that regions but mainly focused on potato fields with the goal of finding EPNs isolates which are probably act as a biological control agents against relevant insect pests in potato fields specially Colorado Potato Beetle.

MATERIAL AND METHODS

Sampling strategy

In total, 320 soil samples were collected randomly from potato fields during 2009. Each soil sample was a composite of 5-20 random sub-samples taken in the same location but at least 10 m away from each other and to a depth of 30 cm,

using a small shovel. Between sampling site, the shovel was thoroughly rinsed with water and air dried to prevent contamination of the next sampling unit. The soil was thoroughly mixed on a plastic sheet and half of each sample was used for extraction of EPN.

Nematodes extraction

EPN were recovered from soil samples using an insect baiting method, described by Bedding and Akhurst (1975). To maximize recovery of nematodes from the soil samples, a second baiting round was done by placing fresh *G. mellonella* into the containers with the same soil.

Taxonomic studies

For morphological studies, nematodes were examined live or heat killed in 60°C Ringer's solution. All nematodes used in this study were reared in *Galleria mellonella* larvae. For isolating mature females and males of the first and second generations, the infected larvae were dissected in Ringer's solution 3 and 5 days after infection, respectively.

Heat killed nematodes were placed in triethanolamin-formalin (TAF) fixative (Kaya and Stock, 1997) and processed to anhydrous glycerine for mounting by a slow evaporation method. Morphology and morphometric studies were made using an Olympus BX41 microscope equipped with differential interference contrast optics. Measurement of specimens was made using UTHSCSA Image Tool software (Vilcox et al., 1995). Morphological identification was made using taxonomic criteria suggested by Stock and Kaya (1996) and Hominick et al. (1997).

Molecular characterization

Total genomic extraction and ITS-rDNA amplification were done as described by Eivazian Kary et al. (2009). Amplified products were purified using a Qiagen Purification kit (Qiagen, Leusden, The Netherlands). Purified DNA was sequenced in IBMP-CNRS, France. The DNA sequences were edited with Chromas 2.01 and aligned using Clustal X 1.64 (Thompson et al., 1997) with the sequences of other *Heterorhabditis* and *Steinernema* species obtained from GenBank.

Molecular phylogenetic relationships were obtained by equally weighted maximum parsimony (MP) and maximum likelihood (ML) using PAUP* 4.0b8 (Swofford, 1998). MP was performed with a heuristic search with the following setting: one hundred replicates of random taxon addition (RTA), tree-bisection-reconnection (TBR) branch swapping, multiple trees retained, no steepest descent and accelerated transformation. All data were assumed to be unordered, all characters were treated as equally weighted, and gaps were treated as missing data. For ML analysis, the appropriate substitution model of DNA evolution that best fitted the data set was determined by the Akaike Information Criterion with Model Test 3.06 (Posada & Crandall, 1998). Bootstrap analysis with 1000 replicates was conducted as a measure of support for individual clades for MP and ML trees. For the phylogeny analysis *Caenorhabditis elegans* (Maupas, 1899) (AF331911) were treated as the outgroup taxon.

RESULTS AND DISCUSSION

From total of 320 samples, entomopathogenic nematodes were recovered from 9 samples (2.81%); 7 isolates (2.18%) were positive for the occurrence of heterorhabditids and 2 (0.62%) for steinernematids. Based on morphological and

molecular characterization two species of *Heterorhabditis* and two species of *Steinernema* were found. Six isolates of *Heterorhabditis* were identified as *Heterorhabditis bacteriophora* and one as *Heterorhabditis* n. sp. Nas7. *Steinernema* species were identified as *Steinernema carpocapsae* and *S. feltiae* (Fig. 1 and 2).

The Colorado Potato Beetle (CPB), *Leptinotarsa decemlineata* (Say) is the most economically damaging pest to potatoes in most areas of the Iran. If potato field left uncontrolled, it can completely defoliate it. The first goal of the present work was to identify species of EPNs which exist in potato fields and probably have impact on CPB population. In biological control programmes, using native biocontrol agents is often preferable to using exotic ones, 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 strains (Choo et al., 1995).

From the results of these surveys, it appears that *Heterorhabditis bacteriophora* is of widespread occurrence in the potato fields of north-west of Iran. This species is widely distributed in the world (Hominick et al., 1996; Adams et al., 2006). The natural hosts of isolated EPN in potato fields are unknown but *Leptinotarsa dcemlineata* is the major insect pest in potato fields in studied regions. Because of the frequent occurrence of *H. bacteriophora* it is possible that CPB is amongst its natural hosts.

The reason for the low recovery of these three species is not known. *Steinernema carpocapsae* appears to have a global distribution. In the first survey in the region it was only appeared from one site (Eivazian Kary et al., 2009). Such a low frequency can be attributed to low ability of the species in colonizing new sites due to limitation in host range and niche conditions. *Steinernema feltiae* was detected from one site too but it was shown that this species has relatively high distribution in north-west of Iran and observed low frequency of the species in the potato fields may be result of unsuitability of human activities on it.

In the studied fields, chemical and cultural controls are two major strategies in deal with CPB. Without chemical control 100% damage will occur. Although chemical control is keystone in CPB managements but it can affect benefit organisms directly or indirectly by reducing available hosts. It seems that infevtice juveniles of heterorhabditids are more resistance to chemical poisons than steinernematids due to stability of second instar larvae sheet and this resistance may be other reason for high distribution of heterorhabditids compare than steinernematids but in order to determine the temporary or permanent existence of *S. carpocapsae* and *S. feltiae* in the potato files and impact of them as a biological control agents on CPB, extensive sampling and laboratory and fields studies are needed.

Molecular analysis of ITS-rDNA of collected isolates showed existence of probably new species from *Heterorhabditis* between them but for more confidence morphology and molecular studies are undertaken.

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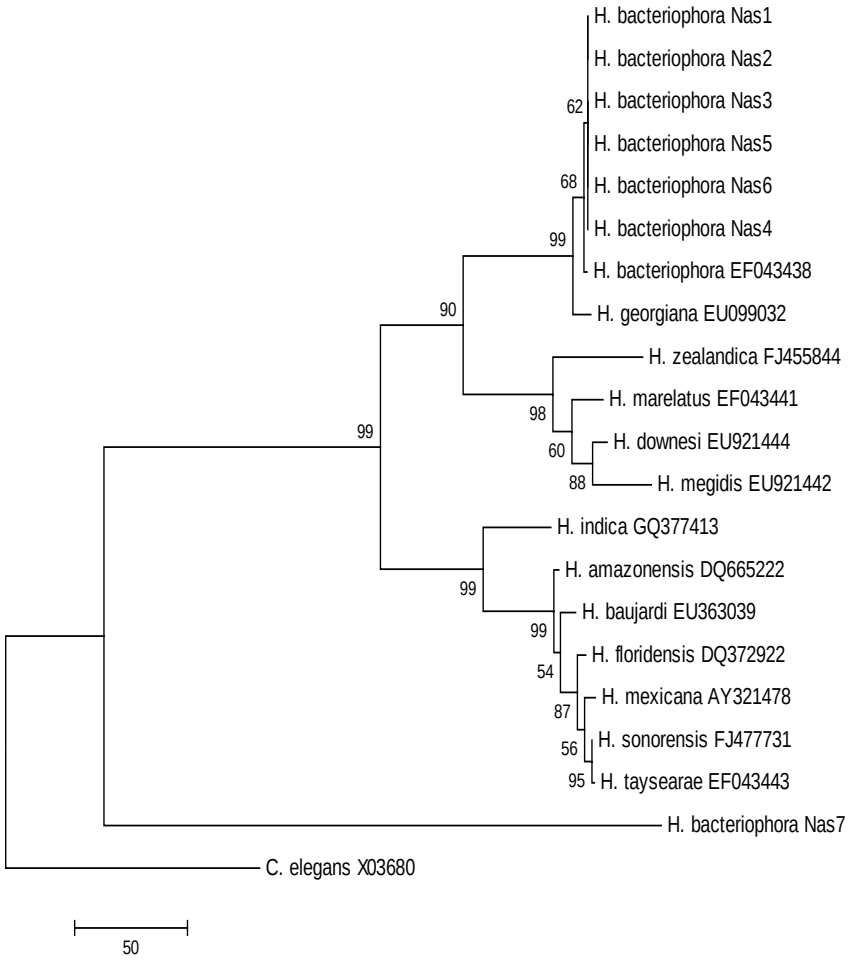

Figure 1. Phylogenetic relationship of studied isolates with other *Heterorhabditis* species based on ITS rDNA. The tree is rooted on *C. elegans*. Bootstraps for MP are shown on nodes.

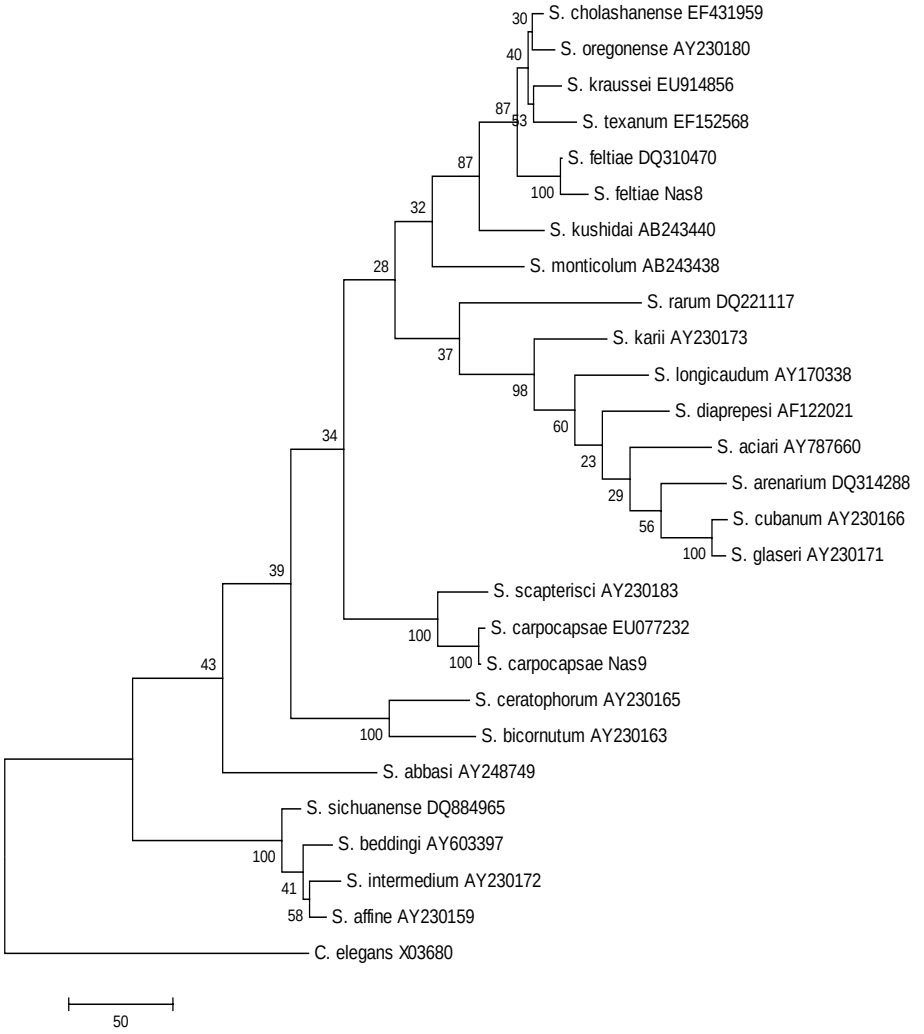

Figure 2. Phylogenetic relationship of studied isolates with other *Steinernema* species based on ITS rDNA. The tree is rooted on *C. elegans*. Bootstraps for MP are shown on nodes.