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Populations of entomopathogenic nematodes in agricultural lands

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ABSTRACT

Entomopathogenic nematodes (EPNs) are known agents in pest biological control. Environmental factors can influence on populations of soil-inhabitant nematodes like EPNs in agricultural lands. In the current study, after selecting a suitable study site (33.20-33.42 N and 48-48.55 E), and soil sampling (250 samples) in May to July 2019, infectious juveniles of EPNs were extracted from soil samples according to the bait method. According to occurrence of EPNs-infected *Galleria mellonella* larvae, natural occurrence (%) of EPNs was estimated, while considering soil characteristics (salinity and acidity), region (altitude) and type of crop. Also, population incidence (%) of EPNs in EPNs-positive soil samples was estimated, according to *G. mellonella* larvae mortality by re-baiting. As results, seven soil samples were found to be EPNs-positive per total samples. Natural occurrence (%) of EPNs, Steinernematidae and Heterorhabditidae was equal to 2.8, 2, and 0.8%, respectively. This was variable under ranges of area altitude, soil salinity and acidity, and crop type, so that the highest to lowest occurrence (%) of EPNs was observed in forage crops (4%), vegetables (4%), industrial crops (4%), fruit trees (2%), and cereals (1.33%), respectively. There was a significant difference ($P<0.05$) in incidence of EPNṡ population (%) in EPNs-positive samples. The lowest-highest incidence of EPNṡ population was obtained as 15.52-33.4% in 7 EPNs-positive samples. As a conclusion, type of crop, altitude and soil acidity and salinity play a role in the level of occurrence and incidence of EPNs populations. These may have affected the biological and ecological properties of the nematodes populations.

Keywords: steinernematidae, heterorhabditidae, natural occurrence, monitoring, environmental and soil factors

INTRODUCTION

Overuse of synthetic insecticides has reduced populations of pests' natural enemies, and has led to incidence of resistance phenomenon, which in turn has resulted in a reduction in efficiency of pests' management programs. Therefore, the use of eco-friendly methods, including biological control, could be an effective long-term strategy to reduce pests' damage (Kumar and Singh, 2015). The use of entomopathogenic nematodes (EPNs) as biological agents to suppress populations of various types of insects, especially soil-associated insects, is common in many European and American countries, and to date, it has been successful (Labaude and Griffin, 2018). *Steinernema feltiae* Filipjev, *S. carpocapsae* Weiser, and *Heterorhabditis bacteriophora* Poinar are known as effective agents for biological control of insects. The third instar juveniles (J_3s) or infective juveniles (IJs) of these EPNs represent a community of soil-inhabitant nematodes (Grewal et al., 2008). So, soil characteristics including texture, acidity, salinity, moisture, and temperature can be expected to have a variety of effects on population and function of this group of nematodes. However, there are few reports on the effect of environmental factors, such as physical and chemical soil characteristics (type of texture, salinity, acidity, and quantity and quality of elements), altitude, and crop type on occurrence and incidence of EPNṡ populations in agricultural lands (Koppenhöfer and Fuzy, 2007). According to Hunt and Subbotin (2016), 100 and 16 valid species have been identified and introduced for *Steinernema* and *Heterorhabditis*, respectively.

In the genus *Steinernema*, *S. feltiae* and *S. carpocapsae* have a worldwide expansion, but it seems that the other species of the genus have a more limited geographic distribution. Studies have shown that some species of *Steinernema* are associated with a specific type of habitat. For example, the species of *S. feltiae*, *S. intermedium* Poinar and *S. affine* Bovien have been found mainly in grasslands. In contrast, other species of *Steinernema* seem to have a wider range of habitats; for instance, *S. kraussei* Steiner has been found mostly in forest lands. This habitat preference may be due to distribution of suitable host or physiological and behavioral requirements of nematodes in specific ecological niches (Kanga et al., 2012). Populations of soil-inhabitant nematodes have a sufficient potential to evaluate biological traits of soil, therefore, investigating their demographic structure can be used as a bio-indicator for natural controlling the soil-associated pests. Tillage operations, organic matter, and pH are important factors in formation of soil-associated nematodeṡ communities in agricultural lands (Moura and Franzener, 2017).

Population characteristics of EPNs and their relationship with the environment are important for successful control of pests in integrated pest management programs on agricultural lands. Accordingly, the current study was done to determine the relationship of occurrence and incidence of EPNṡ populations with crop type, area altitude, and soil acidity and salinity in agricultural lands.

MATERIALS AND METHODS

Study Site

The study site (33.20-33.42 N and 48-48.55 E) was located in Khorramabad City, Lorestan Province, Iran. Agricultural lands of this region, approximately 90000 ha, include a wide variety of cereals, fruit trees, vegetables, forage crops, and industrial crops, hence it is known as a regional base of agricultural industry. According to the Köppen climate classification (Beck et al., 2018), this region has a hot-summer Mediterranean climate, with average annual precipitation of 511.06 mm, average annual temperature of 17.21 °C, and average annual relative humidity of 46.1%. Altitude of the study site is between 980-1 900 m above sea level. The crops have been grown at the study site for at least 5 years. All the studied agricultural lands were covered by integrated pest management (IPM) programs. Agricultural operations and fertilization were used in all crops according to type of crop and soil characteristics. Chemical methods were also used to control pests and pathogens. As a biological control, EPNs were not used to protect plants against pests.

Soil Sampling

At the study site, 250 sampling locations (4 m2) were selected according to accessibility and crop type with a distance of at least 1 km between them. A total of 250 soil samples were taken as representative locations of 27 crop types including alfalfa (*Medicago sativa* L.), apple (*Malus domestica* Borkh.), apricot (*Prunus armeniaca* Koch), barley (*Hordeum vulgare* L.), clover (*Trifolium repens* L.), maize (*Zea mays* L.), eggplant (*Solanum melongena* L.), garlic (*Allium sativum* L.), grapevine (*Vitis vinifera* L.), common bean (*Phaseolus vulgaris* L.), broad bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), peach (*Prunus persica* Batsch), pepper (*Capsicum annuum* L.), pomegranate (*Punica granatum* L.), rice (*Oryza sativa* L.), tomato (*Solanum lycopersicum* L.), rapeseed (*Brassica napus* L.), walnut (*Juglans regia* L.), wheat (*Triticum aestivum* L.), onion (*Allium cepa* L.), spinach (*Spinacia oleracea* L.), parsley (*Petroselinum crispum* Fuss), lentil (*Lens culinaris* Medik.), okra (*Abelmoschus esculentus* Moench), plum (*Prunus domestica* L.), and sugar beet (*Beta vulgaris* L.). Each soil sample (≈ 1.5 kg) was randomly taken as 5 sub-samples from every sampling location and depths of 10-30 cm during growing season in May to July, 2019 (Orozco et al., 2014). The samples were collected using a hand shovel, which was washed at each location with 70% ethanol after sampling to prevent contamination of the next sample. Geographical coordinates as well as altitude of soil sampling were recorded by a global positioning system (GPS) device (model Garmin ETrex Vista HCX). Also, type of crop (cereals, fruit trees, industrial crops, forage crops, and vegetables) was recorded in each sampling location. The samples were placed in polyethylene bags, were transported to laboratory under cold conditions, and were stored at a temperature range of 15-17 °C. Acidity and salinity of soil samples were also determined in laboratory using a pH meter and electrical conductivity (EC) meter, respectively.

Evaluation of EPNs̍ Population

An assay was performed to estimate natural occurrence (%) of EPNs in soil of the study site using the insect bait method according to Bedding and Akhurst (1975). Last-instar larvae of greater wax moth, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) were used as baits, reared using artificial diet containing honey (900 g), bee wax (200 g), glycerin (900 g), rice flour (1 300 g), and yeast (400 g) under temperature of 25±1 °C, relative humidity (RH) of 65±5%, and lighting period of 16 h (Birah et al., 2008). Accordingly, each soil sample was mixed well and then, 250 g of the soil, as sub-sample, was transferred to a plastic container (10×10×5 cm). Ten *G. mellonella* larvae were placed per container and then, the containers were covered with a lid, were inverted, and were kept at a germinator (temperature of 25±3 °C, RH of 70%, and without light). The sub-samples were checked every 2-3 days for 10 days. Due to symbiotic bacteria associated with EPNs, the infected *G. mellonella* cadavers to Steinernematidae turn tan, ochre, gray or dark gray, whereas Heterorhabditidae turn the *G. mellonella* cadavers red, purple, orange, yellow, brown or sometimes green (Kaya and Gaugler, 1993). Infected cadavers were removed and placed individually in White traps for emergence of IJs. The IJs of each cadaver were collected separately, and were used to infect fresh *G. mellonella* larvae for production of a second generation. Emerged IJs of the second generation were preserved in sterile distilled water containing 0.1% formalin and were stored at 15 °C for identification (White, 1927).

Initially, isolates were morphologically identified to sort them into similar family groups using a stereomicroscope (20x) according to their morphological characteristics (Lopez-Nunez et al., 2008). After morphological observations, classification criteria proposed by Stock and Kaya (1996) and Hominik et al. (1997) were applied for classifying them. Natural occurrence (%) of EPNs was estimated based on the presence or absence of the *G. mellonella* cadavers in the soil sub-samples. So, natural occurrence (%) of EPNs was determined according to the number of EPNs-positive samples / number of total samples (Liu and Berry, 1995).

A similar assay was performed to estimate incidence (%) of EPNs̍ populations in EPNs-positive samples using the bait method. Five sub-samples of 250 g soil (as 5 replicates) were prepared in plastic containers with 20 *G. mellonella* larvae. Then, the containers were covered with a lid, were inverted, and were kept at the germinator. After 10 days, based on the number of *G. mellonella* cadavers or mortality of *G. mellonella* larvae, incidence level of EPNs̍ populations was estimated by nematode type. The assay was performed twice (Bedding and Akhurst, 1975).

Data Analysis

The natural occurrence (%) (with two levels: A_1, natural occurrence in same group samples and A_2, natural occurrence in total samples) of EPNs (with three levels: EPNs, Steinernematidae, and Heterorhabditidae) was calculated according to the number of EPNs-positive samples / the total number of samples, with respect to crop type (cereals, fruit trees, industrial crops, forage crops, and vegetables), area altitude (<1000, 1000-1250, 1250-1500, 1500-1750, and 1750-2000 m.a.s.l.) and pH (<6.5, 6.5-7, 7-7.5, 7.5-8, and 8-8.5) and salinity (<0.4, 0.4-0.8, 0.8-1.2, 1.2-1.6, and 1.6-2 ds/ m) of the soil. As an assay of EPNs̍ population incidence (%) of EPNs-positive samples, a factorial experiment was conducted based on a completely randomized design (CRD) with 5 replicates under the controlled conditions. One-way analysis of variance (ANOVA) was used for analysis of the obtained data, and Tukey's post-hoc t-test was used to compare the EPNs-positive samples at a probability level of 5% using SAS software version 9.1. The assay was performed twice, and the diagram was drawn using Microsoft Excel software 2013.

RESULTS

According to observation and checking of *G. mellonella* cadavers, 7 samples were EPNs-positive per 250 soil samples of agricultural lands in the study site. EPNs-positive soil samples included samples from fields of wheat (cereals), apple (fruit trees), sugar beet (industrial crops), rapeseed (industrial crops), clover (forage crops), alfalfa (forage crops) and spinach (vegetables) (Figure 1).

The results show that there is a connection between information obtained from fields and the natural occurrence (%) of EPNs, so that the natural occurrence (%) of EPNs, Steinernematidae, and Heterorhabditidae was different under various conditions (including crop type, area altitude, and soil salinity and acidity). The highest to lowest natural occurrence (%) of EPNs was observed in forage crops, vegetables and industrial crops (4%), fruit trees (2%), and cereals (1.33%), respectively. The highest natural occurrence (%) of Steinernematidae and Heterorhabditidae was obtained in vegetables (4%), and fruit trees and industrial crops (2%), respectively. The highest to lowest natural occurrence (%) of EPNs was observed at altitudes of 1500-1750 (4.16%), 1250-1500 (3.75%), 1000-1250 (2.56%), and 1750-2000 and <1000 (0%), respectively (Table 1).

The highest natural occurrence (%) of Steinernematidae and Heterorhabditidae was obtained at altitudes of 1000-1250 (2.56%) and 1250-1500 (1.25%), respectively. The highest to lowest natural occurrence (%) of EPNs was observed in salinity values of <0.4 (11.11%), 1.2-1.6 (3.57%), 0.4-0.8 (2.88%), 0.8-1.2 (1.98%), and 1.6-2 (0%), respectively (Table 1). The highest natural occurrence (%) of Steinernematidae and Heterorhabditidae was obtained in salinity values of <0.4 (11.11%) and 0.8-1.2 (0.99%), respectively. The highest to lowest natural occurrence (%) of EPNs was observed in pH values of 6.5-7 (7.14%), 7-7.5 (3.2%), 7.5-8 (1.94%), and 8-8.5 and <6.5 (0%), respectively.

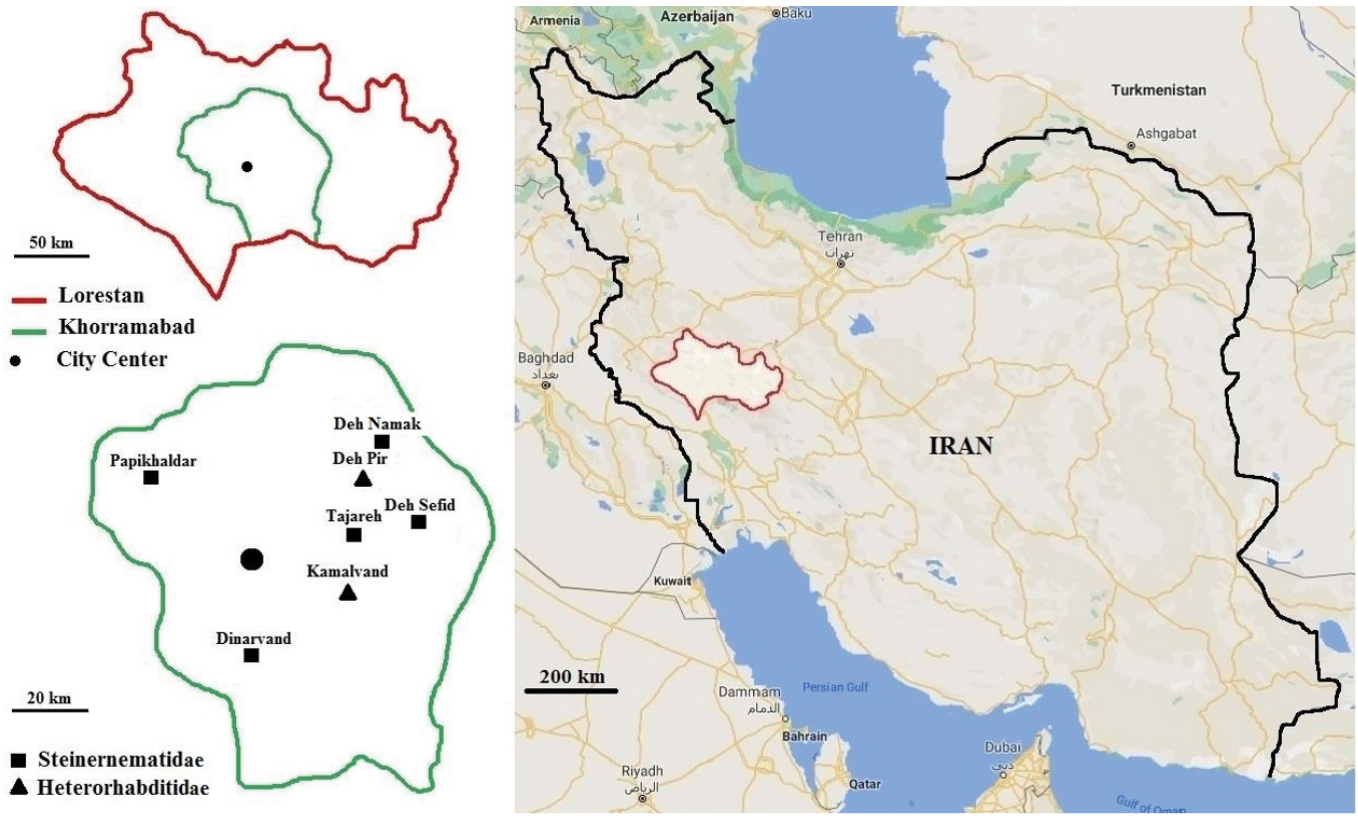

Figure 1. Location of EPNs-positive sites in Khorramabad, Lorestan, Iran

Deh Namak (33.5596, 48.5148), Deh Pir (33.5323, 48.4791), Deh Sefid (33.5012, 48.5345), Dinarvand (33.3986, 48.3661), Kamalvand (33.4560, 48.4522), Papikhaldar (33.5470, 48.3231) and Tajareh (33.4953, 48.4693)

Table 1. Natural occurrence (%) of entomopathogenic nematodes (EPNs) in agricultural land soils of Khorramabad according to cultivated crop type, area altitude, and soil salinity and acidity

Trait		No. of samples	EPNs			Steinernematidae			Heterorhabditidae		
			No. of Positive samples	[1]N_1 (%)	[2]N_2 (%)	No. of Positive samples	N_1 (%)	N_2 (%)	No. of Positive samples	N_1 (%)	N_2 (%)
Crop type	Cereals	75	1	1.33	0.40	1	1.33	0.40	0	0.00	0.00
	Fruit trees	50	1	2.00	0.40	1	2.00	0.40	0	0.00	0.00
	Industrial crops	50	2	4.00	0.80	1	2.00	0.40	1	2.00	0.40
	Forage crops	50	2	4.00	0.80	1	2.00	0.40	1	2.00	0.40
	Vegetables	25	1	4.00	0.40	1	4.00	0.40	0	0.00	0.00
Altitude (m a.s.l.)	<1000	21	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
	1000-1250	78	2	2.56	0.80	2	2.56	0.80	0	0.00	0.00
	1250-1500	80	3	3.75	1.20	2	2.50	0.80	1	1.25	0.40
	1500-1750	48	2	4.16	0.80	1	2.08	0.40	1	2.08	0.40
	1750-2000	23	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
EC (ds/m)	<0.4	9	1	11.11	0.40	1	11.11	0.40	0	0.00	0.00
	0.4-0.8	104	3	2.88	1.20	2	1.92	0.80	1	0.96	0.40
	0.8-1.2	101	2	1.98	0.80	1	0.99	0.40	1	0.99	0.40
	1.2-1.6	28	1	3.57	0.40	1	3.57	0.40	0	0.00	0.00
	1.6-2	8	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
pH	<6.5	3	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
	6.5-7	14	1	7.14	0.40	1	7.14	0.40	0	0.00	0.00
	7-7.5	125	4	3.20	1.60	3	2.40	1.20	1	0.80	0.40
	7.5-8	103	2	1.94	0.80	1	0.97	0.40	1	0.97	0.40
	8-8.5	5	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
Total		250	7	2.80	2.80	5	2.00	2.00	2	0.80	0.80

[1]N_1: Natural occurrence in same group samples
[2]N_2: Natural occurrence in total samples

The highest natural occurrence (%) of Steinernematidae and Heterorhabditidae was obtained in pH values of 6.5-7 (7.14%) and 7.5-8 (0.97%), respectively (Table 1).

In EPNs-positive samples, incidence (%) of EPNs was significantly (*P*<0.05) different according to samples̓ characteristics including crop type, area altitude, and soil salinity and acidity. The highest to lowest incidence (%) of EPNs was obtained in samples No. 3 (33.4%, Steinernematidae), 6 (26.51%, Steinernematidae), 2 (25.3%, Steinernematidae), 4 (21.7%, Heterorhabditidae), 5 (19.98%, Heterorhabditidae), 7 (16.45%, Steinernematidae), and 1 (15.52%, Steinernematidae), respectively.

However, no significant (*P*<0.05) difference was observed in EPNs̓ incidence (%) of samples No. 2 and 6, and 1 and 7 (Figure 2).

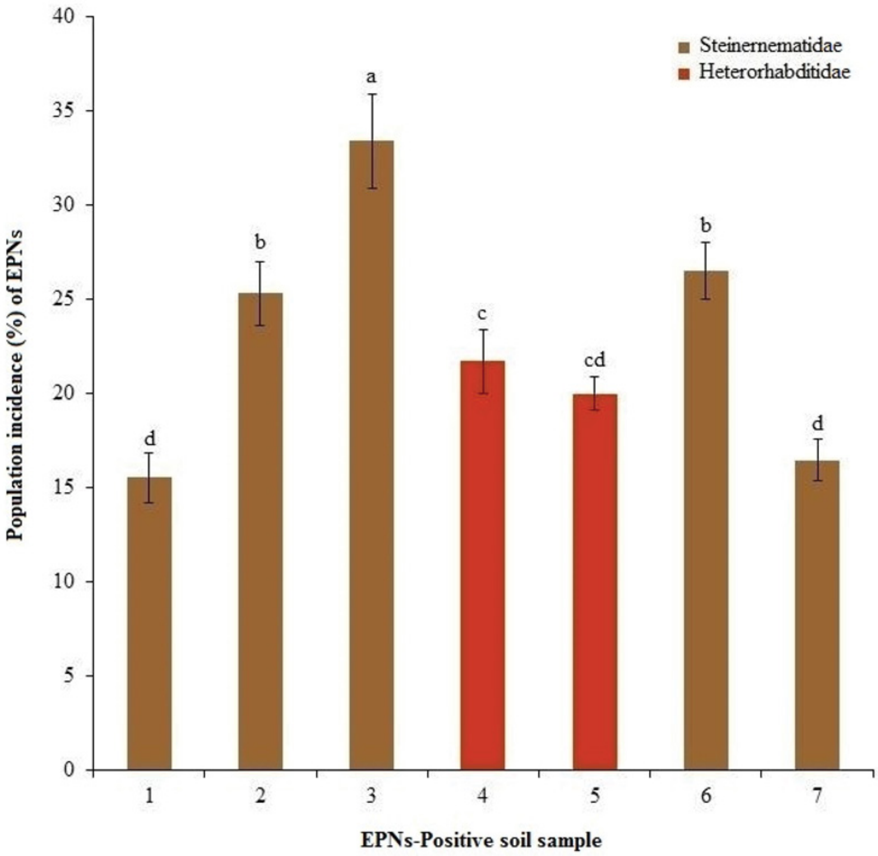

Figure 2. Population incidence[1] (%) of entomopathogenic nematodes in EPNs-positive soil samples[2] collected from Khorramabad agricultural lands, according to *G. mellonella* larvae mortality

[1] Means with the same letters on the same colored columns are not significantly different (*P*<0.05, n=5).

[2] Sample	Crop	Altitude (m a.s.l.)	EC (ds/m)	pH	Location
1	Wheat (Cereals)	1250-1500	0.8-1.2	7-7.5	Tajareh
2	Apple (Fruit trees)	1500-1750	0.8-1.2	7-7.5	Deh Sefid
3	Sugar beet (Industrial crops)	1250-1500	0.4-0.8	7.5-8	Papikhaldar
4	Rapeseed (Industrial crops)	1250-1500	1.2-1.6	6.5-7	Deh Pir
5	Clover (Forage crops)	1250-1500	0.4-0.8	7-7.5	Kamalvand
6	Alfalfa (Forage crops)	1500-1750	0.4-0.8	7-7.5	Deh Namak
7	Spinach (Vegetables)	1000-1250	<0.4	7.5-8	Dinarvand

DISCUSSION

The host-parasite relationship, environment and soil properties have a decisive influence on occurrence, incidence, and distribution of EPNs. Study of occurrence and incidence of EPNs, taking into account characteristics of soil and plant in a geographical area, can increase effectiveness of biological control programs in the area. Improving the potential of natural environmental resistance to pests as an important part of biological control can play an effective role in pest management (Labaude and Griffin, 2018; Abd-Elgawad, 2019). Results of the current study are in agreement with some reports on the EPNś population in Iran (Eivazian Kary et al., 2009; Nikdel et al., 2010). Nikdel et al. (2010) reported that 3% of soil samples were EPNs-positive including 9 samples (1.3%) that were Heterorhabditidae-positive and 12 samples that were (1.7%) Steinernematidae-positive, according to a survey on EPNs in forests and pastures of Arasbaran, Iran. Seven isolates (4 isolates of Steinernematidae and 3 isolates of Heterorhabditidae) were recovered from pastures and 14 isolates (8 isolates of Steinernematidae and 6 isolates of Heterorhabditidae) were recovered from forest soil samples; vice versa, in some reports, occurrence of EPNs was higher than these values. For example, Abdolmaleki et al. (2016) studied 150 soil samples from forest, pastures, and agricultural lands of Kurdistan Province, Iran; and found that 60% of the samples were EPNs-positive. *Heterorhabditis bacteriophora* was the most isolated species of EPN in this study. Occurrence of the EPN in pastures, forests, and alfalfa fields was equal to 6.3, 28.5, and 14%, respectively. In the current study, the total occurrence level of EPNś populations was equal to 2.8%, which varied in ranges of area altitude, soil salinity and acidity, and the cultivated crop type. Seven samples were EPNs-positive at 250 soil samples, which included 5 Steinernematidae samples (2%) and 2 Heterorhabditidae samples (0.8%).

A variation in natural occurrence of EPNs has been observed in various studies. For example, 3.3% in Catalonia (De Doucet and Gabarra, 1994), 2% in Turkey (Hazir et al., 2003), 6.5% in the island of Mallorca, and 0.66-11% in the Canary Islands (Campos-Herrera et al., 2007) were reported as lower occurrence values. Also, higher frequencies have been observed in the southeastern United States (28.5%) (Shapiro-Ilan et al., 2003), Indonesia (20.3%) (Griffin et al., 2000). Various hypotheses have been proposed for this wide range of occurrence of EPNś population in soil including sampling time and method, crop type, and regional climatic conditions (Keshari et al., 2019). Many living and non-living environmental parameters can determine range of activity of EPNs. Soil properties, such as humidity, temperature, and pH are the most important non-living factors influencing biological activity of EPNs. Also, soil type is involved in incidence of nematodes. For example, samples of Steinernematidae have been found on Elateridae beetle larvae in poor soils collected from cereal and potato fields with small amounts of humus and acid (Dzięgielewska and Erlichowski, 2011).

The results of the current study also showed that the increase in soil salinity and acidity reduced the likelihood of incidence and occurrence of EPNs in agricultural lands. Increased salinity as well as soil acidification is associated with the increased solubility and concentration of some elements. Body of nematodes is covered with a selectively permeable cuticle, exposed to different concentrations of elements. In critical situations, cuticle structure is damaged and eventually, the nematodeś life is endangered. Nematodes have been introduced as bio-indicators for environmental systems as well as for assessment of environmental pollutants (Yeates, 2003).

Sarathchandra et al. (2001) found a positive correlation between the increase in plant mass and population density of nematodes in soil of the meadow so that, population density of the soil-inhabitant nematodes was increased by increasing plant density. In the current study, crop type had a significant ($P<0.05$) influence on occurrence and incidence of the EPNs. Accordingly, a significant ($P<0.05$) occurrence of the EPNś population was observed in the field soil of forage crops. Activity of microorganisms, especially nitrogen-fixing bacteria, is clearly observed in rhizosphere of fabaceous plants. Natural occurrence of nematodes in relation to these plants may be due to rich microbial and bacterial population in rhizosphere of these plants. According to the current results, although EPNs were found in agricultural soils, their occurrence and incidence vary depending on type of plant, soil and site characteristics. For example, Steinernematidae are found mostly in forage crops and industrial crops and Heterorhabditidae are found mostly in forage crops and fruit trees. This could be related to diversity and density of insect populations associated with plants grown on agricultural land (Helmberger et al., 2017).

Several species of EPNs have been isolated and identified from soil and insects in different regions of Iran, among which three species of *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* have been greatly reported in the previous studies (Karimi and Salari, 2015). Soil acidity alone has no great effect on activity and population density of soil-inhabitant nematodes, however, survival, pathogenicity, and populations of EPNs have been shown to significantly reduce in acidic and alkaline conditions. Alkalization of soil has been indicated to reduce population of bacterivorous nematodes (Zhao et al., 2015). Low acidity directly, and high temperature indirectly (due to the effect on activity of enzymes, the increased levels of metabolism of body, as well as intensification of proteolytic enzymes) have been found to decrease population of the nematodes (Ingels et al., 2018). Parameters, such as moisture, pH, organic matter, and type of physical structure of the soil have been demonstrated to be effective on population density of the EPNs. Accordingly, the probability regarding the presence of these nematodes in wet, light, and sandy soils, which have low pH levels and organic matter richness, is higher than that of other soils (Kanga et al., 2012). In the current study, EPNs were often found in slightly alkaline (pH 7.5-8) to neutral (pH 7-7.5) soils, i.e., and regardless of plant type, area altitude, and soil salinity, incidence and occurrence of EPNṡ populations were more evident in these soil acidity ranges, which is consistent with other research, in which pH of EPNs-positive soil samples varied from 4.6 to 8 (Hazir et al., 2003).

Studies have shown that Steinernematidae have a wide range of environmental resilience (Abate et al., 2017); hence they have been found in a variety of agricultural lands and soils (Leite et al., 2018). Steinernematidae are relatively cold-loving species (Hazir et al., 2001), however, they have been also found in some tropical (Hawaii, USA) and temperate (Europe) regions (Hominick et al., 1996). Seemingly, Heterorhabditidae belong to subtropical regions (Grewal et al., 1994), and this species of EPNs has been also seen in relatively cold and temperate regions (Griffin et al., 1999). Typically, altitude also influences climatic conditions, especially temperature and consequently, choice of cultivable crop. In the current study, the highest incidence of EPNṡ population was observed at altitude range of 1250-1500 m. Incidence was decreased by increasing altitude from 1500 to 2000 m. The highest incidence level of Steinernematidae was observed at altitudes of 1250-1500 m, and the highest incidence level of Heterorhabditidae was observed at a lower range of altitude, 1250-1500 m. It seems that the probability of observing and isolating the Steinernematidae population has been increased by increasing altitude and decreasing soil temperature.

Apparently, amount and level of EPNṡ occurrence depend on sampling location, soil quantity of sample, and type and number of bait. Regardless of geographical location, temperature, rainfall, and vegetation type of the study site; normally, sampling of soils with a large population of soil-inhabitant pests, increasing quantity of soil samples, and increasing number of sensitive bait can play an effective role in increasing occurrence and incidence of EPNṡ populations. Thus, paying attention to these factors in the future studies can increase and improve the knowledge about characteristics of nematode populations.

CONCLUSIONS

As a conclusion, natural occurrence (%) of EPNṡ populations was estimated as 2.8% in agricultural lands of the area under study. Also, the highest and lowest incidence (%) of EPNṡ populations was obtained as 15.52-33.4% in positive-EPNs samples. Level of natural occurrence and incidence was often correlated with crop type, area height, and soil acidity and salinity. The highest occurrence of EPNs was observed in forage crops, vegetables, and industrial crops, in altitudes of 1000-1250 m, salinity values of <0.4 ds/m, and pH values of 6.5-7, and the lowest occurrence of EPNs was observed in cereals, in altitudes of <1000 and 1750-2000 m, salinity values of 1.6-2 ds/ m, and pH values of <6.5 and 8-8.5. The highest incidence of EPNs was observed in forage crops, in altitudes of 1250-1500 m, salinity values of 0.4-0.8 ds /m, and pH values of 7-7.5, and the lowest incidence of EPNs was observed in cereals, vegetables, and industrial crops, in altitudes of 1750-2000 m, salinity values of 1.6-2 ds/ m, and pH values of <6.5.

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