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Abundance of rhabditid nematodes in agricultural soil of Khorramabad, Iran

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ABSTRACT

This study was undertaken to evaluate the factors affecting the abundance of rhabditid nematodes in the farms of Khorramabad city of Lorestan province, Iran. Nematode communities as well as rhabditids population reacted to the soil pH and the type of crops cultivated at each sample point. Crop type affected nematode populations mainly due to their root systems. Also, the abundance of rhabditid nematodes decreased at lower pH of the soil. Since some of the nematodes are entomopathogenic hence, results of the present study are important in terms of biological control of soil pests which leads to preservation of agro-ecosystem through less use of chemical pesticides and achievement of a sustainable agriculture.

KEY WORDS: NEMATODES, ROOT SYSTEM, SOIL PH

INTRODUCTION

Rhizosphere soil often contains a variety of soil pests that are harmful to the host plant. Potentially, chemical method is the first choice of farmers to cope with this problem. However, in addition to forcing heavy costs, it either endangers the health of human and animals or has harmful side effects on the environment. As stated by Brussaard (2012), soil ecosystem services are benefits derived from ecosystems that are necessary to maintain soil health and productivity; they are delivered by the ecosystem functions of soil organisms. Studies have shown that many soil mesofauna, including nematodes in several trophic levels, are one or two steps higher in the food chain than microbes. Also, their generation time is longer than that of the metabolically-active microbes, making them more temporally stable rather than fluctuating with ephemeral nutrient flushes (Nannipieri et al. 1990) . Furthermore, nematodes have been widely used as indicators of soil biodiversity and functioning and as indicators of environmental disturbances (Bongers & Ferris 1999; Neher

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2001, Ferris et al. 2001; Yeates 2003 Ferris & Tuomisto 2015; Steel & Ferris 2016) .

Soil nematodes play a central role in the soil food web and linkage to ecological processes therefore, they have been considered as a tool for testing ecological hypotheses and understanding biological mechanisms in soil (Neher 2010).Guilds of soil biota are closely associated with different ecosystem functions. In this regard, Carrascosa et al. (2014) reported a positive and significant relationship between soil suppressiveness, soil food web structure and nematode diversity. Suppression of pest and disease organisms is an ecosystem service that is the outcome of the ecosystem function of biological population regulation (Brussaard 2012, Steel & Ferris 2016) .

Rhabditid nematodes are an interesting zoological taxon. They are very abundant in all types of soil and sediments of freshwater bodies and play important ecological roles mainly as primary consumers their free-living forms display saprophagous or bacteriophagous feeding habits but also as animal parasites, in particular enthomopathogenic forms (Abolafia & Peña-Santiago 2003). Previous researches have shown that some soil physico-chemical characteristics such as texture, pH, bulk density, soil water potential, temperature, organic content" can affect the nematodes behavior (Gruner et al. 2007; FAO, 2017).

However, further investigation is still needed on factors affecting their population distribution all over the world (Stuart et al. 2006). Lorestan province with an area of about 28,392 km^2 (1.7% of the country area) is located in the south-western Iran at the Zagros Mountain hillside and is influenced by the Mediterranean climate. According to statistics released by the Ministry of Agriculture in 2015, the province is one of the most important centers of agriculture (535,947 hectares of cultivation area with 2,169,818 tons of agricultural products). Therefore, development of biological control in the province could lead to saving on the costs of agriculture, as well as protection of the environment.The aim of this research was to study the abundance of Rhabditid nematodes under the effect of pH and crop type cultivated in the farms of Khorramabad city of Lorestan, Iran.

MATERIALS AND METHODS

STUDY SITE AND SOIL SAMPLING

The study site (longitude from 48° 0› to 48° 55' E and latitude from 33° 20' to 33° 42' N) is located in the region of the Khorramabad's agricultural lands, Lorestan province, Iran (Fig. 1). Samples were randomly taken from a depth of 15 to 30 cm of rhizosphere soil during

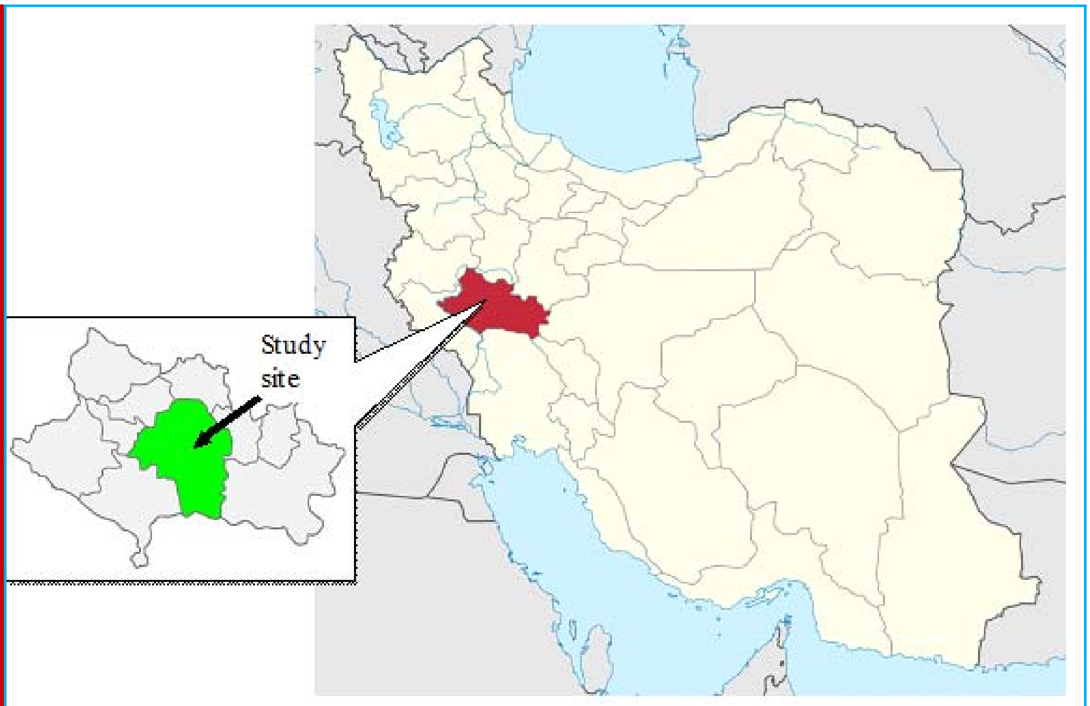

FIGURE 1. Geographical location of the study sitelocated in the region of Khorramabad city (marked with green on the left side) of Lorestan province (colored in red)

the growing season of September and October. Depending on each farm area, sampling intervals was varied from 20 to 25 m. The geographical coordinates as well as altitude were recorded by a GPS device model Garmin ETrex Vista HCX (data are not presented). Also, the type of cultivated crop in each sampling point was recorded. In total, 175 soil samples were taken from 24 different crop types including: alfalfa, apple, apricot, barley, clover, corn, eggplant, garlic, grape, green beans, mung, oak, pea, peach, pepper, pomegranate, radish, red beans, rice, tomato, turnip, vegetable, walnut, wheat. The acidity of the soil samples was also determined in the laboratory by using a pH meter.

NEMATODE EXTRACTION

Nematodes were extracted from soil samples using a modified Baermann funnel procedure (Viglierchio & Schmitt 1983). For this purpose, plastic trays having a sieve (mesh no 10) were used. First, trays were filled with water and covered with a layer of tissue paper. Afterward, soil samples were spread on tissue paper. After four days, nematodes were extracted using a sieve (mesh no 400) from the water and were transferred to Petri

TABLE 1. ANOVA of frequency of nematode populations studied in some different farms/gardens of Khoramabad of Lorestan province, Iran.

		Mean Square	
Source	DF	Total Nematodes	Rhabditids
Type of cultivated crop	23	4910.86 *	15.06 *
Error	151	2572.58	8.50
Total	174		

*significant difference at the 0.05 of probability level

TABLE 2. Descriptive statistics related to the frequency of nematode populations studied in some different farms/gardens of Khoramabad of Lorestan province, Iran.

Type of cultivated crop	Number of studied farms/gardens	All nematodes				Rhabditid nematodes			
		Sum	Mean ± SE	Min	Max	Sum	Mean ± SE	Min	Max
Alfalfa	9	993	110.33±18.58 ab	27	230	47	5.22±0.81 ab	1	9
Apple	13	1394	107.23±18.4 abc	9	264	44	3.38±0.59 ab	0	7
Apricot	5	523	104.6±18.85 abc	53	157	22	4.4±1.5 ab	1	10
Barley	25	2257	90.28±10.57 abc	8	205	82	3.42±0.6 ab	0	10
Clover	7	593	84.71±16.16 abc	16	146	32	4.57±1.56 ab	0	13
Corn	16	1894	118.38±14.37 ab	30	288	67	4.19±0.44 ab	2	7
Eggplant	7	528	75.43±9.34 abc	43	104	21	3±0.72 ab	1	7
Garlic	2	46	23±18 c	5	41	2	1±1 b	0	2
Grape	7	426	60.86±18.33 abc	3	136	20	2.86±1.08 ab	0	8
Green beans	3	129	43±16.77 bc	13	71	4	1.33±0.88 b	0	3
Mung	6	391	65.17±11.99 abc	18	97	20	3.33±0.56 ab	1	5
Oak	6	331	55.17±15.9 abc	4	100	6	1±0.37 b	0	2
Pea	3	112	37.33±4.91 bc	31	47	8	2.67±0.88 ab	1	4
Peach	2	236	118±75 ab	43	193	10	5±3 ab	2	8
Pepper	3	322	107.33±22.7 abc	62	132	8	2.67±1.33 ab	0	4
Pomegranate	3	280	93.33±22.58 abc	56	134	10	3.33±1.33 ab	2	6
Radish	2	228	114±38 ab	76	152	13	6.5±0.5 a	6	7
Red beans	2	75	37.5±5.5 bc	32	43	3	1.5±0.5 b	1	2
Rice	8	336	42±15.59 bc	2	122	5	0.71±0.42 b	0	3
Tomato	5	403	80.6±11.95 abc	58	122	16	3.2±0.73 ab	1	5
Turnip	12	1562	130.17±12.05 a	36	188	78	6.5±1.25 a	1	16
Vegetable	6	500	83.33±17.69 abc	43	152	11	1.83±0.54 ab	0	3
Walnut	2	230	115±30 ab	85	145	9	4.5±0.5 ab	4	5
Wheat	21	1883	89.67±13.52 abc	10	247	80	3.81±0.99 ab	0	18

Means have been compared using Duncan multiple range method.
Means with the same letter are not significantly different.

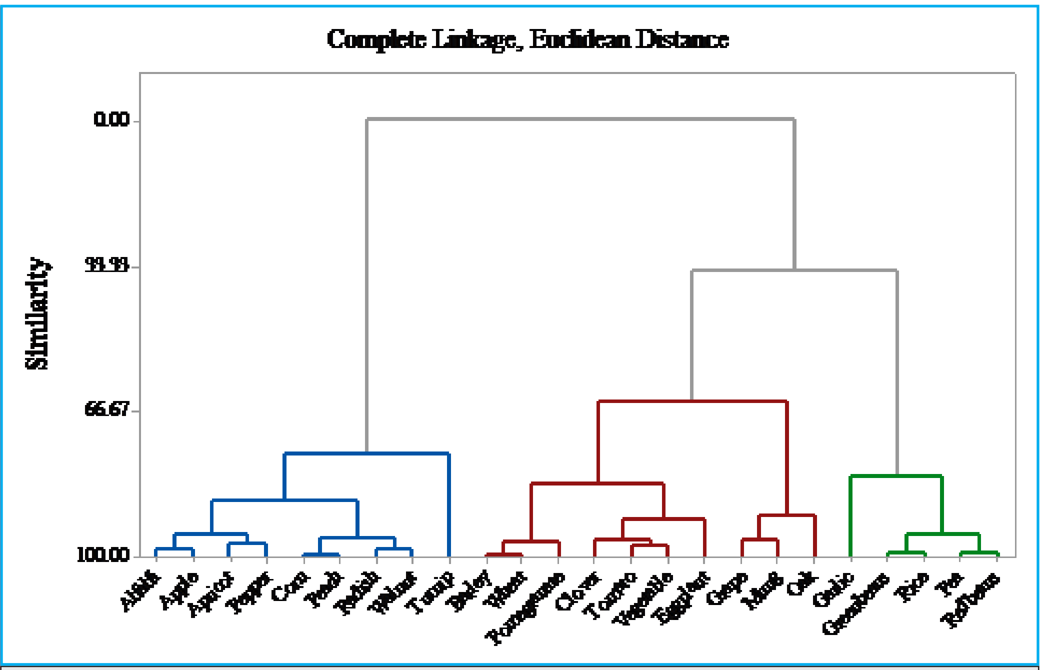

FIGURE 2. Dendrogram obtained based on complete linkage method and Euclidean similarity matrix by using data recorded for abundance of all nematodesas well asRhabditid nematodes in some different farms/gardens of Khoramabad of Lorestan province, Iran. Dnedrogram was drawn using Minitab software.

dishes. The total number of nematodes as well as the number of Rhabditids were counted using a stereomicroscope at 40x of magnification. Statistical analyses were carried out using SAS, Excel and Minitab software.

RESULTS AND DISCUSSION

ASSOCIATION BETWEEN NEMATODE ABUNDANCE AND CROP TYPE

Soil ecosystem functioning is of topics in ecological and agricultural studies (Steel & Ferris 2016). Researches have shown that plant composition, soil properties, and microclimates cause changes in composition and structure of nematode populations among soil ecosystems (Neher 2010). The results of counting nematodes revealed that the overall mean abundance of all nematodes in the study site was 89.33±3.94 nematode per 100 g of the soil (n = 175). Also, the mean abundance of Rhabditid nematodes was 3.55±0.23 nematode per 100 g of the soil. According to this result, approximately 4% of the total population of assayed nematodes belonged to the Rhabditids. This ratio was 7.12% (highest) for pea and 1.70% (lowest) for rice. One way ANOVA showed that the abundance of all nematode types as well as Rhabditid nematodes was statistically significantly different among studied crop types (Table 1).

Averagely, the highest and lowest mean abundance of all nematodes was found in the rhizosphere soil of farms of turnip (130.17±12.05, n=12) and garlic (23±18,

TABLE 3. Properties of clusters obtained on the basis of data recorded for Nematode and Rhabditids abundance in some different farms/gardens of Khoramabad of Lorestan province, Iran.

Cluster	Number of observations	Cluster membership (crop type)	All nematodes mean ± SE	Rhabditid nematodes mean ± SE
1	9	Alfalfa, Apple, Apricot, Corn, Peach, Pepper, Radish, Turnip, Walnut	114.16±2.53	4.71±0.43
2	10	Barley, Clover, Eggplant, Grape, Mung, Oak, Pomegranate, Tomato, Vegetable, Wheat	77.85±4.20	3.02±0.31
3	5	Garlic, Green beans, Pea, Red beans, Rice	36.57±3.58	1.43±0.34

Cluster analysis has been carried out using complete linkage method and Euclidean similarity matrix

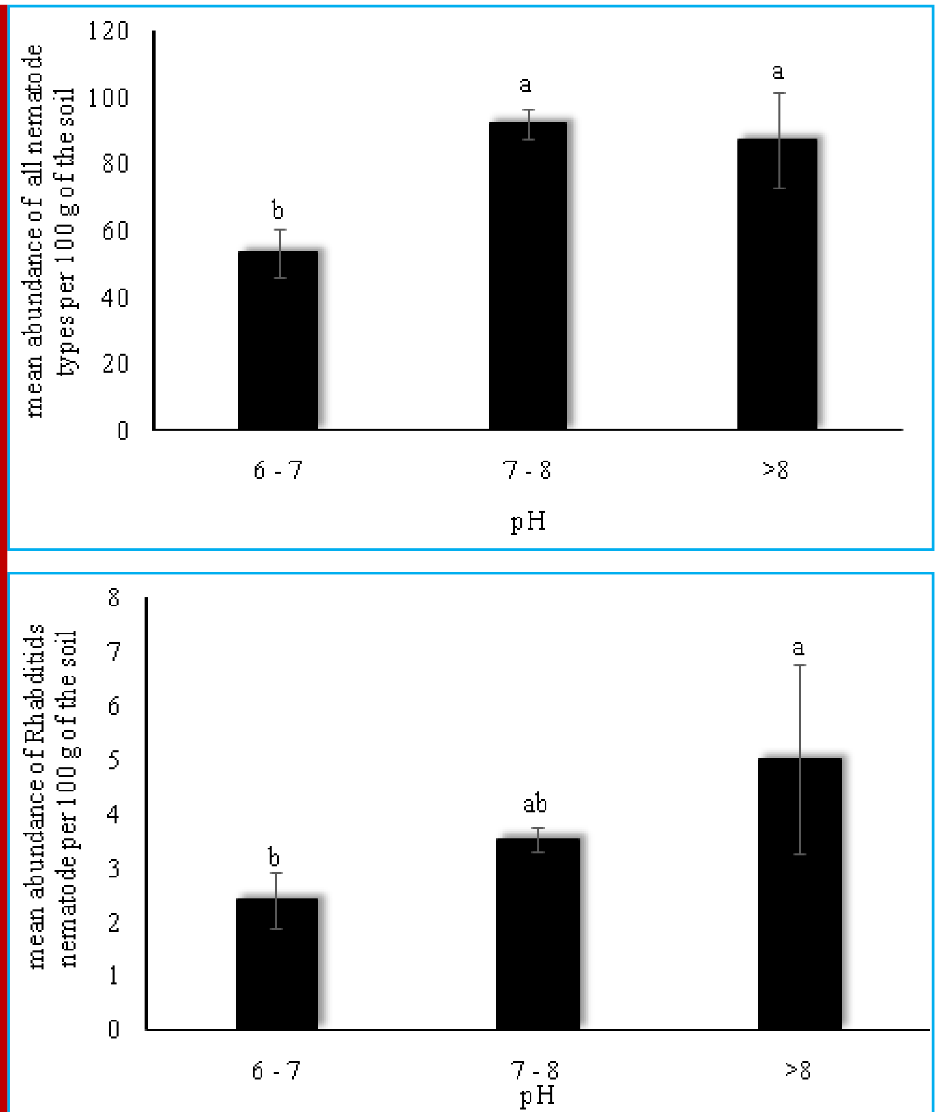

FIGURE 3. The effect of pH on mean abundance of all nematode types (above) and Rhabditid nematodes (below) per 100 g of the soil extracted from some different farms of Khoramabad city of Lorestan province, Iran.

n=2), respectively. Also, the highest and lowest mean abundance of Rhabditid nematodes belonged to radish (6.5±0.5, n=2) and rice (0.71±0.42, n=8), respectively (Table 2). The result revealed that rice rooting system and/ or paddy soil was not a suitable environment for reproduction and growth of Rhabditida nematodes. Also, it seems that glandular root system provided a more suitable environment for the growth and mobility of Rhabditid nematodes than a superficial root system.

As demonstrated earlier, crop type affects the soil ecosystem in different ways. For instance, breeding programs for improving yield lead to the introduction of new high-yielding varieties. Such modified crops have a high potential of absorption of minerals from the soil as well as higher photosynthetic capacity that affect the quantity and quality of nutrients and energy flowing through the soil (Neher 2010). Also, plant life cycle has been known as another factor affecting soil ecosystem. Perennial crops have a more extensive root web and rooting depth than annual crops leading to support a soil community with many omnivores and predators. Populations of terrestrial ecosystems in perennial crops is more similar to that of natural soil ecosystem as compared to annual crops (Freckman & Ettema 1993; Neher & Campbell 1994). Wardle et al. (2003), believe that plant species have greater effects on microbes and

TABLE 4. The pH measured in the soil cultivated with various crops in different farms of Khoramabad city of Lorestan province, Iran.

Crop type	pH				
	Mean	Standard error	Minimum	Maximum	Count
Apricot	7.16	0.21	6.51	7.66	5.00
Grape	7.18	0.15	6.50	7.55	7.00
Radish	7.21	0.09	7.12	7.30	2.00
Rice	7.22	0.07	6.99	7.60	8.00
Green beans	7.28	0.31	6.65	7.60	3.00
Garlic	7.29	0.01	7.27	7.30	2.00
Peach	7.31	0.11	7.20	7.42	2.00
Tomato	7.33	0.02	7.30	7.40	5.00
Barley	7.33	0.04	6.90	8.01	25.00
Mung	7.36	0.08	7.01	7.57	6.00
Corn	7.41	0.09	6.64	7.97	16.00
Alfalfa	7.41	0.09	7.00	7.66	9.00
Pea	7.42	0.19	7.07	7.70	3.00
Wheat	7.44	0.06	7.10	8.00	21.00
Eggplant	7.47	0.14	7.00	8.00	7.00
Turnip	7.48	0.10	7.02	8.06	12.00
Clover	7.52	0.10	7.30	8.06	7.00
Apple	7.56	0.11	6.65	8.05	12.00
Pepper	7.57	0.13	7.30	7.70	3.00
Red beans	7.58	0.13	7.45	7.70	2.00
Pomegranate	7.65	0.05	7.60	7.74	3.00
Oak	7.67	0.11	7.34	8.10	6.00
Walnut	7.68	0.20	7.48	7.87	2.00
Vegetable	7.78	0.09	7.50	8.16	6.00

plant-parasitic nematodes than they do on predatory nematodes. Moreover, rooting pattern of different functional groups of plants (i.e., legumes, forbs) constructs habitats which are more favorable to some species of nematodes than others (Neher 2010). Furthermore, Piś kiewicz et al. (2008) reported that communities of plant-parasitic nematodes can be complex within the rhizosphere of a single plant species. Such as those obtained in this study, the above reports confirm that there are significant differences in soil ecosystems arising from various crops cultivation.

Figure 2 shows the results obtained from cluster analysis of 24 different crops based on nematode abundance observed in each cultivated crop. On this basis, studied crops were classified into 3 groups. The average abundance of nematodes along with the standard error corresponding to each group has been shown in Table 3. Clusters 1, 2 and 3 had 9, 10 and 5 members, respectively. The highest frequency of nematodes belonged to cluster 1 where some crops such as alfalfa, apple, apricot, corn, peach, pepper, radish, turnip and walnut were grouped in it. Also, cluster 3 comprising of garlic, green beans, pea, red beans and rice had the lowest frequency of nematodes (Table 3). With respect to the members of cluster 1 "which had the highest abundance of Rhabditid nematodes" it can be concluded that the root systems arisen by perennial crops (alfalfa), glandular plants (turnip, radish) and trees (apple, apricot, peach, walnut) were more favorable for Rhabditid nematodes. Therefore, in agreement with Freckman & Ettema (1993) and Neher (2010), the result showed that perennial plants had an impact on Rhabditid nematodes population.

EFFECT OF SOIL PH ON NEMATODE ABUNDANCE

Data obtained from the study showed that soil pH in the area of study ranged from 6.5 (Grape) to 8.16 (Vegetable) (Table 4). Figure 2 shows mean abundance of nematodes at different amounts of pH of the soil. Results revealed that the mean abundance of nematodes rose up in soils with slightly alkaline property (soil pH more than 7). The average frequency of Rhabditid nematodes in the soil with pH more than 8 was numerically higher than that of 7-8. However, this superiority was not statistically significant. In general, results of this study showed that the nematode populations significantly decreased at the soil pH less than 7.

As described by Garbeva et al. (2004) and Šalamún et al. (2014), changes in soil pH could have led to direct and indirect effects on the nematode community. Likewise, results of this study revealed that the abundance of Rhabditid nematodes decreased at lower pH of the soil. Korthals et al. (1996), believe that a lower pH enhances the toxicity of heavy-metals through increase of their adsorption to the soil.

Korthals et al. (1996), have shown that nematodes exchange several ions through their cuticle in order to regulate their osmotic pressure. It has been suggested that soil acidification can lead to increasing ion concentrations in the soil pore water to such an extent that nematodes might experience problems in regulating their water status. They also stated that, soil pH indirectly affected the nematode community by influencing food availability, by interfering with the competitive interactions between species, or by affecting the abiotic environment.

In conclusion, results of the present study indicated that crop type as well as soil pH could affect Rhabditid nematodes communities. The outcome is important in terms of biological control of soil pests which leads to preservation of agro-ecosystem through less use of chemical pesticides and achievement of a sustainable agriculture.

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