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Original Research

Occurrence, prominence and severity of root-knot nematodes (*Meloidogyne* sp) associated with okra in Najaf province

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ABSTRACT:

Root-knot nematode *Meloidogyne* sp were investigated for their occurrence, incidence and severity in 41 okra fields and farms located in six different districts of Najaf. Only two species, *Meloidogyne javanica* and *M. incognita*, were detected in all the districts under this survey. Of all samples identified, 69% were *M. javanica* while *M. incognita* constituted 31%. All the districts were infested with the two nematode species, but none of the fields were infected with mix population of both species. The infection was undetectable in only 2% of the investigated fields. The perineal pattern was analyzed for 80 *M. incognita* and 140 *M. javanica* females. Morphological variations within each nematode species were observed. *M. incognita* showed high dorsal arch that slightly shouldered or squarish in most samples (72%), or medium dorsal arch closely surrounding the tail terminus 24%, while the rest (4%) were rounded with smooth striae. The prineal pattern of *M. javanica* on the other hand was found to possess more variations. Distinct lateral lines were detected in all perineal patterns. High proportion (40%) exhibited low to medium high dorsal arch with moderate wavy to smooth striae and 34% had much higher dorsal arch with squarish, while oval to round shaped with wavy striae were found in the lowest proportion. Most okra fields were highly infested and infection severity was affected by plant cultivar, irrigation method and cropping system. Winter surveys on other vegetable crops might be required to confirm the absence of other *Meloidogyne* sp.

Keywords:
Perineal pattern, Gall index, *M. javanica*, *M. incognita*, Okra.

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INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench.) is an important summer vegetable crop grown almost in all parts of Iraq. Regarding to acreage cultivated in the growing season of 2016, okra ranked third after water-melon and cucumber and followed by melon, tomato and eggplant. This comprises 14% of total area cultivated with those crops (ICSO, 2016). The total summer production of okra in 2016 was 46071 metric ton yielded from 28401 donum (≈7100 ha), approximately 4 ton/ hectare. This is very low in comparison with the okra production of Jordan (17.7 t), Egypt (14.1 t) and Barbados (11.1 t) (FAO, 2016). This low production of okra in Iraq is due to many biotic and abiotic factors. Yield loss by the plant parasitic nematodes is one of the most important issues. Amongst these nematodes, the common root-knot nematode *Meloidogyne* sp is the most prevalent nematode associated with the vegetable crops in Iraq (Stephan, 1988; Stephan *et al.*, 1998). Significant yield losses occurred usually due to the frequent high population of this nematode (Barker and Olthof, 1976) and its complicated reproduction mechanism (Perry and Wright, 1998). Moreover, root-knot nematode invasion usually gives rise to infection with some other secondary pathogens (Caperton *et al.*, 1986; Castillo *et al.*, 2003), and may breaks down resistance in resistant plants to certain pathogens (Taylor, 1979).

In spite of okra importance, studies on problems associated with its cultivation are few, especially those related to plant nematodes, including root-knot nematodes. Okra yield losses due to different population densities of *M. incognita* were assessed in Jordan (Younis, 1995). The damage threshold by this nematode was found to be as very low as 15 J2/100 g soil. Also in Jordan, there was another study to screen available okra varieties for resistant or less susceptible to *M. javanica* (Karajeh and Salameh, 2015). In Iraq, most studies on root-knot nematodes were focusing on controlling this nematode mainly in eggplant and tomato (Stephan and Trudgill, 1983; Stephan *et al.*, 1998; Jumaah, 2015) as well as in okra (Alwaily *et al.*, 2011). Very few surveys on root-not nematodes association with vegetable crops were conducted so far. Most of them, if not all, were limited to solanaceous plants, tobacco (Stephan *et al.*, 1977) and eggplant (Al-Kubaicy and Al-Sabe'a, 2014), or beans (Al-Hakeem and Mohamed, 2011). A field survey that provides information on root-knot nematodes species prominence and density (disease severity) associated with vegetable crops spatially okra is needed and beneficial. Such data could be useful for better cropping plans, making the right decision in terms of controlling practices and selecting resistant or less susceptible cultivar, if available, to grow in field infested with a known nematode species. This study, therefore, was done to investigate root-knot nematodes occurrence, incidence, severity and species prominence associated with okra plants grown at the different districts of the province of Najaf.

MATERIAL AND METHODS

Occurrence, incidence and severity of root-knot nematodes

Forty-one okra fields were randomly selected from the total okra fields of six different districts of the Najaf province during 2015-2016 growing season to determine Root-Knot Nematodes (RKN) occurrence (prevalence), incidence and severity. Sample of ten plants /acre (1/4 hectare) were selected from each field in a zigzag pattern**.** Adhering soil to the root system was gently removed and roots were observed for root-knot nematodes infection (presence of galls) evidence. RKN infection severity on each root system was rated (0-8 rating scale) according to Daulton and Nusbaum (1961) galling index, where 0 = Free from galls, 1 = Trace less than 5 galls, 2 = Very slight up to 25 galls, 3 = Slight, 26 to 100 galls, 4 = Moderate, galls numerous, mostly discrete, 5 = Moderately heavy, numerous galls, many are coalesced, 6 = Heavy, galls very numerous, mostly

Table 1. Occurrence, incidence and gall rating of root-knot nematode (*Meloidogyne* sp) associated with okra in the different districts of Najaf

S. No	District	Total No. of fields/infested	Disease occurrence (%)	Total No. of observed plants/ infected	Disease incidence (%)	Gall Rating Range	Gall Rating Mean
1	Abbasiya 1	8/6	66	80/57	71	0-8	7.3
2	Abbasiya 2	9/8	88	90/77	85	3-8	8.3
3	Almuash	4/4	100	50/46	92	3-7	4.2
4	Albonoman	4/4	100	40/32	80	0-6	3.6
5	Hewatim	5/5	100	50/46	92	4-8	8.3
6	Bahar Al-Najaf	11/9	81	110/82	74	0-7	7.6

coalesced with slight retarded root growth, 7 = Very heavy, mass invasion with slight root growth and 8 = Extremely heavy, mass invasion with no root development. This gall rating, based on observations in this survey, was the most fitted in case of okra roots infected with root-knot nematodes.

The occurrence (%) of RKN in each district was calculated as follows (Sipes and Arakaki, 1997):

$$\text{Occurrence (\%)} = \frac{\text{Number of RKN infected fields}}{\text{Total number of fields surveyed}} \times 100\%$$

The percent incidence of RKN for each okra field was determined as follows (Sipes and Arakaki, 1997) :

$$\text{Incidence (\%)} = \frac{\text{Total No. of RKN infected plants}}{\text{Total number of observed plants}} \times 100\%$$

For RKN *Meloidogyne* sp identification

Three infected root system out of each ten were selected and packed in polythene bags, labeled and brought to the laboratory of nematology at the department of plant protection, Faculty of agricultural, University of Kufa.

Root-knot nematodes (*Meloidogyne* sp) were identified according to Eisenback *et al*. (1981) after processing the female perineal pattern as described by Taylor and Netscher (1974). Out of each infected root system, a three galled piece was cut and subjected to female dissection. Galled root tissue around the mature RKN females was gently teased out and females were picked out and placed on a glass slide in a drop of glycerin. Lactic acid and lactophenol (Taylor and Netscher, 1974) were not used as we experienced that the female body tissue worn and ruptured where it was placed in 45% lactic acid for more than 30 min. New razor blade was used to cut off the female posterior end and the body tissues, then were removed by lightly brushing the

Table 2. Prominence of *Meloidogyne* sp associated with okra in different districts of Najaf

S. No	District	No. of *Meloidogyne* populations	*Meloidogyne* sp (%)	
			M. incognita	*M. javanica*
1	Abbasiya 1	2	34	66
2	Abbasiya 2	2	38	62
3	Almuash	2	25	75
4	Albonoman	2	25	75
5	Hewatim	2	20	80
6	Bahar Al-Najaf	2	44	56
7	Average		31	69

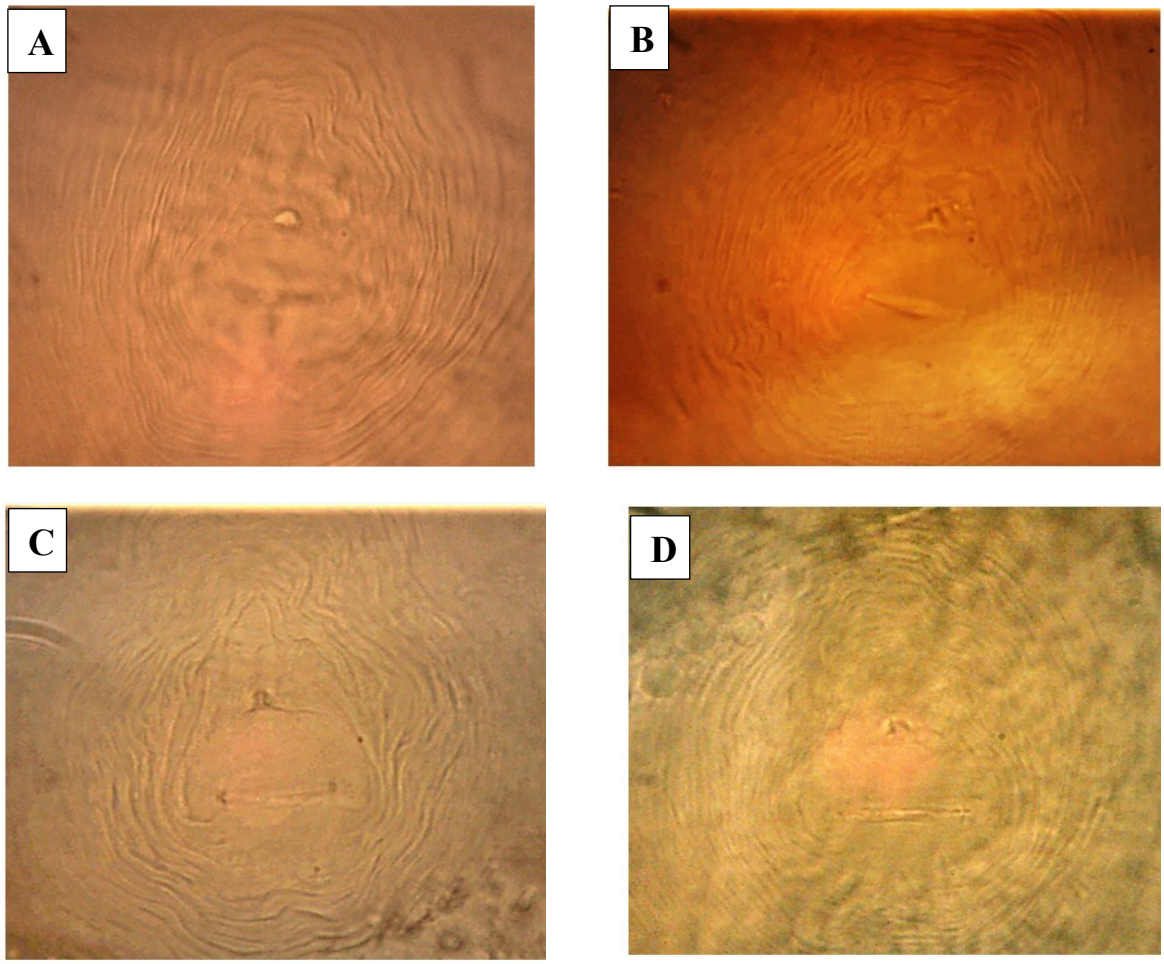

Figure 1. Representative variation of female perineal patterns of *M. incognita* associated with okra from Najaf. A and B: most frequent perineal patterns from surveyed fields; C: perineal pattern with medium high dorsal arch closely surrounding the tail terminus; D: Round perineal pattern with smooth striae.

inner surface of the cuticle using two handmade eyelash brush. The cleaned cuticle was then transferred to a small drop of glycerin on a new glass slide and carefully trimmed keeping the peace with the vulva portion in the center of the specimen. Another micro slide with a drop of glycerin was used to transfer the final peace of cuticle with the typical perineal pattern. After applying a cover slip which was sealed with nail polish, the slide was observed under compound microscope. Following Eisenback *et al*. (1981), standard diagrams were used to compare perineal patterns and *Meloidogyne* sp were identified. Perineal patterns of nine females were subjected and identified from each infected okra field and *Meloidogyne* sp distribution was calculated for each district.

RESULTS

Occurrence of root-knot nematodes: 36 of 41 surveyed fields were infested with root-knot nematodes (Table 1). Maximum occurrence (100%) was found in the districts of Almuash, Albonoaman and Alhwatim although the number of fields were relatively low (4-5 fields), while the minimum (66%) was recorded in Abbasiya 1 (city side). Districts of Abbasiya 2 and Bahar-Alnajaf had RKN occurrence of 88% and 81%, respectively. Disease incidence of root knot nematode differed among fields within each district (Table 1). Districts of Almuash and Alhwatim had the highest root galling disease incidence of 92% for both districts. Lowest disease incidence (71%) was recorded in Abbas-

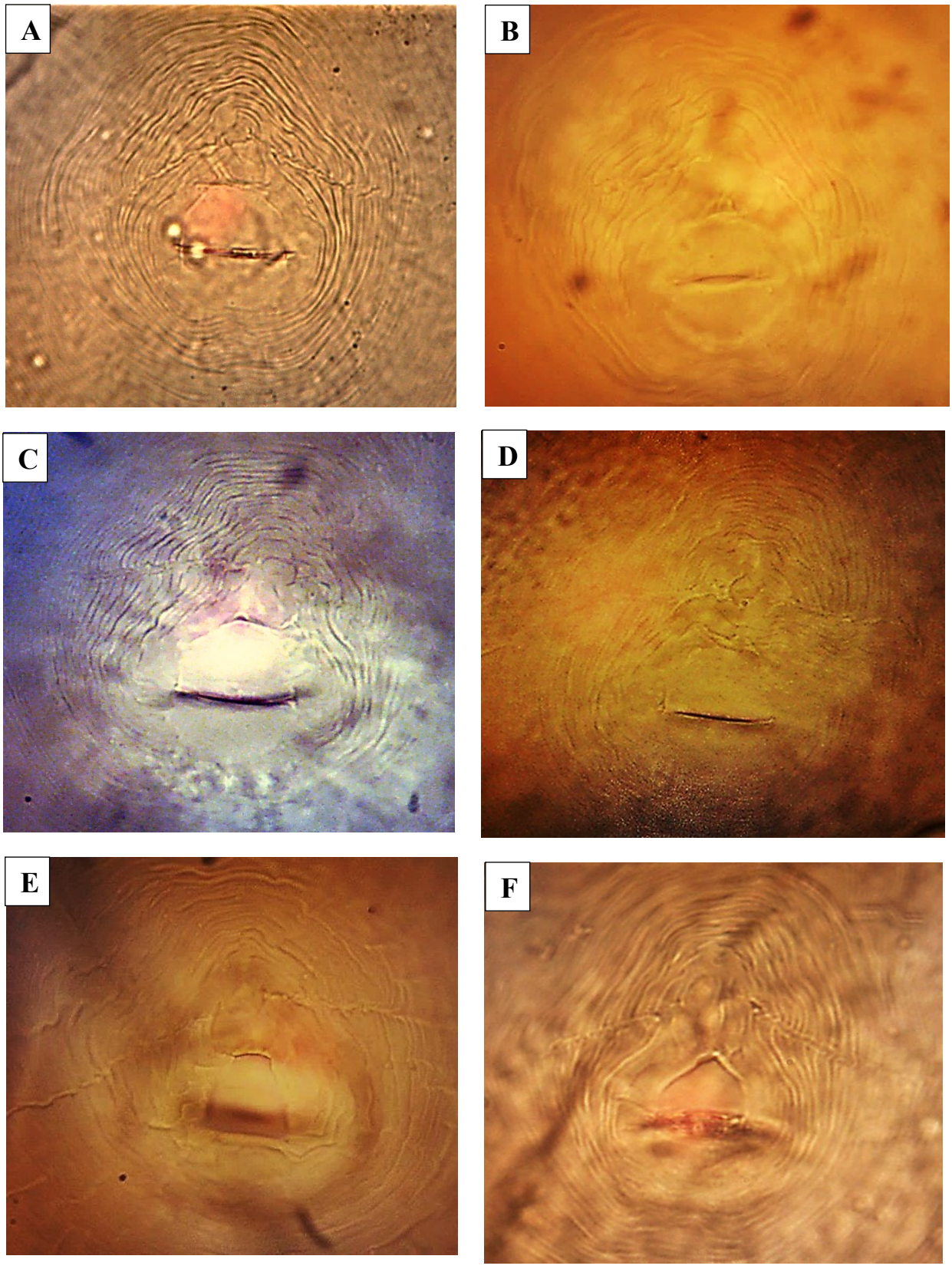

Figure 2. Representative variations of female perineal patterns of *M. javanica* with distinct lateral lines; A and B: low to medium high dorsal arch with moderate wavy to smooth striae are the most frequent shapes; C,D and E: perineal pattern with high squarish dorsal arch and wavy (C), slightly wavy (D) or smooth (E) striae; F: oval to round shape with wavy striae.

iya 1 followed by similar value (74%) from the district of Bahar-Alnajaf, while districts of Albonoaman and Abbasiya 2 had relatively high disease incidence of 80% and 85%, respectively. Based on a scale from 0 to 9, gall rating differed among all the districts ranging from 0 to 8. Generally, except in some cases, a high disease incidence reflects high gall rating value. Although districts of Almuash and Alhewatim had similar disease incidence, but gall rating of the first was almost half of the second. Districts of Abbasiya 1, Abbasiya 2, Hewatim and Bahar-Alnajaf had higher gall rating means ranging from 7.3 to 8.3 than the other two districts with gall rating means ranged from 3.6 to 4.2.

Prominence (frequency of occurrence) of root-knot nematodes species.

Results showed only two RKN species *Meloidogyne incognita* and *M. javanica* were detected and found associated with okra plants (Table2). All the districts showed predominance of *M. javanica* over *M. incognita*, the prominence total average was 69% for *M. javanica* and 31% for *M. incognita*. Results in Table2 also showed that *M. javanica* was at maximum (80%) in Hewatim while the minimum (56%) was found in Bahar-Alnajaf. Mix population of root-knot nematodes species could not be detected in this survey. The perineal pattern was analyzed for 80 *M. incognita* and 140 *M. javanica* females. Morphological variations were observed in both nematode species populations. In case of *M. incognita*, the shapes of the perineal pattern were generally (72%) with high dorsal arch with squarish appearance or slightly shouldered (Figure 1 A and B).

Oval shape of perineal pattern was observed in 24% of the samples, most of them had moderately high dorsal arch with distinct whorl tail terminal area and wavy striae that often bending toward the vulva (Figure 1 C). Few cases (4%) were round shape with low dorsal arch and mostly smooth striae (Figure 1 D). Distinct lateral lines were not observed. Regarding to *M. javanica*, all the observed perineal patterns contained distinguishable lateral ridges (lateral lines) that dividing the striae to dorsal and ventral area. The lateral incisures crossing the entire body length almost meet each other (Figure 2 A, B) or gradually disappear before the tail terminus area. 42% of the perineal patterns showed low and rounded dorsal arch (Figure 2 A, B), 33% were high and slightly squarish (Figure 2 C, D) and contained a whorl in the tail terminal area while 25% had oval to round shape with medium high dorsal arch and wavy striae (Figure 2 E, F).

DISCUSSION

The results of this survey showed that Root-knot nematodes occurrence, incidence and infection severity varied in the different districts of Najaf. Al-Kubaicy *et al*., (2014) reported similar results on *Meloidogyne* sp association with eggplant cultivated in the different fields in Musol. Likewise Taylor *et al*. (1982) based on the results of International *Meloidogyne* Project, which reported the prominence of *M. javanica* over other root-knot nematode species in mid the and south of Iraq. This is confirming our study findings of prevalence and occurrence of *Meloidogyne* sp on okra plants representing summer vegetable crops. The geographical location within a country, which is mostly affected by certain temperature change over the year, seems to have more significance in *Meloidogyne* sp distribution than the soil type (Taylor *et al.,* 1982). For instance, *M. javanica* was found to be most predominant species in Jordan on most vegetable crops including tomato, eggplant and okra (Younis, 1995; Karajeh and Salameh, 2015) regardless the soil type. In our observations, we could not determine a clear relationship of *Meloidogyne* sp and soil type, irrigation method and/or cropping system. However, infection severity (gall rating) was observed to be higher in plant grown in sandy soils especially when the farm is regularly over irrigated. The cropping system was noticed to have effects on infection severity

(McSorley *et al.*, 1994; Sipes and Arakaki, 1997). Okra plants that grown right after a winter crop or weedy fallow showed heavier gall rating and more stunting symptoms (Bhan *et al.*, 2010) than those grown after one year fallow including summer clean fallow (McSorley, 2011).

Morphological features of a *Meloidogyne* adult female including perineal pattern, stylet and head structure can be used for species identification (Eisenback *et al.*, 1980). Although perineal pattern is a reliable feature to identify *Meloidogyne* sp (Chitwood 1949; Eisenback *et al.*, 1980; Hirschmann 1985), significant variability was observed even among females of the same population from a single egg mass (Netscher, 1978; Karssen and Van Aelst, 2001; Carneiro *et al.*, 2004). In our study, however, perineal patterns variability for both *M. incognita* and *M. javanica* were found to be with in possible morphological variability of each nematode species (Eisenback *et al.*, 1980; Eisenback, 1985). *M. javanica* was the most abundant species followed by *M. incognita*. Association of *M. arenaria* with okra roots was reported in Egypt (Ibrahim *et al.*, 1982) and north of Iraq (Al-Sabe and Ami, 1990). These results did not support our findings in Najaf (southwest of Iraq). Since *M. arenaria* usually performs better in cool humid weather (Brown, 1962; Taylor *et al.*, 1982), its absence in this survey may need to be confirmed on some other winter vegetables.

CONCLUSION

Data of our study confirmed that most vegetables cultivation fields in Najaf are infested with root-knot nematodes *Meloidogyne* sp. All okra cultivated varieties or cultivars can be affected by *Meloidogyne* infection to varying degrees depending on the nematode species. Prevalence of root-knot nematodes species are affected by the cultivation and irrigation methods, okra cultivated variety and the cropping system.

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