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MORPHOLOGY ROOT-KNOT NEMATODE *MELOIDOGYNA INCOGNITA* ON CUCUMBER PLANT IN SOUTHERN IRAQ

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Abstract

Isolated root-knot nematodes sp. Meloidogyne from the roots of the cucumber plant from different regions of southern Iraq, and the morphological study showed the presence of type M. incognita. It showed the perineal pattern characterized by the presence of a squarish dorsal arch located at the dorsal apex of the perineal pattern, and jagged and jagged cuticle lines, in addition to the unclear lateral fields and the presence of wrinkles or breaks instead. The measurements of the second larvae phase showed that the average body length (L) was 444 µm, while the largest body length was 496 µm and the lowest body length was 386 µm, the average length of the spear was 11.8 µm, the largest length of the shaft was 12.5 µm and the minimum length was 11 µm. The average tail length was 61.9 µm, the largest tail length was 66 µm, and the minimum tail length was 60 µm. As for hyaline, the largest length was 13 µm and the lowest length was 11 µm.

Keywords: Root-knot nematode, Meloidogyna incognita, cucumber plant

Introduction

The cucumber crop L. Cucumis sativus is one of the most important and widely spread vegetable crops of the Cucurbitaceae family due to its rapid growth, early maturity, abundant production, and constant need to consume it fresh. The fruits contain a quantity of dry matter 4-6%, carbohydrates 2-3%, protein 1-1.5%, 0.6% fiber and 0.1 fat, in addition to 6% mineral salts (Al-Bahash, 2005), It is widely cultivated in greenhouses and open fields, where the number of greenhouses planted with cucumber in Iraq reached 2516.4 dunums, with a total production estimated at 138,400 tons, (Central Statistics Organization, 2018). Cucumbers are infected with many insect and disease pests, and the pathogens of downy and powdery mildew and root knot are among the pathogens. Meloidogyne root-knot nematode is the most dangerous among the types of plant pathogenic worms due to its global spread and wide host range, where it causes a reduction in the quantity and quality of production and greatly affects the marketing of these crops (Gharabadiyan et al., 2013). The genus Meloidogyne is the most important and widespread genera of caecilians. Vegetable crops, including cucumbers grown in warm climates, suffer great losses as a result of infection with root-knot nematodes, which may cause the loss of the entire crop in case of severe infection (Abu Gharbeh et al., 2010). Globally there are more than 90 species belonging to the genus Meloidogyne (Hunt and Handoo, 2009), of which four are widespread and represent 95% of root-knot nematodes in agricultural lands, namely, M. javanica, M. hapla, M.arenaria and M.incognita, to which they are attributed. 90% of the losses are caused by infection with root-knot nematodes (Casagnone-Sereno, 2002). It infects up to 3000 plant families, and in

Iraq, 120 plant families have been recorded to be infected with this pathogen, which made it occupy the first place in the group of nematode plant pathogens (Antoine, 2014).Ahmed et al (1997) confirmed the presence of these species on cucumber, in addition to the type M. hapla, which was recorded for the first time in Iraq by the aforementioned researcher.

Materials and methods

After washing the roots infected with nematodes with tap water to remove dust and suspended matter, the mature females were extracted from them after removing the egg mass from their ends by means of dissection needles and using a stereo microscope. After placing the female on a clean glass slide, her rear end was cut with a sharp scalpel designed for this purpose and it was cleaned from the inner viscera in a drop of lactophenol (phenol + lactic acid + glycerin + distilled water in a ratio of 1: 1: 2: 1) (Southey, 1970).Then it was transferred to another glass slide in a drop of lactophenol and after placing the slide cover, the circumference of the cover and the slide were coated with an adhesive (nail dye) and left to dry. A light microscope examined the sections under magnification of 40 and 100 times. Depending on the characteristics of the perineal pattern of mature females, the types were diagnosed (Tylor and Netscher, 1974) and ten sections were made for each affected root (sample).

Killing, fixing and transferring nematodes

In order to prepare permanent slides and study nematodes, the following steps were conducted: M.incognita egg sacs were isolated directly from the infected plant parts and from the females that were thumb-printed. Eggs were extracted according to Stetina et al. (1997) method. The eggs were placed in a 100 ml flask in an incubator (Binder) at a temperature of 25 ° C for 4 days, after which the eggs hatched to the second larval stage, which was drawn using the De Grisse method (1969). After removing the excess water from the flask, the nematodes were transferred to a small Petri dish 5 cm and by a syringe, a few mml of the fixing solution number one containing 91 parts of distilled water, 7 parts of formalin 40% and 2 parts of triethanolamine were added. Petri dishes containing solution I were kept in a desiccator containing 96% ethyl alcohol in an incubator at 37 °C for 24 h. after this period, The solution was emptied into the Petri dishes first with a syringe and then solution No. 2 containing 95 parts ethanol 96% alcohol and 5 parts glycerine into the Petri dishes.These Petri dishes were kept in the same desiccator in the incubator for at least four hours. After this time, the solution was emptied with a syringe and solution number three with a combination of 50 parts ethyl alcohol and 50 parts glycerine was added to the Petri dish. Petri dishes were placed in the incubator for at least four hours. After complete evaporation of the alcohol, the Petri dishes were stored in a desiccator containing calcium chloride ($CaCl2$) to prevent the absorption of air moisture.

Measurement and drawing of the second instar of root-knot nematodes

To do this, an Olympus BH2 microscope (Tokyo-Japan) fitted with a calibrated drawing tube was used to do so. To prepare a circular slide, a glass slide was placed on it and a circle of paraffin wax was placed on it, transferred by a hollow metal tube after being heated with a flame and immersed

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in paraffin wax. A small drop of aqueous glycerine was placed in the centre of the wax circle on the slide. on her. Then we gently heat the slide until the paraffin melts and surrounds a drop of glycerine (De Grisse, 1969). Eight second-stage larvae of root-knot nematodes were measured. After measuring and identifying the species, the shapes of the nematodes were first drawn on plain paper using a drawing tube and then mirrored onto calcareous paper (gear paper) to complete and show the details of Figure (3).

Results and discussion

Results of the phenotypic diagnosis of root knot worms

The results of the phenotypic diagnosis of the root knot worm Meloidogyne sp. Isolated from infected cucumber roots according to the perrineal pattern of the back of the body of mature females, the presence of a type of worm is M. incognita (Al-Saadi 1985, Al-Sharjabi & Ammi 2006) This is the first study to indicate the presence of the M type. incognita in southern Iraq, where all previous studies indicated the presence of the species was M. javanica (Al-Waeli et al., 2011. Al-Munsha, 2014). This is consistent with Al-Saadi (1985), who found that the most common species were M. javanica, M. incognita and M. arenaria on cucumber in Iraq. Studies indicated the presence of 4 main species in Iraqi soils (M. javanica, M. incognita and M. incognita) arenaria and M. hapla. Figure 1 shows the type diagnosed based on the perineal pattern. The figure shows the perineal pattern of the type M.incognita, which is characterized by the presence of a squarish dorsal arch located at the apex of the dorsal side of the perineum pattern, and jagged and jagged cuticle lines, in addition to the lateral fields are not clear and the presence of wrinkles or breaks instead (Eisenback et al., 1981) .

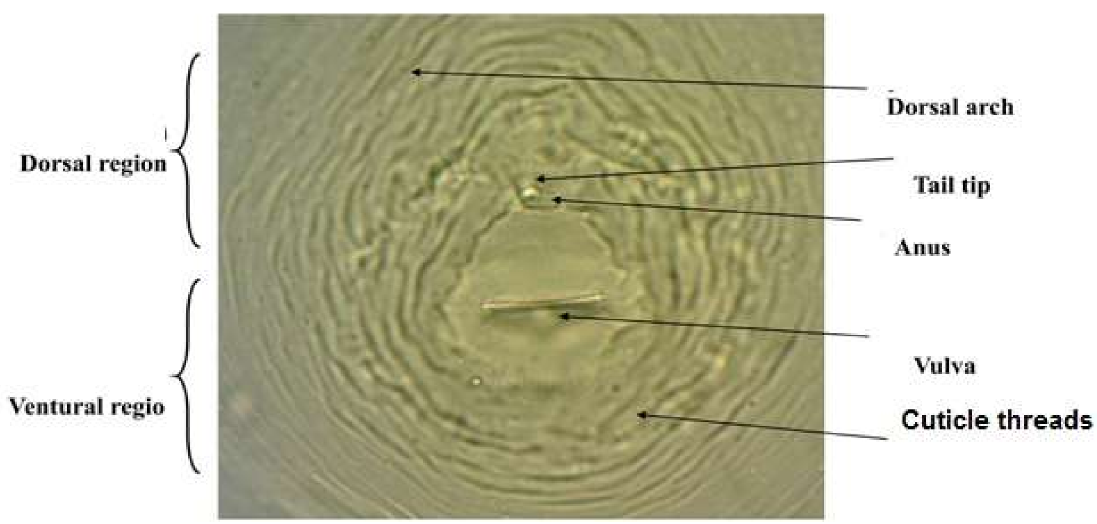

Figure 1. The perineal pattern of the root-knot nematode Meloidogyne incognita

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Figure (2) The head and tail region of the second instar larvae of Meliodogyna incognita

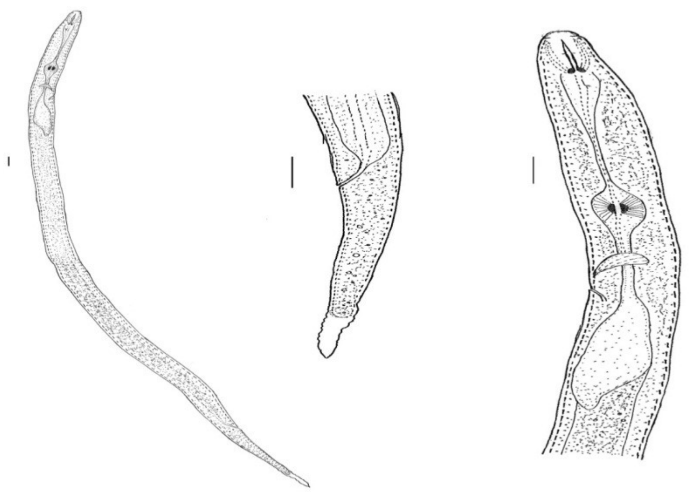

Figure (3) Morphological drawing of second-stage larvae of Meloidogyna incognita. A represents the length of the body B represents the tail area C represents the head Scale (A, B, C 10 micrometers)

Table(1) shows the description of the second larvae phase of the M. incognita nematode. The average body length (L) was 444 µm, while the largest body length was 496 µm, and the least body length was 386 µm, the average length of the spear was 11.8 µm, and the largest length of the shaft was 12.5 µm The minimum length is 11 µm. The average length of the Excretory Pore was 91.6 µm, interaction21.8 µm, DGO was 3.6 µm, the average tail length was 61.9 µm, the

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largest tail length was 66 µm, and the minimum tail length was 60 µm, while the hyaline had the largest length of 13 µm and the lowest length 11 µm.

Table (1) The morphometrics of the second instar larvae of the root-knot nematode Meloidogyna incognita

character	Holotype	Paratype (8n)
L	386.0	444 ± 33.9(386.0 -496.0)
L'	324.0	382.1± 33.8 (324.0 - 430.0)
a	29.7	33.1± 3.7 (29.7 - 39.3)
b	3.6	3.9± 0.5 (3.2 - 4.7)
b'	2.9	3.3± 0.4 (2.7 - 3.7)
c	6.2	7.2± 0.6 (6.2 - 8.1)
c'	6.2	6.4± 0.6 (5.7 - 7.3)
Head Height	3.0	2.3± 0.5 (2.0 – 3.0)
Head Width	5.0	4.9± 0.2 (4.5 –5.0)
Stylet	12.0	11.8± 0.5 (11.0 – 12.5)
Conus	5.0	5.3± 0.5 (5.0 – 6.0)
m	41.7	44.4± 3.3 (41.7 -50.5)
DGO	4.0	3.6± 0.4 (3.0 – 4.0)
O	33.3	30.7± 3.9 (25.0 - 36.4)
Median bulb	65.0	60.5± 3.2 (55.0 –65.0)
MB	60.2	52.9± 5.7 (42.3 - 60.2)
Excretory Pore	94.0	91.6± 7.6 (81.0 - 105.0)
Oesophagus	108.0	115.1± 9.0 (105.0 – 130)
End Of Glands	135.0	136.9± 10.0 (130.0 –155.0)
Overlapping	27.0	21.8± 6.6 (10.0 - 30.0)
Body Width(BW)	13.0	13.5± 1.3 (11.0 – 15.0)
Anal Bady Width	10.0	9.8± 0.5 (9.0 – 10.0)
Tail	62.0	61.9± 2.9 (57.0 - 66.0)
Hyalin	11.0	12.0± 1.0 (11.0 – 13.0)

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