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Characterization of the Root-Knot Nematodes Infecting Olive Trees

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A B S T R A C T

This study was conducted in 2022–2023 to identify the plant parasitic nematodes that infect olive trees. A field survey was conducted in the most olive-growing areas in Bashiqa and Al-Fadhiliya, Nineveh Governorate, Iraq. The results recorded the parasitism of the nematode *Meloidogyne javanica* on the olive roots. The formation of root knots on the roots was the main disease symptom. The phenotypic characteristics and perineal pattern of adult females indicated *M. javanica*, which was used to identify the species. DNA barcoding of the 18SrDNA gene with the primer sets 18S-530R and 18S-CL-F3 yielded a 494-bp fragment to identify *M. javanica*. The sequence blasted with the GenBank database gave a maximum similarity of 97% to the global isolates. The sequence was submitted and deposited with the NCBI as an Iraqi isolate under accession number PP273501. This is considered the first record of *M. javanica* on olive trees in Nineveh Governorate.

Introduction

Root-knot nematodes are highly adaptive obligate pathogens that infect thousands of plants and crops grown in tropical, subtropical, and mild climates. They cause various economic damages and are considered the most economically important plant parasitic nematode populations [1], [2]. The genus *Meloidogyne* spp. generally stimulates the formation of root knots on the roots of a wide range of plants, which is why they are referred to as root-knot nematodes [3]. Root-knot disease is considered one of the main diseases affecting olive trees, especially in nurseries and high-density orchards. This disease is characterized by its rapid spread globally, leading to significant economic losses [4]. This spread is attributed to its wide family range and its interaction with other pathogens, such as fungi and bacteria. This pathogen's cooperation contributes to its complex effects on plants, including creating diseases that are difficult to control. In addition, its ability to break and weaken plants' resistance to other diseases further complicates control efforts [5]. Many species of the genus *Meloidogyne* infect olive trees, like *M. javanica*, *M. incognita*, *M. arenaria*, *M. hapla*, and *M. spartelensis* [6]. Hanoon [7] and Al-Hakeem [8] point out that the first recording of the root-knot nematodes on cucumber roots was in England by Berkeley, while Al-Azami is the first to point out their presence in Iraq. Methods based on morphology, perineal pattern, and molecular diagnosis have been the most accurate way to identify root-knot nematode species over the past few decades [9]. The study aims to identify and describe the morphological characteristics of the root-knot nematodes infecting olive trees.

Materials and Methods

A field survey and sample collection have been conducted in the most important olive-growing areas in Bashiqa and Al-Fadhiliya districts of Nineveh Governorate on trees that show symptoms of general weakness in plant growth, yellowing of the leaves, wilting of some branches, a decrease in yield, and weakness and distortion in the roots. It also includes disease signs such as root galls, egg masses, and different stages of the nematode's juveniles and adults.

The samples were placed in polyethylene bags, the required information was recorded on them, and they were transferred to the plant pathology laboratory at the College of Agriculture and Forestry, University of Mosul, and kept in the refrigerator at 4 degrees Celsius until use [10]. The roots have been washed using a gentle stream of tap water to remove the soil and other plant roots.

Morphological Diagnosis:

Under a dissecting microscope and using a dissecting needle, adult females have been extracted from the root galls by tearing the root tissue. The females have been isolated, killed, and fixed for microscopic examination using heating and 4% formalin [11]. The adult female has been placed on a clean glass slide containing a drop of 4% formalin solution. Its posterior end has been cut using a small, sharp, sterile scalpel designed for this purpose. The cut part has been cleaned using a soft brush to obtain the perineal pattern of the adult female. Then it has been transferred to another clean, sterile glass slide containing 4% formalin. The sample has been closed with a slide cover and sealed [11]. They have been diagnosed under a compound light microscope according to the classification key developed by [12].

Molecular Diagnostics:

Genomic DNA has been extracted from adult female nematode isolates isolated from the roots of olive trees using the AddPrep Tissue genomic DNA extraction kit. PCR reactions have been performed using the forward and reverse primers, 18S-CL-F3 CTTGTCTCAAAGATTAAGCCATGCAT and 18S-530R GCGGCTGCTGGCACCACACTT, to partially amplify the 18SrDNA region [13]. The following thermal cycling conditions have been used: 10 min at 95°C, then 35 cycles of 45 s at 95°C; 54°C; 45 seconds to 1 minute at 72°C; and a 5-min extension step at 72°C. The PCR product has been evaluated on a 1.5% agarose gel, and DNA bands have been detected under UV light (Gel Doc EZ Image, Bio-Rad, USA). DNA has been sequenced by Macrogen, a Korean company. Pathogenicity was tested by preparing *M. javanica* inoculum from the single egg mass method of Hussey [14], where the eggs were incubated at a temperature of 30 °C until they hatched and the second-stage juveniles were obtained. After incubation, two-year-old olive seedlings have been inoculated with 200±5 newly hatched second-stage juveniles by making three small holes approximately 2.5 cm deep near the base of the plant. They have been grafted into sterile soil pots [14].

Results and Discussion

Morphological Diagnosis:

Meloidogyne javanica was diagnosed based on the taxonomic key [12]. The female is characterized by its spherical body shape, which is white in color. The cuticle is striped with transverse lines throughout most of the body. The neck appeared

clear, overlapping the end of the head (Figure 1-A), while the posterior end of the body is rounded and slightly prominent. The stylet is well structured, stomatostylet type, cylindrical, slightly curved towards the dorsal region, and has three nobs, in addition to the presence of egg masses at the posterior end, as in Figure (1-B). This result is one of the most important distinguishing characteristics of *M. javanica* [12] and [15].

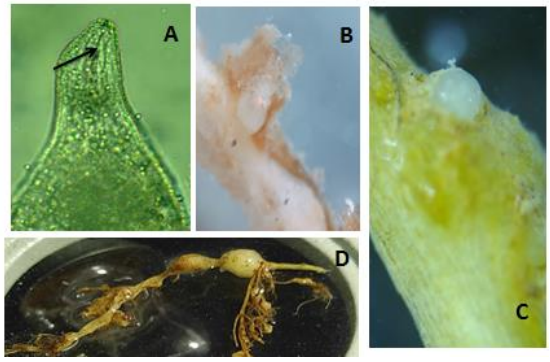


Figure 1. Morphological characteristics of the *M. javanica* female: (A) the head region and (the arrow points to the stylet); (B) the female with egg masse; (C) the female embedded in the root tissue; (D) the root galls.

The perineal pattern of mature females of *M. javanica* shows the presence of two lateral fields. These fields divide the perineal pattern into a dorsal and a ventral part. The dorsal part is characterized by the presence of straight or wavy curved cuticle lines surrounding the end of the cylindrical tail, ending with the vulva and anus clearly visible and close together (Figure 2). These features are consistent with the perineal section, and it is evident that these females belong to the genus *Meloidogyne* and specifically to the species *javanica*. The diagnosis of these characters is in agreement with what has been found [7], [12], and [16].

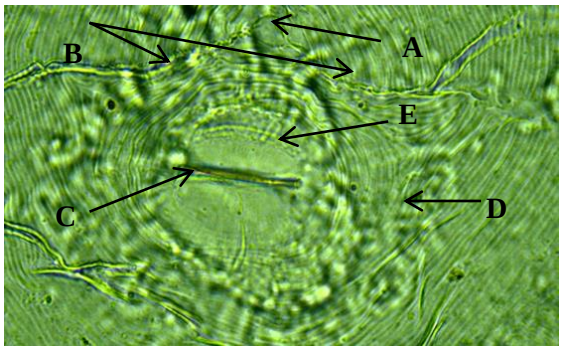
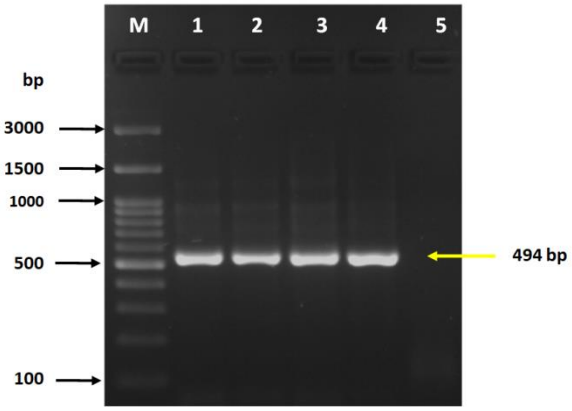


Figure 2. Perineal pattern type of root-knot nematode *Meloidogyne javanica* (a) Tail; (b) Lateral field; (c) Vulva; (d) cuticle lines; (e) Anus**.**

Molecular Diagnostics:

DNA barcoding of the 18S rDNA gene with the primer sets 18S-530R and 18S-CL-F3 generated a 494-bp fragment (Figure 3) that was used to identify *Meloidogyne javanica*. When compared to the GenBank database, the sequence showed a 97% similarity to isolates from both Northeast Brazil and North Carolina, USA [17] and [18]. The sequence was submitted and deposited in GenBank under the accession number PP273501 as an Iraqi isolate. This study marks the first recorded instance of *M. javanica* affecting olive trees in the Nineveh Governorate, as our sources did not mention any previous records of this nematode on olives.

Figure 3. Electrophoresis of the polymerase chain reaction product in agarose gel. (M) DNA ladder, (1-4) *M. javanica*, (5) negative control.



Conclusions:

The results of this research recorded the parasitism of root-knot nematodes on olive trees in the Bashiqa and Al-Fadhiliya regions of Nineveh Governorate. The results of the morphological and molecular diagnosis of the nematode indicated that it belongs to the *M. javanica* species, which is widespread in Nineveh Governorate and Iraq.

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Conflicts of interest

The authors stated that there are no conflicts of interest in the publication of this work.

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