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First Record of the Root-Knot Nematode *Meloidogyne africana* on Albizia Trees in Karbala, Iraq

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Abstract

Albizia (*Albizia odoratissima*) trees in (Al-Husayniyah, University of Karbala, and Al-Hur,) Karbala province, Iraq were found with symptoms of yellowing, stunting, and galls in the roots, symptoms typical of infestation with root-knot nematodes. Morphological and molecular methods were used to identify the nematode species. Morphological characteristics and perineal patterns of the adults fit the description of *Meloidogyne africana*. Moreover, the molecular analysis (PCR), of all screened samples with the specific primer 194/195 set also produced a 667-bp fragment, confirming that the root-knot nematodes isolated from albizia roots were *M. africana*. To the best of our knowledge, this is the first report of *M. africana* infecting albizia trees in Iraq.

Keywords: *Albizia, Meloidogyne africana, Iraq.*

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Introduction

Albizia is a large, fast-growing deciduous tree belonging to the family Mimosaceae, it can grow to 72-85 feet. It is native to Bangladesh, China, Myanmar, and Sri Lanka with many herbal medicines used (Jain and Häggman, 2007; Dinesh et.al, 2011). This tree is infected with many pathogens, including root-knot nematodes (*Meloidogyne* spp.). It is an obligate endoparasitic nematode and is considered the most damaging genus for many agricultural crops worldwide. Infestation with root-knot nematode reduces the quality and the quantity of crops (Moens et al. 2009). Albizia trees have not formerly been recorded to be infected with any root-knot nematode species in Iraq. Diagnosis of root-knot nematode species is the first step in designing effective nematode management practices (Perry & Moens, 2013; Le, et al., 2019). Root-knot nematode species are normally identified using morphological features, and the perineal patterns of mature females (Blok & Powers, 2009; Eisenback, 1985). Moreover, molecular approaches are a substantial tool for the identification of nematodes, which remain fundamental for the accurate diagnosis and can strongly support morphological approaches to root-knot nematodes (Perry & Moens, 2013).

This study aimed to describe the root-knot nematode, *Meloidogyne africana*, infecting the albizia tree, by using morphological and molecular characters.

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Materials and Methods

A survey was conducted in 2021, on albizia trees that displayed above-ground symptoms of yellowing, and stunting, which was observed on farms and gardens at (Al-Husayniyah, University of Karbala, and Al-Hur) within Karbala province. The collected plants showed high infection rates of root galls, which were symptoms of root-knot nematode species.

Sample Collection

Galled roots of albizia trees, with symptoms of yellowing, stunting, and root galls (see Figure 1), were collected (five samples for each area), put in labeled plastic bags, and transferred to the Department of Plant Protection Faculty of Agriculture, Karbala University. The infection percentage was calculated according to the following equation:

$$\text{Infection percentage} = \frac{\text{Number of infected plants}}{\text{Total number of plants examined}} \text{x } 100 \quad (1)$$



Figure 1. Symptoms of Collected Albizia Plant Samples

Morphological Diagnosis

The infested roots samples of albizia trees were washed carefully, and the Female nematodes were dissected from the infected roots under a light microscope using a scalpel and forceps (Fig 2). The egg masses were removed from the posterior end of the females. Perineal patterns slides were prepared by removing the body tissues from the posterior third of the female and mounted in lactophenol as outlined in (Taylor & Netscher, 1974), and examined under a compound light microscope with a magnification of 40x and 100x and identified.

Molecular Diagnosis

Molecular diagnosis was carried out in the advanced scientific bureau (Baghdad) according to the following steps:

DNA Extraction

DNA was extracted from the mature female-root-knot nematodes infected Albizia trees, by the DNA Easy Tissue kit (Genet Bio, Korea) using the manufacturer's protocol, and kept at -70 °C until it was used.

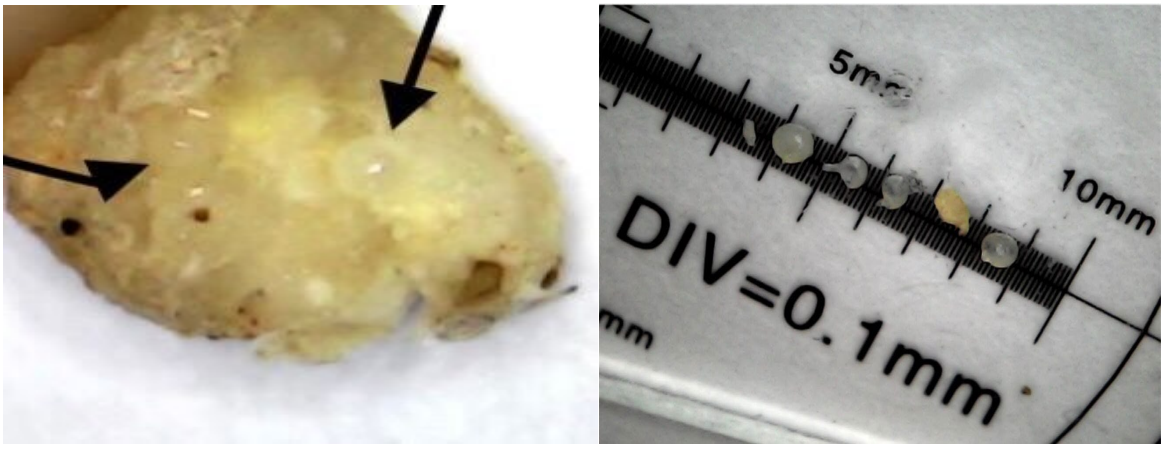


Figure 2. Nematode Females on Albizia Plant

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed by using primer pairs of 194 (5"TTAACTTGCCAGATCGGACG3") and 195 (3"TCTAATGAGCCGTACGC"5), which amplify 720 bp fragments in 18S – 5S region. PCR was performed by a thermal profile with an initial denaturing at 98 °C for 2 min, followed by 35 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 45 sec, terminating with 7 min at 72 °C.

Electrophoresis

Agarose gel electrophoresis was used to visualize the PCR products following amplification. The PCR product was loaded on a (1.5% agarose-gel dissolved in 100 ml of TBE1X * solution and 2 microliters of Red stain) for 60 min at 70 volts. The gels were then visualized under UV light with the help of a 20 µg/ml (EcoDey) stain present within the gels.

Sequence and Phylogenetic Analysis

The sequences' results were verified using the BLAST program in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequences were deposited into the GenBank and compared with the corresponding sequence in GenBank. Phylogenetic trees were constructed based on the nucleotide sequence of Iraqi ON168929 isolates, by the maximum-likelihood method implemented in MEGA6 (Hanoon, and Altememe, 2018).

The Field Experience

The seedlings aged 3-4 true leaves were transferred to plastic pots filled with the soil mixture and peatmoss prepared previously, at a rate of 3 seedlings / pot according to a Complete Randomized Design (CRD) with three replications and left for a week and then inoculated with RKN galls with a rate of 2000 ± 20 second-stage juveniles (J2) and eggs for each pot. The inoculum of nematode was added to the soil by a sterile pipette and inserted during making 4 holes near roots incision 2 cm away from the plant and 3 cm deep, then covered with light soil and watered when needed. After at same concentration of 400ppm for them in three intervals between each of others 15 days. While chemical pesticide (Velum Prime), German company BAYER, was used at concentrations of 400, ppm / liter according to the recommendation each. Em 1400 ppm pesticide, American company Em 1, plant extraction (Palizin) 400 ppm.

Positive control treatment was using inoculum of nematode only while negative control was without inoculum only watering. After the 65-days trial period, the number of knots and gall index were calculated

according to the scale developed by (Taylor and Sasser (1978), which consists of five degrees as in the table below.

Table 1. Root Gall Index According to Taylor and Sasser scales (1978)

Description degree	Description
0	There are no knots on the roots
1	Number of nodes 1 - 2 knots on the root
2	Number of nodes 3-10 nodes on the root
3	Number of nodes 11-30 nodes on the root
4	Number of nodes 31-100 nodes on the root
5	Number of nodes more than 100 nodes on the root

Note: Abdalredaet al., 2024

Statistical Analysis

A Complete Randomized Design (CRD) was applied in the experiment, and the averages were tested using Duncan's Multiple Range at a probability level of 0.05 using the SAS program for statistical analysis (S.A.S., 2012; Ismail, 2022).

Results and Discussion

This survey showed there were different levels in the nematodes infection percentage on albizia plants in the surveyed regions, as the highest percentage of infection (92.3%) existed in the University of Karbala. In contrast, the lowest percentage (38.3) was found in the Al-Hur district, whereas, the Al-Husayniyah district recorded (72.6) of the infection percentage. The discrepancy in the infection percentage may be due to the variation in the number of nematodes that exist in the soil of these areas, and this may be due to the soil texture, pH, or types of microorganisms (fungi or bacteria) in these soils, which some of them may represent natural enemies of nematodes.

The morphological features of the female's perineal pattern (presented below) matched the description for *Meloidogyne africana*, where the perineal pattern with low dorsal arch, lateral lines absent, and the cuticle lines are coarse, wavy, and not separated (Fig 3) (Alinizi, Mehrvar, & Zakiaghl, 2021). The vulva is clear, and the anus is distinguished, this type of root-knot nematode spreads in Africa and infects coffee trees (Hunt, 2018; Whitehead, & Kariuki, 1960; Alinizi, Mehrvar, & Zakiaghl, 2021.).

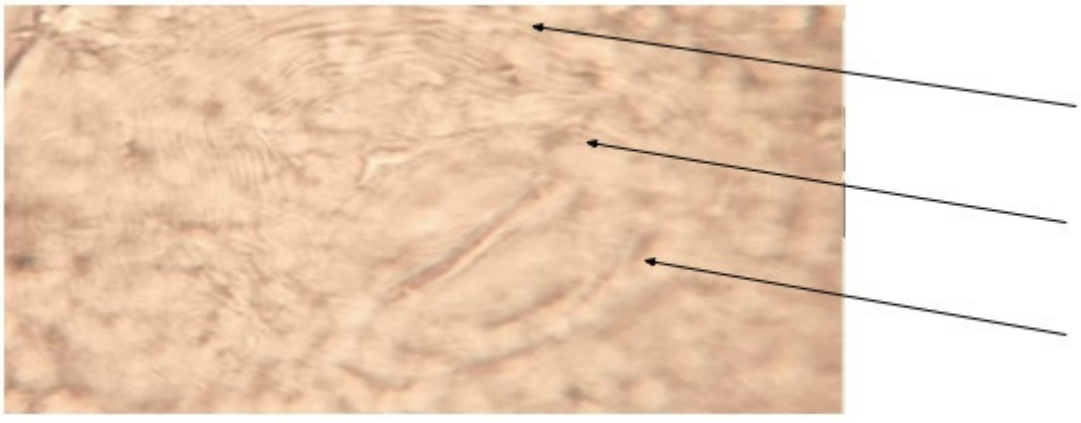


Figure 3. Female's Perineal Pattern of *Meloidogyne africana*

The results of PCR amplification with primers pair (194, 195), which targeted the 18S – 5S region confirmed the morphological diagnosis of *Meloidogyne africana*, which was deposited in the GenBank database under the accession number ON168929. To the best of our knowledge, this is the first report of *M. africana* in Iraq. Moreover, the sequence of our isolate was compared with the corresponding sequences of other *Meloidogyne africana* isolates available in the GenBank. The Iraqi isolate ON168929 shared identity of 99% with Belgian (KY433428, KY433429, and KY433430) isolates.

Multiple sequence alignments were achieved using maximum likelihood (ML) implemented in MEGA6 software (8). The phylogenetic tree was constructed based on the nucleotide sequence of Iraqi ON168929 isolates and the three Belgian (KY433428, KY433429, and KY433430) isolates obtained from the GenBank (Fig 4).

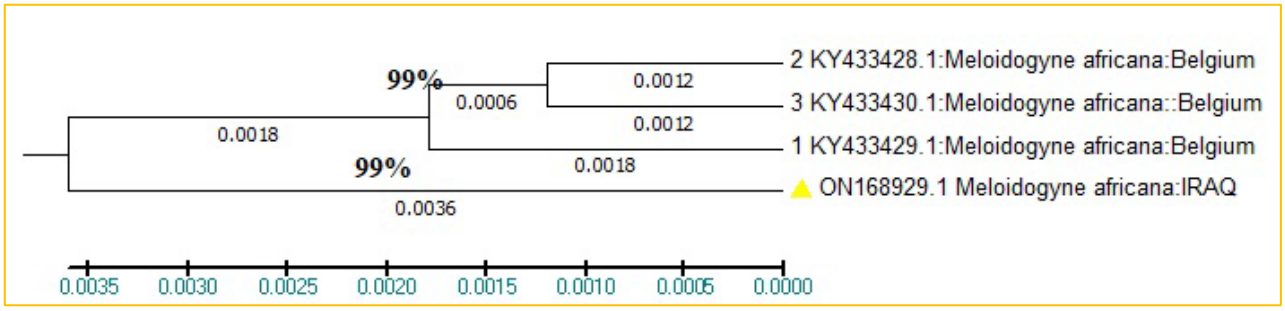


Figure 4. The Phylogenetic Tree was Constructed Based on Nucleotide Sequences of Iraqi ON168929 Isolates and the Three Belgian Isolates Obtained

Table 2. Effect of Using Same Chemical Materials and Plant Extraction, Em1 on the Degree of Infestation and Infection Percentage of Root Knot Galls RKN

Tretment	Infection percentage %	degree of infestation
Velum prime	16.66 bc	1.00 bc
Em1	38.11 b	2.33 b
Pazilen	36.44 b	2.33 b
Con-	0.00 c	0.00 c
Con+	72.22 a	4.00 a

Note: * Similar letters within the same column are not significantly different from each other according to Duncan's test at the 0.05 probability level; *Each value in the table represents an average of three replicates.

Maximum were observed in both negative controls, treatmentsV. was the best treatment which reduced the Infection percentage 16.66%. Then Em1 38.11% , Pazilen 36.44% and con+highe Infection percentage.

Conclusion

Biological effects produced by the degradation of organic matter in the soil could result in some adverse effects or antagonistic action on phytonematode populations in the ecosystem (Bello et al., (2002) indicated that bio-fumigation (gases produced during the bio-decomposition of organic matter in the

soil) is a control alternative based on the use of local organic sources and that could reduce environmental impact from agricultural waste and increases the quality of plant production (Ghazalbash, & Abdollahi, 2012).

The main advantage of plant amendments is that they can be easily and cheaply produced by farmers and small-scale industries in the form of chopped leaves and stems, powders, or partially purified extracts (Ghazalbash, & Abdollahi, 2012). The application of plant materials to soil is an inexpensive and effective technique, and its ease of adaptation will provide additional benefits, leading to the acceptance of this technology by farmers. However, it is important to note that plant materials carry certain risks, such as toxicity to beneficial soil organisms. Further investigations are required to evaluate the potential hazardous effects (Mandal, H. R. et al., 2021).

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