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Research Article

Molecular Characterization of Root-Knot Nematodes on Selected Vegetables Grown in Kano State, Nigeria

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ABSTRACT
Root-Knot Nematode species are morphologically very similar to each other and identification to the species level is difficult. More than one root knot nematode species is sometimes found together in the same plant root. Therefore, fast and accurate identification is needed for management and breeding. *Meloidogyne incognita* is the predominant root-knot nematode species infecting tomato, okra, and capsicum in the surveyed vegetable fields of Kano State. An extensive field survey was carried out from June to September 2025 across four vegetable fields located within Kano State (Bichi, Bagwai, Dawakin/Tofa, and Ungogo). Tomato, okra, and capsicum plants were inspected for above-ground symptoms of nematode infestation. The results revealed that Ungogo LGA was the only location among the surveyed areas with severe and widespread infestation of root-knot nematodes, characterized by profuse galling, chlorosis, wilting, and general plant decline. Infected roots and rhizosphere soil samples were collected from heavily infested fields in the Centre for Dryland Agriculture (CDA, BUK New Campus), located in Ungogo LGA. Nematodes were extracted from soil and Morphological identification was performed using Perennial pattern analysis under compound microscopy, indicating *Meloidogyne incognita*. Molecular analysis further validated this identification; the genomic DNA was extracted from single adult female nematodes using worm lysis buffer, followed by PCR amplification with species-specific SCAR primers SEC1F/SEC2R. The amplified products were separated on 1.5% agarose gel electrophoresis for species confirmation, which produced a distinct amplicon of approximately 502 bp, a diagnostic fragment widely reported for *M. incognita* in species-specific identification studies.

Keywords: *Meloidogyne incognita*; Molecular characterization; PCR; Root-knot nematodes (RKN); SCAR primers; Vegetables

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INTRODUCTION

The name nematode was derived from the Greek word nemas (thread) and adios (resembling), they are triploblastic, bilaterally symmetrical, multicellular, unsegmented, generally microscopic worms having single cavity and reproduce either sexually, hermaphroditically and through parthenogenesis (Deshmukh, 2021). Plant parasitic nematodes are microscopic, thread-like roundworms that inhabit agricultural soils and feed on plant roots using specialized needle-like stylet to puncture and extract cell contents (Senger *et al.*, 2024). Root-knot nematodes (*Meloidogyne* spp.) are among the most destructive PPN, posing major constraints to the cultivation of *Solanaceous* crops such as pepper, tomato, and eggplant worldwide (Santhosh, 2023).

They are obligate biotrophic organisms that cause significant phytoeconomic losses in vegetable production (Senger *et al.*, 2024). The identification of species of RKN by using morphological characters is time consuming and requires skilled personnel (Bogale *et al.,* 2020). Traditionally, RKN populations are identified and counted by nematologists using a microscope, this method is a specialized, time-consuming process and prone to errors (Bahadur *et al.,* 2022). Molecular characterization of nematodes involves a process of using molecular markers to determine the genetic characteristics of nematodes, these markers can include DNA, RNA, and Proteins, some molecular methods used to characterize nematodes include; PCR, DNA Sequencing, Denaturing Gradient Gel Electrophoresis (DGGE), and Pyrosequencing (Ye *et al.,* 2021). Therefore, this research aims to determine the molecular characterization of root-knot nematodes isolated from selected vegetable fields in Kano State, Nigeria.

MATERIALS AND METHODS

Study Site

The study was conducted in Kano State, northwestern Nigeria. The state lies between latitudes 10°03'N and 12°04'N and longitudes 7°04'E and 9°03'E**,** positioning it within the Sudan–Sahel savanna ecological zone of West Africa (Abdullahi *et al.,* 2023).

Survey

An extensive field survey, as described by Sachan *et al.* (2023), was conducted between June to September 2025 to determine the presence, incidence, and distribution of root-knot nematodes (*Meloidogyne* spp.) in major vegetable-producing areas of Kano State, Nigeria. The survey covered four selected Local Government Areas (LGAs), including Bichi, Dawakin/tofa, Bagwai, and Ungogo. During the survey, Tomato, okra, and capsicum plants were carefully inspected in the field for typical above-ground symptoms of root-knot nematode infection, including stunted growth, chlorosis, and general plant decline, infected field found at Ungoggo L.G.A of Kano State were uprooted, and their entire root systems were inspected for gall formation and other signs of nematode infection.

Sample Collection

Soil and root samples were collected from infected vegetable fields found at Ungoggo L.G.A, at a depth of 15–20 cm using a zigzag sampling pattern to ensure representative collection from 10–12 plants per field. From each sampled plant, approximately 1g of infected root material and a composite soil sample were collected. All samples were placed in labeled polythene bags and securely sealed using rubber bands to minimize moisture loss during transport. The samples were then transported to the Department of Biological Sciences, Northwest University, Kano, and stored at 7°C prior to further nematological extraction and laboratory analysis.

Determination of Root-Knot Nematode Species from the Collected Soil Sample

A 100cc soil sample was processed using Cobb's sieving and decanting technique (Coyne *et al.,* 2007; Camacho *et al.,* 2018), followed by the Baermann funnel method (Hooper *et al.,* 2005). After 24 hours, the nematode suspension was collected in a beaker and allowed to settle. The supernatant was carefully decanted to obtain a concentrated nematode suspension, and the final volume was adjusted to 100 ml. Following method of Isa *et al.,* (2024). Air was introduced into the suspension using an aquarium pump to enhance nematode activity and mobility. The suspension was thoroughly homogenized, and 10 mL aliquots were pipetted into a Doncaster counting dish for nematode enumeration.

Morphological Identification

As described by Manzoor *et al.* (2022), 10mL of the nematode suspension was transferred into a Petri dish and examined under a compound microscope. A single female nematode was isolated using an entomological needle and mounted in a drop of water on a microscope slide. The slide was placed on a hot plate at 60°C for a few seconds to straighten the nematode body. The posterior region containing the anus and vulva was dissected using an ophthalmic scalpel and transferred into a watch glass containing lactophenol solution (a mixture of lactic acid, glycerol, distilled water, and phenol crystals in a 1:2:1 ratio) for clearing. Root-knot nematode genera were identified under a compound microscope at 40–100× magnification based on external and internal morphological characteristics, using standard taxonomic keys, photographic references, and published species descriptions.

Molecular Identification of Root-Knot Nematodes

Nematode Extraction from Roots samples

As described by Manzoor *et al.* (2022); Sachan *et al.* (2023); Isa *et al.* (2024), infected root galls were thoroughly washed under running tap water to remove adhering soil particles. The roots were then chopped into small pieces and placed in a Petri dish containing distilled water. The tissue was gently macerated for approximately 5seconds and then rinsed thoroughly to release nematodes into suspension. The resulting suspension was examined under a compound microscope to confirm the

presence of root-knot nematodes and was subsequently used for further molecular analysis.

DNA Extraction

DNA extraction was performed following the method described by Boonrin *et al.* (2024), using nematodes isolated from pure suspensions obtained above. A single adult female nematode was transferred into a 0.2 mL PCR tube containing 20 µL of sterile water. Subsequently, 20 µL of worm lysis buffer (0.2 M NaCl, 0.2 M Tris-HCl, pH 8.0), 800 µg/mL proteinase K, and 1% β-mercaptoethanol were added. The mixture was incubated at 65°C for 1 hour 30 minutes, followed by 99°C for 5 minutes in a Biometra Gradient Thermoblock PCR thermocycler. The resulting DNA was quantified using a Nanodrop spectrophotometer to confirm the present of DNA in the sample and immediately used for PCR analysis.

Polymerase Chain Reaction (PCR)

According to Sowmya and Kalaiarasan (2024), PCR amplification was performed in a 25 µL reaction volume containing 3 µL of extracted DNA and 25 pmol of each species-specific SCAR primer (SEC1F/SEC2R): SEC1F: 5′-GGGCAAGTAAGTAAGGATGCTCTG-3′/ SEC2R: 5′-CGTGGCTATGAAAGAGGTGC-3′ The reaction mixture also contained 3 mM $MgCl_2$, 1× PCR buffer, 0.2 mM of each dNTP, and 0.25 µL (1.25 units) of Taq polymerase (Qiagen, Germany). The PCR cycling conditions were as follows: Initial denaturation: 94°C for 5 minutes, Denaturation: 95°C for 45 seconds, Annealing: 55°C for 45 seconds, Extension: 72°C for 1 minute 40 seconds, Final extension: 72°C for 5 minutes. (Steps 2 to 4 were repeated for 35 cycles).

Gel Electrophoresis

Following PCR amplification, 3 µL of PCR products were separated through 1.5% agarose gel in a 1× TAE buffer (Tris-acetate-EDTA) at 120 volts for 45 minutes. A 100 bp DNA ladder was used as a molecular size marker. The gel was stained with 0.01% ethidium bromide and visualized under UV light. Photographs were taken to document banding patterns. Allele sizes were estimated by comparing fragment migration distances with the 100 bp ladder (Boonrin *et al.,* 2024).

RESULTS AND DISCUSSION

Survey of Root-Knot Nematodes (RKN) in Selected Vegetables (Tomato, Okra, and Capsicum) in Kano State

The results of the field survey conducted from June to September 2025 across selected tomato, okra, and capsicum farms and irrigation sites in Kano State, Nigeria specifically in Bichi, Dawakin/tofa, Bagwai, and Ungogo Local Government Areas (LGAs) confirmed the presence and widespread distribution of root-knot nematodes (*Meloidogyne* spp.) in vegetable production fields of Centre for Dryland Agriculture (CDA), Bayero University, Kano, Ungogo LGA of Kano State. Among the four surveyed locations Three L.G.A Bichi, Bagwai, Dawakin/tofa shows no sign of *Meloidogyne* infections while Ungogo LGA of Kano State, was identified as one of the most heavily infested sites. CDA, field's revealed severe infestation characterized by widespread root galling, plant decline, wilting, and chlorosis. The infected root systems showed abundant and well-developed galls, which are typical diagnostic symptoms of *Meloidogyne* infection (**Fig. 1**). These findings are in agreement with surveyed findings of Boorin *et al.* (2024) and Bacic *et al.* (2025).

Figure 1: **Field survey and sample collection of root-knot nematode-infested vegetable plants in Kano State: (a) root samples exhibiting characteristic galling caused by *Meloidogyne* spp. and (b) infected vegetable field showing symptoms of nematode infestation**

Determination of Root-Knot Nematode Species in the Soil Sample Collected

The results of nematode population assessment showed an average of 20 juveniles per 0.1 mL of suspension extracted from each 100cc soil sample. Within the examined samples, different developmental stages of root-knot nematodes (*Meloidogyne* spp.) were observed, including males, juveniles, and females, as well as characteristic perennial patterns (Figure 2).

Morphological examination revealed that the nematodes exhibited typical diagnostic features of root-knot nematodes. The body was generally saccate and spheroid in shape, with a distinct neck region. The stylet was slender with prominent basal knobs. The oesophageal median bulb was clearly visible, and the excretory pore was located between the stylet knobs and the median bulb. The perennial pattern, which is a key diagnostic feature for species identification, was observed around the vulva and anus and resembled a fingerprint-like structure. On the side opposite the neck region, the striations of the perennial pattern were smooth and wavy, lacking distinct lateral lines. The dorsal arch was high and well developed, with a clearly defined whorl around the tail terminus (Figure 3). These perennial pattern characteristics closely matched those described for *Meloidogyne incognita*. Based on the observed morphological features, the root-knot nematode species associated with tomato, okra, and capsicum plants in the Centre for Dryland Agriculture (CDA), Kano State, Nigeria, was identified as *Meloidogyne incognita*. This was in consistent with the findings of Hajihassani *et al.* (2024) and Yusuf *et al.* (2026).

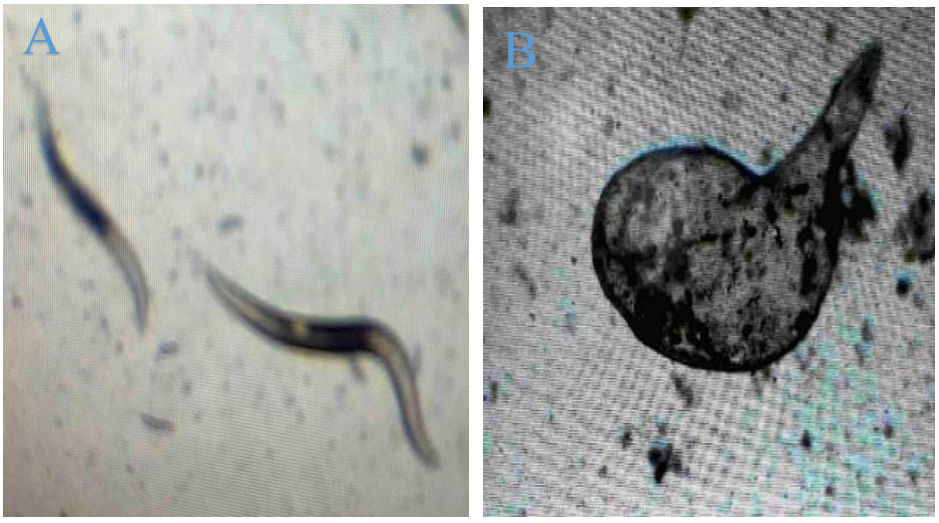

Figure 2: Photomicrographs of developmental stages of root-knot nematodes recovered from infected vegetable fields in Kano State showing: (a) adult male, (b) adult female

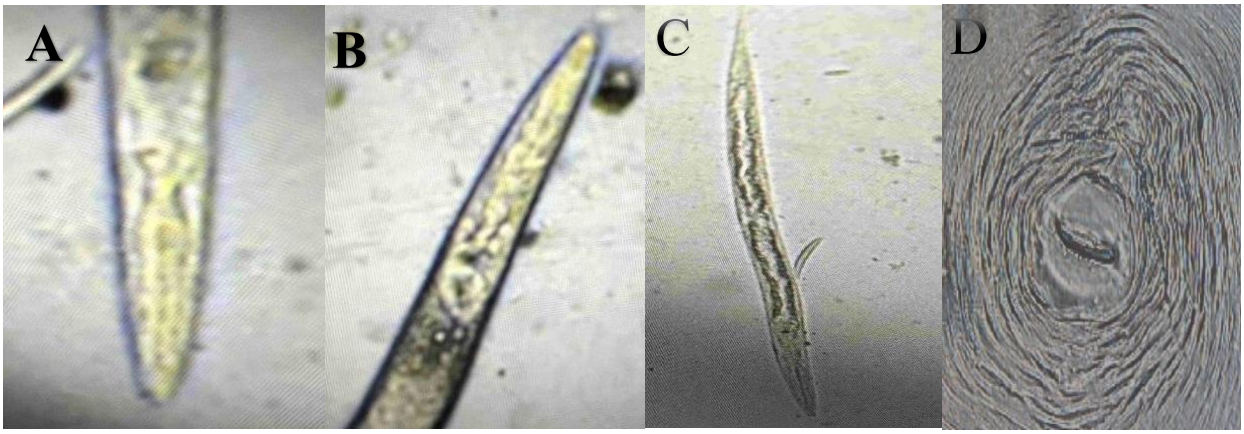

Figure 3: Diagnostic morphological characteristics of *Meloidogyne incognita*: (a) smooth anterior perennial pattern without distinct lateral lines, (b) high dorsal arch with distinct whorl around the tail terminus, and (c) full body view showing the stylet region (d) perineal pattern surrounding the vulva and anus

Molecular Identification of Root-Knot Nematodes

The results of molecular identification of root-knot nematodes infecting tomato, capsicum, and okra in Kano State, Nigeria, corroborated the morphological findings and confirmed that the predominant species present in the study area was *Meloidogyne incognita*. This confirmation was achieved through DNA-based analysis using species-specific molecular techniques. Genomic DNA was extracted from nematode samples using a conventional worm lysis buffer method containing proteinase K and β-mercaptoethanol. The extracted DNA was subsequently quantified using a NanoDrop spectrophotometer to assess concentration and purity.

The rDNA was amplified using the species-specific SCAR primers SEC1F (5'-GGGCAAGTAAGTAAGGATGCTCTG-3') and SEC2R (5'-CGTGGCTATGAAAGAGGTGC-3'), which were synthesized by INQABA Biotec Africa Ltd. PCR products were then subjected to gel electrophoresis in 1× TAE buffer using a 1.5% agarose gel at 120 V for 45 minutes. The amplified DNA fragments were visualized under a UV transilluminator and photographed for analysis. The rDNA region produced an amplicon of approximately 502 bp **(Figure 4).** Using a 100 bp DNA ladder as a molecular size marker, the banding patterns were analyzed and compared with reference profiles of root-knot nematode species. The results confirmed the identity of the nematode population as *Meloidogyne incognita*. This was in accordance with the findings of (Sowmya and Kalairasan, 2024) who used the same primer SEC1F/SEC2R and found *M. incognita* at 502bp, which was also in accordance with the findings of Devran and Sogut (2009); Hajihassani *et al*. (2024) who also used SEC1F/SEC2R Primer and identified *M*. incognita.

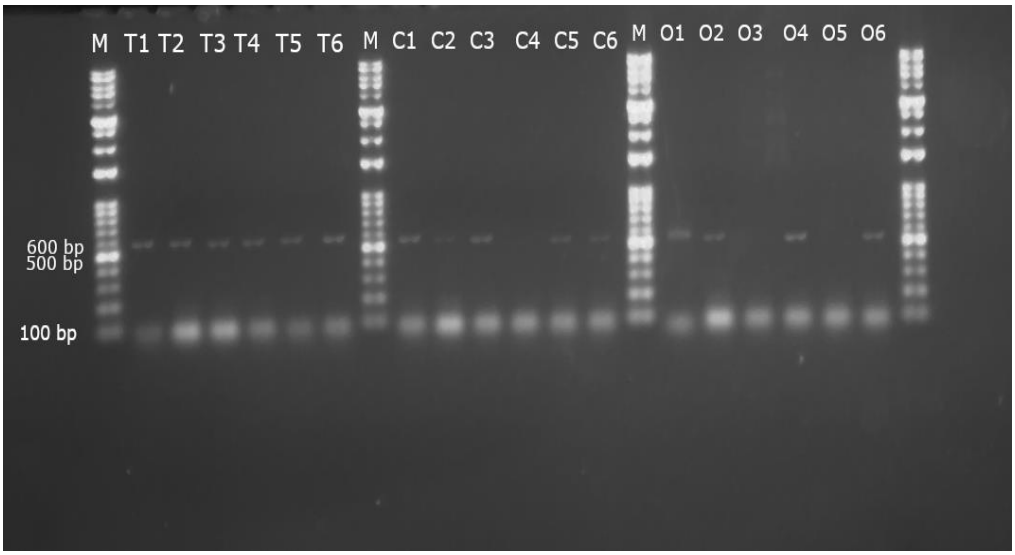

Figure 4: Agarose gel electrophoresis of PCR-amplified DNA from root-knot nematodes isolated from tomato (T1–T6), capsicum (C1–C6), and okra (O1–O6) using species-specific SCAR primer SEC1F/SEC2R showing the diagnostic 502 bp amplicon of *Meloidogyne incognita*; M = 100 bp DNA ladder

DISCUSSION

This study provides the first comprehensive assessment of molecular characterization of RKN on tomato, capsicum and okra in Kano State Nigeria. four local governments were selected for surveying (BICHI, BAGWAI, DAWAKIN-TOFA, UNGOGGO) among the four local govt. selected, symptoms of RKN were only presents in Ungoggo LGA in the vegetable fields of CDA, BUK the symptoms shows damage on tomato, pepper and okra plants which included plant decline, wilt and chlorosis and abundant large galls presented in the almost entire root systems, this proves the finding of (Boorin *et al.,* 2024; Bacic *et al.,* 2025)

The species analysis we conducted based on the analysis of morphological and molecular genetic indicators of root-knot nematodes is important because it was conducted for the first time in Kano State Nigeria, which confirmed the identity of the *Meloidogyne incognita*. Molecular analyses based on rDNA region, corroborated the morphological identification findings. The molecular data confirms the identity of *Meloidogyne incognita* rDNA region yielded approximately 502bp amplicons. The rDNA data revealed that the Root-Knot Nematode affecting

Kano CDA vegetable populations was *M. incognita.* This is in line with the findings of (Hajihassani *et al.,* 2024: Yusuf *et al.,* 2026) who also used a primer to identify *M*. *incognita*. The integration of morphological and molecular approaches provides a robust framework for accurate identification, particularly of the closely related *Meloidogyne spp*. these findings are consistent with previous studies that showed these rDNA regions exhibit limited inter specific variability among these closely related taxa (Rashidifard *et al.,* 2019; Ye *et al.,* 2019*)*. Sownya and Kalairasan (2024) reported that with the SEC1F/SEC1R primers used in identification of nematodes the amplification products of *M. incognita* are of very similar sizes (502bp, respectively).

CONCLUSION

This study provides the first comprehensive assessment of molecular characterization of RKN on tomato, capsicum and okra in Kano State Nigeria. The advent of DNA molecular diagnostic techniques is facilitating faster and more efficient on RKN detection. PCR optimization in this study helps in identification of RKN infecting okra, tomato and capsicum in Kano Nigeria, *M*. *incognita* possess a substantial threat to tomato, okra and capsicum cultivation by impeding both growth and yield, In northern Nigerian vegetable production (Kano State), the Southern root-knot nematode (*M. incognita*) is the most common species, because weather conditions are highly conducive for their rapid development under a host crop (tomato, okra and capsicum) with sufficient soil moisture.

Highly Nematode infested fields should be treated before sowing in order to ensure high qualitative harvest. Further molecular studies should incorporate the use of sequencing to identify root-knot nematode species. The highly impacting of *M*. *incognita* in tomato, okra, capsicum and other plant host in CDA, Bayero University, Kano requires an urgent integration management for this plant pathogen.

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