

***Meloidogyne* Species (Root Knot Nematodes) Associated with Different Climatic Conditions of the *Sorghum bicolor* Production Sites in Telangana, India**

Short Title: Climatic Conditions Associated with *Meloidogyne* Species

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Abstract Although crop damage due to nematodes in Telangana, *Sorghum bicolor* crops has been suspected and sampled occasionally and no study on *Meloidogyne* species (root-knot nematodes) associated with different climatic conditions of the soil has yet to be reported. In the present study, we report the *Meloidogyne* species associated with different climatic conditions (seasonal variations) and edaphic factors like soil moisture, soil pH, organic matter, sand, silt, clay, and soil types of the *Sorghum bicolor* crop production sites, which could be useful to get more knowledge and develop appropriate management strategies. A total of 60 rhizosphere soil samples along with roots were collected from 60 fields of 4 villages to provide comprehensive coverage of the *S. bicolor* production sites in four agro-climatic districts of Telangana. An aliquot from each of the 60 composite soil samples was processed for estimation of the soil conditions like moisture, organic content, pH, sand, silt, and clay. Subsequently, nematodes were isolated by Baermann

funnel and Cobb's method, identified by root dissection, and verified using a microscope. Out of 60 soil samples, 21.2% of *Meloidogyne*-like species were observed at 5.0-7.5% of soil moisture, 27.3% at 17.5-20% of soil organic matter, 30.3% at pH between 7.0 and 7.5, 51.5% at 15-20% of silt, 36.4% at 20 to 25% of the rhizosphere clay and 45.5% of *Meloidogyne*-like species at 50-55% of rhizosphere sand. The distributions of other root-knot nematode species between different soil types were statistically significant (p-value = 0.039). The distributions of *Meloidogyne*-like species between different soils were statistically insignificant (p-value = 0.212). However, the infested roots were often carved in the shape of a hook, horseshoe, or a complete spiral without excessive proliferation of secondary roots which was common in *Meloidogyne*-like species. The results of this study can be used as an advisory to the farmers who intend to take only a particular variety of crops in their field but were unaware of the damage that was happening underground due to

biotic and abiotic factors.

Keywords *Meloidogyne* Species, Root-Knot Nematodes, *Sorghum bicolor*, Different Climatic Conditions

1. Introduction

Sorghum bicolor (L.) Moench belongs to the Andropogoneae tribe of the panicoideae subgroup of the Poaceae grass family [1,2]. Warm-season crop *S. bicolor* performs best in rainy and post-rainy seasons with temperatures from 13 to 38°C, with 32 to 34°C being the ideal range [3]. For optimal growth and yield, loam and sandy loam soil with temperatures above 18°C and a pH of around 5.8 are recommended [4]. The subtropical regions of Uttar Pradesh and Uttaranchal in central and southern India, including Telangana, are most suited for the production of *Sorghum* [5]. The crop is easy to grow, competes readily with weeds, produces reasonably large amounts of food grains, and biomass within 2-3 months at any time of the year, and is proven as a break crop for winter forages and cereals [5,6]. It appears to be a hopeful source for sugar as well as lignocellulosic biofuel production [7]. Improved varieties and hybrids that revolutionized *Sorghum* production in India have evidenced 100% susceptibility to diseases due to varied genetic backgrounds, cultivation time, soil quality, climatic conditions, and other environmental factors [8-10].

Out of all the disease-causing agents, root-knot nematodes (RKNs) of the genus *Meloidogyne* are serious pathogens of paramount importance to *S. bicolor* cultivation with symptoms exhibiting galling, forking, stubbing, and fasciculation of the roots that may reduce crop yield [11-13]. The members of the genus *Meloidogyne* consist of *M. incognita*, *M. arenaria*, *M. javanica*, and *M. hapla* [14,15]. *M. hapla* is the most common *Meloidogyne* species in open fields and cool temperate climatic regions, while the others are tropical RKN species found in hot tropical or warm climates [16]. Human activities such as the introduction of nematode-infected planting materials or seedlings or tubers or young plants into a new field or diffusion of infested soil with nursery practices contribute to the spreading or redistribution of RKNs [17]. They can also be spread to new areas by running water [18], wind [19], cultivation tools, machinery, animals, and footwear [20]. Managing the negative effects produced by RKNs is a difficult task due to the biological traits of both crop and RKN species, including the high complexity of abiotic factors like pH, soil type, organic content, moisture [21], and local climatic conditions [13,22]. Many studies have been designed to understand *Meloidogyne* species,

concerning a wide host range, varied geographical regions, and their occurrence [23]. Similarly, *S. bicolor* crop damage due to nematodes and their distribution in Telangana has been suspected and sampled occasionally; no study on root-knot nematodes of the genus *Meloidogyne* at different climatic conditions of the cultivation sites has yet been reported. Understanding the drivers of community structures at different climatic conditions of *S. bicolor* crop production sites is necessary to increase the knowledge and development of appropriate environmental management strategies for root-knot nematodes [24]. In the present study, we report the *Meloidogyne* species (root-knot nematodes) associated with different climatic conditions (seasonal variations) and edaphic factors like soil moisture, soil pH, organic matter, sand, silt, clay, and soil types of the *Sorghum bicolor* crop production sites.

2. Material and Methods

Collection and Preparation of Soil Samples

Based on the distribution of *Sorghum bicolor* fields, travel, and lodging facilities, four agro-climatic districts Mahabubabad, Ranga Reddy, Khammam, and Sericella were selected, surveyed, and sampled from the start of 2015 to the end of 2021. Permission for sampling from the *Sorghum* field was granted verbally by the landowners. No special permission was required for the indicated fieldwork and sampling. The frequency and abundance of *S. bicolor* fields and incidences of root-knot plant-parasitic nematode disease associated with *Sorghum* crops were determined (**Figure 1**). Details of the preceding crops, use of pesticide, fertilizer, manure, and lesion/ symptoms were recorded in standard sample collection performa. Pre-selected fields of *Sorghum* were sampled to a depth of 15 cm. A total of 60 rhizosphere soil along with root samples were collected from the *S. bicolor* production areas. All such composite rhizosphere soil samples along with root samples collected at different seasons were used for the isolation of root-knot nematodes. The presence of galls was thoroughly investigated in the laboratory using plant and root samples that had been brought in and correctly labeled in polythene bags. Each sample was divided four times through a sample divider after being sieved through a screen with 6-mm holes and properly mixed. Immediately following sample collection, processing began. Utilizing a microscope, the number of galls and egg masses per plant were counted. Gall index (GI) and egg mass index (EMI) were determined on the following scale: 0=0, 1=1-2, 2=3-10, 3=11-30, 4= 31-100, and 5=greater than 100 galls or egg masses per plant [25]. The frequency of occurrence (percentage) of the infection in each locality was calculated using the standard formula [25].

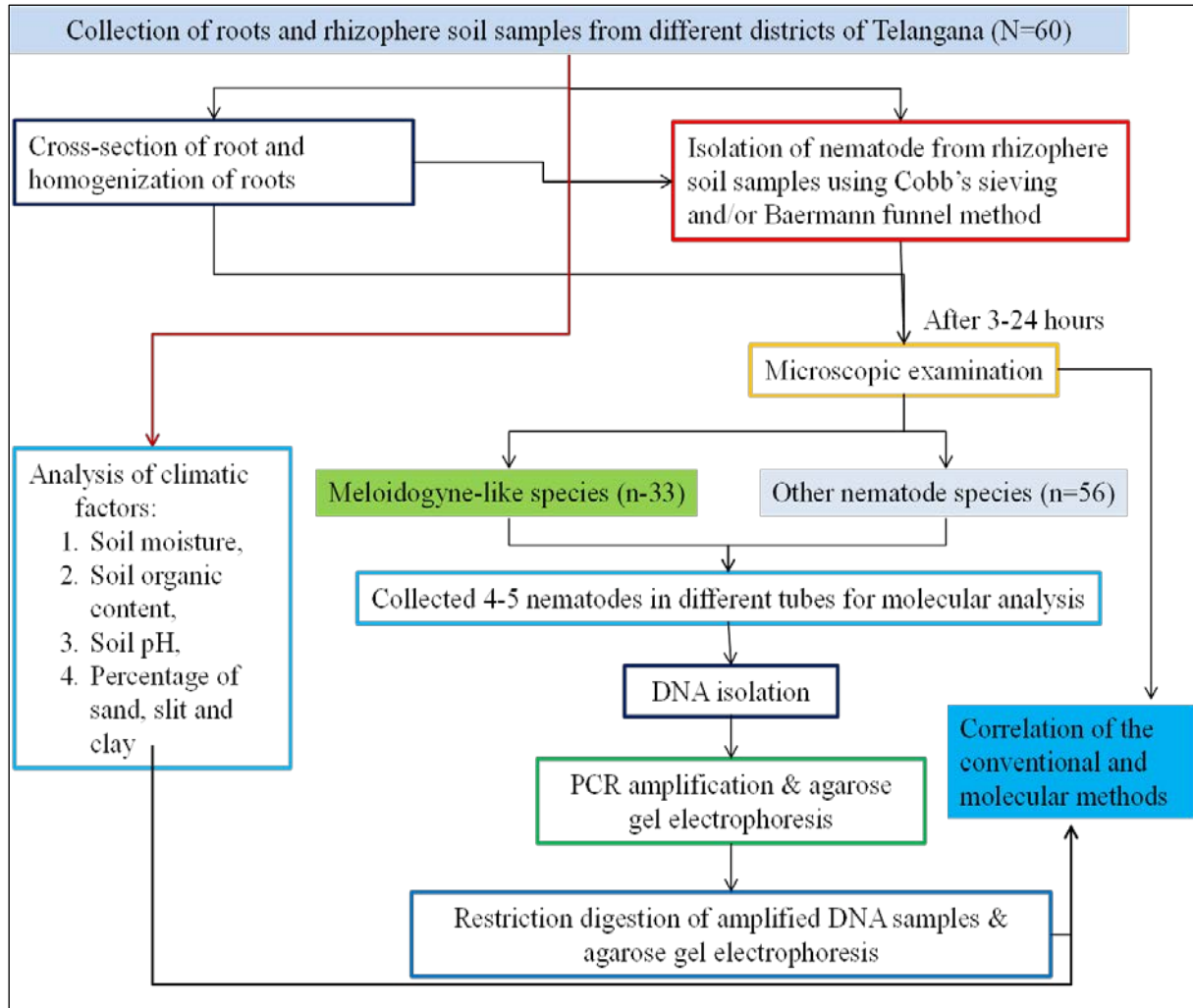


Figure 1. Implementation plan

Assessment of Climatic Conditions Related to the Sorghum Field

Climatic conditions like soil type, soil moisture, organic matter of the soil, soil pH, sand, silt, and clay content of different agro-climatic districts of Telangana were analyzed [26,27].

Soil Moisture

The moisture of the soil was determined by weighing a sample of wet soil (4 grams) that had been taken from the field and dried for 4 hours at 105 degrees Celsius in an oven. The dry soil was then weighed. Gravimetric water content is calculated as the difference between the wet and dry soil masses divided by the dry soil mass. The bulk of the soil was split by the mass of the water, in other words.

The formula for soil moisture:

$$\frac{[\text{Wet soil (g)} - \text{Dry soil (g)}]}{\text{Dry weight of soil (g)}} * 100$$

Soil pH

The pH meter was calibrated before using it with known

pH buffer solutions (weak acid and its conjugate base). To measure soil pH, 10 ml of distilled water was added to a flask containing 10 gms of soil. The mixture was stirred with a glass rod and allowed to stand for 10 minutes. The mixture was then filtered using filter paper to separate the soil from the water. The electrode was then placed into the filtrate to measure the pH levels [28]. The pH value was recorded in the datasheet from the automatic display of the pH meter.

Soil Organic Matter

Four (4) grams of soil collected from the field were kept in the clean, dry, and empty porcelain dish and the mass of the dish was determined and recorded. The soil samples were kept in an oven at 110°C for 2 hours to initiate the gradual burning of the soil and allowed to cool in desiccators before weighing. The dish was placed in a muffle furnace and the temperature was gradually increased to 440°C and kept for 3 hours and then allowed to cool overnight. The incinerated soil was again placed in the desiccators before it was weighed [29]. The mass of the dish containing the ash (burned soil) was determined and

recorded.

The organic matter of the soil sample was obtained by using the formula below:

$$\frac{[\text{Dry soil (g)} - \text{incinerated soil (g)}]}{\text{Dry soil (g)}} * 100$$

Sand, Silt, and Clay Separation Method

Fifteen (15) centimeters (cm) of soil was dug down to collect a cup of soil sample. Soil samples were spread on newspaper and pebbles, and bits of roots, and fluffy organic matter were removed. Soil lumps were also crushed. 25 grams of soil was taken into a measuring jar and filled up to 100 ml with water. The content was mixed well for 5 minutes to break aggregates into components. The jar was kept undisturbed overnight to make the different components settle in layers. The sand, being the biggest and heaviest particle, settled first. The silt layer settled next, usually after one to two hours. The clay layer took a long time to fully sink. Two sand layers, a coarse one and a finer were observed. It was sand, the silt had a tiny bit of texture and the clay was very dense. Clay was colloidal, meaning it tends to stay in suspension. Two days later, the water in the other jar was still cloudy with suspended clay. The layer of organic matter that floats on top of the water was measured.

Extraction and Identification of Nematodes

Root dissecting method: Carefully cleaned roots were put in Petri dishes with water. Using forceps and dissecting needles, the samples were dissected. Under a microscope with a 15–50x magnification, infected plant sections were checked for the presence of nematodes. (Figure 2). The emerging nematodes, egg masses, nematode-induced

expansion of root cells (galls), and damage to the vascular tissues of roots were evident from the suspension with a handling needle or painting brush.

Baermann Funnel technique: Nematodes were allowed to emerge from infected samples and sink when they were mixed in water [30]. Active nematodes were obtained from soil and plant material after 48 hours of extraction.

Cobb's sieving or Gravitation technique [31]: This technique uses many characteristics of nematodes and soil particles, such as size, shape, sedimentation rate, and nematode mobility.

This helps in the removal of active nematodes from soil and sediments.

Statistical Analysis

Simple frequency distribution with methods of central tendency (mean) and dispersion SEM (standard error of the mean) was determined. The data were presented as mean \pm SEM and percentage (%) was determined. One-way ANOVA (between the districts) was performed using SPSS Version 20 for Windows. If the p-value was less than 0.05, then the data was considered statically significant. Distribution of *Sorghum* plants, rhizosphere soil with roots, affected root knots per plant, egg density or egg mass index, and overall nematode population per field by soil type were recorded. The distribution of *Meloidogyne*-like species and other phytonematode species per field by soil type, soil moisture, soil organic matter sand, silt, and clay was recorded. The occurrence of *Sorghum* crop-associated root-knot nematodes (*Meloidogyne* species) and other phytonematodes at different seasons, fields applied with pesticides, fertilizers, and manure, and preceding *Sorghum* crops were recorded.

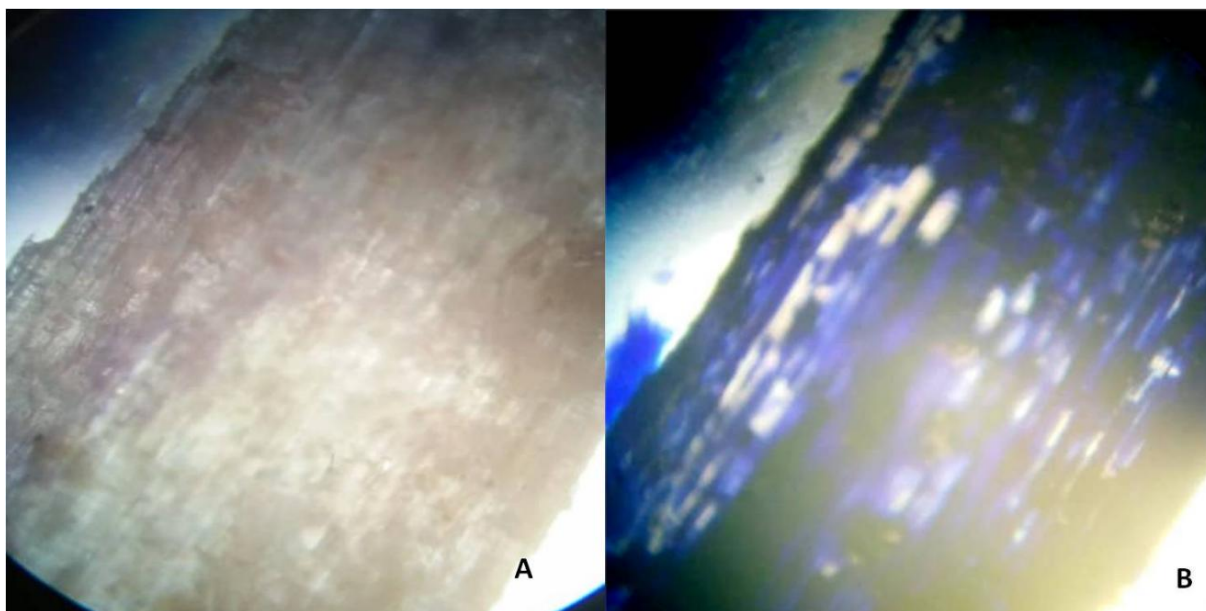


Figure 2. Light microscopic picture “A” represents an unstained cross-section of the root-knot and “B” represents a methyl blue-stained cross-section of the *Sorghum* root-knot

3. Results

The study relied on 60 fields from 4 agro-climatic districts of Telangana. The single-nucleus egg stage to multiple-nucleus egg stage of nematodes along with juvenile stages (1 to 4) of nematodes was shown in **Figure 3**. A female produces a one-cell zygote in an egg, which develops into a vermiform first-stage juvenile (J1) that is contained within the egg. This is the beginning of the life cycle of *Meloidogyne*. The J1 changes into the vermiform, infectious stage (J2), which hatches, travels through the soil or host tissue (for example, in the roots of the plants in which it hatched), penetrates the roots, and then begins eating in the roots and other below-ground sections of a host plant. After the motile J2 becomes immotile and enlarged, it goes on to create the third (J3) and fourth-stage

juvenile stages (J4). The J4 eventually turns into a bloated female or a vermiform male. Female *Meloidogyne* has inflated saccate-like bodies in general. Females were inactive and did not move like J2 or males. However, females have a strong neck area that permits them to alter positions during feeding and consume a variety of enormous cells. The *Meloidogyne* species were characterized by well-developed stylets and overlapping esophagus without longitudinal striations on ventral and lip regions. When at rest or dead, this nematode tends to form a different shape [32,33]. The *Meloidogyne*-like nematodes with curve-shaped, flat bodies with visible intestines, hook or sickle-shaped bodies and spiral or coiled bodies with intestines were observed (**Figures 4 & 5**) along with other phytonematodes with stout, short and rough bodies (**Figure 6**).

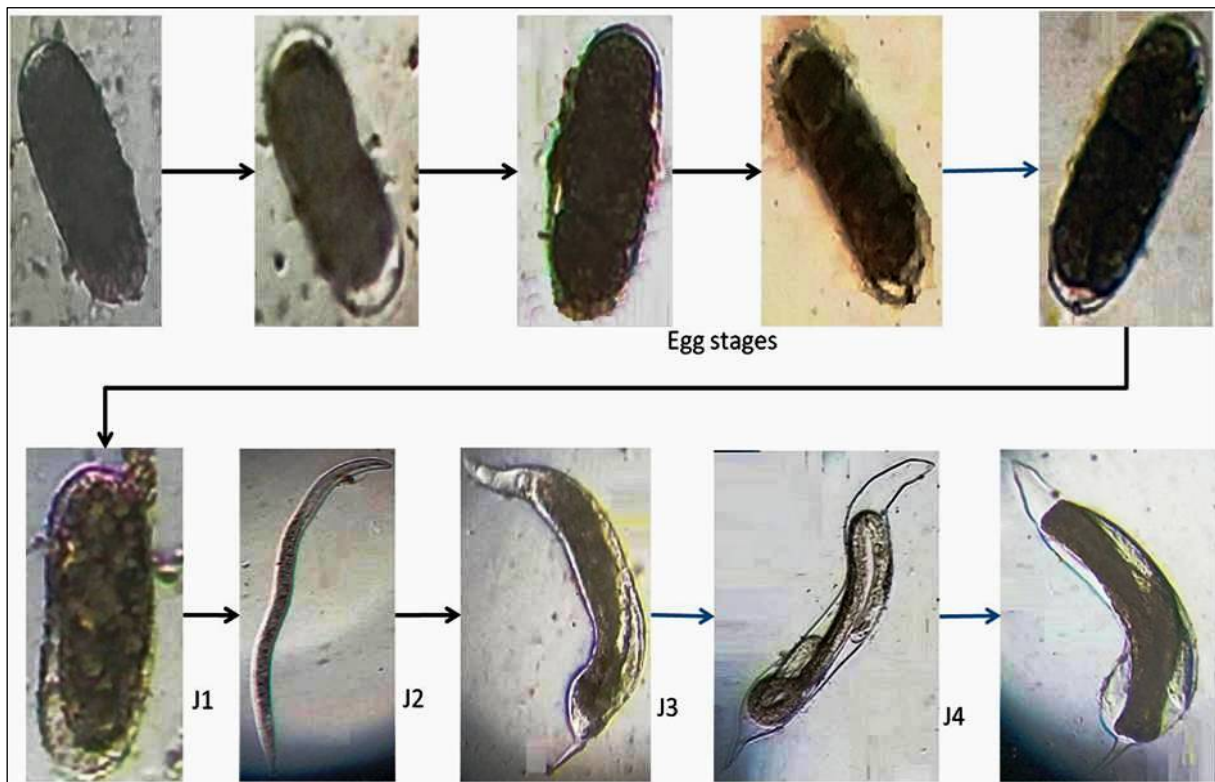


Figure 3. Light microscopic pictures representing single nucleus egg stage to multiple nucleus egg stage (upper lane) and juvenile stages (1 to 4) of nematodes (lower lane)

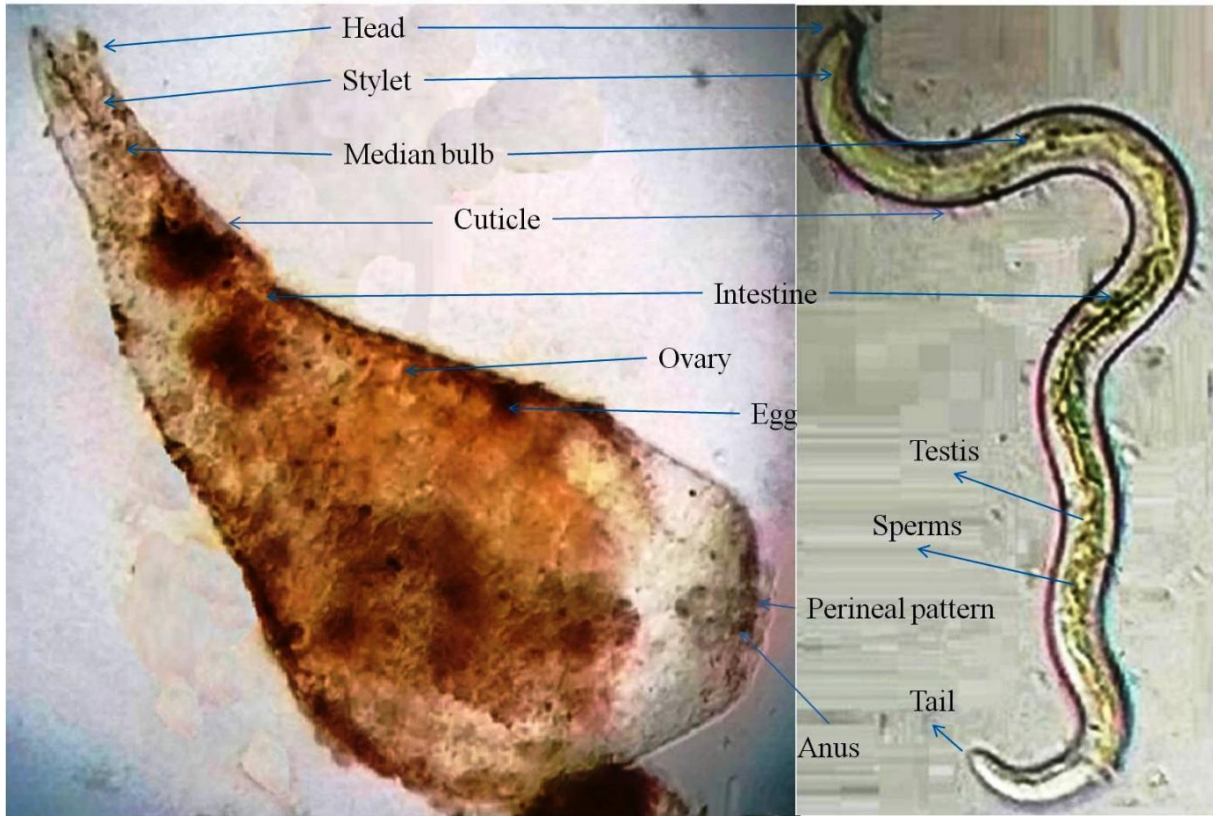


Figure 4. *Meloidogyne incognita* female (A) and male (B) associated with the *Sorghum* crop

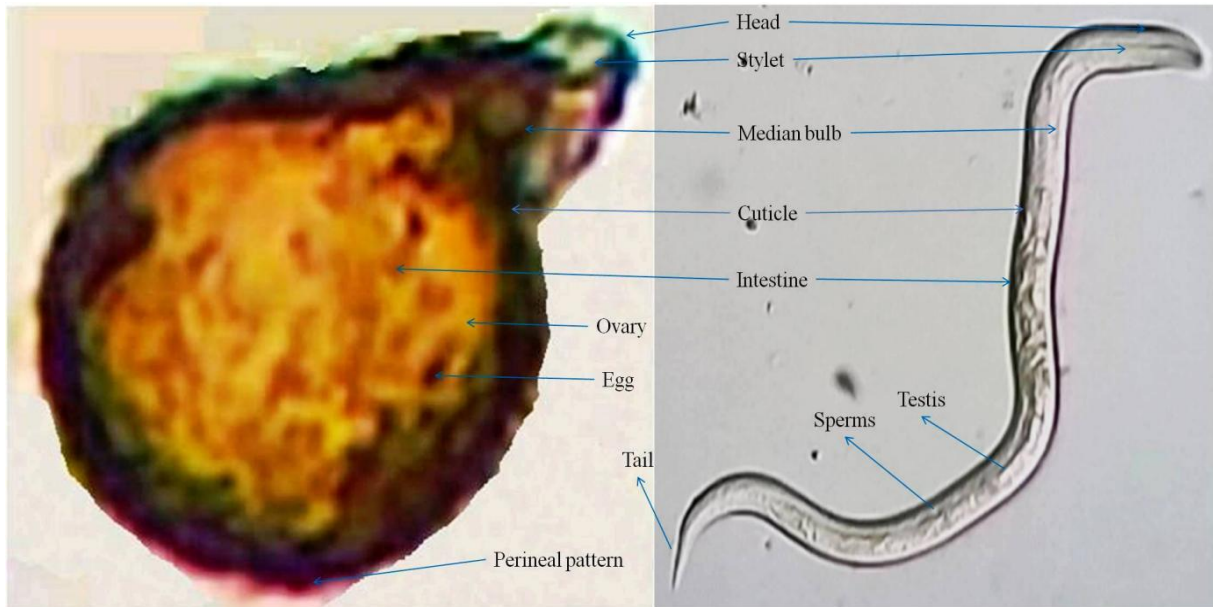


Figure 5. *Meloidogyne javanica* female (A) and male (B) associated with the *Sorghum* crop



Figure 6. Other phytonematodes [34]: “A” represents the genus *Criconemoide*, “B” represents the genus *Pratylenchus*, “C” represents the genus *Tylenchorhynchus*, “D” represents the genus *Rotylenchus*, “E” represents the genus *Dolicodorus*, “F” represents the genus *Psilenchus*, “G” represents the genus *Xiphinema*, “H” represents the genus *Helicotylenchus*

The primary morphological characteristics of males, adult females, and second-stage juveniles were used to characterize the species. It was noted that the female body had an annulated cuticle and a pear-shaped, rounded posterior portion. The head and body were in good alignment. Stylet appears to be transversely oval, narrow, and straight. The oval valved medial bulb is situated at the anterior end. As it approaches the lining of the median bulb, the oesophageal lumen narrows. The perineal pattern was circular to oval in shape, the dorsal arch was medium to high, the apex of the dorsal arch was broadly rounded to square, and the junction of the lateral lines was typically y-shaped. We noticed vague phasmids, rectal punctuations, scarce, typically big, vague lateral lines or striae, and prominent tail termini. The male body is seen to be lengthy and vermiform. The head area is in line with the body and not distinguished by a high and spherical cap. Stylet noticed that they had long, straight, big, ovoid, backward-sloping knobs. The entrance of the dorsal esophageal gland was seen behind the stylet knobs, the tail to the middle bulb areolated lateral fields. It was noted that the narrow middle bulb elongated and was followed by a narrow isthmus. The anterior end is where the excretory pore is situated. The tail is not annular and is lengthy. On the side opposite the cloaca, long, arcuate phasmid pore-like spicules were seen.

District-wise Distribution of the Nematodes

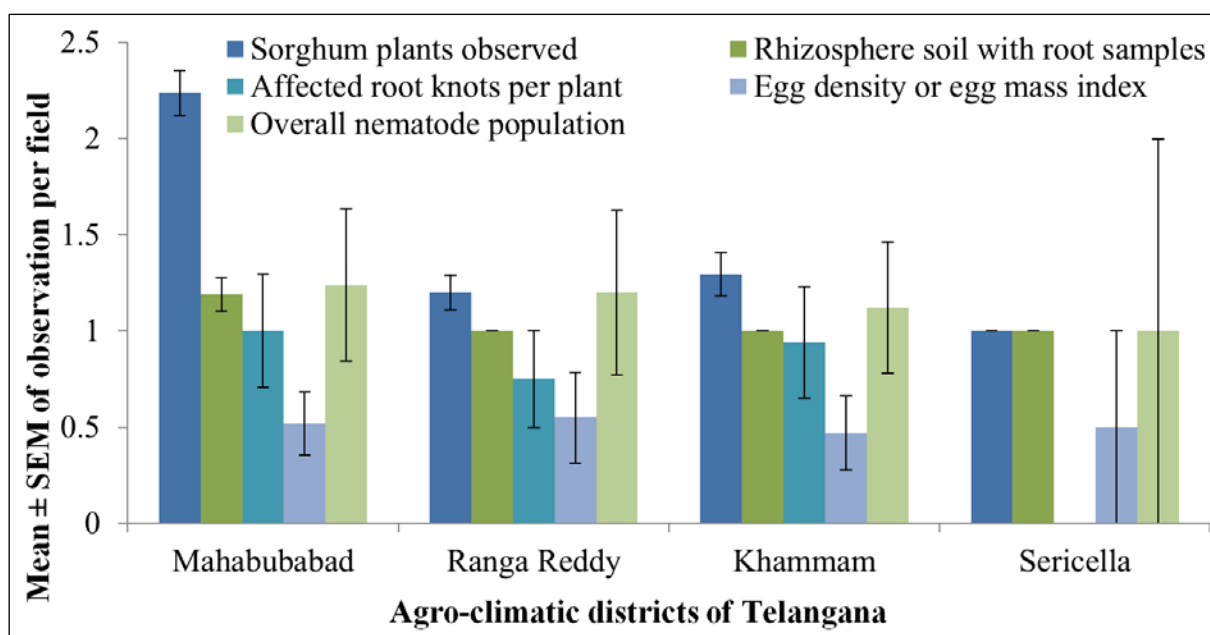
A total of 60 fields, distributed in all 4 districts, were

studied. Out of which, 35% of samples were collected from the Mahabubabad district, 33.3% of samples were collected from Ranga Reddy district, and only 3.3% of samples were collected from the Sericella district. The higher numbers of affected *Sorghum* plants per field in the Mahabubabad district (2.238 ± 0.118) compared to the overall mean per field (1.58 ± 0.087) were recorded. Similarly, more numbers of rhizosphere soil with root samples per field from the Mahabubabad district (1.190 ± 0.088) compared to the overall mean per field (1.07 ± 0.032) were studied. The affected root knots per plant (1.000 ± 0.293), nematode egg density or egg mass index (0.52 ± 0.164), and overall nematode population (1.24 ± 0.396) per sample and field were higher in the Mahabubabad compared to other districts (**Figure 7**). Observed plants and rhizosphere soil sample collection showed statistical significance. Distribution of fields by district, mean number of observed *Sorghum* plants, rhizosphere soil with roots, affected root knots per plant, egg density or egg mass index, and overall nematode population per field and statistical significance were given in **Table 1**. The one-way ANOVA test of *Sorghum* plants (p value < 0.0001) and rhizosphere soil with roots (p -value = 0.045) per field between the districts showed statistical significance. However, affected root knots per plant (p -value = 0.680), egg density or egg mass index (p -value = 0.994) and overall nematode population per sample/ per field (p -value = 0.995) were statistically insignificant.

A higher number of *Meloidogyne*-like species were recorded in the fields of Ranga Reddy district (0.90 ± 0.289)

compared to other districts. A lower number of *Meloidogyne*-like species were recorded in the fields of Khammam (0.24 ± 0.136) and Sericella (0.000 ± 0.000) districts compared to other districts. However, higher numbers of other nematode species were recorded in the fields of Khammam district (1.12 ± 0.342) compared to other districts (Figure 8 & Table 2). A lower number of other nematode species were recorded in the agro-climatic fields of the Mahabubabad district compared to other districts. The distributions of root-knot nematodes (p -value = 0.190) and other types of nematodes (p -value = 0.763) between different districts were statistically insignificant. The winter season was found more suitable for all *Sorghum* crop-associated root-knot nematodes (66.7%) including other phytonematodes (80.4%). The use of pesticide/fertilizer was found to provide more favorable conditions for the growth of the *Sorghum* crop-associated root-knot

nematodes (45.5%) including other phytonematodes (46.4%) (Table 3). In most of the cases, the rainy season and summer season was found unfavorable for the survival and growth of the *Sorghum* crop-associated root-knot nematodes (87.87%) including other phytonematodes (91.07%). Similarly, more use of manure was found to help manage the *Sorghum* crop-associated root-knot nematodes (84.84%) including other phytonematodes (69.64%) (Table 4). A higher number of other phytonematodes (41.1%) were found to be associated with the paddy crop. Similarly, *Meloidogyne* species were found high in the *Sorghum* crop (24.2%) (Table 5). Lesions/ symptoms of *Sorghum* crops like yellowing, wilting, and stunted growth was found to be associated with other phytonematodes (41%). However, indications like wilted and stunted growth were found to be associated with *Meloidogyne* species (51.5%) (Table 6).



Note: The values were represented as Mean \pm SEM (Standard error of the mean)

Figure 7. District-wise distribution of fields, *Sorghum* plants, rhizosphere soil with roots, affected root knots per plant, egg density or egg mass index, and overall nematode population per field

Table 1. Distribution of fields by district, mean number of observed *Sorghum* plants, rhizosphere soil with roots, affected root knots per plant, egg density or egg mass index, and overall nematode population per field

Agro-climatic districts and field distribution (N, %; 60, 100)	<i>Sorghum</i> plants observed	Rhizosphere soil with root samples	Affected root knots per plant	Egg density or egg mass index	Overall nematode population
One-way ANOVA (between the districts)					
Sum of squares	14.044	0.495	2.242	0.060	0.209
df value	3	3	3	3	3
Mean square	4.681	0.165	0.747	0.020	0.070
F value	20.908	2.855	0.506	0.026	0.023
Significance	0.000*	0.045*	0.680	0.994	0.995

Note: "N" represents the total number of fields, "%" represents a percentage, and "*" represents the mean difference that was significant at the 0.05 level

Table 2. District-wise distribution of fields, mean number of root-knot nematodes (*Meloidogyne* species), and other phytonematodes per field

Agro-climatic districts with field distribution (N, %; 60, 100)	Other nematode species	<i>Meloidogyne</i> -like species
One-way ANOVA (between the districts)		
Sum of squares	2.352	4.753
df value	3	3
Mean square	0.784	1.584
F value	0.387	1.640
Significance	0.763	0.190

Note: P value > 0.05 was considered significant

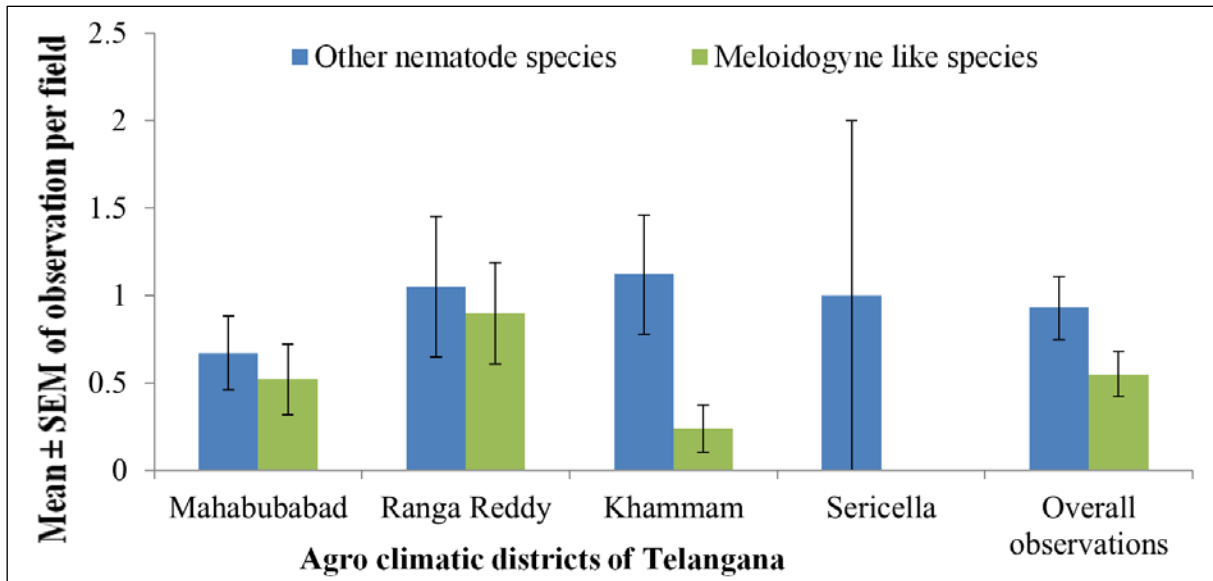


Figure 8. District-wise distribution of fields, mean number of *Meloidogyne*-like species, and other phytonematodes per field. Data represented in mean ± SEM

Table 3. The occurrence of *Sorghum* crop-associated root-knot nematodes (*Meloidogyne* species) and other phytonematodes at different seasons and fields applied with pesticides, fertilizers, and manure

Characteristics	Grown using/ During (N; %, 60; 100)	Other nematode species (N; %, 56; 100)	<i>Meloidogyne</i> -like species (N; %), 33; 100
Pesticide/ fertilizer	37; 61.7	26; 46.4	15; 45.5
Manure	14; 23.3	17; 30.4	5; 15.2
Both	5; 8.33	3; 2.57	0; 0
Season			
Rainy	9; 15.0	5; 8.93	7; 21.2
Winter	44; 73.3	45; 80.4	22; 66.7
Summer	7; 11.7	6; 10.7	4; 12.1

Note: "N" represents the total number of fields, and "%" represents the percentage

Table 4. The occurrence of *Sorghum* crop-associated root-knot nematodes (*Meloidogyne* species) and other phytonematodes in nonspecific seasons and fields applied with no pesticides, fertilizers, or manure

Characteristics	Not grown using/ During (N; %) 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
Pesticide/ fertilizer	23 (38.3)	30; 53.57	18; 54.54
Manure	46 (76.7)	39; 69.64	28; 84.84
Both	14; (23.3)	8; 14.24	7; 21.2
Season			
Rainy	51 (85.0)	51; 91.07	26; 78.78
Winter	16 (26.7)	11; 19.64	11; 33.33
Summer	53 (88.3)	50; 89.28	29; 87.87

Note: "N" represents the total number of fields, "%" represents the percentage

Table 5. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with preceding and *Sorghum* crop

Preceding crop	No of field (N; %) 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
Brinjal	3; 5	0; 0	5; 15.15
Chilli	1; 1.7	0; 0	0; 0
Cotton	5; 8.3	11; 19.6	0; 0
Groundnut	5; 8.3	3; 5.36	0; 0
Lady's finger	1; 1.7	0; 0	2; 6.06
Paddy	17; 28.3	23; 41.1	7; 21.2
Pulses	10; 16.7	6; 10.7	5; 15.2
<i>Sorghum</i>	9; 15.0	4; 7.14	8; 24.2
Tomato	9; 15.0	9; 16.1	6; 18.2

Note: "N" represents the total number of fields, "%" represents the percentage

Table 6. Lesions/symptoms-wise distribution of *Sorghum* crop-associated root-knot nematodes (*Meloidogyne* species) and other phytonematodes

Lesions/symptoms of the <i>Sorghum</i> crop	No of field (N; %) 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
Normal	2; 3.33	0; 0	0; 0
Stunted	11; 18.3	2; 3.6	5; 15.2
Yellowing	8; 13.3	9; 16	3; 9.09
Scaly, yellowing	2; 3.33	2; 3.6	0; 0
Stunted, wilted	13; 21.7	13; 23	17; 51.5
Stunted, skeletal, wilted	1; 1.67	4; 7.1	0; 0
Stunted, yellowing	8; 13.3	2; 3.6	0; 0
Stunted, wilted, yellowing	8; 13.3	23; 41	2; 6.06
Wilted, yellowing	6; 10	0; 0	4; 12.1
Wilted, skeletal	1; 1.67	1; 1.8	2; 6.06

Note: "N" represents the total number of fields, "%" represents the percentage

Soil Type and Distribution of Nematodes

Out of 60 samples, 36.7% were collected from red sandy loams, 33.3% were from red loamy sands, 18.3% were

from medium black soils, and 11.7% were collected from lateritic soils. Red sandy loam soils (2.045±0.139) showed the highest number of affected *Sorghum* plants per field compared to other soil types. Similarly, more numbers of

rhizosphere soil with root samples per field were recorded from red sandy loam soil (1.182 ± 0.084) compared to other soil types. Whereas, a lower number of affected *Sorghum* plants per field (1.143 ± 0.143) from lateritic soil compared to other soil types were recorded. The same number of samples per field (1.000 ± 0.000) from all soil types except red sandy loam soil was collected. In medium black soil, a higher number of affected root knots per plant (1.450 ± 0.39), egg density or egg mass index (0.910 ± 0.285), and overall nematode population (2.270 ± 0.662) per sample and field were recorded in medium black soil compared to other soil types. However, a lower number of affected root knots per plant (0.290 ± 0.184), egg density or egg mass index (0.140 ± 0.143), and overall nematode population (0.710 ± 0.359) per sample and field were recorded in lateritic soil compared to other soil types (Figure 9).

The one-way ANOVA test for *Sorghum* plants per field between different soil types showed statistical significance (p value < 0.0001). However, rhizosphere soil with root samples (p value = 0.59), affected root knots per plant (p -value = 0.225), egg density or egg mass index (p -value = 0.260) and overall nematode population per sample/ per field (p value = 0.123) were statistically insignificant (Table 7). A higher number of other phytonematode species in the medium black soils (1.91 ± 0.547) compared to other soil types were recorded. A lower number of other phytonematode species in the red sandy loams (0.45 ± 0.158) compared to other districts were recorded. However, higher numbers of *Meloidogyne*-like species in the red loamy sands (0.90 ± 0.271) compared to other soil types were recorded. A lower number of *Meloidogyne*-like species (0.14 ± 0.143) in lateritic soils than in other soil

types was reported (Figure 10). The distributions of other phytonematode species between different soil types were statistically significant (p -value = 0.039). However, the distributions of *Meloidogyne* species between different soil types were statistically insignificant (p -value = 0.212) (Table 8).

Distribution of Nematodes with Varied Climatic Conditions

A higher number of other phytonematodes (28.6%) was recorded at 27.5-30% of soil moisture. Whereas a higher number of *Meloidogyne* species (21.2%) were recorded at 5.0-7.5% of soil moisture (Table 9). A higher number of other phytonematodes (41.1%) was recorded at 5-7.5% of soil organic matter. Whereas a higher number of *Meloidogyne* species (27.3%) were recorded at 17.5-20% of soil organic matter (Table 10). A higher number of other phytonematodes (44.6%) was recorded at a pH range of 5.5 to 6.0. Whereas a higher number of *Meloidogyne* species (30.3%) were recorded at a pH range of 7.0 to 7.5 (Table 11). A higher number of other phytonematodes (76.8%) was recorded at 20 to 25% of the rhizosphere silt. Whereas at 15-20% silt, a higher proportion of *Meloidogyne*-like species (51.5%) were observed (Table 12). At 20 to 25% of the clay, a greater number of other phytonematodes (62.5%) and *Meloidogyne*-like species (36.4%) were recorded (Table 13). At 50 to 55% of the rhizosphere sand, a greater number of other phytonematodes (63%) and *Meloidogyne*-like species (45.5%) were observed (Table 14).

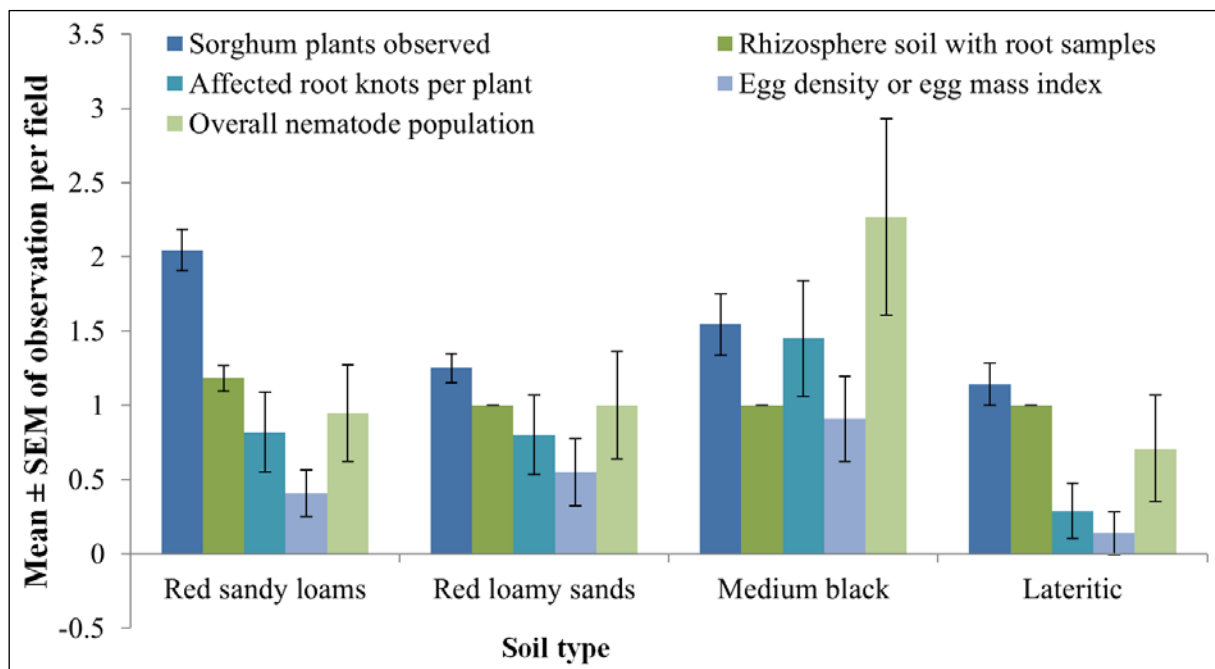


Figure 9. Distribution of *Sorghum* plants, rhizosphere soil along with root knots per plant and egg density or egg mass index, and overall nematode population per field by soil type

Table 7. Distribution of *Sorghum* plants, rhizosphere soil with roots, affected root knots per plant, egg density or egg mass index, and overall nematode population per field by soil type

Soil type with field distribution (N; %), 60, 100	<i>Sorghum</i> plants observed	Rhizosphere soil with root samples	Affected root knots per plant	Egg density or egg mass index	Overall nematode population
One-way ANOVA (Between the soil types)					
Sum of squares	8.294	0.461	6.305	2.949	16.418
df value	3	3	3	3	3
Mean square	2.765	0.154	2.102	0.983	5.473
F value	8.466	2.627	1.497	1.375	2.009
Significance	0.000*	0.059	0.225	0.260	0.123

Note: *The mean difference was significant at the 0.05 level

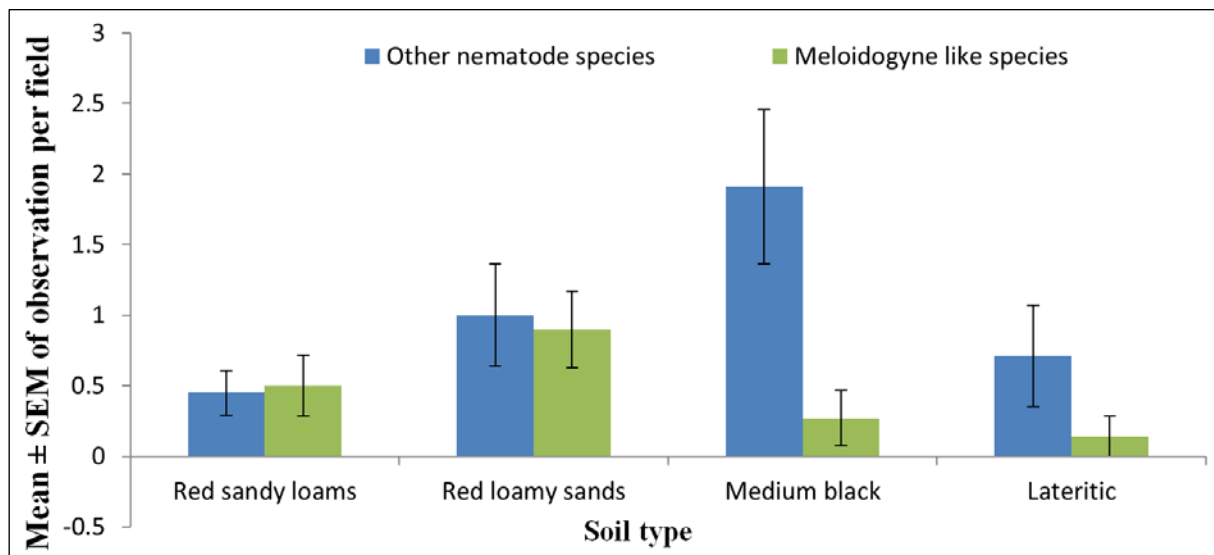


Figure 10. Distribution of *Meloidogyne*-like species and other phytonematode species per field by soil type

Table 8. Distribution of *Meloidogyne*-like species and other phytonematode species per field by soil type

Soil type with field (N; %), 60, 100	Other nematode species	<i>Meloidogyne</i> like species
One-way ANOVA (Between different soil)		
Sum of squares	15.941	4.511
df value	3	3
Mean square	5.314	1.504
F value	2.982	1.550
Significance	0.039*	0.212

Note: *The mean difference was significant at the 0.05 level

Table 9. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with *Sorghum* crop at the varied percentage of soil moisture

Percentage (%) of soil moisture	The observed number of samples (N; %) 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
1.0- 2.5	0; 0	0; 0	0; 0
2.5- 5.0	15; 25	6; 10.7	1; 3.03
5.0- 7.5	12; 20	4; 7.14	7; 21.2
7.5- 10.0	5; 8.3	1; 1.79	3; 9.09
10.0– 12.5	5; 8.3	1; 1.79	2; 6.06
12.5-15.0	0; 0	0; 0	0; 0
15.0-17.5	0; 0	0; 0	0; 0
17.5-20.0	5; 8.3	2; 3.57	4; 12.1
20.0-22.5	4; 6.7	5; 8.93	4; 12.1
22.5- 25.0	4; 6.7	8; 14.3	3; 9.09
25.0- 27.5	0; 0	0; 0	0; 0
27.5- 30.0	6; 10	16; 28.6	6; 18.2
30.0-32.5	0; 0	0; 0	0; 0
32.5-35.0	4; 6.7	13; 23.2	3; 9.09

Note: “N” represents the total number of fields, “%” represents the percentage

Table 10. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with *Sorghum* crop at varying percentages of soil organic matter

Percentage (%) of organic matter	The observed number of samples (N; %) 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
1.0- 2.5	0; 0	0; 0	0; 0
2.5- 5.0	13; 21.7	15; 26.8	6; 18.2
5.0- 7.5	16; 26.7	23; 41.1	4; 12.1
7.5- 10.0	7; 11.7	7; 12.5	1; 3.03
10.0– 12.5	6; 10	6; 10.7	6; 18.2
12.5-15.0	8; 13.3	2; 3.57	2; 6.06
15.0-17.5	0; 0	0; 0	0; 0
17.5-20.0	4; 6.7	3; 5.36	9; 27.3
20.0-22.5	4; 6.7	0; 0	2; 6.06
22.5- 25.0	2; 3.3	0; 0	3; 9.09

Note: “N” represents the total number of fields, “%” represents the percentage

Table 11. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with *Sorghum* crop at a varied range of soil pH

Range of Soil pH	The observed number of samples (N; %), 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
5.5 -6.0	10; 16.67	25; 44.6	8; 24.2
6.0- 6.5	11; 18.33	24; 42.9	7; 21.2
6.5-7.0	3; 5	7; 12.5	2; 6.06
7.0-7.5	13; 21.67	0; 0	10; 30.3
7.5-8.0	22; 36.67	0; 0	6; 18.2
8.0-8.5	1; 1.67	0; 0	0; 0

Note: “N” represents the total number of fields, “%” represents the percentage

Table 12. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with *Sorghum* crop at varying percentages of Silt

Percentage (%) of Silt	The observed number of samples (N; %), 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
15-20	24; 40	11; 19.6	17; 51.5
20-25	33; 55	43; 76.8	12; 36.4
25-30	3; 5	2; 3.57	4; 12.1

Note: "N" represents the total number of fields, "%" represents the percentage

Table 13. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with *Sorghum* crop at varied percentages of Clay

Percentage (%) of Clay	The observed number of samples (N; %), 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
15-20	2; 3.33	5; 8.93	0; 0
20-25	13; 21.67	35; 62.5	12; 36.4
25-30	21; 35	16; 28.6	9; 27.3
30-35	22; 36.67	0; 0	9; 27.3
35-40	2; 3.33	0; 0	3; 9.09

Note: "N" represents the total number of fields, "%" represents the percentage

Table 14. Distribution of root-knot nematodes (*Meloidogyne* species) and other phytonematodes associated with *Sorghum* crop at varied percentages of Sand

Percentage (%) of Sand	The observed number of samples (N; %), 60; 100	Other nematode species (N; %), 56; 100	<i>Meloidogyne</i> -like species (N; %), 33; 100
40-45	6; 10	0; 0	1; 3.03
45-50	23; 38.33	3; 5.4	13; 39.4
50-55	24; 40	35; 63	15; 45.5
55-60	7; 11.67	18; 32	4; 12.1

Note: "N" represents the total number of fields, "%" represents the percentage

4. Discussion

The most significant root-knot nematode genus to harm crops worldwide is *Meloidogyne* Göldi, 1887 [35]. The root-knot nematodes are ubiquitous, obligatory parasites of numerous below-ground components of many crops. Particularly in warm climes and tropical and subtropical regions, this genus severely reduces the economic value of crops [35,36]. Numerous studies revealed that *Sorghum* was a poor host for *M. incognita*, although some reproduction took place [37]. The response of *Sorghum* to populations of *Meloidogyne* spp. may vary by location, according to variations in the host compatibility of other graminaceous crops to populations of *Meloidogyne* spp. [38]. In Kolar and Bagepalli, Karnataka, India, cucumber fields, the root-knot nematode *Meloidogyne incognita* was assessed for its impact on yield losses. Both locations had significant incidence and severity levels of root-knot nematodes [39]. Due to the diverse climatic and edaphic conditions in Telangana, the root-knot nematodes constitute a major and endemic problem in various crops

farmed in India. At present, this study reports the abundance, distribution, and community indices of root-knot nematodes associated with different edaphic factors and climatic conditions (rainfall and temperature) in the form of seasonal variations. The study relied on 60 fields from 4 agro-climatic districts of Telangana. Our data on sample collection for *Sorghum* plants (p value<0.0001) and rhizosphere soil with roots (p-value = 0.045) per field between the districts showed statistical significance. Root galls may be elongated swellings or discrete knots that are relatively small in comparison to other root-knot galls [37]. Infested roots were often carved in the shape of a hook, horseshoe, or a complete spiral without excessive proliferation of secondary roots. *Sorghum* plants with root-knot infestations have been seen to be stunted, blossom later, and have lower yields. Small root systems and galls were visible on the lateral roots of the infected plants. Small galls only had pieces of nematodes; larger ones had no trace of developing larvae, and druses developed in both the resistant and susceptible plant roots' galls but not in healthy tissue. Root-knot nematodes and

phytonematodes were identified when the soil and roots of infected plants were examined. The affected root knots observed per plant (p-value = 0.680), egg density or egg mass index (p-value = 0.994), and overall nematode population per sample/ per field (p-value = 0.995) was statistically insignificant.

Many of the soil's physical, chemical, and biological properties are a function of organic matter content and quality. It is critical to monitor the effects of organic matter additions on the activities of major and minor plant-parasitic nematodes in the production system. Nematodes occupy every ecosystem but are considered aquatic animals and depend on moisture for their activities and survival. Although soil edaphic and climatic factors such as moisture, temperature, humidity, soil pH, and nutrients affect the biology and physiology of nematodes, they have better survival strategies (generally anhydrobiosis) [40]. A higher number of other phytonematodes (44.6%) was recorded at pH values of 5.5 to 6.0. Whereas a higher number of *Meloidogyne* species (30.3%) were recorded at pH values of 7.0 to 7.5. The results of this study suggest that the densities of most plant-parasitic nematodes did correlate with measured soil factors (organic matter, pH, texture). In numerous studies, the pH was well within the range acceptable by the plant nematodes and reports state that most agroecosystems tend to fall within a range of 4-8 [41], thus *Sorghum* crops are appropriate environments for nematode reproduction. The effects of pH on nematode behavior have been examined in several species, and the range of 4.5-6.0 was found to be quite tolerable by entomopathogenic nematodes (EPNs). Other results designate that extreme pH is harmful to soil nematodes [41].

Different types of soil have different nematode holding capacities and showed significant influence on the damage to the crop. Nematodes normally choose sandy soils, e.g. *Meloidogyne* spp., however, several genera can effectively live and survive in other soil types, including clayish soils [42]. The nematode population was found to be adaptable and proliferative in all seasons. This study revealed that the soil type and climatic conditions have a significant influence on the extent of damage caused by the root-knot nematode. The one-way ANOVA test for *Sorghum* plants per field (p value<0.0001) between different soil types showed statistical significance. However, rhizosphere soil with root samples (p value=0.59), affected root knots observed per plant (p-value =0.225), egg density or egg mass index (p-value =0.260) and overall nematode population per sample/ per field (p value=0.123) were statistically insignificant. The distributions of other nematode species between different soils were statistically significant (p-value = 0.039). However, the distributions of *Meloidogyne* species between different soils were statistically insignificant (p-value = 0.212). Out of 60 soil samples, 21.2% of *Meloidogyne*-like species were observed at 5.0-7.5% of soil moisture, 27.3% at 17.5-20%

of soil organic matter, 51.5% at 15-20% of silt, 36.4% at 20 to 25% of the rhizosphere clay and 45.5% of *Meloidogyne*-like species at 50-55% of rhizosphere sand. Some of the aids of organic matter comprise the development of soil structure, erosion control, water relations, obtainability of plant nutrients, ion exchange, chelation, buffering capacity, energy for soil organisms, and conquest of plant pathogens. Plant-parasitic nematodes can cause varying degrees of harm, depending on the worm species, host plant, crop rotation schedule, season, and soil type. Soil type may decide the type of nematode species present according to the water-holding capacity and mineral composition of the soil [43].

Species identification in *Meloidogyne* spp. has been a major component of taxonomic research in nematology. Numerous researchers have found that host plant species as well as nematode number and species affect the size and general appearance of galls [44]. Despite the numerous studies about their biology and taxonomy, their identification at the species level still poses a huge challenge to many diagnosticians [15] mostly because of their very small inter-specific morphological variation [45]. Nematode taxonomic investigations typically involve counting, measuring, and evaluating a variety of features under various types of microscopes. Chitwood's 1949 article, which compared the distinctions between *M. arenaria*, *M. hapla*, *M. incognita*, and *M. javanica*, was a significant contribution to the identification of *Meloidogyne* [46]. Sexual dimorphism is the term used to describe how the bodies of J2 men (vermiform) and females (swollen) differ from one another. In actuality, differential host tests and microscopic examination of the perineal patterns of adult females are frequently used to attempt to identify the most prevalent and agriculturally significant species.

In conclusion, this study understands that the abundance of *Meloidogyne* species varies with climatic conditions and edaphic factors of the soil. Therefore, farmers are suggested to check the edaphic factors of the soil and climatic condition at which they are planning to cultivate the crop for a better yield. In order to comprehend the biology of *Meloidogyne* spp., we will need to use a variety of approaches, including morphological, biochemical, and molecular methodologies [49]. The findings of this study can serve as a warning to farmers who want to plant only a specific type of crop on their field but are unaware of the damage being done below ground. The methods employed in this study to find root-knot nematodes are simple to utilize and don't require any special expertise. Thus, this could be advantageous for entomology, farming, or nematology.

Declaration

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable

Availability of data and materials: Data made available online

Competing interests: No competing interests exist

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Authors' contributions: BV has designed and performed the experiments. BV drafted the manuscripts and communicated. VB has helped in the data analysis and reviewed the manuscript. VVD has reviewed and approved the manuscript. RP has reviewed the manuscript and approved the manuscript. BVT has reviewed the manuscript.

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