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Survey and Assessment of Occurrence and Infestation level of Plant -Parasitic Nematodes in Banana Orchards of Vaishali District, Bihar

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ABSTRACT

Banana (*Musa* spp.) is a vital fruit crop of Bihar, especially in the Vaishali district, where it plays a crucial role in the rural economy and supports the livelihood of thousands of farmers. Despite its economic significance, banana cultivation is often hampered by several soil-borne pests, among which plant-parasitic nematodes are one of the most overlooked yet highly damaging factors. In particular, *Meloidogyne incognita* (root knot nematode) is known to cause severe root damage, nutrient imbalance, plant stunting and significant yield loss. A systematic survey was conducted in seven major banana-growing blocks of Vaishali: Bidupur, Hajipur, Vaishali Goraul, Bhagwanpur, Raghopur and Mahnar. Soil and root samples collected from banana fields were processed and analysed for nematode diversity and population density. The study identified five major plant-parasitic nematode genera: *Meloidogyne incognita*, *Rotylenchulus* spp., *Hoplolaimus* spp., *Helicotylenchus* spp. and *Xiphinema* spp. Among them, *Meloidogyne incognita* was found to be the most prevalent and aggressive species across all surveyed sites, with severe root galling and plant health decline observed in infested fields. Population as low as 1,000 J_2 per plant was sufficient to cause significant reductions in plant height, pseudo stem girth, number of leaves, root length, root weight, corm weight and an increase in root gall index and egg masses, establishing the threshold level of infestation. In conclusion, the study confirms that *Meloidogyne incognita* is the most damaging nematode species in banana-growing regions of Vaishali, Bihar.

Keywords: Banana, Plant Parasitic Nematodes, Threshold ,Survey, Analysis

INTRODUCTION

Banana, botanically known as *Musa* spp., is among the major fruit crops around the globe and belongs to the family Musaceae (Hinge *et al*., 2022). Bananas are thought to have originated in the tropical regions of Southeast Asia (Simmonds, 1962). It's cultivation is extensively practiced across more than 130 countries situated in tropical and subtropical zones worldwide. As per the latest statistics from FAOSTAT (2022), global banana production exceeds 179.26 million tonnes annually, cultivated over an area of more than 12.67 million hectares. Among the banana-growing nations, India stands out as the leading producer, contributing approximately 35 million tonnes per year from nearly 994,000 hectares of cultivated land. Within India, the state of Andhra Pradesh is recognized as the foremost region for banana production and productivity. The state produces around 6.1 million tonnes annually from approximately 111,000 hectares

of banana plantations. In addition, Bihar contributes substantially to the national banana output, with an estimated production of 2 million tonnes from about 44,000 hectares under cultivation. According to India Stat District Agriculture data for 2023-24, Vaishali district ranks third in banana production within Bihar, with an area under cultivation of approximately 3.75 thousand hectares. The district produces around 157.50 thousand tonnes of bananas annually, achieving an average productivity of 42.00 tonnes per hectare. Plant-parasitic nematodes are microscopic roundworms that attack plant roots, causing significant yield losses in banana and other crops (Moens *et al*., 2009). Plant pathogens are a major factor contributing to the decline in banana production. Among these pathogens, nematodes are particularly harmful as they attack the roots, interfering with the plant's ability to absorb water and nutrients and stunting its growth. Such damage can cause yield losses exceeding 75%, often without any obvious symptoms showing on the plant's appearance (Nasution *et al*., 2025). The major nematode species known to affect banana include the burrowing nematode (*Radopholus similis*), root-knot nematodes (*Meloidogyne* spp.), lesion nematodes (*Pratylenchus* spp.), spiral nematodes (*Helicotylenchus* spp.) and cyst nematodes (*Heterodera* spp.) (Nasution *et al*., 2025). *Meloidogyne incognita* is more prevalent in subtropical areas, including many banana-growing belts of India, where it causes 19–32 % yield loss by inducing gall formation in roots and affecting water and nutrient uptake (Karunakaran *et al*., 2011; Koshy & Nagesh, 2000). *Helicotylenchus multicinctus* and *Pratylenchus coffeae* have also been reported to cause chronic damage, particularly under mixed infestations, leading to cumulative yield suppression (Sikora *et al*., 2008). *Meloidogyne incognita* is a sedentary endoparasitic nematode with a wide host range and rapid reproductive capacity, making it highly adaptable to diverse agro-ecosystems (Sasser & Carter, 1985). It infects the roots of banana plants by penetrating the root tips and migrating intercellularly toward the vascular cylinder, where it induces the formation of multinucleate giant cells that serve as feeding sites (Abad *et al*., 2008). These alterations lead to the formation of large root galls, disruption of xylem and phloem tissues, and reduction in water and nutrient absorption (Williamson & Gleason, 2003). Above-ground symptoms include yellowing of leaves, stunting, wilting, and reduced bunch size (Bridge *et al*., 2005). The nematode has a short life cycle of 25-30 days and can produce several generations per year under favourable conditions, leading to rapid population build-up (Jones *et al*., 2013). Eggs are laid in gelatinous matrices on the root surface, where they hatch into second-stage juveniles that actively seek host roots (Perry *et al*., 2009). This life cycle ensures persistence in the soil. The damage was more pronounced in fields with continuous banana monoculture and poor organic matter input, conditions favourable for nematode proliferation (Reddy *et al*., 2010). These nematodes not only cause direct damage by impairing root function but also predispose banana plants to secondary infections by fungi and bacteria, creating complex disease interactions (Rajendran *et al*., 2012). Understanding the threshold levels—the nematode population density above which significant yield loss occurs—is critical for effective management and sustainable banana production (Nasution *et al*., 2025). Threshold study allows for timely intervention strategies that can minimize economic losses and improve crop health (Gowen *et al*., 2005).

Objectives of investigation:

1. To survey plant parasitic nematodes associated with banana in Vaishali district of Bihar
2. To assess threshold level of predominant parasitic nematodes associated with banana

MATERIALS AND METHODS

Survey -A field survey was carried out in the major Banana growing areas in and around Vaishali district to investigate the presence of various plant parasitic nematodes affecting Banana crops. During the survey,

Samples were collected from seven blocks of Vaishali district viz. Bidupur, Hajipur, Vaishali, Goraul, Bhagwanpur, Raghopur and Mahnar. Banana plants showing symptoms such as yellowing leaves, stunted and weak growth, toppled plants, small and slow-developing fruits, low yield, severe root galling, root decay and a reduced root system were identified and selected for sample collection. Soil and root samples were collected from banana fields to assess nematode infestation following standard sampling protocols to obtain

Collection of soil and root samples

For soil samples, surface debris and plant litter were removed first. Soil was taken from the banana root zone, typically 0-30 cm deep, where nematodes are most concentrated. About 250-300 g of soil per individual sample was collected, with 10 such subsamples pooled into a composite sample for each site. Root samples were dug out carefully from the rhizosphere, targeting roots that showed symptoms of nematode damage such as galling, lesions or decay. Around 25 to 50 g of roots per sample were collected depending on root size. Samples were immediately placed in labelled sealed bags and transported to the laboratory under cool conditions to maintain nematode viability

Estimation of nematode population in soil

To estimate the nematode population in soil, samples were collected from field sites and brought to the laboratory for analysis. 200 cc of soil was taken and processed using Cobb's decanting and sieving method (Cobb, 1918), followed by the Baermann funnel technique (Christie and Perry, 1951) to extract nematodes. The soil sample was placed in a bowl half-filled with water and thoroughly mixed to create a uniform suspension, then left undisturbed for 5 to 10 minutes to allow heavier particles to settle at the bottom. The mixture was then carefully poured through a course 20 mesh sieve to remove debris and unwanted materials into a clean bowl. The resulting liquid suspension was further passed sequentially through finer sieves of 60 and 325 mesh sizes to separate nematodes from soil particles. The final residue collected on the 325mesh sieve was gathered into a beaker.

The collected suspension was then poured onto a wire gauge lined with double layers of tissue paper, positioned on top of a Baermann funnel filled with enough water to contact the bottom of the sieve. After 24 hours, the nematode suspension was collected from the funnel into a beaker and allowed to settle for some time. Finally, nematodes were counted under a stereoscopic binocular microscope using a counting dish, where 5 ml of the suspension from each sample was examined to enumerate various plant parasitic nematode species.

Estimation of nematode population in root

To observe nematodes within banana roots, infected roots weighing 5 g are first collected, cleaned thoroughly to remove soil and cut into small 1 cm segments (or kept whole if the plant is small). These pieces are wrapped in muslin cloth and immersed in a boiling solution of lactophenol containing 0.1 % stain (acid fuchsin or cotton blue) for about 1–3 minutes, based on root thickness. After cooling, the roots are transferred to fresh lactophenol and left overnight for detaining, which clears the tissues and makes them almost transparent. Under a stereomicroscope, the nematodes inside the roots appear as distinct red-stained structures, making them easy to detect and count.

Estimation of Population of Nematode

The counting dish containing 1 ml of the nematode suspension was observed under an inverted compound microscope at low magnification to count the nematode population. For root-knot nematodes, only second-stage juveniles (j_2) were enumerated. The mean count from three dishes was calculated to determine the

number of nematodes per ml, which was then multiplied by the total suspension volume to estimate the total nematode population in 200 cc of soil.

Killing, fixing and clearing of nematodes

Nematode suspensions were heated at 50 °C and fixed using 4 % formaldehyde (FA) either separately or by combining equal volumes of 8 % formaldehyde and nematode suspension to achieve a final concentration of 4 %. For clearing, two methods were used. In the Rapid Lactophenol Method, nematodes were transferred into a warmed drop of lactophenol (55–60 °C) on a slide, where they turned transparent within 1–2 minutes and were suitable for semi-permanent mounting. In the Glycerol-Ethanol Method, nematodes were first incubated in Seinhorst's solution I in a cavity block placed in a desiccator with 96% ethanol, then kept at 40 °C for 12 hours. They were then transferred into Seinhorst's solution II and further incubated at 40 °C for 4 hours before being moved into pure glycerin. In another variation, nematodes were fixed in a glycerin-alcohol mixture (5:95) and incubated at 55 °C for two days, then refilled and maintained at the same temperature for 4–5 days. Fully transparent nematodes were then mounted permanently in glycerin for microscopic study.

Identification of Nematodes

The extracted nematodes were then identified using morphological characters and identification key provided by **Taylor *et al*. (1955)**

Identification of *Meloidogyne* spp. using perineal pattern

McBeth *et al*. (1941) prepared the perineal patterns of *Meloidogyne* females by first staining them in 0.1 % Acid Fuchsin dissolved in lactophenol, heating the mixture to about 80 °C to allow proper penetration of the stain. After staining, the specimens were cleared in pure lactophenol to make the internal structures more visible. Using a fine scalpel, the posterior cuticular region of adult females, which contains the perineal pattern, was carefully excised. These delicate patterns were then transferred onto clean glass slides with the help of a soft pigeon hair brush (No. 1) as suggested by Taylor and Netscher (1974), ensuring the structures were not damaged during handling. The mounted patterns were examined under a high-power compound microscope for species identification. The perineal pattern of *Meloidogyne incognita* is typically recognized by its distinct high dorsal arch, along with the absence of punctations and lateral lines, which helps distinguish it from other root-knot nematode species.

Per cent occurrence

The frequency of occurrence of the plant parasitic nematode at each location was determined using the following calculation:

(a) Percentage of occurrence = (Number of samples in which the species was found ÷ Total number of samples collected) × 100

Blocks	Latitude	Longitude
Bidupur	25.64287° N	85.33395° E
Hajipur	25.63410° N	85.24570° E
Vaishali	26.00403° N	85.08201° E
Goraul	25.95910° N	85.28417° E
Bhagwanpur	25.85319° N	85.29811° E
Raghopur	25.64000° N	85.33000° E
Mahnar	25.60380° N	85.4923° E

Figure 1. Surveyed blocks under Vaishali district

a b c

Figure 2. *Meloidogyne incognita*; (a) Mature female (b)Egg mass & male (c) Perineal pattern

To assess threshold level of predominant parasitic nematodes associated with banana

For the experiment, soil was collected from an uneven field and transported to the laboratory for sterilization. The moist soil was autoclaved at 121 °C under 15 psi pressure for one hour to eliminate any pathogens and pests. After sterilization, the soil was left exposed to open air for 24 hours to cool and aerate. Subsequently, the sterilized soil was filled into pots, each with 5 kg of soil per pot for crop planting.

Disinfection and filling of po The earthen pots were thoroughly washed, cleaned and disinfected by rinsing them with a 4% formalin solution. After rinsing, the pots were left to air dry so the formalin could evaporate completely. For all the experiments, pots of nearly the same size and volume were chosen to ensure uniformity. Each pot had a drainage hole at the bottom, The experiment was conducted using pots with a 5 kg soil capacity, each of which was planted with one healthy banana plant to maintain uniformity. After a growth period of 45 days, the plants were inoculated with varying densities of infective juveniles, ranging from 10 to 5000, of *Meloidogyne incognita*. The banana plants will be grown for the period of 90 days after which they were uprooted for observation. Detailed recordings of growth parameters and nematode infestation levels were recorded.

The following treatments were used:

T_1: 10 J_2/pot, T_2: 100 J_2/pot, T_3: 1000 J_2/pot, T_4: 5000 J_2/pot, T_5: Uninoculated (control)

Replications: 04

Design: Completely Randomized Design (CRD)

Figure 3. Pot experiment setup for pathogenicity testing of *Meloidogyne incognita* at graded inoculum levels. (a) Autoclaving of soil (b) Planting (c) Inoculation (d) Recording observations (e) Uprooting (f) Washing of plants for final observation

Preparation and Maintenance of pure culture of nematodes For establishing a pure culture, the root-knot nematode species was first isolated from cucumber roots exhibiting galls collected from an infested field. The galled roots were brought to the laboratory, where the nematode species was identified microscopically, and a pure culture was prepared for further research. To extract eggs, the galled roots were gently stirred in a sodium hypochlorite solution (0.4–0.5 % free chlorine) for about six minutes, which facilitated the release of egg masses. The eggs were then rinsed thoroughly to remove residual hypochlorite and collected on a 500-mesh sieve. The material retained on the sieve was transferred to a beaker and placed on a Modified Baermann Funnel Technique (MBFT) setup for 24 hours to allow juvenile hatching. Concurrently, 10–20 mature females were dissected from the galls, and their perineal patterns were carefully excised using a fine blade for species identification. These patterns were mounted on glass slides with a drop of lactophenol, covered with a cover slip, and sealed with nail polish to prevent drying. Multiple slides were prepared and examined under a compound microscope using an oil immersion lens, following the identification keys provided by Taylor *et al*. (1955). The species was confirmed as *Meloidogyne incognita* (Kofoid & White) Chitwood, 1949. For culture propagation, egg masses obtained via the sodium hypochlorite method were placed on double-folded tissue paper supported by a molded aluminium wire net inside Petri plates, maintained at 28 ± 2 °C. Sufficient water was added to keep the egg masses partially submerged. By the following day, freshly hatched second-stage juveniles (J_2s) were collected from the water in the Petri plates, quantified in three replications to determine juveniles per ml and these freshly hatched J_2s were subsequently used as inoculum for further experiments. Which was covered with broken pieces of clay pot to prevent soil loss. Finally, every pot was filled with an equal amount of soil.

Planting of banana

Healthy and uniform banana tissue culture plant, free from diseases and pests, were selected for the experiment. Prior to planting, the dry leaves were removed and the roots were pruned to promote vigorous growth. The pots were then filled evenly with the prepared soil. Individual banana plants were carefully planted in the Centre of each pot, positioning the base of the pseudo stem slightly above or level with the

soil surface to avoid deep planting. The soil around the plants was gently firmed without compacting to ensure good root-soil contact. After planting, the pots were thoroughly watered to settle the soil and provide adequate moisture. The planted pots were maintained with regular irrigation, nutrients and care throughout the experimental period.

Nematode inoculation

Egg masses were handpicked from the cleaned roots and transferred to a Petri dish for hatching. The collected egg masses were placed on a double layer of tissue paper supported by coarse aluminum wire gauze, positioned over a 10 cm diameter Petri dish containing enough water to keep the tissue moist by contact. After 24 hours, the hatched larvae were collected and used for inoculation. Prior to inoculation, the concentration of juveniles in the suspension was standardized by determining the average number of juveniles per ml based on three counts. As per the specificity of inoculum levels of different experiments, the inoculation was done 45 days after the planting of Banana crop. Several small holes (4-5) were made around the plant's root zone and a specific volume of the1000 juvenile suspension was pipetted into the holes as per the experimental design for management. The holes were then covered with soil using a glass rod, followed by light watering.

Uprooting of banana plant

The banana plants were uprooted 90 days after transplanting, taking special care to minimize the loss of roots and nematodes adhering to the soil. Various growth parameters, including plant height, pseudo stem girth, number of leaves, root length, corm weight and root weight, were recorded immediately. For the assessment of nematode infestation, the roots were stained using 0.1% acid fuchsin in lactophenol by heating at 80 ºC for 2 to 3 minutes (McBeth *et al.*, 1941). After gentle washing, the roots were placed in clear lactophenol for 24 hours and then examined under a microscope to detect nematode infection. Observations included counting the number of galls per plant and egg masses per plant. Additionally, the final nematode population per 200 cc of soil was determined.

RESULTS AND DISCUSSION

Survey and Sample Collection

To evaluate the nematode diversity associated with banana cultivation, a comprehensive survey was conducted in seven major banana-growing blocks of Vaishali district, Bihar, namely Bidupur, Hajipur, Vaishali, Goraul, Bhagwanpur, Raghopur and Mahnar. From each block, 10 soil and root samples were collected, resulting in a total of 70 samples. The sampling sites were carefully selected to include fields showing typical symptoms of nematode infestation, such as yellowing, stunting, poor root development and reduced bunch weight. Each collected sample comprised both rhizosphere soil and banana feeder roots, representing the root zone environment of actively growing banana plant.These samples were brought to the laboratory for further processing and nematode extraction.

Sample Processing and Species Identification

All samples were processed using a modified Baermann funnel technique for nematode extraction. Extracted nematodes were killed, fixed, cleared and permanent mounts were prepared for microscopic observation. Identification of nematodes was performed based on standard morphological characters using stereoscopic and compound microscope. Based on these diagnostic features, a total of five plant-parasitic nematode genera were identified across all blocks. (**Fig. 4**.) These include:

1. ***Meloidogyne* spp.** (Root knot nematode) (Fig 4.a)
2. ***Rotylenchulus* spp.** (Reniform nematode) (Fig 4.b)

3. ***Hoplolaimus* spp.** (Lance nematode) (Fig 4.c)
4. ***Helicotylenchus* spp.** (Spiral nematode) (Fig 4.d)
5. ***Xiphinema* spp.** (Dagger nematode) (Fig 4.e)

Block-wise nematode scenario

Bidupur In Bidupur, plant parasitic nematode population was moderately high. *Meloidogyne* spp. was dominant, with an average population (A) of 1178 nematodes/200 cc soil, and frequency (F) of 80 %, indicating widespread infection. *Rotylenchulus* spp. was also present in significant numbers (A = 453, F = 70 %), suggesting co-existence. *Hoplolaimus* spp. (A = 180, F = 60 %). Few *Xiphinema* spp. were detected with an average population (A) of 16 nematodes/ 200 cc soil and frequency (F) = 60 %. The total nematode count in Bidupur was 1827. (Table 1. & Fig.4)

Hajipur

Hajipur emerged as the most heavily infested block in the entire district. *Meloidogyne* spp. was extraordinarily high, with an average population (A) of 2180 nematodes/200 cc soil and frequency (F) of 100 %, the highest recorded across all surveyed blocks. This extremely high density of root knot nematodes reflects an advanced level of infestation, possibly due to continuous cultivation of susceptible banana varieties or lack of proper nematode management practices. *Rotylenchulus* spp. (A = 468, F = 80 %) and *Helicotylenchus* spp. (A = 57, F = 60 %) were also abundant. *Hoplolaimus* spp. were notably absent. *Xiphinema* spp. were detected with an average population (A) of 10 and frequency (F) of 60 %. The total nematode population reached 2715, which was highest among all blocks. (Table 1. & Fig.4)

Vaishali

In Vaishali, nematode population was comparatively lower. *Meloidogyne* spp. were present (A = 625, F = 70), but in reduced numbers compared to other blocks. *Rotylenchulus* spp. (A = 345, F = 70 %) and *Hoplolaimus* spp. (A = 165, F = 60 %) were moderately present, while *Helicotylenchus* spp. and *Xiphinema* spp. were completely absent in this block. The total nematode count was 1135. (Table 1. & Fig.4)

Goraul

An average population of *Meloidogyne* spp. was 786 nematodes/200 cc soil and frequency of 70 %, which signifies a notable infection. *Rotylenchulus* spp. (A = 201, F = 60 %) and *Helicotylenchus* spp. (A = 68, F = 60 %) were also detected. However, *Hoplolaimus* spp. and *Xiphinema* spp. were absent, which may suggest limited nematode diversity in this region. The total nematode population was 1055, reflecting moderate infestation levels. (Table 1. & Fig.4)

Bhagwanpur

Bhagwanpur had a *Meloidogyne* spp. population of A = 1075 and F = 80 %, which is high. *Hoplolaimus* spp. (A = 115, F = 60 %), *Helicotylenchus* spp. (A = 81, F = 60 %) and *Xiphinema* spp. (A = 29, F = 60 %) indicated a diverse nematode population. Interestingly, *Rotylenchulus* spp. was present only in low numbers (A = 55, F = 60 %). The total nematode population was 1355, suggesting a broad-spectrum nematode infestation in this block. (Table 1. & Fig.4)

Raghopur

Raghopur showed very high nematode densities. *Meloidogyne* spp. were present in large numbers (A = 1232, F = 90 %), *Rotylenchulus* spp. (A = 442, F = 80 %), *Hoplolaimus* spp. (A = 189, F = 70 %) and *Helicotylenchus* spp. (A = 113, F = 70 %) added to the nematode load. *Xiphinema* spp. (A = 40, F = 60 %) were also present. Total nematode count was **2016**, which was significantly higher. (Table 1. & Fig.4)

Mahnar

Mahnar was the second most infested block, following Hajipur. *Meloidogyne* spp. were present in very high numbers (A = 1559, F = 100 %), indicating widespread infestation. *Rotylenchulus* spp. (A = 547, F = 80 %) was the second most dominant. *Hoplolaimus* spp. (A = 151, F % = 70 %), *Helicotylenchus* spp. (A = 75, F = 60 %) and *Xiphinema* spp. (A = 19, F = 60 %) contributed to the high total nematode population of 2351. The presence of all five nematode genera suggests that Mahnar represents a hotspot of nematode diversity and intensity. (Table 1. & Fig.4)

Predominance of *Meloidogyne* spp.

Analysis of nematode populations across all surveyed blocks revealed that *Meloidogyne* spp. (root knot nematodes) were the most predominant genus, found in all seven blocks, in both average population and frequencies. Their population ranged from 625 (Vaishali) to 2180 (Hajipur). The mean population density of *Meloidogyne* spp. was 1233.57 nematodes per 200 cc of soil, which was significantly higher than any other genus. Blocks such as Hajipur, Mahnar, Raghopur and Bidupur recorded extremely high *Meloidogyne* populations, making them the most vulnerable to root knot nematode damage. Even in blocks with lower overall populations like Vaishali and Goraul, *Meloidogyne* spp. were still the leading genus. (Table 1,2 & Fig.5,6,7)

Survey and Sample Collection

To evaluate the nematode diversity associated with banana cultivation, a comprehensive survey was conducted in seven major banana-growing blocks of Vaishali district, Bihar, namely Bidupur, Hajipur, Vaishali, Goraul, Bhagwanpur, Raghopur and Mahnar. From each block, 10 soil and root samples were collected, resulting in a total of 70 samples. The sampling sites were carefully selected to include fields showing typical symptoms of nematode infestation, such as yellowing, stunting, poor root development and reduced bunch weight. Each collected sample comprised both rhizosphere soil and banana feeder roots, representing the root zone environment of actively growing banana plants. These samples were brought to the laboratory for further processing and nematode extraction.

Sample Processing and Species Identification . All samples were processed using a modified Baermann funnel technique for nematode extraction. Extracted nematodes were killed, fixed, cleared and permanent mounts were prepared for microscopic observation. Identification of nematodes was performed based on standard morphological characters using stereoscopic and compound microscope Based on these diagnostic features, a total of five plant-parasitic nematode genera were identified across all blocks. (**Fig. 7**.) These include

a b c

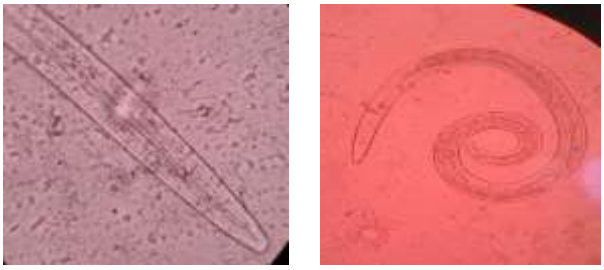

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Figure 4. Microscopic view of nematodes identified– (a) *Meloidogyne* spp. (b) *Rotylenchulus* spp. (c) *Hoplolaimus* spp. (d) *Helicotylenchus* spp. (e) *Xiphinema* sp

1 ***Meloidogyne* spp.** (Root knot nematode) (Fig4.a)
2 ***Rotylenchulus* spp.** (Reniform nematode) (Fig 4.b)
3 ***Hoplolaimus* spp.** (Lance nematode) (Fig 4.c)
4 ***Helicotylenchus* spp.** (Spiral nematode) (Fig 4.d)
5 *Xiphinema spp*. (Digger nematode) (Fig.4 e)

Table1. Population of plant parasitic nematodes in banana orchards of Vaishali district

Blocks	No of samples	*Meloidogyne* spp	*Rotylenchulus* spp	*Hoplolaimus* spp	*Helicotylenchus* spp	*Xiphinema* Spp.	Total
Bidupur	A	1178	453	180	0	16	1827
	F	80	70	60	0	60	
Hajipur	A	2180	468	0	57	10	2715
	F	100	80	0	60	60	
Vaishali	A	625	345	165	0	0	1135
	F	70	70	60	0	0	
Goraul	A	786	201	0	68	0	1055
	F	70	60	0	60	0	
Bhagwanpur	A	1075	55	115	81	29	1355
	F	80	60	60	60	60	
Raghopur	A	1232	442	189	113	40	2016
	F	90	80	70	70	60	
Mahnar	A	1559	547	151	75	19	2351
	F	100	80	70	60	60	
	Mean	1233.57	358.71	114.29	56.29	16.29	1779.14

Where A* =Average population, F*=Frequency of occurrence (%)

Table 2. Frequency of occurrence of *Meloidogyne* spp. in different blocks of Vaishali

S. No.	Location	Total number of soil samples		Frequency of occurrence of Meloidogyne spp.(%)
		Collected	Infested	
1	Bidupur	10	8	80
2	Hajipur	10	10	100
3	Vaishali	10	7	70
4	Goraul	10	7	70
5	Bhagwanpur	10	8	80
6	Raghopur	10	9	90
7	Mahnar	10	10	100

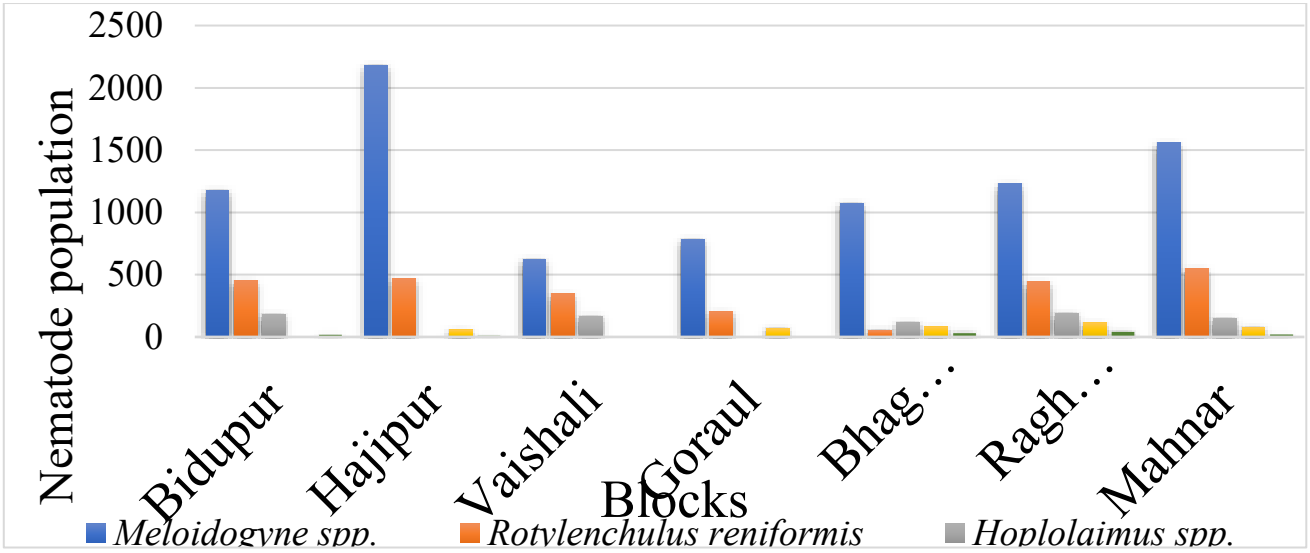

Figure 5 Population of plant parasitic nematodes in banana orchards of Vaishali district

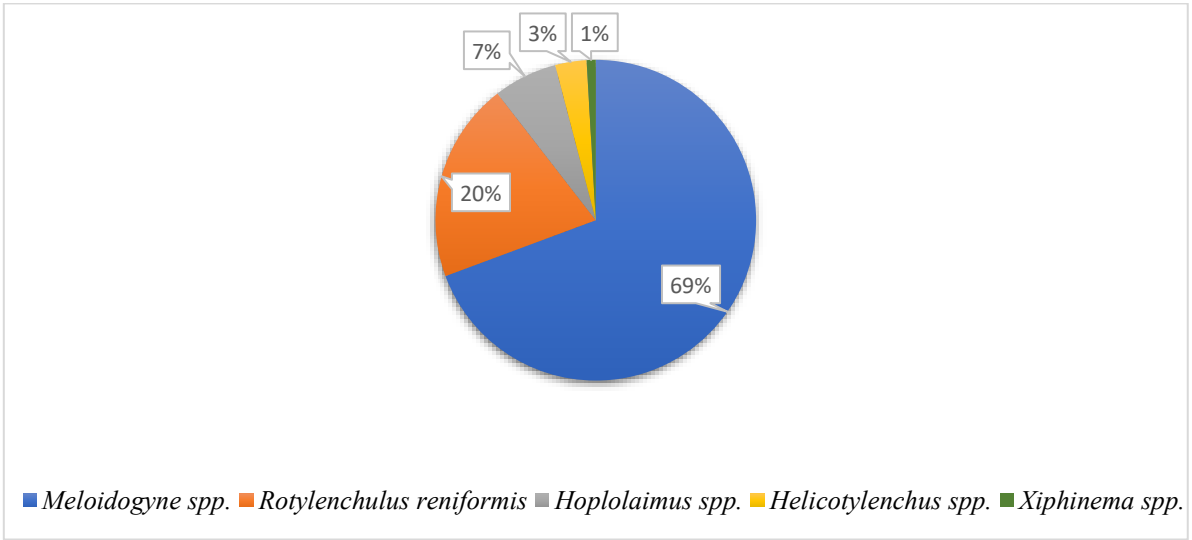

Figure 6. Status of plant parasitic nematodes in banana orchards of Vaishali district

Predominance of *Meloidogyne* spp.

Analysis of nematode populations across all surveyed blocks revealed that *Meloidogyne* spp. (root knot nematodes) were the most predominant genus, found in all seven blocks, in both average population and frequencies. Their population ranged from 625 (Vaishali) to 2180 (Hajipur). The mean population density of *Meloidogyne* spp. was 1233.57 nematodes per 200 cc of soil, which was significantly higher than any other genus. Blocks such as Hajipur, Mahnar, Raghopur and Bidupur recorded extremely high *Meloidogyne* populations, making them the most vulnerable to root knot nematode damage. Even in blocks with lower overall populations like Vaishali and Goraul, *Meloidogyne* spp. were still the leading genus. (Table 1,2 & Fig.8,9,10)

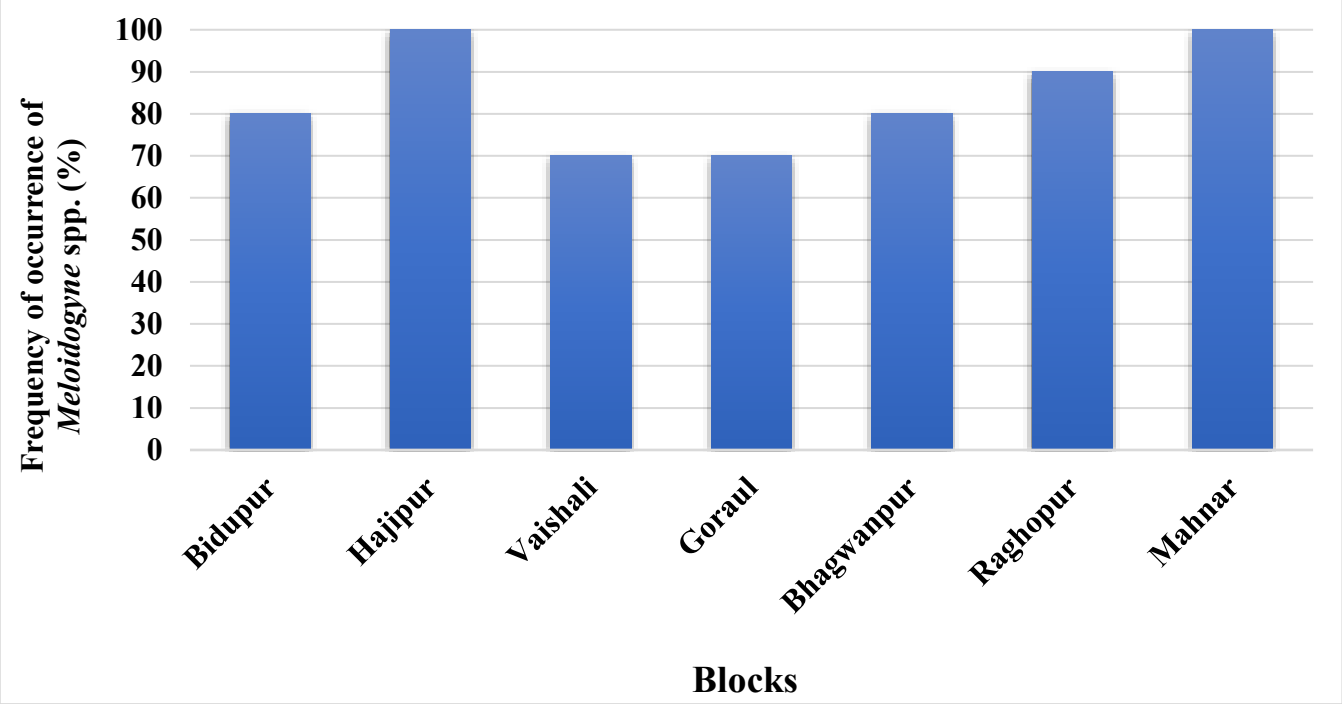

Figure 7. Frequency of occurrence of *Meloidogyne* spp. in different blocks of Vaishali

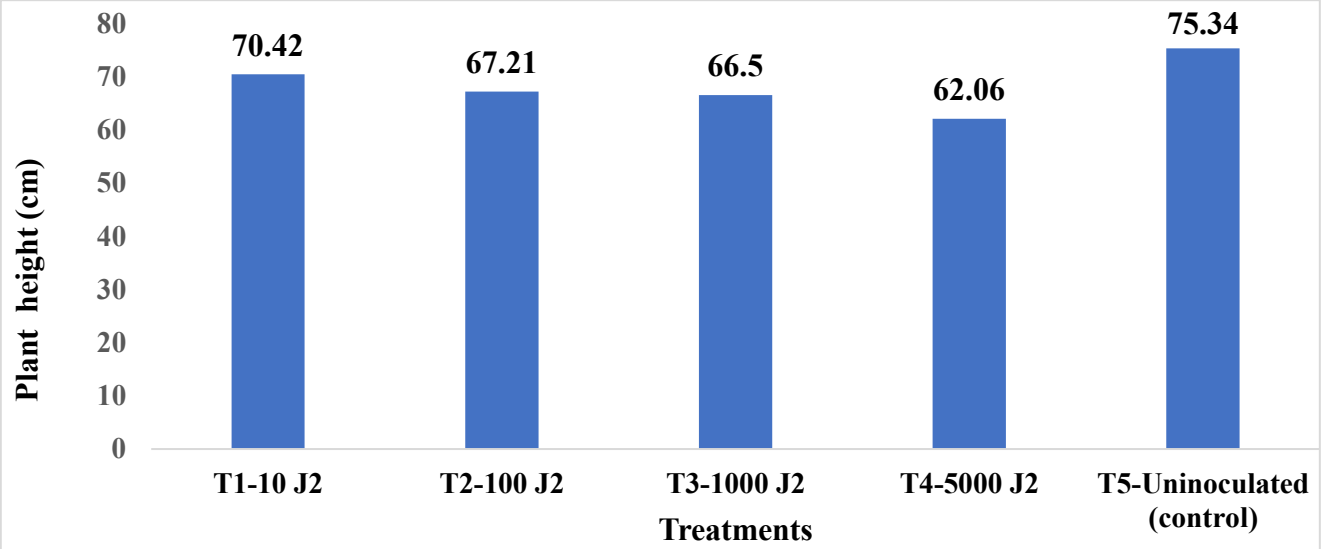

Figure 8. Effect of different inoculum levels of *Meloidogyne incognita* on plant height

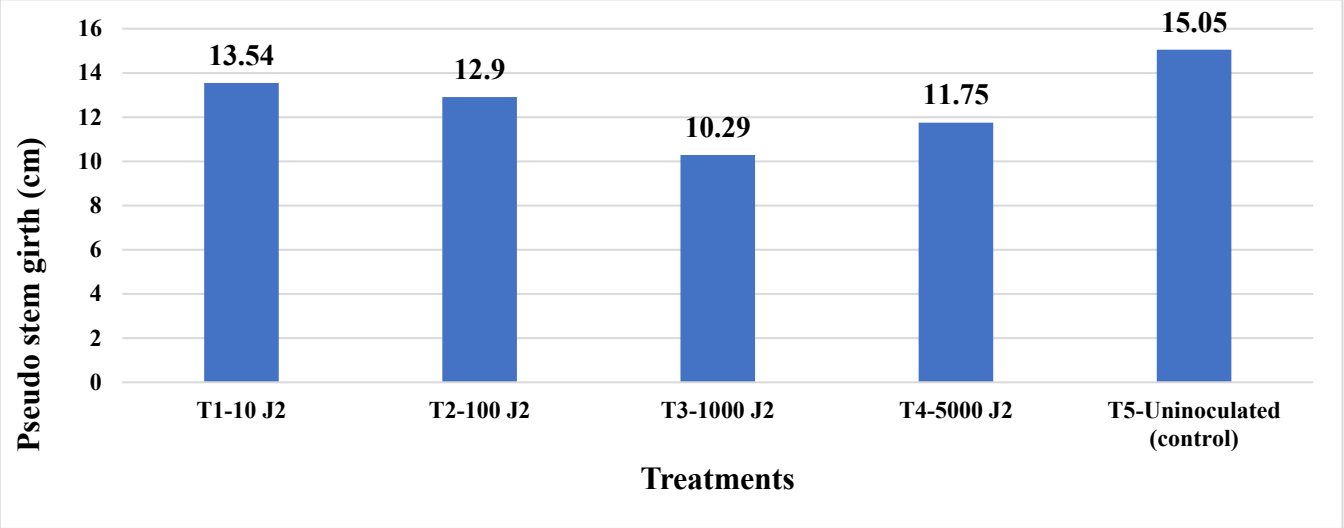

Figure 9. Effect of different inoculum levels of *Meloidogyne incognita* on pseudo stem girth

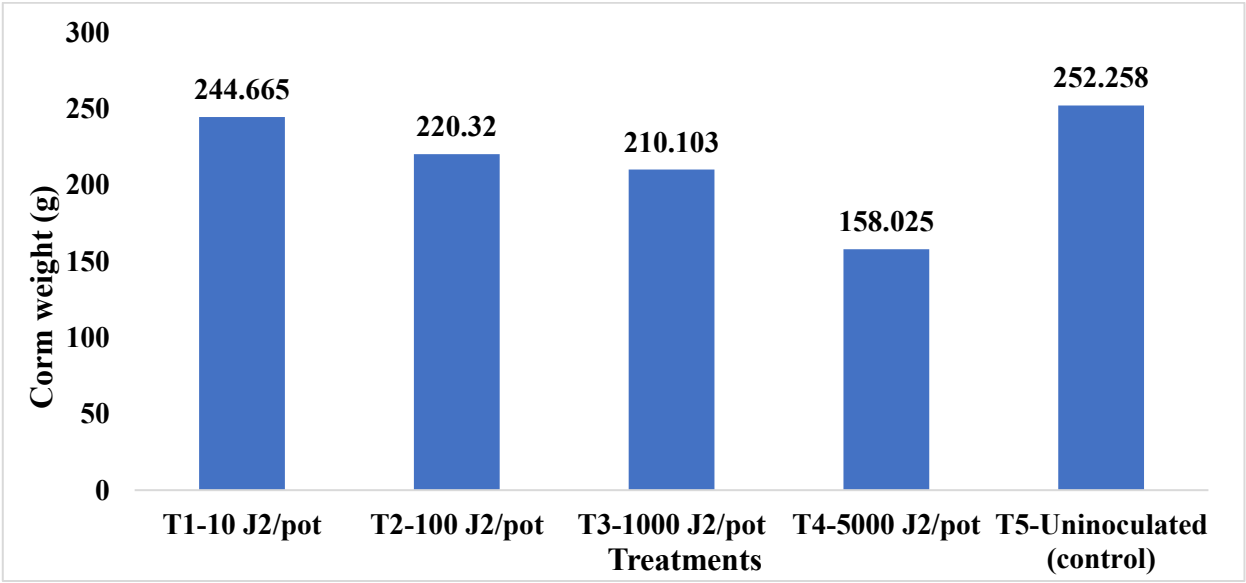

Figure 10. Effect of different inoculum levels of *Meloidogyne incognita* on corm weight

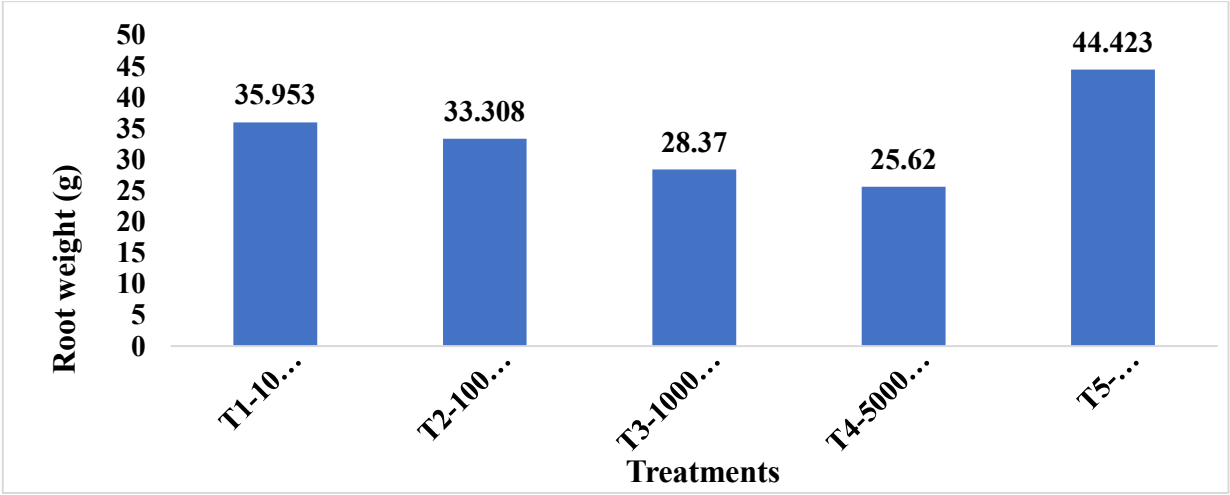

Figure 11. Effect of different inoculum levels of *Meloidogyne incognita* on root weight

Observations

Plant growth parameters

To determine the overall growth and vigor of the banana plants, the following parameters were measured:

- **Plant height (cm)** The height of each plant was measured from the base of the pseudo stem at the soil level up to the tip of the tallest fully opened leaf using a measuring scale.
- **Girth of the pseudo stem (cm)**The circumference of the pseudo stem was recorded at 10 cm above the soil surface using a flexible measuring tape.
- **Number of leaves per plant** All fully developed leaves present on each plant were counted manually.
- **Weight of the corm (g)** At the time of harvest, the corm of each plant was carefully uprooted, cleaned, and weighed using an electronic balance.
- **Weight of the roots (g)** Roots were carefully separated from the soil, thoroughly washed to remove debris, and weighed fresh using an electronic balance.
- **Length of the roots (cm):** The maximum length of the primary roots was measured using a measuring scale.

Nematode multiplication parameter To assess nematode infestation levels and their multiplication within the plant-soil system, the following observations were recorded

- **Nematode population in soil (200 cc)** Soil samples weighing 200 cc were collected from the rhizosphere of each plant and processed using Cobb's sieving and decantation method followed by Baermann funnel technique. The extracted nematodes were counted under a stereoscopic microscope, and the total population per 200 cc soil was recorded.
- **Nematode population in roots (5g):** Five gram of fine feeder roots from each plant were stained with acid fuchsin using the modified Byrd's method. The stained nematodes (primarily juveniles and females) were counted under a compound microscope, and the population per 5 g root sample was noted.
- **Counting root galls per plant** The roots were thoroughly washed with tap water to remove any soil, and excess moisture was gently blotted with tissue paper. The cleaned roots were then placed in a Petri dish and examined under a stereoscopic microscope. The number of root galls per plant was recorded using a hand tally counter, with the results noted manually.
- **Counting egg masses per plant** Counting the egg masses on the roots required careful observation and concentration. Since nematode eggs are deposited in a gelatinous mass forming egg clusters, the roots were examined under a stereoscopic microscope for this purpose. Three replicates were performed to ensure accuracy, following a standardized counting procedure to maintain consistency.
- **Final nematode population in soil (200 cc)** After harvesting the plants, soil samples from the experimental pots were processed by Cobb's sieving and decanting method (Cobb, 1918) combined with Baermann funnel extraction (Christie and Perry, 1951). Following a 24-hour incubation, the suspension containing nematodes was poured into a beaker and allowed to settle. Excess water was gently removed without disturbing the nematodes, the volume was adjusted to 100 ml, and 10 ml aliquot was transferred to a counting dish. Nematodes were counted under a stereoscopic microscope, with counts averaged from three replicates to estimate the final soil nematode population.
- **Reproduction factor** To quantify the reproductive capacity of the nematode population, the reproduction factor (Rf) was calculated utilizing the following mathematical formula Rf = Pf/Pi

Where, Pf = Final nematode population, denoting the total number of nematodes at the conclusion of the study

Pi = Initial nematode population, signifying the initial number of nematodes at the commencement of the study

Root knot index (RKI)

Calculated as per Hartman and Sasser (1985) used scale of 1-5 based on the number of root galls/plant.

To assess threshold level of predominant parasitic nematodes associated with banana

The experiment was conducted using pots with a 5 kg soil capacity, each of which was planted with one healthy banana plant to maintain uniformity. After a growth period of 45 days, the plants were inoculated with varying densities of infective juveniles, ranging from 10 to 5000, of *Meloidogyne incognita*. The banana plants will be grown for the period of 90 days after which they were uprooted for observation. Detailed recordings of growth parameters and nematode infestation levels were recorded.

The following treatments were used:

T_1: 10 J_2/pot, T_2: 100 J_2/pot, T_3: 1000 J_2/pot, T_4: 5000 J_2/pot, T_5: Uninoculated (control)

Replications: 04

Table 3 .Effect of different inoculum levels of *Meloidogyne incognit*a on shoot parameters of banana

	Plant height(cm)		Pseudo stem girth(cm)		No. of leaves	
Treatments	Mean	Decrease over control(%)	Mean	Decrease over control(%)	Mean	Decrease over control(%)
T_1 -10 J_2	70.42	6.53	13.54	15.27	8.00	8.57
T_2 -100 J_2	67.21	10.79	12.90	9.83	7.75	11.43
T_3 -1000 J_2	66.50	11.74	10.29	12.41	8.00	8.57
T_4 -5000 J_2	62.06	17.63	11.75	28.07	7.75	11.43
(T_5– Uninoculated (control)	75.34		15.05		8.75	
Sem (+-)	0.73		0.26		0.19	
CD at 5 %	2.22		0.67		0.58	
CV (%)	2.14		4.16		4.81	

Where J_2 * is the second stage juvenile of *Meloidogyne incognita*

Design: Completely Randomized Design (CRD)

Statistical analysis and data interpretation

The statistical evaluation of the data collected during the study was performed using OPSTAT software . Design (CRD). Parameters such as root and shoot lengths and weights, number of root galls per plant, egg masses per plant, and final nematode population in the soil were analyzed. Treatment means were compared by calculating standard error of the mean (SEM) and critical difference (CD). When the difference between the means of any two treatments exceeded the CD value, it was

Table 4. Effect of different inoculum levels of *Meloidogyne incognita* on root parameters of banana

Treatments	Root Length(cm) Mean	Decrease over control (%)	Corm weight (g) Mean	Decrease over Control(%)	Fresh root weight (g) Mean	Decrease over Control(%)
T_1 -10 J_2	37.22	4.93	244.66	3.01	35.95	19.06
T_2-100 J_2	34.85	10.99	220.32	12.66	33.30	25.01
T_3-1000 J_2	30.31	22.60	210.10	16.71	28.37	36.13
T_4-5000 J_2	27.07	30.84	158.02	37.35	25.62	42.33
T_5— Uninoculated control	39.15		252.25		44.42	
Sem(+=)	0.59		4.84		1.11	
CD at 5%	1.79		14.73		3.39	
CV(%)	3.49		4.46		6.66	

Where J_2 is the second stage of *Meloidogyne incognita*

T_1 T2 T3 T4 T5

Figure 12 Effect of different inoculum levels of *Meloidogyne incognita* on banana

Considered statistically significant, indicating a meaningful difference between those treatment

A total of 70 samples were collected from seven banana-growing blocks—Bidupur, Hajipur, Vaishali, Goraul, Bhagwanpur, Raghopur and Mahnar. Samples were taken from fields where banana plants were showing symptoms like yellow leaves, poor root development, stunted growth and small bunch size—common signs of nematode damage.The nematodes were extracted from the soil and roots and were studied under a microscope to identify them. Five main types (genera) of harmful nematodes were found: *Meloidogyne* spp. (root knot nematodes), *Rotylenchulus* spp. (reniform nematodes), *Hoplolaimus* spp. (lance nematodes), *Helicotylenchus* spp. (spiral nematodes) and *Xiphinema* spp. (dagger nematodes).Among these, root knot nematodes (*Meloidogyne* spp.) were the most common and were

found in all blocks in high numbers. Nematode fauna associated with banana has also been been reported from Muzaffarpur, Bihar by Fatma *et al*. (2023) Different blocks showed different levels of nematode infestation. Hajipur had the highest number of root knot nematodes (2180 per 200 cc soil) and the highest total nematode population (2715). Mahnar and Raghopur also had very high populations. These high numbers could be due to continuous banana farming, lack of crop rotation and no proper control methods. Similar results were reported by Gowen *et al*. (2005), who also found that *Meloidogyne incognita* causes major damage in banana-growing areas.

Moderate levels of nematodes were found in Bidupur, Bhagwanpur and Vaishali, while Goraul had the lowest population. Even in these areas, *Meloidogyne* spp. was the most dominant type. This suggests that root knot nematodes can survive and multiply under many different soil and weather conditions. This observation agrees with the work of Koshy and Nagesh (2000), who said that root knot nematodes are well adapted to different environments and remain a big threat.

Some blocks showed more types of nematodes than others. For example, Bhagwanpur had all five genera, which means many types of nematodes may be attacking the plants at the same time, leading to more serious damage. On the other hand, blocks like Goraul had only a few types. According to Seenivasan and Senthilnathan (2015), when several nematode types are present together, they can cause more harm to plants by attacking different parts of the root system.

In all blocks, the root knot nematode was found to be the main problem. Symptoms like gall formation on roots, yellowing of leaves and poor plant growth were seen, especially in heavily infected areas. These symptoms are typical of *Meloidogyne* damage and match what Karunakaran *et al*. (2004) reported in their study on banana nematodes in Tamil Nadu

A pot experiment was conducted using different amounts of *Meloidogyne incognita* juveniles (J_2) – specifically 0 (control), 10, 100, 1000 and 5000 J_2 per plant. The results showed that as the number of nematodes increased, the growth of the banana plants decreased. This included plant height, pseudo stem girth, number of leaves, root length, fresh root weight and corm weight. At the same time, nematode damage signs like root galls, egg masses and nematode population increased.

Plants in the control group (no nematodes) were the healthiest, with the tallest height (75.34 cm), thickest girth (15.05 cm) and longest roots (39.15 cm). As nematode numbers increased, these values dropped. The lowest growth was seen in the group that received 5000 J_2 (T_4), where plant height fell to 62.06 cm, and root length dropped to 27.07 cm. This shows that nematodes directly affect plant growth and health. These results align with Khan and Hasan (2008), who demonstrated major reductions in banana plant growth at 2,000 J_2/plant. Bhatti and Jain (1977) also showed that a nematode density above 10,000 J_2 could lead to over 70 % crop loss. The relationship between nematode density and plant damage also supports the theoretical model proposed by Seinhorst (1965), which explains how yield loss is proportional to the initial nematode population (Pi).

The number of galls and egg masses per plant also increased with more nematodes. For instance, there were 146.5 galls and 137.75 egg masses per plant at 1000 J_2 (T_3). But interestingly, increasing the nematode number to 5000 (T_4) did not significantly raise gall or egg mass numbers. This shows that 1000 J_2 might be the threshold beyond which plant damage becomes very serious and the nematodes start facing overcrowding, which limits further multiplication.

The final nematode population and reproduction factor (Rf) followed a similar pattern. The highest reproduction rate (Rf = 16.12) was recorded at 100 J_2 (T_2), meaning this was the level where nematodes multiplied the fastest. But at 1000 and 5000 J_2, the Rf dropped to 4.31 and 5.38, likely because too many

nematodes caused overcrowding and less food availability in roots. Bridge and Page (1980) also observed that moderate initial populations result in higher reproduction rates than high populations.

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