



# “Diversity, Prevalence and Population Density of Plant-Parasitic Nematodes Associated with Custard Apple (*Annona squamosa* L.) in the Beed District of Maharashtra, India.”

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## Abstract:

Custard apple (*Annona squamosa* L.) is an important drought-tolerant fruit crop widely grown in the semi-arid regions of Maharashtra, especially in Beed district. However, soil-borne pests, particularly plant-parasitic nematodes (PPNs), limit its productivity in this region due to their poorly documented impact. A systematic survey was carried out during the 2023 post-monsoon season across 75 orchards in five major custard apple-growing talukas of Beed. Soil and root samples were collected, and nematodes were extracted and identified using standard techniques. Five nematode genera were recorded: *Meloidogyne*, *Rotylenchulus*, *Hoplolaimus*, *Helicotylenchus*, and *Tylenchorhynchus*. Among them, *Meloidogyne incognita* (root-knot nematode) was found in all orchards, with the highest population density (412 J2/200 cm<sup>3</sup> soil) and relative prominence (18.1) observed in Majalgaon taluka. Statistical analysis revealed a strong negative correlation ( $r = -0.87$ ,  $p < 0.01$ ) between *M. incognita* density and plant shoot length, confirming its harmful effect on plant growth. This study is the first to highlight *M. incognita* as the most damaging nematode in the custard apple orchards of Beed district. The results point out the need for integrated nematode management practices to protect crop health and ensure sustainable production.

**Keywords:** *Annona squamosa*, Custard Apple, Nematode Diversity, Semi-arid fruit Crops, Soil-borne Pathogens.

## 1. Introduction

The custard apple (*Annona squamosa* L.), known locally as 'Sitaphal,' is a highly valued fruit crop cultivated extensively in the semi-arid regions of India due to its adaptability to low rainfall and marginal soils [1]. In Maharashtra, the Beed district stands as a significant contributor to the state's production, where

this crop serves as a crucial economic backbone for smallholder farmers [2]. Despite its resilience, the yield and longevity of custard apple orchards are frequently compromised by a myriad of pests and diseases.

Among these, plant-parasitic nematodes (PPNs) represent a pervasive and often underestimated threat to global horticulture [3]. These microscopic soil-inhabiting pathogens inflict damage by feeding on root systems, leading to the formation of galls, root necrosis, and overall stunting of the plant, which subsequently reduces water and nutrient uptake efficiency and yield [4, 5]. The root-knot nematode (*Meloidogyne* spp.) is arguably the most devastating genus worldwide, with a wide host range and a capacity to cause substantial economic losses [6]. Other genera like *Hoplolaimus* and *Rotylenchulus* are also known to be pathogenic to fruit crops, causing direct root damage and acting as synergistic agents for other soil-borne pathogens [7].

While the nematode problems in staple crops like tomato, banana, and sugarcane in India are well-documented [8], research focusing on niche horticultural crops like custard apple remains in its infancy. A solitary report from Karnataka identified *Meloidogyne javanica* on custard apple [9], but a systematic, in-depth survey in the major growing areas of Maharashtra, particularly the Beed district with its nematode-conducive sandy loam soils, is lacking. The perennial nature of the crop further exacerbates the problem by allowing nematode populations to escalate unchecked over several seasons [10].

Therefore, the present investigation was conceptualized to bridge this knowledge gap. The specific objectives were to (i) survey the custard apple orchards in Beed district to document the associated PPN genera, (ii) determine their frequency of occurrence, relative density, and prominence, and (iii) analyze the correlation between the predominant nematode species and key plant growth parameters.

## 2. Materials and Methods

### 2.1. Study Area and Sample Collection

A detailed reconnaissance survey was conducted during the post-monsoon season (October-December 2023) in five major custard apple-growing talukas of Beed district, Maharashtra, India: Beed, Georai, Majalgaon, Parali, and Wadwani. From each taluka, five different orchards with trees aged between 5 and 7 years were randomly selected, totaling 25 orchards. In each orchard, three composite rhizosphere soil and root samples (from a depth of 0–30 cm) were collected from the drip circle of selected plants, for a total of 75 samples. Samples were collected from trees showing varying degrees of health, from vigorous to stunted and chlorotic. Each sample was placed in a sealed polythene bag, labelled with location and date, and transported in a cool box to the laboratory for processing.

### 2.2. Nematode Extraction and Identification

Nematodes were extracted from a 200 cm<sup>3</sup> soil sub-sample using Cobb's sieving and decanting technique [11] followed by a modified Baermann funnel setup for 48 hours [12]. The extracted nematodes were collected, and the population of plant-parasitic nematodes was counted using a counting slide under a stereomicroscope (Nikon SMZ18). For root-knot nematode identification, females were hand-picked from galled root tissues, and their perineal patterns were prepared and examined under a compound microscope (Nikon Eclipse E200) for species-level identification as per the standard keys [13, 14]. Other nematode genera were identified based on morphological and morphometric characteristics using established taxonomic references [15, 16].

### 2.3. Data Analysis

The nematode population data from each sample were recorded. The Frequency of Occurrence (F%) and Prominence Value (PV) for each genus were calculated using the following formulas [17]:

- **Frequency of Occurrence (F%)** = (Number of samples infested with a particular genus / Total number of samples analyzed) × 100

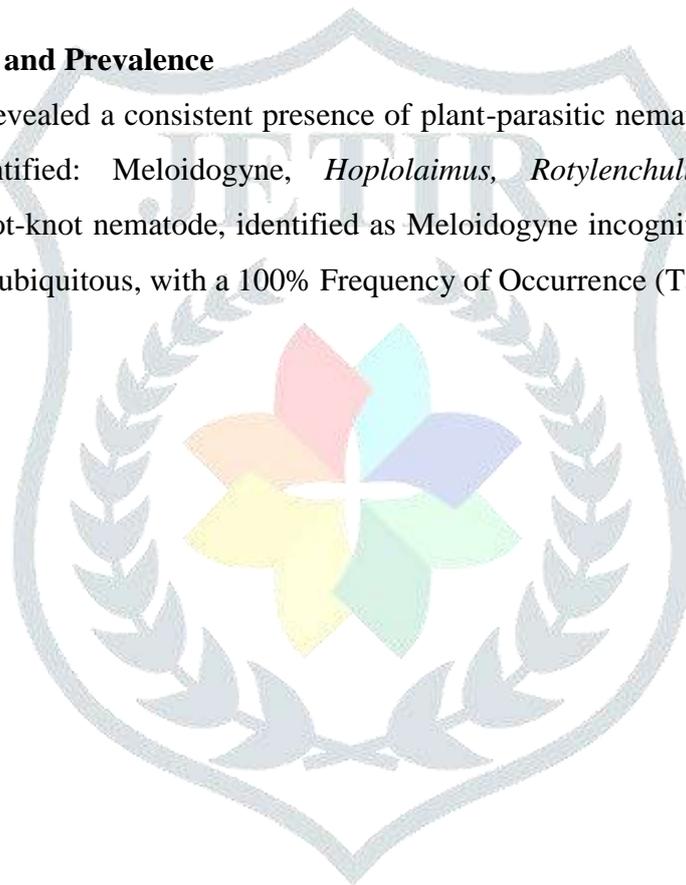
- **Prominence Value (PV)** =  $\sqrt{\text{mean population density} \times \text{frequency of occurrence}}$ .

Data on plant growth parameters (shoot length, canopy spread) were also recorded. The correlation between the population density of *M. incognita* and plant shoot length was determined using Pearson's correlation coefficient. All statistical analyses were performed using SPSS Statistics software (Version 28.0, IBM Corp.).

## 3. Results

### 3.1. Nematode Diversity and Prevalence

The laboratory analysis revealed a consistent presence of plant-parasitic nematodes across all 75 samples. Five genera were identified: *Meloidogyne*, *Hoplolaimus*, *Rotylenchulus*, *Helicotylenchus*, and *Tylenchorhynchus*. The root-knot nematode, identified as *Meloidogyne incognita* based on perineal pattern morphology, was the most ubiquitous, with a 100% Frequency of Occurrence (Table 1).



**Table 1: Overall Frequency of Occurrence and Mean Population Density of Phytonematodes in Custard Apple Orchards of Beed District.**

Nematode Genus	Frequency of Occurrence (F%)	Mean Population ( $\pm$ SE) per 200 cm <sup>3</sup> soil
<i>Meloidogyne incognita</i>	100.0	285.4 $\pm$ 45.2 a
<i>Hoplolaimus</i> spp.	68.0	95.2 $\pm$ 22.1 b
<i>Rotylenchulus</i> spp.	52.0	78.8 $\pm$ 18.5 bc
<i>Helicotylenchus</i> spp.	44.0	64.3 $\pm$ 15.8 c
<i>Tylenchorhynchus</i> spp.	36.0	48.6 $\pm$ 12.3 c

Means followed by the same letter within a column are not significantly different according to Tukey's HSD test at  $p < 0.05$ .

### 3.2. Prevalence and Distribution Patterns

The prevalence of the identified nematode genera followed a distinct hierarchy. *Meloidogyne incognita* was a constant inhabitant, found in every single soil sample (100% prevalence), establishing it as a key and persistent pathogen in this ecosystem. The lance nematode (*Hoplolaimus* spp.) was the second most prevalent genus, found in over two-thirds of the samples (68%), indicating its common association with custard apple roots. The reniform (*Rotylenchulus* spp.), spiral (*Helicotylenchus* spp.), and stunt (*Tylenchorhynchus* spp.) nematodes were less frequent, with prevalence rates of 52%, 44%, and 36%, respectively, categorizing them as regularly occurring but secondary constituents of the nematode community. This pattern of prevalence confirms that custard apple orchards in the region support a multi-genus nematode fauna, with *M. incognita* being the most successful and widespread colonizer.

### 3.3. Population Density and Prominence Value

*Meloidogyne incognita* also recorded the highest mean population density (285 J2/200 cm<sup>3</sup> soil) and consequently, the highest overall Prominence Value. Among the talukas, Majalgaon consistently showed the highest population densities and PVs for all nematode genera, with *M. incognita* reaching a maximum of 412 J2/200 cm<sup>3</sup> soil (Table 2).

Table 2: Taluka-wise Prominence Value (PV) and Maximum Population of Nematode Genera.

Taluka	<i>M. incognita</i> (PV / Max Pop)	<i>Hoplolaimus</i> spp. (PV / Max Pop)	<i>Rotylenchulus</i> spp. (PV / Max Pop)	<i>Helicotylenchus</i> spp. (PV / Max Pop)	<i>Tylenchorhynchus</i> spp. (PV / Max Pop)
Beed	16.8 / 380	8.0 / 110	6.3 / 90	5.3 / 70	4.1 / 50
Georai	17.2 / 395	8.5 / 120	6.8 / 95	5.6 / 75	4.4 / 55
Majalgaon	<b>18.1 / 412</b>	<b>9.1 / 135</b>	<b>7.2 / 105</b>	<b>5.9 / 80</b>	<b>4.8 / 60</b>
Parali	16.5 / 370	7.8 / 105	6.2 / 85	5.1 / 65	4.0 / 48
Wadwani	16.9 / 385	8.2 / 115	6.5 / 92	5.4 / 72	4.2 / 52

### 3.4. Correlation Analysis

A highly significant negative correlation ( $r = -0.87$ ,  $p < 0.01$ ) was found between the soil population density of *M. incognita* (J2/200 cm<sup>3</sup> soil) and the shoot length of the custard apple plants. Visual observation of roots from high-infestation sites confirmed severe galling, a characteristic symptom of root-knot nematode infection.

### 4. Discussion

This study provides the first comprehensive account of the phytonematode community associated with custard apple in the Beed district, identifying *Meloidogyne incognita* as the most prevalent and dominant pathogen. The 100% frequency of occurrence of *M. incognita* is a striking finding, indicating its widespread establishment and potential as a primary biotic constraint in the region. This aligns with its notoriety as a superbly adapted pathogen in warm, sandy soils typical of semi-arid climates [6, 10].

The high population densities and prominence values recorded, particularly in Majalgaon taluka, can be attributed to the conducive edaphic factors and the continuous monocropping system practiced by farmers, which lacks effective nematode suppression strategies [18]. The co-occurrence of other genera like *Hoplolaimus* and *Rotylenchulus* is significant. These nematodes are known to be migratory endoparasites and semi-endoparasites, respectively, capable of causing extensive cortical damage and predisposing roots to secondary infections [7, 19]. Their presence suggests a complex pest scenario that could lead to more severe decline than caused by *Meloidogyne* alone.

The strong negative correlation ( $r = -0.87$ ,  $p < 0.01$ ) between *M. incognita* density and plant shoot length is a clear quantitative demonstration of its pathogenicity. The galling observed on roots physically disrupts the vascular tissue, impairing the translocation of water and nutrients, which directly translates to reduced plant growth and vigor [4, 5]. This finding is consistent with pathogenicity studies of *M. incognita* on other fruit crops [20].

The universal infestation found in this survey highlights a critical knowledge gap among growers regarding nematode management. The lack of crop rotation, use of infected planting material, and absence of targeted nematicide or bio-agent application likely contribute to the rampant proliferation of these pathogens [21]. Our results underscore the urgent need to integrate nematological assessments into the standard horticultural advisory for custard apples.

## 5. Conclusion

This research unequivocally establishes that plant-parasitic nematodes, led by *Meloidogyne incognita*, are a major and widespread production constraint in the custard apple orchards of Beed district, Maharashtra. The high prevalence, population density, and significant negative impact on plant growth warrant immediate attention.

**Future Perspectives:** Based on these findings, future work should focus on:

- (i) Conducting pathogenicity assays to establish economic threshold levels for *M. incognita* on custard apple,
- (ii) Screening custard apple rootstocks for sources of resistance, and
- (iii) Developing and validating integrated nematode management (INM) modules incorporating cultural practices, organic amendments, and biocontrol agents like *Purpureocillium lilacinum* and *Trichoderma* spp. for sustainable management.

## 6. References

- [1] Pareek, S., Yahia, E. M., & Kaushik, R. A. (2011). Postharvest physiology and technology of Annona fruits. *Food Research International*, 44(7), 1741-1751.
- [2] Kadam, S. S., Patil, R. B., & Desai, U. T. (2018). Fruit crop production in drought-prone areas of Maharashtra: An economic analysis. *Journal of Arid Land Studies*, 28(3), 123-128.
- [3] Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., ... & Perry, R. N. (2013). Top 10 plant-parasitic nematodes in molecular plant pathology. *Molecular Plant Pathology*, 14(9), 946-961.
- [4] Moens, M., Perry, R. N., & Starr, J. L. (2009). *Meloidogyne* species—a diverse group of novel and important plant parasites. In R. N. Perry, M. Moens, & J. L. Starr (Eds.), *Root-knot nematodes* (pp. 1-17). CAB International.
- [5] Sikora, R. A., Coyne, D., Hallmann, J., & Timper, P. (2018). *Plant parasitic nematodes in subtropical and tropical agriculture* (3rd ed.). CAB International.

- [6] Abad, P., Gouzy, J., Aury, J. M., Castagnone-Sereno, P., Danchin, E. G., Deleury, E., ... & Wincker, P. (2008). Genome sequence of the metazoan plant-parasitic nematode *Meloidogyne incognita*. *Nature Biotechnology*, 26(8), 909-915.
- [7] Castillo, P., & Vovlas, N. (2007). *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, biology, pathogenicity and management. *Nematology Monographs and Perspectives*, 6, 1-529.
- [8] Khan, M. R., & Jairajpuri, M. S. (2010). Nematode infestation in horticultural crops: Indian scenario. In M. R. Khan & M. S. Jairajpuri (Eds.), *Nematode infestation in horticultural crops* (pp. 1-48). National Academy of Sciences.
- [9] Khan, M. R. (2015). Nematode diseases of horticultural crops in India. *Indian Phytopathology*, 68(1), 1-21.
- [10] Nicol, J. M., Turner, S. J., Coyne, D. L., den Nijs, L., Hockland, S., & Maafi, Z. T. (2011). Current nematode threats to world agriculture. In J. Jones, G. Gheysen, & C. Fenoll (Eds.), *Genomics and molecular genetics of plant-nematode interactions* (pp. 21-43). Springer.
- [11] Cobb, N. A. (1918). Estimating the nema population of soil. *Agricultural Technology Circular*, 1, 48.
- [12] Southey, J. F. (Ed.). (1986). *Laboratory methods for work with plant and soil nematodes*. Her Majesty's Stationery Office (HMSO).
- [13] Hartman, K. M., & Sasser, J. N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In K. R. Barker, C. C. Carter, & J. N. Sasser (Eds.), *An advanced treatise on Meloidogyne, Vol. II* (pp. 69-77). North Carolina State University Graphics.
- [14] Eisenback, J. D., & Hirschmann, H. (1981). Identification of *Meloidogyne* species on the basis of head shape and stylet morphology of the male. *Journal of Nematology*, 13(4), 513-521.
- [15] Jairajpuri, M. S., & Ahmad, W. (1992). *Dorylaimida: Free-living, predaceous and plant-parasitic nematodes*. E.J. Brill.
- [16] Siddiqi, M. R. (2000). *Tylenchida: Parasites of plants and insects*. CABI Publishing.
- [17] McSorley, R., & Frederick, J. J. (1996). Nematode community structure in agroecosystems: A regional overview. *Journal of Nematology*, 28(4S), 435-443.
- [18] Bridge, J., & Page, S. L. J. (1980). Estimation of root-knot nematode infestation levels on roots using a rating chart. *Tropical Pest Management*, 26(3), 296-298.
- [19] Robinson, A. F. (2007). \*Reniform nematode: *Rotylenchulus* spp.”. In: *Nematology advances and perspectives, Vol. 2* (pp. 893-919). CAB International.
- [20] Nyczepir, A. P., & Becker, J. O. (2010). Fruit and nut tree hosts of *Meloidogyne* spp. with emphasis on *M. incognita* and *M. javanica*. In A. P. Nyczepir & J. O. Becker (Eds.), *Plant Nematology* (pp. 345-367). CAB International.
- [21] Stirling, G. R. (2014). *Biological control of plant-parasitic nematodes: Soil ecosystem management in sustainable agriculture*. CAB International.