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RESEARCH ARTICLE

Survey-driven insights into the diversity and prevalence of nematode-fungus complex in guava

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Abstract

A systematic survey during 2022–2024 was conducted in guava orchards and nurseries of Jind, Hisar and Bhiwani districts in Haryana to assess the incidence, diversity and community structure of plant-parasitic nematodes (PPNs) and fungi associated with guava decline. A total of 150 samples of guava orchards were collected from Hisar, Jind and Bhiwani districts of Haryana, 105 were found infested with *Meloidogyne incognita* with a 70.0 % frequency of occurrence. Among 105 samples, 88 were found infested with *M. incognita* and *Fusarium oxysporum* f.sp. *psidii* both with a disease incidence of 58.6 % during 2022–24. In guava nurseries, 44 samples (out of 60) were infected with *M. incognita* having a frequency of occurrence of 73.0 % and 27 were infected with nematode and fungus (45.0 %). Results revealed that *M. incognita* and *F. oxysporum* f.sp. *psidii* were identified as the most prominent pathogens causing guava decline. Community analysis confirmed *M. incognita* as the most frequent and dominant nematode species, with relative frequencies up to 24.6 % and densities up to 60.2 %, followed by *Helicotylenchus* and *Hoplolaimus*. Morphometric observations of *M. incognita* obtained from the current study were similar to those of its original description. Morphological identification of the fungus associated with guava decline was identified as *F. oxysporum* f.sp. *psidii* . The results revealed a strong association between *M. incognita* and *F. oxysporum*, contributing to the guava decline complex and highlight the role of nursery-borne inoculum in disease spread.

Keywords: community analysis; *Fusarium oxysporum*; guava decline; Haryana; *Meloidogyne incognita*

Introduction

Guava (*Psidium guajava* L.) is an economically important tropical fruit crop belonging to the family *Myrtaceae* and is widely cultivated across tropical and subtropical regions of the world. In India, it is often referred to as the “poor man’s fruit” for its affordability, high nutritional value and adaptability to diverse agroclimatic conditions. The crop thrives well in well-drained sandy loam to clay loam soils with a pH range of 6.5–8.5 and is known for its high content of ascorbic acid, pectin, antioxidants and polyphenols that contribute to its recognized antimutagenic, antimicrobial and antiviral properties (1). India ranks among the world’s leading producers of guava, with substantial cultivation in Uttar Pradesh, Bihar, Madhya Pradesh, Maharashtra and Haryana. However, despite its economic potential, guava productivity has been steadily declining due to the prevalence of biotic and abiotic stresses, particularly those caused by soil-borne pathogens. Among the biotic factors, insect pests such as fruit fly (*Bactrocera dorsalis*), fruit borer (*Deudorix isocrates*), tea mosquito bug (*Helopeltis antonii*) and guava aphid (*Aphis gossypii*) are of concern, while fungal pathogens and plant-parasitic nematodes (PPNs) represent major threats to both yield and fruit quality. Root-knot nematodes (RKNs) belonging to the genus *Meloidogyne* are among the most damaging PPNs of guava, causing characteristic root galling and disrupting water and nutrient uptake. The predominant species reported from India are *Meloidogyne incognita*, *M. javanica*, *M. arenaria* and *M. hapla*, which together account for 10–69 % annual yield losses in horticultural crops (2). These sedentary endoparasites establish permanent feeding sites (giant cells) in the vascular cylinder, resulting in physiological stress and reduced plant vigor (3). In guava, *M. incognita* and *M. javanica* are the most widespread species in nurseries and orchards across northern India (4), while the occurrence of the aggressive species *M. enterolobii* has been sporadically reported (5, 6). Equally significant is the role of soil-borne fungi, particularly *Fusarium oxysporum*, *Rhizoctonia solani* and *Macrophomina phaseolina* , which cause wilt, root rot and damping-off diseases in guava. *Fusarium oxysporum* is the principal causal agent of guava wilt in India. The pathogen survives in soil for extended periods as chlamydospores and infects plants through wounds or lateral roots, leading to vascular blockage and wilting (7, 8). Recent investigations have revealed that the simultaneous infection of guava roots by *Meloidogyne spp.* and *F. oxysporum* produces a severe nematode –fungus (NF) complex, which results in rapid wilting, defoliation

and premature plant death (9). This complex interaction intensifies the disease severity beyond that caused by either pathogen alone, leading to major economic losses. The guava wilt complex has emerged as one of the most critical challenges to sustainable guava production in India. Since planting material is largely derived from nurseries, the presence of soil-borne pathogens in nursery soils facilitates the early establishment and spread of the disease (10). Effective management therefore depends on a comprehensive understanding of the nematode–fungus association, their distribution and the ecological factors influencing their co-occurrence. The main objective of this survey was to determine the incidence, diversity and distribution of PPNs and soilborne fungal pathogens associated with the guava wilt complex in major guava-producing districts of Haryana. The study also aimed to analyze the nematode community structure and explore the synergistic interaction between nematodes and fungi responsible for guava decline, providing a scientific basis for eco-friendly management strategies.

Materials and Methods

The survey was carried out for the incidence of guava decline in the Jind, Hisar and Bhiwani districts of Haryana during 2022–24. The GPS coordinates of surveyed locations were recorded. Twenty-five samples of guava orchards and twenty samples of guava nurseries from each district (Jind, Hisar and Bhiwani) were collected randomly each year. Composite samples of soil (500 g) and root (5 g) were collected randomly from the rhizosphere of guava plants (Fig. 1). The number of wilted and dried guava plants was also counted in the orchard and disease incidence was expressed as a percentage.

Sample collection

Soil and root samples were collected in polythene bags, with proper labeling and were stored in the refrigerator at 4 ± 1 °C before processing. The samples were analyzed for the infestation of PPNs and fungi. Data on nematode population densities were analyzed to assess the average density of RKNs and frequency of occurrence in each district. The severity of the infection was recorded on the basis of root-knot index (Table 1).

Nematode extraction from soil (J2s/200 cc soil)

Cobb's decanting and sieving with the Modified Baermann's Funnel Technique was employed to extract nematodes from soil. A 200-cc soil sample was thoroughly mixed with 1000 mL of water in a pan and allowed to settle for 10 to 15 sec. A series of 20, 100 and 300 mesh sieves was used to extract nematodes from the soil. To remove roots, stubbles and other inert material, the soil suspension was run through a 20-mesh sieve. Filtrate from a 20-mesh sieve was collected in a different pan and the process was repeated for 60, 100 and 300 mesh sieves. The 300-mesh sieve was disposed of and the remains were collected in the beaker by backwashing the sieve. After that, the contents of the 300-mesh sieve were poured into a Petri dish with two layers of tissue paper held on a molded wire gauge and they were left undisturbed for 24 hr. After 24 hr, the nematode suspension was collected, its volume was increased to 100 mL and it was used to analyze the nematode population using a stereo binocular microscope.

Counting of nematodes

The nematode population in the suspension was examined under a stereo binocular microscope. The nematode suspension was made up to a known volume (100 mL). Nematode counting was done by bubbling the water suspension with a pipette to disperse the nematodes. 1 mL of the suspension was poured into a counting dish and the number of nematodes was counted. An average of three counts was taken and multiplied by a known volume to calculate the total population.

Nematode extraction from guava roots

The roots were gently washed under running tap water to remove adhering soil. The roots were weighed to 5 g and chopped into small pieces (1 cm or smaller) using a sterile blade. The chopped roots were blended with 100–200 mL of distilled water in a blender for 10–15 sec. The macerated root suspension was poured through a 60-mesh sieve (250 µm) to remove large debris. The filtrate was passed through a 500-mesh sieve (25 µm) to retain nematodes. The nematodes were backwashed from the sieve into a Petri dish using a small amount of water.

Preparation of slides for nematode identification

The killing, fixing and clearing method was used to process the nematode suspension that was extracted from the samples to prepare permanent slides (11). An equivalent volume of boiling fixative (8 % formalin) was added to the vials to kill and fix the nematodes. Nematodes were killed and fixed for additional processing to prepare permanent slides after the lid was securely closed and left for a full day. After transferring nematodes to a cavity block that contained Seinhorst's solution I, the partially covered cavity blocks were kept in the oven for 12 hr at 40 °C. The cavity block was then partially covered, filled with Seinhorst's solution II and placed in the oven for 4 hr at 40 °C. Then the nematode was transferred from the hollow block to a sanitized glass slide and a drop of glycerol was placed to make permanent slides. A circular coverslip was carefully fitted over the glycerol drop after placing the tiny pieces of wax.

Identification of root-knot nematode species

The root samples infected with RKNs were washed in running tap water. After being chopped into tiny pieces of 2 cm, infected roots were boiled in 0.1 % acid fuchsin lactophenol for 2–3 min. To de-stain the roots, the roots were kept in plain lactophenol overnight after being well-cleaned under running water. Under a stereo binocular microscope, adult females were removed from the root galls, their posterior portion was cut and their internal organs were cleansed. The perineal pattern was put on a glass slide with a drop of lactophenol and a cover slip was placed on it and sealed with nail polish (Fig. 2). The species confirmation was done based on the perineal pattern.

Table 1. Root-knot index on 1–5 scale

Sl. No.	No. of galls/plant	Root-knot index (RKI)
1.	No galls	1.0
2.	1–10 galls	2.0
3.	11–30 galls	3.0
4.	31–100 galls	4.0
5.	More than 100 galls	5.0

Fig. 1. a, b & c: Nematode + fungus infested nurseries; d & e: nematode + fungus infested guava orchard samples.

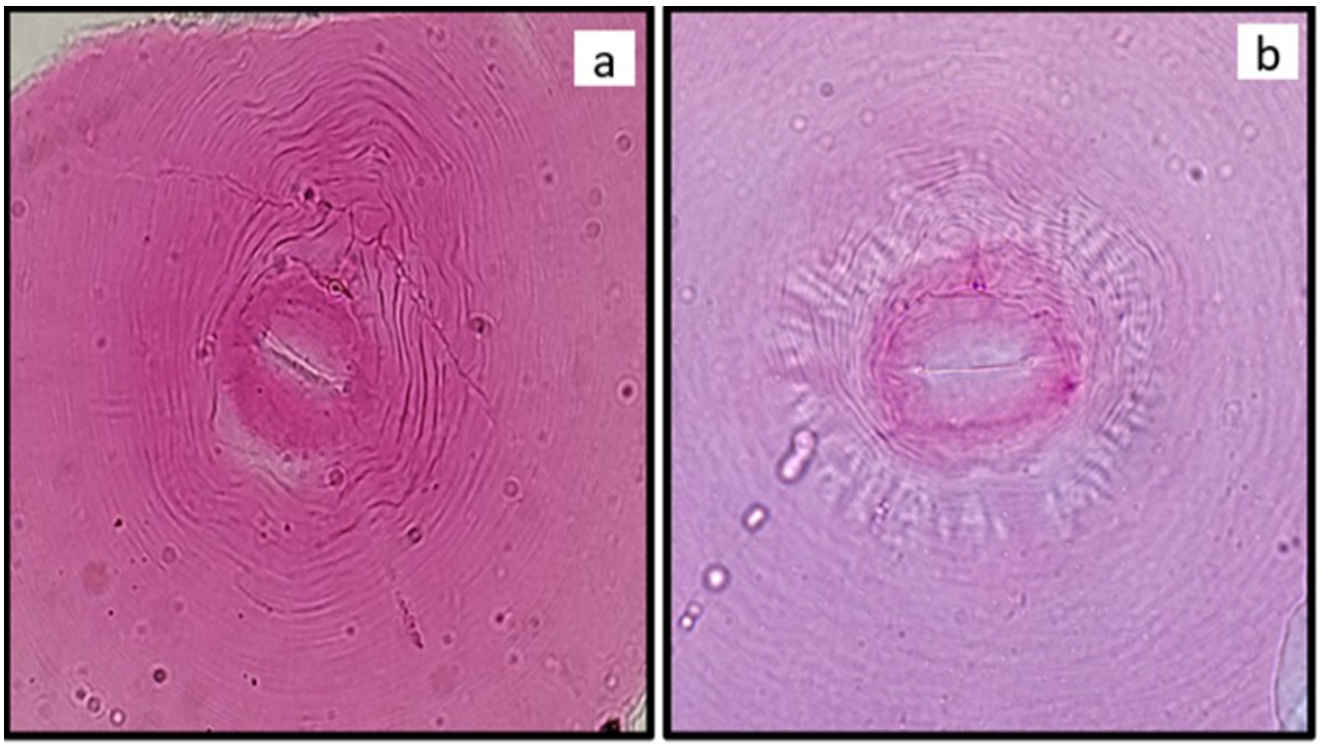

Fig. 2. Perineal pattern of RKNs found during the survey. a: *M. javanica* b: *M. incognita*.

Isolation and identification of fungi

The associated fungi were isolated from infected guava plant parts and rhizospheric soil on potato dextrose agar medium (PDA). Pure culture was obtained from the single spore method and was maintained on PDA slants. The roots were cleaned and washed in water to remove soil particles and were surface sterilized by using 0.1 % sodium hypochlorite for 2–3 min and then rinsed with sterile distilled water. The small bits of roots were transferred to Petri plates containing sterilized PDA medium and they were then incubated for seven days at 26 ± 1 °C. Colonies were examined under a compound microscope for identification of fungus (Fig. 3). The fungus was identified based on mycological observations such as colony color, mycelial growth and micro and macro conidia (Fig. 4–6) (12).

Community analysis of plant parasitic nematodes

To extract nematodes, 150 samples were taken from guava orchards. PPNs were identified by making permanent slides and using a compound microscope. The absolute frequency, relative frequency, absolute density, relative density and prominence value of each nematode species were calculated by using the following formulae (13).

$$\text{Absolute frequency (\%)} = \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency (\%)} = \frac{\text{Frequency of species}}{\text{Sum of frequencies of all species}} \times 100$$

Absolute density (%) = Total number of species in known volume of soil

$$\text{Relative density (\%)} = \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

Prominence value = Absolute density × √ Absolute frequency

Statistical analysis

Statistical analysis was performed using two-way ANOVA (F-test) to compare nematode and fungal parameters among districts and years. Data were analyzed for variance homogeneity and significance at $p \leq 0.05$ and $p \leq 0.01$. In addition, a Random Forest model was trained to predict disease incidence using nematode-related variables (frequency, density, RKI). Model accuracy and feature importance were calculated to identify key predictors influencing guava wilt incidence. All analyses were carried out using R (v4.3.2) and Python (scikit-learn library).

Results

Guava orchards

The results revealed that RKN species, viz. *M. incognita* and other PPNs viz. *Rotylenchulus reniformis*, *Helicotylenchus* spp., *Hoplolaimus* spp., *Pratylenchus* spp. and *Tylenchorhynchus* spp., were associated with the guava during 2022–23. Parasitic fungi viz. *F. oxysporum*, *R. solani*, *F. solani* and *M. phaseolina* were found to be associated with guava orchards during the survey (Table 2). A total of 75 soil and root samples were collected from guava orchards of Hisar, Jind and Bhiwani, out of which 51 samples (42 were above ETL) were found infested with *M. incognita*, with a frequency of occurrence of 68 %. Out of 51 samples, 43 were infested with nematode and fungus with an incidence of 57 %. The maximum frequency of occurrence of nematode among the three districts was found in the Jind (84 %), followed by Bhiwani (64 %) and Hisar district (56 %). Among all the districts surveyed, Jind had the highest number of samples (17 out of 21) that were infected with nematode and fungus, with a disease incidence of 68 %, followed by Bhiwani (56 %) and Hisar (48 %) districts. The maximum density range of *M. incognita* was recorded in the Jind (250-870 J2s/200 cc soil) district, followed by Bhiwani (150-767 J2s/200 cc soil) and Hisar (154-610 J2s/200 cc soil) districts. The RKI was recorded to be 2.0–5.0 in the samples surveyed. Within the villages of different districts, the maximum RKI (5.0) was observed in the villages Jeetakheri and Dhani Miran of Bhiwani, Hindwan and Matershyam of Hisar and Sudkain Kalan of Jind during 2022–23 (Table 3–5).

The results revealed that out of 75 soil and root samples collected from all the districts, 54 (47 were above ETL) were found infected with RKNs having 72 % frequency occurrence. *M. incognita* was the predominant and more pathogenic to guava than *M. javanica*. The nematode *M. javanica* was found in the orchards where tomato and okra crops were intercropped with guava. Out of 54 samples found infested, 45 were found infested with nematode and fungus with a disease incidence of 60 %. The maximum frequency of occurrence of *M. incognita* was found in Hisar (88 %), followed by Jind (76 %) and Bhiwani (52 %). Among all the districts surveyed, the maximum disease incidence was found in the Jind (72 %), followed by Hisar (60 %) and Bhiwani (48 %). The maximum density range of *M. incognita* was found in the Jind (250-870 J2s/200 cc soil), followed by Hisar (165-685 J2s/200 cc soil) and Bhiwani (104-527 J2s/200 cc soil) districts (Table 6). The maximum RKI was recorded in the Jind (3.0–5.0). Within the villages of all districts, the maximum incidence of guava decline was found in the Jind district followed by Hisar and Bhiwani districts during 2022–24. The decline in guava was prominent throughout the survey in the orchards where guava plants were infected with *M. incognita* and *F. oxysporum*. During the survey, both the young as well as old orchards were found infected with the guava decline. Among the villages, Dumarekha Khurd of Jind, Dharan of Bhiwani, Jagan and Sabarwas of Hisar have the highest nematode infestation with RKI of 5.0 during 2023–24 (Table 7–9). The combined ANOVA for the two survey years (2022–24) revealed highly significant district-level differences ($p < 0.01$) for nematode frequency, population density, RKI and disease incidence (Table 10). Jind district consistently showed the highest infestation levels across both years, confirming its status as a major hotspot for *M. incognita* and *F. oxysporum* f.sp. *psidii* complex infection. A moderate but significant year effect ($p < 0.05$) was observed for nematode frequency, suggesting inter-annual variability influenced by climatic or soil factors. However, other parameters such as nematode density, RKI and disease incidence remained statistically stable across years, indicating persistent endemicity of the nematode-fungus complex.

Fig. 7 depicts the distribution of major fungal pathogens associated with guava wilt in the orchards of Bhiwani, Hisar and Jind during 2022–23 and 2023–24, showing that *F. oxysporum* is the most dominant pathogen across all districts and years, with the highest incidence recorded in Hisar and Jind. *Fusarium solani* appears as the second most prevalent fungus, with consistently

Table 2. Diversity of economically important plant parasitic nematodes and fungi infecting guava orchards in Haryana during 2022–23

Districts	GPS location	Surveyed	Nematode parameters						Fungus parameters			
			Infected	Frequency of occurrence (%)	Density range (J2s/200 cc soil)	RKI*	Root-knot nematode identified	Other plant parasitic nematodes associated	Infected (Nematode +Fungus)	Disease incidence (%)	Major fungi identified	Other fungi associated
Hisar	28°93'49" N-29°49'97" N 75°47'56" E-75°96'93" E	25	14	56	154–610	2.0–5.0	*M. incognita*	*Hoplolaimus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Pratylenchus* spp.	12	48	*F. oxysporum*	*Macrophomina phaseolina*, *Rhizoctonia solani*, *F. solani*
Bhiwani	28°81'57" N-29°67'57" N 75°73'65" E-76°72'13" E	25	16	64	150–767	2.0–5.0			14	56		
Jind	29°19'76" N-29°58'31" N 76°10'93" E-76°42'22" E	25	21	84	250–870	3.0–5.0			17	68		
Total		75	51**	68	150–870	2.0–5.0			43	57		

*RKI: Root-knot index; **Out of 51 samples, 42 were found above ETL.

Table 3. Distribution of root-knot nematode and fungi associated with guava roots in the orchards of Bhiwani district during 2022–23

Sl. No.	Villages	GPS coordinates	RKNs identified	Pathogenic fungus identified	RKI
1.	Sagban	28°84'17" N 75°93'59" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0.
2.	Sagban	28°84'17" N 75°93'59" E	-	-	1.0
3.	Sagban	28°84'82" N 75°93'36" E	*M. incognita*	*F. oxysporum*	2.0
4.	Sagban	28°84'87" N 75°93'43" E	*M. incognita* *M. javanica*	*F. solani*	3.0
5.	Kharakk	28°84'05" N 75°91'63" E	*M. incognita*	-	2.0
6.	Kharakk	28°83'98" N 75°87'06" E	-	-	1.0
7.	Nigna kalan	28°77'40" N 75°93'96" E	-	-	1.0
8.	Nigna kalan	28°77'98" N 75°93'86" E	*M. incognita* *M. javanica*	*F. oxysporum*	4.0
9.	Nigna kalan	28°77'96" N 75°93'56" E	-	*M. phaseoli*	1.0
10.	Riwasa	28°79'89" N 75°95'95" E	*M. incognita*	*F. solani*	2.0
11.	Riwasa	28°80'81" N 75°95'88" E	-	-	1.0
12.	Riwasa	28°82'70" N 75°94'46" E	*M. incognita*	*F. oxysporum* *M. phaseoli*	4.0
13.	Dharan	28°82'76" N 75°94'46" E	*M. incognita*	*F. oxysporum*	4.0
14.	Dharan	28°82'32" N 75°93'31" E	*M. incognita*	*F. oxysporum*	2.0
15.	Dharan	28°82'33" N 75°93'51" E	*M. incognita*	*F. oxysporum*	3.0
16.	Dharan	28°82'34" N 75°92'78" E	*M. incognita* *M. javanica*	*F. oxysporum* *M. phaseoli* *R. solani*	5.0
17.	Khak	28°82'39" N 75°91'92" E	*M. incognita*	*F. oxysporum*	3.0
18.	Khak	28°83'10" N 75°89'56" E	-	*F. oxysporum*	1.0
19.	Khak	28°83'10" N 75°89'55" E	*M. incognita*	*F. oxysporum* *R. solani*	4.0

20.	Khak	28°85'60" N 75°94'28" E	-	*F. oxysporum*	1.0
21.	Tosham	28°86'95" N 75°92'18" E	*M. incognita*	*F. oxysporum*	2.0
22.	Tosham	28°87'60" N 75°93'68" E	*M. incognita*	*F. solani*	3.0
23.	Tosham	28°87'60" N 75°93'68" E	*M. incognita*	-	2.0
24.	Pinjo	28°89'89" N 75°81'00" E	-	*R. solani*	1.0
25.	Pinjo	28°86'28" N 75°90'91" E	-	-	1.0

RKNs- Root-knot nematodes; RKI- Root-knot index.

Table 4. Distribution of root-knot nematode and fungi associated with guava roots in the orchards of Hisar district during 2022–23

Sl. No.	Villages	GPS coordinates	RKNs identified	Pathogenic fungus identified	RKI
1.	Saharwa	28°93'49" N 75°72'54" E	-	-	1.0
2.	Matershyam	29°16'27" N 75°56'79" E	*M. incognita*	*F. oxysporum* *M. phaseoli* *F. solani*	5.0
3.	Salemgarh	29°18'80" N 75°54'46" E	-	-	1.0
4.	Shiswal	29°20'46" N 75°50'13" E	*M. incognita*	-	3.0
5.	Shiswal	29°23'42" N 75°48'66" E	*M. incognita*	*F. oxysporum*	4.0
6.	Shiswal	29°23'53" N 75°48'03" E	*M. incognita*	*F. oxysporum*	3.0
7.	Shiswal	29°22'91" N 75°48'95" E	-	-	1.0
8.	Adampur	29°26'23" N 75°47'56" E	*M. incognita*	*F. oxysporum*	4.0
9.	Nangthala	29°33'07" N 75°68'55" E	-	-	1.0
10.	Jaggabara	29°16'41" N 75°96'08" E	*M. incognita*	*F. oxysporum*	3.0
11.	Jaggabara	29°16'41" N 75°96'20" E	-	-	1.0
12.	Suleman Bara	29°15'85" N 75°96'93" E	-	-	1.0
13.	Balsamand	29°10'95" N 75°49'38" E	*M. incognita*	*F. oxysporum*	3.0
14.	Rawalwas	29°08'10" N 75°57'99" E	-	-	1.0
15.	Rawalwas	29°08'72" N 75°57'97" E	-	-	1.0
16.	Aryanagar	29°13'04" N 75°66'50" E	*M. incognita*	*F. oxysporum* *M. phaseoli*	4.0
17.	Aryanagar	29°13'04" N 75°66'50" E	*M. incognita*	*F. oxysporum*	3.0
18.	Aryanagar	29°12'72" N 75°66'62" E	*M. incognita*	-	2.0
19.	Patan	29°49'97" N 75°91'03" E	-	-	1.0
20.	Hindwan	29°13'39" N 75°60'44" E	*M. incognita*	*F. oxysporum* *F. solani* *M. phaseoli*	5.0
21.	Hindwan	29°12'27" N 75°60'44" E	*M. incognita*	*F. oxysporum*	3.0
22.	Hindwan	29°11'17" N 75°62'46" E	-	-	1.0
23.	Adampur	29°26'23" N 75°47'56" E	*M. incognita*	*F. oxysporum* *F. solani*	3.0
24.	Balsamand	29°10'95" N 75°49'38" E	-	-	1.0
25.	Hisar	29°14'87" N 75°70'79" E	*M. incognita*	*F. oxysporum*	4.0

RKNs- Root-knot nematodes; RKI- Root-knot index.

Table 5. Distribution of root-knot nematode and fungi associated with guava roots in the orchards of Jind district during 2022–23

Sl. No.	Villages	GPS coordinates	RKNs identified	Pathogenic fungus identified	RKI
1.	Baroda	29°42'54" N 76°42'22" E	*M. incognita*	*F. oxysporum*	3.0
2.	Baroda	29°44'41" N 76°20'07" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
3.	Baroda	29°44'41" N 76°20'14" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
4.	Palwan	29°49'11" N 76°18'04" E	-	-	1.0
5.	Palwan	29°49'03" N 76°18'04" E	*M. incognita*	*F. oxysporum*	3.0
6.	Palwan	29°29'46" N 76°10'82" E	*M. javanica* *M. incognita*	*F. oxysporum* *M. phaseoli*, *R. solani*	3.0
7.	Palwan	29°29'58" N 76°10'93" E	*M. incognita*	-	2.0
8.	Sapaakheri	29°50'29" N 76°17'12" E	*M. incognita*	*F. oxysporum*	2.0
9.	Sapaakheri	29°50'36" N 76°17'11" E	*M. incognita*	-	2.0
10.	Sapaakheri	29°50'40" N 76°17'08" E	*M. incognita*	*R. solani*	3.0
11.	Ghaso Khurd	29°34'62" N 76°16'89" E	*M. incognita*	*F. oxysporum*	3.0
12.	Ghaso Khurd	29°54'25" N 76°15'57" E	-	-	1.0
13.	Dumarkha Khurd	29°55'66" N 76°18'02" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
14.	Dumarkha Khurd	29°55'68" N 76°18'02" E	-	-	1.0
15.	Dumarkha Khurd	29°55'71" N 76°18'02" E	*M. incognita*	*F. oxysporum*	3.0
16.	Kheri Lohchab	29°32'90" N 76°11'47" E	*M. incognita*	*F. oxysporum*	3.0
17.	Sudkain Kalan	29°58'31" N 76°20'26" E	*M. incognita*	*F. oxysporum* *F. solani*	5.0
18.	Sudkain Kalan	29°55'68" N 76°18'02" E	*M. incognita*	-	3.0
19.	Sudkain Kalan	29°58'31" N 76°20'23" E	*M. incognita*	*F. oxysporum*	3.0
20.	Haibatpura	29°32'26" N 75°20'56" E	*M. incognita*	*F. oxysporum* *F. solani*	5.0
21.	Dilluwala	29°24'52" N 76°24'85" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
22.	Dilluwala	29°25'32" N 76°24'97" E	-	-	1.0
23.	Nagura	29°27'32" N 76°22'63" E	*M. incognita*	*F. oxysporum*	3.0
24.	Sangatpura	29°19'76" N 76°15'02" E	*M. incognita*	-	2.0
25.	Jind	29°54'25" N 76°16'98" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0

RKNs- Root-knot nematodes; RKI- Root-knot index.

Table 6. Diversity of economically important plant parasitic nematodes and fungi infecting guava orchards in Haryana during 2023–24

			Nematode parameters						Fungus parameters			
Districts	GPS location	Surveyed	Infected	Frequency of occurrence (%)	Density range (J2s/ 200 cc soil)	RKI*	Root-knot nematode identified	Other plant parasitic nematodes associated	Infected (Nematode +Fungus)	Disease incidence (%)	Major fungi identified	Other fungi associated
Hisar	29°27'79" N-29°39'96" N 75°16'59" E-75°66'01" E	25	22	88	165–685	2.0–5.0	*M. incognita*, *M. javanica*	*Hoplolaimus* spp., *Helicotylenchus* spp., *Tylenchorhynchus* spp., *Pratylenchus* spp.	15	60	*F. oxysporum*	*R. solani*, *F. solani*
Bhiwani	28°77'40" N-28°89'89" N 76°87"06" E-76°95'95" E	25	13	52	104–527	2.0–5.0			12	48		
Jind	29°31'52" N-29°57'46" N-76°07'90" E-76°32'17" E	25	19	76	208–895	3.0–5.0			18	72		
Total		75	54**	72	104–895	2.0–5.0			45	60		

*RKI: Root-knot index; **Out of 54 samples, 47 were found above ETL.

Table 7. Distribution of root-knot nematode and fungi associated with guava roots in the orchards of Jind district during 2023–24

Sl. No.	Villages	GPS coordinates	RKNs identified	Pathogenic fungus identified	RKI
1.	Intal Kalan	29°31'52" N 76°24'87" E	*M. incognita*	*F. oxysporum*	3.0
2.	Intal Kalan	29°31'54" N 76°24'88" E	*M. incognita*	*F. oxysporum*	2.0
3.	Intal Kalan	29°31'62" N 76°25'36" E	*M. incognita*	*F. solani*	3.0
4.	Intal Kalan	29°31'62" N 76°25'32" E	*M. incognita*	*F. oxysporum*	2.0
5.	Intal Kalan	29°32'03" N 76°24'80" E	*M. incognita*	*F. oxysporum*	4.0
6.	Jalalpur	29°32'20" N 76°26'41" E	*M. incognita* *M. javanica*	*F. oxysporum* *M. phaseoli*	4.0
7.	Amarheri	29°34'39" N 76°32'17" E	-	-	1.0
8.	Ahiraka	29°34'61" N 76°30'29" E	*M. incognita*	*F. oxysporum*	3.0
9.	Ahiraka	29°34'61" N 76°30'26" E	*M. incognita*	*R. solani*	3.0
10.	Ahiraka	29°35'49" N 76°29'09" E	*M. incognita*	*F. oxysporum*	4.0
11.	Baroda	29°44'62" N 76°20'42" E	*M. incognita*	*F. oxysporum*	2.0
12.	Uchana	29°49'18" N 76°18'02" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
13.	Uchana	29°49'07" N 76°18'06" E	-	-	1.0
14.	Uchana	29°49'09" N 76°18'04" E	*M. incognita*	*F. oxysporum* *M. phaseoli* *R. solani*	3.0
15.	Ghasso Khurd	29°53'46" N 76°16'07" E	*M. incognita*	*F. oxysporum*	3.0
16.	Ghasso Khurd	29°53'50" N 76°16'07" E	-	-	1.0
17.	Ghasso Khurd	29°54'27" N 76°15'59" E	-	-	1.0
18.	Dumarekha Khurd	29°54'29" N 76°15'50" E	*M. incognita*	*F. oxysporum* *M. phaseoli*	5.0
19.	Dumarekha Khurd	29°54'29" N 76°15'50" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
20.	Dumarekha Khurd	29°54'34" N 76°15'61" E	-	-	1.0
21.	Dumarkha Khurd	29°54'25" N 76°17'02" E	*M. incognita*	*F. oxysporum*	3.0
22.	Dumarkha Khurd	29°57'46" N 76°13'39" E	*M. incognita*	-	3.0
23.	Badowala	29°57'12" N 76°07'90" E	*M. incognita*	*F. oxysporum* *R. solani*	3.0
24.	Kheri Chopta	29°32'90" N 76°11'47" E	-	-	1.0
25.	Kheri Lohchab	29°33'06" N 76°08'39" E	*M. incognita*	*F. oxysporum*	3.0

RKNs- Root-knot nematodes; RKI- Root-knot index.

Table 8. Distribution of root-knot nematode and fungi associated with guava roots in the orchards of Hisar district during 2023–24

Sl. No.	Villages	GPS coordinates	RKNs identified	Pathogenic fungus identified	RKI
1.	Jagan	29°27'79" N 75°62'27" E	*M. incognita*	-	2.0
2.	Jagan	29°27'79" N 75°62'30" E	*M. incognita*	*F. oxysporum* *F. solani*	5.0
3.	Jagan	29°27'79" N 75°62'30" E	*M. incognita*	-	2.0
4.	Jagan	29°27'81" N 75°62'85" E	*M. incognita*	*F. oxysporum* *F. solani*	3.0
5.	Jagan	29°27'78" N 75°62'85" E	*M. incognita*	-	2.0
6.	Landhari	29°28'59" N 75°65'77" E	*M. incognita* *M. javanica*	*F. oxysporum*	4.0
7.	Landhari	29°28'68" N 75°65'77" E	*M. incognita*	-	3.0
8.	Landhari	29°31'05" N 75°66'01" E	-	-	1.0
9.	Landhari	29°31'05" N 75°66'01" E	*M. incognita*	*F. oxysporum*	2.0

10.	Mirpur	29°31'56" N 75°16'59" E	-	-	1.0
11.	Mirpur	29°31'52" N 75°16'59" E	*M. incognita*	*F. oxysporum*	3.0
12.	Mirpur	29°32'47" N 75°65'48" E	*M. incognita*	-	2.0
13.	Mirpur	29°32'47" N 75°65'90" E	*M. incognita*	*F. oxysporum*	3.0
14.	Sabarwas	29°38'16" N 75°63'38" E	*M. incognita*	*F. oxysporum*	2.0
15.	Sabarwas	29°39'22" N 75°63'90" E	*M. incognita*	*F. oxysporum*	3.0
16.	Sabarwas	29°39'22" N 75°63'90" E	*M. incognita*	*F. oxysporum*	3.0
17.	Sabarwas	29°39'22" N 75°63'90" E	-	-	1.0
18.	Sabarwas	29°39'96" N 75°63'19" E	*M. incognita*	*F. oxysporum* *F. solani* *M. phaseoli*	5.0
19.	Sabarwas	29°38'96" N 75°64'16" E	*M. incognita*	*F. oxysporum*	3.0
20.	Sabarwas	29°38'96" N 75°64'16" E	*M. incognita*	-	2.0
21.	Kuleri	29°36'92" N 75°65'65" E	*M. incognita*	*F. oxysporum* *F. solani*	3.0
22.	Kuleri	29°36'91" N 75°65'65" E	*M. incognita*	-	2.0
23.	Kuleri	29°36'19" N 75°65'73" E	*M. incognita*	*F. oxysporum*	2.0
24.	Kuleri	29°34'92" N 75°64'83" E	*M. incognita*	*F. oxysporum*	3.0
25.	Kuleri	29°34'91" N 75°64'80" E	*M. incognita*	*F. oxysporum*	3.0

RKNs- Root-knot nematodes; RKI- Root-knot index.

Table 9. Distribution of root-knot nematode and fungi associated with guava roots in the orchards of Bhiwani district during 2023–24

Sl. No.	Villages	GPS coordinates	RKNs identified	Pathogenic fungus identified	RKI
1.	Dhani Miran	28°82'78" N 75°73'67" E	*M. incognita*	*F. oxysporum* *F. solani* *M. phaseoli*	5.0
2.	Dhani Miran	28°82'71" N 75°73'65" E	*M. incognita*	*F. oxysporum*	3.0
3.	Dhani Miran	28°88'27" N 75°73'65" E	-	-	1.0
4.	Kharleri	28°81'58" N 75°85'23" E	*M. incognita* *M. javanica*	*F. oxysporum*	3.0
5.	Kharleri	28°81'57" N 75°85'24" E	-	-	1.0
6.	Thilor	28°82'03" N 75°85'48" E	*M. incognita*	-	2.0
7.	Thilor	28°81'58" N 75°85'24" E	*M. incognita*	*F. oxysporum*	2.0
8.	Biran	28°83'61" N 75°99'11" E	*M. incognita* *M. javanica*	*F. oxysporum*	3.0
9.	Bapora	28°81'51" N 76°07'75" E	-	-	1.0
10.	Talu	28°96'72" N 76°16'84" E	-	-	1.0
11.	Talu	28°96'66" N 76°17'38" E	-	-	1.0
12.	Talu	28°96'65" N 76°16'84" E	*M. incognita*	*F. oxysporum* *M. phaseoli*	4.0
13.	Jatai	28°96'67" N 76°17'21" E	*M. incognita*	*F. oxysporum* *F. solani*	4.0
14.	Jatai	28°96'67" N 76°72'13" E	-	-	1.0
15.	Jeetakheri	28°99'58" N 75°01'00" E	*M. incognita*	*F. oxysporum*	3.0

16.	Jeetakheri	28°99'58" N 76°01'00" E	*M. incognita* *M. javanica*	*F. oxysporum* *F. solani* *M. phaseoli*	5.0
17.	Milakpur	29°04'99" N 75°98'72" E	-	-	1.0
18.	Milakpur	29°06'75" N 75°97'73" E	-	-	1.0
19.	Dhani Khushal	29°06'75" N 75°97'73" E	*M. incognita* *M. javanica*	*F. oxysporum* *F. solani*	4.0
20.	Kungar Bhaini	28°99'04" N 76°09'82" E	-	-	1.0
21.	Bhiwani	28°79'55" N 75°72'41" E	*M. incognita*	*F. oxysporum*	3.0
22.	Biran	28°83'61" N 75°99'11" E	-	-	1.0
23.	Sagban	28°85'09" N 75°93'43" E	*M. incognita*	*F. oxysporum*	3.0
24.	Sagban	28°84'87" N 75°93'43" E	-	-	1.0
25.	Sagban	28°84'82" N 75°93'36" E	-	-	1.0

RKN- Root-knot nematode; RKI- Root-knot index.

Table 10. ANOVA for district- and year-wise variation in nematode and fungal parameters associated with guava decline in Haryana (2022–24)

Parameter	Source of variation	df	Mean square	F-value	p-value	Significance	District/Year with maximum value
Frequency of nematode occurrence (%)	District	2	602.7	10.81	0.00012	Highly significant ($p < 0.01$)	Jind (84 % in 2022–23; 76 % in 2023–24)
	Year	1	256.0	4.59	0.038	Significant ($p < 0.05$)	2023–24
Nematode density (J2s/200 cc soil)	District	2	67825	9.47	0.00028	Highly significant ($p < 0.01$)	Jind (870 J2s/200 cc in 2022–23; 895 in 2023–24)
	Year	1	8012	1.12	0.296	NS	–
Root-knot index (RKI)	District	2	0.395	7.21	0.0017	Significant ($p < 0.01$)	Jind
	Year	1	0.042	0.76	0.385	NS	–
Disease incidence (%)	District	2	346.7	9.80	0.00023	Highly significant ($p < 0.01$)	Jind (68 % in 2022–23; 72 % in 2023–24)
	Year	1	54.7	1.54	0.225	NS	–

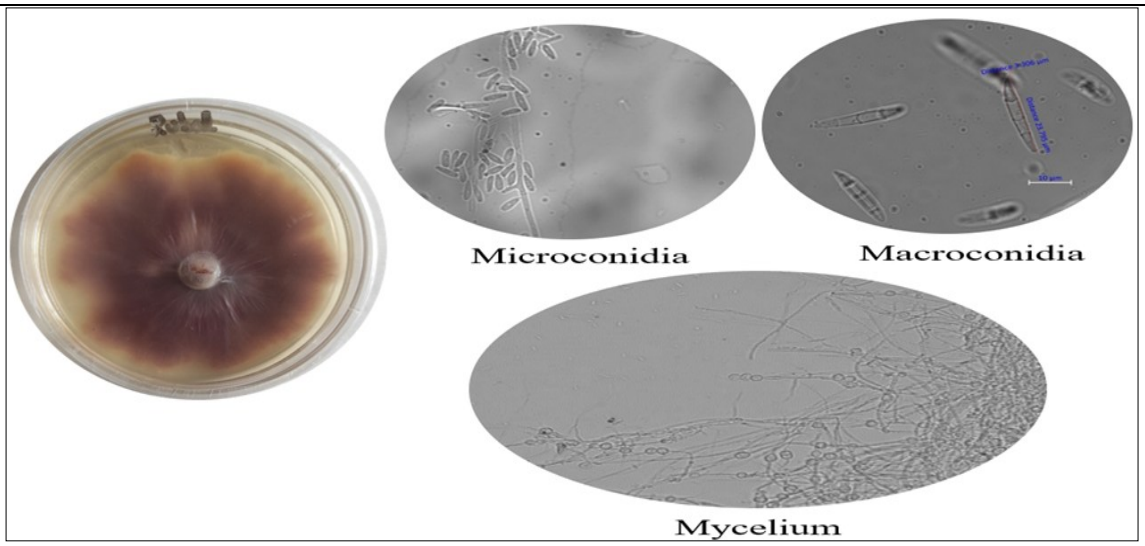

Fig. 3. Morphological characteristics of fungus associated with the disease (*F. oxysporum* f.sp. *psidii*).

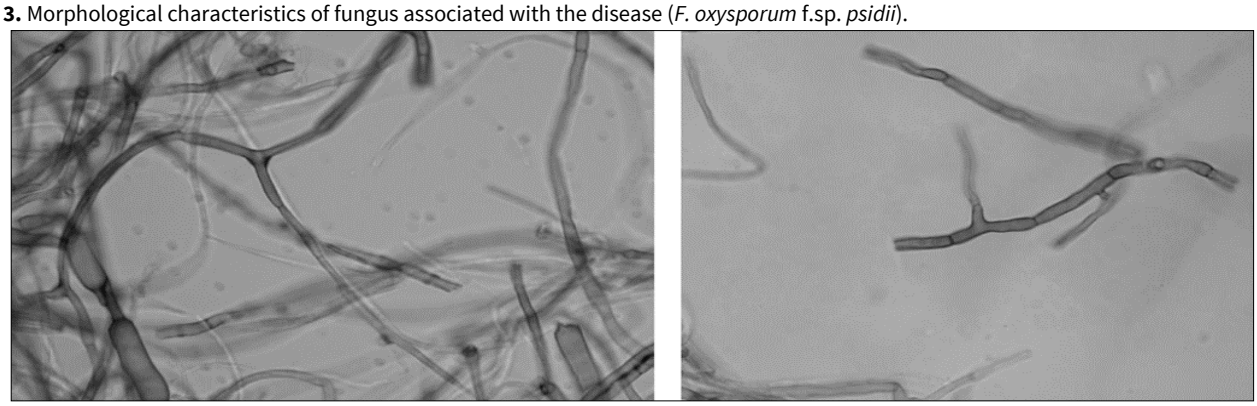

Fig. 4. Morphological characteristics of *R. solani* (The branches arise at 90° below the septa).

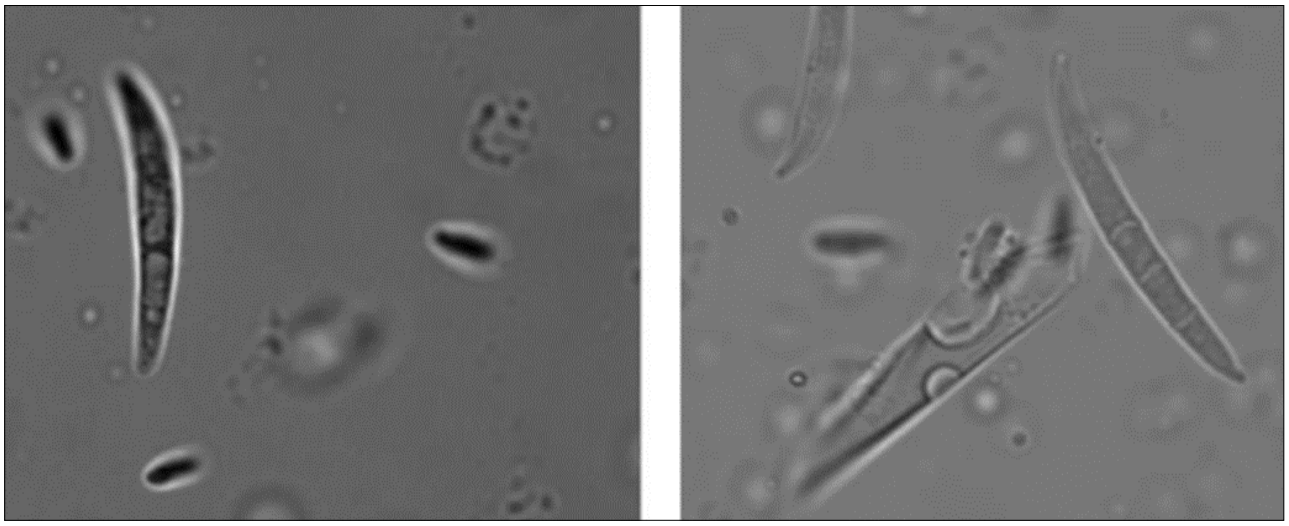

Fig. 5. Morphological characteristics of *F. solani* (conidia tapered, having septa).

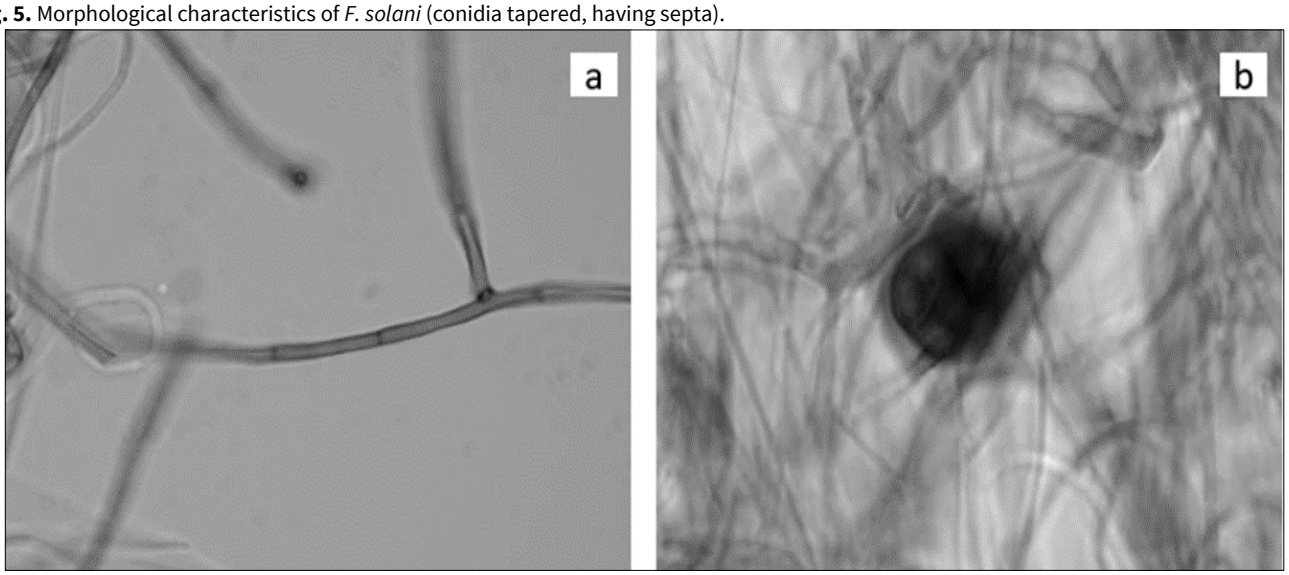

Fig. 6. Morphological characteristics of *M. phaseolina* a: branched mycelium; b: black sclerotia.

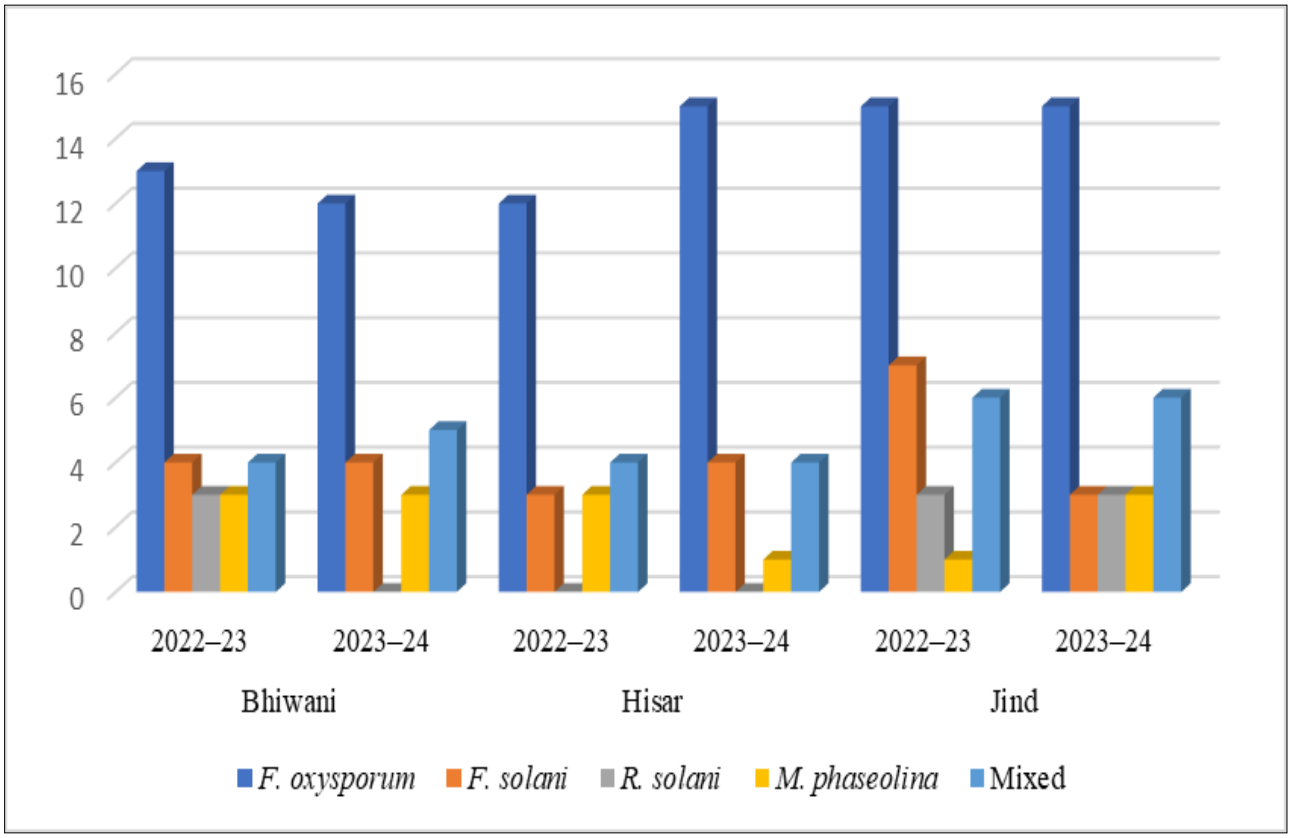

Fig. 7. Occurrence and association of fungal pathogens in guava orchards of Hisar, Jind and Bhiwani.

moderate levels, while *R. solani* and *M. phaseolina* occur at comparatively lower but steady frequencies, indicating their contributory role in disease development. Mixed infections involving more than one pathogen were also common, especially in Jind, highlighting the complex and synergistic nature of the guava wilt disease. Overall, the figure indicates that guava wilt in these districts is caused by a multi-pathogenic complex dominated by *F. oxysporum*, necessitating integrated and broad-spectrum management strategies.

Guava nurseries

Out of 60 samples collected (20 from each district), 44 were found infested with a frequency of occurrence of 73 % and a density of 110-485 J2s/200cc soil (Table 11). The maximum frequency of occurrence of *M. incognita* was found in Hisar (64 %) followed by Jind (56 %) and Bhiwani (56 %). Out of 44 infested samples, 27 were found to be infested with both nematodes and fungus, resulting in a disease incidence of 61.4 %. The maximum disease incidence was observed in the Jind (50 %) followed by Hisar (45 %) and Bhiwani (40 %). Among all the districts, the maximum density was observed in Jind (225-475 J2s/200 cc soil) followed by Hisar (165-440 J2s/200 cc soil) and Bhiwani (110-390 J2s/200 cc soil). The maximum range of RKI was recorded in Hisar (3.0–5.0) district, followed by Jind (2.0–5.0) and Bhiwani (2.0–5.0). Among the detected PPNs, *M. incognita* was the most prevalent, often found in association with wilted and stunted plants. Similarly, the fungal pathogens *F. oxysporum, F. solani* and *R. solani* were commonly isolated from symptomatic plant samples, highlighting their significant role in guava decline. Statistical analysis using ANOVA revealed significant district-wise variation ($p < 0.05$) in nematode infection, density, root-knot index and disease incidence in guava nurseries of Haryana (Table 12). Machine learning through a Random Forest classifier achieved 92.8 % accuracy, identifying nematode density and root-knot index as the most influential parameters predicting wilt incidence. These results collectively confirm substantial spatial heterogeneity in pathogen pressure and emphasize the potential of data-driven approaches for early risk assessment and targeted management.

Community analysis of plant parasitic nematodes associated with guava orchards

To ascertain the distribution and prevalence of various PPN genera, community analysis was carried out (Fig. 8). The findings showed that the most common nematodes were *Meloidogyne* spp. followed by *Helicotylenchus* spp., *Hoplolaimus* spp., *Pratylenchus* spp. and *Tylenchorhynchus* spp. with absolute frequencies of 64.0, 52.0, 48.0 and 40.0 % respectively, in Bhiwani district during 2022–23. *Meloidogyne* spp. had the maximum absolute frequency, relative frequency, absolute density, relative density and prominence value in all the districts (Jind, Hisar and Bhiwani) and was minimum in *Tylenchorhynchus* spp. *Meloidogyne* spp. had the highest relative frequency (24.6 %) in Hisar, followed by *Helicotylenchus* spp. (23.1 %), *Pratylenchus* spp. (20.2 %), *Hoplolaimus* spp. (18.8 %) and *Tylenchorhynchus* spp. (13.0 %). During 2023–24, *Meloidogyne* spp. had the maximum (88.0 %) absolute frequency in Hisar district, followed by Jind (76.0 %) and Bhiwani (52.0 %). Among all the nematode species, *Tylenchorhynchus* spp. had the lowest absolute frequency (40.0 %) both in Hisar and Jind followed by Bhiwani (44.0 %). *Meloidogyne* spp. had the highest relative frequency (24.6 %) and relative density (60.2 %) in Hisar followed by *Helicotylenchus* spp. (22.2 %) and *Hoplolaimus* spp. (20.8 %) in Jind. *Tylenchorhynchus* spp. had the maximum absolute density (1.6 %) and prominence values (10.1 %) in Hisar.

Discussion

The study aimed to determine the incidence and diversity of nematodes and fungi associated with guava decline. Additionally, nematode community analysis was performed to identify the predominant PPNs affecting guava plantation. It was evident from the survey results that the presence of *M. incognita* and *F. oxysporum* f.sp. *psidii* and their interaction were the predominant reasons for disease incidence and the main cause of guava decline in Haryana. *M. incognita* was shown to be significantly present in guava orchards, together with other PPNs

Table 11. Incidence of root-knot nematode and fungi in guava nurseries in different districts of Haryana (2022–24)

Districts	GPS location	Surveyed	Nematode parameters					Fungus parameters		
			Infected	Frequency of occurrence (%)	Density range (J2s/ 200cc soil	RKI*	Major nematode identified	Infected (Nematode +Fungus)	Disease incidence (%)	Major fungi identified
Hisar	29°42'10" N-29°54'12" N 76°15'57" E-76°20'24" E	20	16	64	165-440	3.0-5.0		9	45	
Bhiwani	28°81'50" N-28°96'79" N 75°71'41" E-76°73'84" E	20	14	56	110-390	2.0-5.0	*M. incognita*	8	40	*F. oxysporum*
Jind	29°42'51" N-29°58'68" N 76°15'14" E-77°20'20" E	20	14	56	225-475	2.0-5.0		10	50	
Total		60	44**	73	110-485	2.0-5.0		27	45	

*RKI: Root-knot index; **Out of 44 samples, 35 were found above ETL.

Table 12. Statistical and machine learning (ML) analysis of nematode and fungal incidence in guava nurseries across Haryana (2022–2024)

Parameter	F-value	p-value	Significance	ML Feature Importance Rank
Frequency of nematode occurrence (%)	6.40	0.009	Significant	3
Nematode density (J2s/200 cc soil)	8.90	0.005	Highly Significant	1
Root-knot index (RKI)	4.70	0.045	Significant	2
Disease incidence (%)	5.92	0.021	Significant	4
Model (Random Forest)	—	—	Accuracy = 92.8 %, $R^2 = 0.89$	—

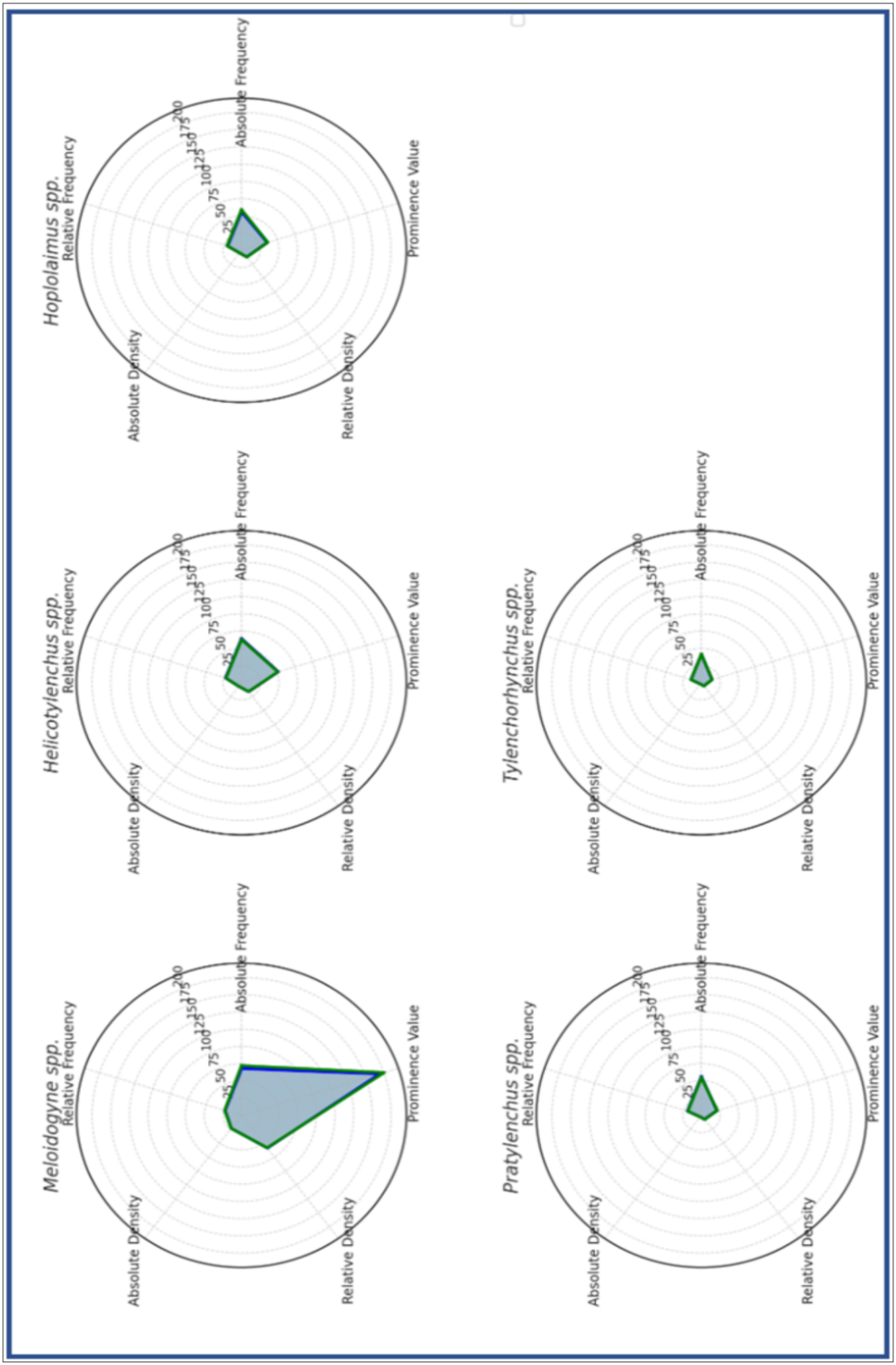

Fig. 8. Comparative radar of plant parasitic nematodes community structure (2022–24).

such as *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Hoplolaimus* spp. and *Pratylenchus* spp. Guava decline was most common in Jind, followed by Hisar and Bhiwani districts. This pattern aligns with previous studies that demonstrated the prevalence of fungal infections and RKNs in tropical and subtropical regions, where their proliferation was facilitated by soil and climate (14).

Similar findings were observed when major guava growing districts of Tamil Nadu were surveyed for the incidence of *M. enterolobii* (15). Results indicated that *M. enterolobii* was found positive in all districts of the state. Similarly, different districts of Haryana were surveyed to determine the frequency and abundance of PPNs associated with guava orchards. The incidence of guava decline was found to be the highest in Jind followed by Sirsa, Hisar and the least disease was observed in Fatehabad district (16). Our results were consistent with those of previous findings (17), that evaluated the diversity, occurrence and distribution of PPNs associated with guava orchards in Haryana. *Meloidogyne incognita* was found in 60 out of the 95 samples, with a frequency of occurrence of 63.2 % and a density range of 50–785 J2s/200 cc soil. *Meloidogyne incognita* was considered the most significant PPN based on occurrence, population density and related guava damage. The high infestation rate of *M. incognita* in Jind district suggests that nematode infestation plays a crucial role in predisposing guava plants to secondary infections, particularly by *F. oxysporum*, which is known to cause wilt in guava (18). In the present investigation, the RKI was highest in Jind (3.0–5.0), correlating with the higher density of *M. incognita*. A large number of RKNs cause significant gall formation and root malfunction, which makes plants more vulnerable to fungal diseases. The highest disease incidence (45.0 %) was recorded in Jind nurseries, reinforcing the hypothesis that pathogen pressure is established early in the plant growth cycle, leading to increased fecundity in mature orchards (19).

Understanding the diversity, distribution and ecological interactions of PPNs within agroecosystems necessitates comprehensive community analyses. Such investigations provide critical insights into nematode dominance, prevalence and functional roles, thereby enabling the development of targeted and sustainable management strategies (20). In the present study, community analysis of PPNs revealed that *Meloidogyne* spp. exhibited the highest absolute and relative frequencies across all three surveyed districts, whereas *Tylenchorhynchus* spp. recorded the lowest absolute density during 2022–2024, reflecting distinct regional variations in nematode population composition. The coexistence of multiple nematode genera alongside pathogenic fungi suggests a synergistic relationship, wherein nematode-induced root injuries facilitate subsequent fungal invasion and enhance disease severity (16). *Rotylenchulus reniformis* was found to be the most prominent nematode species in guava orchards, followed by *Helicotylenchus* and *Tylenchorhynchus.* Other PPNs, including *Pratylenchus*, *Xiphinema* and *Longidorus*, were also discovered in the guava rhizosphere and played a role in the guava decline, either alone or in conjunction with soilborne fungal diseases (21). A study on the nematode community in guava orchards also identified several genera, including *Meloidogyne*, *Helicotylenchus*, *Hoplolaimus*, *Pratylenchus* and *Tylenchorhynchus* (22). In a study, *Tylenchorhynchus* had the highest prominence value, followed by *Aphelenchoides.* This shows how important community analysis is for finding important nematode genera associated with various crops. These results underline how crucial it is to carry out evaluations of nematode communities to identify the dominant and ecologically important nematode species that need specific control approaches. In the surveyed guava orchards, symptoms observed included yellowing of leaves with marginal necrosis and the presence of simple or compound galls on the roots, depending on the severity of infestation. Interestingly, in some newly established orchards, nematode infection was detected in the roots even though no above ground symptoms were visible. Additionally, a few orchards had healthy roots without any signs of root-knot nematode infection. Examination of the perineal patterns and their comparison with closely related species confirmed that all patterns corresponded to those characteristics of *M. incognita*.

The observed perineal patterns of *M. incognita* were oval in shape, with smooth, wavy striations and lacking lateral lines. In contrast, *M. javanica* was characterized by distinct lateral lines that divide the pattern into dorsal and ventral regions. The perineal patterns of *M. javanica* are typically flat to round, with few or no striations crossing the lateral lines. Besides the fungal pathogens contributing to the decline of guava orchards, the rapid deterioration may also result from nematode infestation, which can predispose the plants to secondary infections. Although earlier studies have indicated that other root-knot nematode species, such as *M. enterolobii*, can also infect guava plants (23), this species was not detected in the present survey. The observed perineal pattern characteristics closely resembled the original description of *M. incognita* provided by previous researchers (24). In some samples, slight variations in the patterns were noted compared to previous descriptions; however, these differences are likely due to geographic and environmental factors specific to the surveyed locations (25). The likely reason for *M. incognita* emerging as a major problem in newer guava orchards appears to be the use of infested planting material. Nurseries supplying guava seedlings seem to play a key role in disseminating the nematode infection. Therefore, there is an urgent need to conduct surveys in more districts and regions to better understand the extent of nematode distribution.

Conclusion

This study elucidates the synergistic interaction between *M. incognita* and *F. oxysporum* f.sp. *psidii* as the principal cause of guava decline in Haryana, confirming the research hypothesis. Beyond establishing etiology, the work advances understanding of soilborne pathogen complexes and their cumulative impact on perennial fruit crop health under subtropical conditions. The findings highlight the necessity of transitioning from single-pathogen control to integrated, eco-sustainable soil health management, emphasizing nematode-free nursery stock, microbiome restoration and biologically based interventions. These approaches align with climate-smart, low-input agriculture and ensure economic viability by reducing chemical dependence and minimizing yield losses. From an industrial standpoint, the study identifies promising avenues for investment in certified planting material systems, microbial bioformulations and rapid diagnostic platforms, offering scalable, environmentally responsible and economically resilient solutions for the guava industry.

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Authors' contributions

RK[1], VK, SSM and RK[2] performed the survey. SK and PK[1] contributed to manuscript writing. PK[2], HC and DK participated in analysis and coordination. All authors read and approved the final manuscript [RK[1]- Rubal Kamboj; RK[2]- Rohit Kumar; PK[1]- Parveen Kumar; PK[2]- Pawan Kumar].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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