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**Research Article**

# GUAVA NURSERIES: PREVALENCE OF ROOT-KNOT NEMATODE AND FUNGAL PATHOGENS IN HARYANA

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## ABSTRACT

Guava is one of the most important fruit crops grown in Haryana. Its orchards are affected by various pathogens. Among them, plant parasitic nematodes (PPNs) and fungi play a major role in causing guava decline. An extensive survey of guava nurseries was carried out to determine the incidence of guava, decline in four districts of Haryana *viz.*, Jind, Hisar, Bhiwani, and Sirsa. In survey conducted, root-knot nematode *Meloidogyne incognita* was the major PPN associated with guava decline. Furthermore, fungus, *Fusarium oxysporum* has also been associated with guava decline symptoms in guava nurseries. During 2022-24, 80 soil and root samples were collected, 25 were infested with *M. incognita* alone with a 31.2 % frequency of occurrence. Apart from this, 39 samples were found infested with root-knot nematode and fungi, with a disease incidence of 48.7 %. Among all the districts, the maximum incidence was found in Sirsa district (40%) followed by Hisar (35%), Bhiwani (30%), and Jind (20%). Based on the survey results, it is clear that the primary cause of guava declines and disease incidence is the association of *M. incognita* and *F. oxysporum*.

**Keywords:** *Fusarium oxysporum*, Guava nursery, *Meloidogyne incognita*, Plant-parasitic nematodes, Survey.

## INTRODUCTION

Guava (*Psidium guajava* L.) is one of India's important commercial fruit crops and is known as poor man's fruit. In India, 4.469 million MT of guava is produced annually on an area of 3.10 lakh hectares (Anonymous, 2020). With a production share of about 21.78% of India's total guava production, Uttar Pradesh has the maximum production followed by Madhya Pradesh, Bihar, Andhra Pradesh, Haryana, and Punjab (APEDA AgriXchange, 2020-2021). Its cultivation is affected by various biotic and abiotic factors. Among biotic factors, insect pests *viz.*, fruit fly, fruit borer, tea mosquito bug, and guava aphid; fungal diseases *viz.*, guava wilt, anthracnose, rust, damping-off and plant parasitic nematodes (PPNs) *viz.*, root-knot nematodes (*Meloidogyne* spp.), spiral nematode (*Helicotylenchus dihystera*), lance nematode (*Hoplolaimus indicus*) and lesion nematode (*Pratylenchus coffeae*) are major constraints for profitable guava production. Among these, the complex infestation of root-knot nematodes, *Meloidogyne* spp., and fungus, *Fusarium oxysporum* f. sp. *psidii* causes the sudden death of guava plants and severe loss in terms of quality and quantity of fruits (Khan *et al*., 2001). The cosmopolitan anamorphic species *Fusarium oxysporum* is found all over India (Agrios, 2005; Ashwathi *et al*., 2017). *Fusarium oxysporum* is a fungus that enters plants by their roots and settles in the intercellular spaces and root cortical cells before invading the vascular tissue through the xylem pits. The typical dark brown staining caused by this invasion frequently covers the entire upper stem of the affected plant, obstructing the xylem ducts. The plant wilts, collapses, and eventually dies due to toxins or enzymes carried in the plant sap.

*M. incognita* is well-known for severe damage to a variety of agricultural and horticulture crops. Their second-stage juveniles ($J_{2s}$) penetrate through the cortex and reach the root vascular tissues via the elongation zone. They establish permanent feeding sites where they complete their life cycle. PPNs cause the development of specialized structures called multinucleate and hypertrophied giant cells once feeding sites are established. The plant root system is damaged by the constant feeding of nematodes, which lowers the plant's capacity to absorb nutrients and water (Maqsood *et al*., 2020). The fungus and nematode develop a synergistic relationship leading to a root-knot

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wilt disease complex (Patel *et al*., 2000). Nematode infestation in nurseries is indeed a significant concern because infected seedlings can serve as primary sources for the spread of nematodes to the field. To understand the complexity of guava decline, the present investigation involves the survey of guava nurseries in different districts of Haryana for the incidence of PPNs and pathogenic fungi associated with the occurrence of disease.

## MATERIAL AND METHODS

### Sample collection

A survey of guava nurseries was carried out for the guava decline in different districts (Jind, Hisar, Sirsa, and Bhiwani) of Haryana during 2022-24 (Plate 1). Soil and root samples were collected in polythene bags, with proper labeling and were stored in the refrigerator at $10\pm1^{0}$C before processing. The samples were analyzed for the infestation of plant parasitic nematodes (PPNs) and fungi. Data on nematode population densities were analyzed to assess the average density of root-knot nematode and frequency of occurrence in each district. The severity of the infection was recorded on the basis of root-knot index (Table 1).

### Identification of root-knot nematode species

After being obtained during the survey, the root samples that were infected with root-knot nematodes were washed in running tap water. After being chopped into tiny pieces of two centimeters, infected roots were boiled in 0.1% acid fuchsin lactophenol for two to three minutes. To de-stain the roots, the roots were kept in plain lactophenol for an overnight period 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 (Plate 2). 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. The species confirmation was done based on the perennial pattern as described by Chitwood (1949).

**Table 1.** Root-knot index (RKI) on 1-5 scale (Hartman and Sasser, 1985).

| Sr. 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 |

**Plate 1**. Dissemination of nematode and fungus infested guava nurseries in the state.

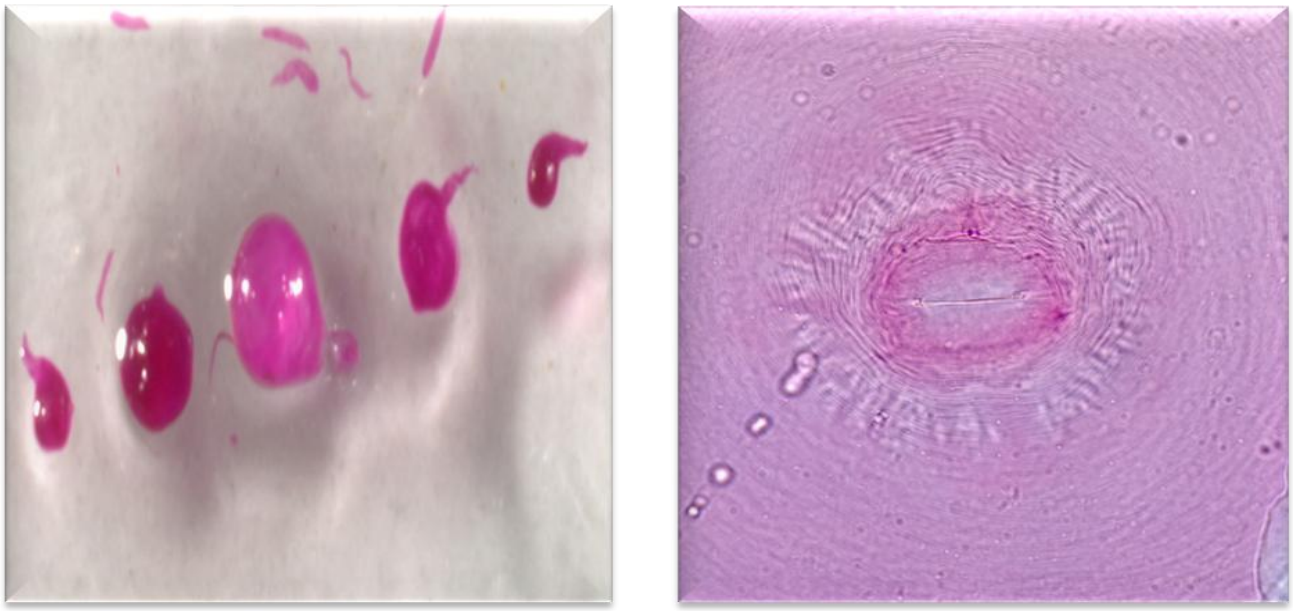

**Plate 2**. (a) Females of root-knot nematode (b) perineal pattern of root-knot nematode (*M. incognita)*

**Isolation and identification of fungi**

The roots were cleaned and washed in water to remove soil particles and were surface sterilized by using 0.1% sodium hypochlorite for two to three minutes and then rinsed with sterile distilled water. The small bits of roots were transferred to Petri plates containing sterilized potato dextrose agar (PDA) medium, and they were then incubated for seven days at $27\pm1^0$ C. Colonies were examined under a compound microscope for identification of fungus (Plate 3). The sporulation and mycelia characteristics were used to identify the fungi according to Booth (1971).

**Plate 3**. (a) Growth of *Fusarium oxysporum* f. sp. *psidi* (b) microscopic view of *Fusarium oxysporum* f. sp. *psidi*.

## RESULTS AND DISCUSSION

Data represented the documentation of nematode infested guava nurseries in different districts of Haryana (Table 2). The survey was conducted during 2022-24, soil and root samples of guava nurseries were collected from the Hisar, Jind, Bhiwani, and Sirsa districts. Out of 80 samples, 25 were infested with *M. incognita* with 31.2 % frequency of occurrence (Table 2). The results revealed that out of 20 samples (Hisar), 7 were found infested with the *M. incognita* with 35 % frequency of occurrence and a density range of 165-440 $J_{2s}$/200 cc soil. In Bhiwani district, this nematode had 30 % frequency of occurrence (6 out of 20 samples) with a density range of 110-390 $J_{2s}$/200 cc soil. Out of 20 samples from the Sirsa district, 8 were found infested with *M. incognita* with 40 % frequency of occurrence. Out of which 20 samples collected from Jind, 4 were found infested with *M. incognita* with 20 % frequency of occurrence. The results revealed that out of 80 samples 25 were found infested with nematode alone. Other PPNs found associated with the guava were *Rotylenchus reniformis, Hoplolaimus* sp., *Helicotylenchus* sp. and *Pratylenchus* sp. Among all the districts, maximum nematode infestation was found in Sirsa followed by Hisar and Bhiwani. Out of 80 samples from Hisar Jind, Bhiwani, and Sirsa districts, 39 were found infested with *M. incognita* and *Fusarium oxysporum*. In Bhiwani district, this nematode had 40 % disease incidence (8 out of 20 samples). Results revealed that out of 20 samples, 9 were infested with nematode and fungus (N+F), with 45.0 % disease incidence in the Hisar district. Out of 20 samples from Jind district 10 were found infested with *M. incognita* and *Fusarium oxysporum*. In Sirsa district, 12 samples were infested with nematode and fungus with 60 % disease incidence. Based on the incidence, population density, and associated damage to affected crops, *M. incognita* and *Fusarium oxysporum* were considered the most important pathogens causing guava decline and damaging the guava seedlings. The major fungus found associated with guava decline was *Fusarium oxysporum*. Other fungi associated with guava nurseries were *Fusarium solani*, *Macrophomina phaselina*, *Rhizoctonia solani*. Among all the districts, maximum infestation was found in Sirsa (60%) followed by Jind (50%), Hisar (45%), and Bhiwani (40%).

Throughout the survey, guava seedlings showed the presence of simultaneous and individual presence of *M. incognita* and *F. oxysporum*. *M. incognita* and *F. oxysporum* alone caused less disease incidence and severity than *M. incognita* and *F. oxysporum* association. According to Gomes *et al*. (2011), guava decline is a complex disease caused by the combined effects of *M. mayaguensis* and *F. solani*, wherein the nematode parasitism predisposes the plants to the fungus root rot. Singh, (2020) reported that nematodes parasitic on plants were a co-factor in the decline of guavas.

**Table 2.** Incidence of root-knot nematode and fungus in guava nurseries in different districts of Haryana (2022-24).

| Districts | GPS location | Surveyed | Nematode parameters | | | | | Fungus parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Infected (Nematode alone) | Frequency of occurrence (%) | Density range ($J_{2s}$/200 cc soil) | RKI* | Major Nematodes identified | Infected (Nematode +Fungus) | Percent disease incidence | Major fungi identified |
| Hisar | $29^0$42’10’’ - $29^0$54’12’’ N $76^0$15’57’’ - $76^0$20’24’’ E | 20 | 7 | 35 | 165-440 | 3.0-5.0 | *M. incognita* | 9 | 45 | *Fusarium oxysporum* |
| Bhiwani | $28^0$81’50’’ - $28^0$96’79’’ N $75^0$71’41’’ - $76^0$73’84’’ E | 20 | 6 | 30 | 110-390 | 2.0-5.0 | | 8 | 40 | |
| Jind | $29^0$42’51’’ - $29^0$58’68’’ N $76^0$15’14’’ - $77^0$20’20’’ E | 20 | 4 | 20 | 225-475 | 2.0-5.0 | | 10 | 50 | |
| Sirsa | $29^0$77’17’’ - $29^0$91’07’’ N $76^0$95’94’’ - $77^0$00’90’’ E | 20 | 8 | 40 | 135-485 | 2.0-5.0 | | 12 | 60 | |
| **Total** | | **80** | **25** | **31.2** | **110-485** | **2.0-5.0** | | **39** | **48.7** | |

*RKI=Root-knot index

According to the results of the current investigations, the root-knot nematode was the most harmful and caused significant damage to guava plants as compared to other PPNs. The guava plant was more affected by the root-knot nematode species, *M. incognita* than by *M. javanica*, which was mostly found in guava orchards where vegetables were intercropped.

Kumar and Poornima (2019) surveyed major guava-growing districts of Tamil Nadu for the incidence of *M. enterolobii*. Results indicated that *M. enterolobii* was found positive in all districts of the state. *M. enterolobii* infested guava orchards in each district surveyed revealed that appropriate symptoms such as bronzing of leaves with marginal necrosis, simple and compound galls in the roots, and browning of younger and older leaves which finally resulted in wilting of the infested plants. A survey was conducted in different districts of Haryana to determine the frequency and abundance of PPNs associated with guava orchards (Madhu *et al*., 2019). It was evident from the survey results, that the presence of *M. incognita*, *F. oxysporum*, and their interaction was the predominant reason for disease incidence and the main cause of guava decline. The frequency of occurrence of *M. incognita* was 72.2 percent in Fatehabad, 63.2 % in Hisar, 56.3 percent in

Jind, and 53.3 percent in Sirsa district, respectively. Guava decline incidence was found to be the maximum in Jind (51.6%) followed by Sirsa (49.4%), and Hisar (40.4%), and the least disease was observed in Fatehabad district (36.6%). Kumar *et al*. (2023) conducted a survey in 2019-22 in the major guava-growing regions of Haryana to evaluate the diversity, occurrence, and distribution of PPNs associated with guava plantations. *M. incognita* was found in 60 out of the 95 samples, with a frequency of occurrence of 63.2 percent and a density range of 50-785 nematodes/200 cc soil. *M. incognita* was considered to be the most significant PPN based on occurrence, population density, and related guava damage.

Ansari and Khan (2012) also found that *M. incognita* exhibited signs of uneven growth, dieback, yellowing and wilting, stunted growth, and numerous small to large-size galls in the guava orchards of Aligarh district of Uttar Pradesh. Accurate identification of nematodes is essential for implementing precise and effective management practices. This holistic approach can help minimize nematode damage while ensuring sustainable agricultural practices. These studies will guide the development of effective management strategies by incorporating nematicides and fungicides, with a focus on determining their optimal dosages and appropriate timing for application.

## CONCLUSION

A complex disease epidemic known as "guava decline" poses a major threat to guava production in India. The guava declines as observed in all four districts that were surveyed. Both *F. oxysporum* and *M. incognita* alone caused less disease incidence and severity in orchards; however, both *F. oxysporum* and *M. incognita* showed higher disease severity. The rapid and severe decline of guava plants throughout all regions is mostly caused by the simultaneous infection by both pathogens.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

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