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Distribution, prevalence and intensity of guava decline in western Haryana

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Abstract
Guava is an important fruit crop and is cultivated in large areas of Haryana. A lot of guava orchards are wilting up, so an intensive survey was conducted in four districts of western Haryana for the guava decline incidence. The survey results revealed the prevalence of *Meloidogyne incognita, M.javanica, Rotylenchulus reniformis, Helicotylenchus* spp., *Hoplolaimus* spp. and *Pratylenchus* spp. In its addition, fungi associated in the orchards of guava decline symptoms were *Fusarium oxysporum, Macrophomina phaseoli* and *Rhizoctonia solani.* The frequency of occurrence of *M. incognita* was 72.2% in Fatehabad, 63.2% in Hisar, 56.3% in Jind and 53.3% in Sirsa district respectively. Guava decline incidence was maximum in Jind (51.6%) followed by Sirsa (49.4%), Hisar (40.4%) and least disease was observed in Fatehabad district (36.6%). It is evident from the survey results, that the presence of *M. incognita* and *F. oxysporum* and their interaction is predominant reason for disease incidence and the main cause of guava decline.

Keywords: *Fusarium oxysporum,* guava decline**,** *Meloidogyne incognita*

Introduction
Guava (*Psidium guajava* L.) is an important fruit crop in India which belongs to the family Myrtaceae. It is known as 'Poor man's apple' for its nutritionally rich in vitamins and minerals. Guava decline is a complex disease gaining importance at national and state level due to many factors involved in causing the disease and also for their management. Plant parasitic nematodes acts as co-factor by predisposing plants to soil borne fungi which leads to severe cause of 'guava decline' (Suarez *et al.,* 1999; Khan *et al*., 2001) [14, 9]. Misra and Shukla (2002) [11] reported 5-60 per cent losses due to guava wilt around Lucknow. The root-knot nematode (*M. mayagaunesis*) infested guava area is estimated to be 5000 ha and cause economic loss of US$ 66 million in Brazil (Pereira *et al.,* 2009) [12]. To understand the complexity of guava decline disease and its distribution and severity, the present investigation involves survey of guava orchards in western Haryana, identification of plant parasitic nematodes and pathogenic fungi associated and prevalence of disease occurrence.

Materials and Methods
The systematic survey was conducted in four districts of western Haryana viz., Hisar, Jind, Sirsa and Fatehabad for the guava decline incidence. The list and address of old and newly established guava orchards was made available by state horticulture department. Composite samples of soil and root were collected randomly from rhizosphere of disease infected guava trees. Soil samples were collected in polythene bags, labeled, handled and refrigerated at 7-10^0 C before processing. Number of wilted and dried guava trees or plants was counted in the orchard and disease incidence was expressed in per cent. The nematode population in soil and roots was counted and identified and elaborated as the number of second stage juveniles (j_2) per 200 cc soil and 5 g of roots. 0-5 scale root-knot index was recorded according to the following scale: 0 = no galls or egg masses, 1=1-2 galls or egg masses, 2=3-10, 3=11-30, 4 = 31-10, and 5=over 100 galls or egg masses (Hartmant & Sasser, 1985) [8].

Extraction of nematodes from soil samples
Cobb's decanting and sieving combined with Modified Baermann's funnel technique (Schindler, 1961) was used for the extraction of nematodes from soil samples collected during survey. Killing and fixing of nematodes was done by adding boiling fixative (8% formalin) to nematode suspension.

The nematode suspension further processed by glycerol-ethanol method for identification of nematodes.

Identification of root-knot nematode species

The root samples infected with root-knot nematodes collected during the survey were washed in running tap water to remove the soil particles. Infected roots were cut into small bits of 2 cm size and boiled in 0.1% acid fuchsin lactophenol stain for 2 to 3 minutes. Root bits were further washed under running tap water to remove the excess stain and kept overnight in plain lactophenol for destaing the roots. Then matured females were dissected out from galls of the root under stereo binocular microscope and the posterior portion of the female was cut and the body contents were cleaned. The posterior portion of the female was further trimmed and perennial pattern was mounted on glass slide in a drop of lactophenol and cover slip was placed on it, sealed with nail polish. The species confirmation was done based on the perennial pattern as described by Chitwood (1949) [3]. Estimation of root population of root-knot nematode was made by counting under microscope.

Isolation and identification of fungi

The roots were washed properly in water to remove the soil particles and cut into small pieces of 5 mm size from each samples collected during survey. The roots were cleaned and surface sterilized by using about 0.1 per cent sodium hypochlorite for two to three minutes and rinsed two to three times with sterile distilled water (Dhingra and Sinclair, 1995) [4]. The small root samples were transferred aseptically to Petri plate containing sterilized potato dextrose agar (PDA) medium with 500 ppm of streptomycin sulfate and incubated at 27 ± 1^0 C for seven days. After incubation period of seven days, colonies were checked under compound microscope for the identification. Fungi were identified based on the sporulation, mycelia character according to the descriptions by Booth (1971) [2], Ellis (1971) [6].

Results

The survey conducted in guava orchards of Hisar, Jind, Sirsa and Fatehabad districts and revealed that the presence of several plant parasitic nematodes viz., *Meloidogyne incognita, M. javanica, Rotylenchulus reniformis, Helicotylenchus* spp., *Hoplolaimus* spp., *Pratylenchus* spp. *Tylenchorhynchus, Xiphinema, Longidorus* and many parasitic fungi viz., *Fusarium oxysporum, Macrophomina phaseoli* and *Rhizoctonia solani* in guava orchards.

Table 1: Relative abundance of *M. incognita* and other nematodes associated with guava in four districts of Haryana

Districts	Nematode recorded	Frequency of occurrence (%)	Density range	Other plant parasitic nematodes
Hisar	*M. incognita*	63.2	98-710	*Tylenchus* spp. *Longidorus* spp.
	Helicotylenchus spp.	50.0	25-350	
	Hoplolaimus spp.	36.8	20-200	
	Rotylenchulus reniformis	47.4	50-140	
	Pratylenchus spp.	31.6	30-100	
Jind	*M. incognita*	56.3	310-930	*Xiphenima* spp. *Longidorus* spp. *Tylenchorhynchus* spp.
	Helicotylenchus spp.	25.0	80-425	
	Hoplolaimus spp.	62.5	50-450	
	Rotylenchulus reniformis	32.7	45-80	
	Pratylenchus spp.	42.1	60-95	
Fatehabad	*M. incognita*	72.2	280-820	*Tylenchus* spp. *Xiphenima* spp. *Longidorus* spp.
	Helicotylenchus spp.	33.3	40-300	
	Hoplolaimus spp.	44.4	25-475	
	Rotylenchulus reniformis	28.5	35-230	
	Pratylenchus spp.	39.3	25-190	
Sirsa	*M. incognita*	53.3	525-940	*Longidorus* spp. *Tylenchorhynchus* spp.
	Helicotylenchus spp.	66.0	70-290	
	Hoplolaimus spp.	60.0	15-320	
	Rotylenchulus reniformis	24.8	40-170	
	Pratylenchus spp.	38.6	60-280	

The frequency of occurrence of *M. incognita* among four districts was recorded maximum in Fatehabad (72.2%) followed by Hisar (63.2%), Jind (56.3%) and Sirsa district (53.3%). The maximum frequency of occurrence 66.0 per cent of *Helicotylenchus* sp. was in Sirsa district, whereas, high frequency of *Hoplolaimus* spp. (62.5%) was observed in Jind district. Among four districts, the occurrence of *Rotylenchulus reniformis* and *Pratylenchus* sp. varies from 24.8 to 47.4 and 31.6 to 42.1 per cent respectively (Table 1).

The results from Table 1 summarize the density range (j_2/200cc soil) of plant parasitic nematodes in four districts. The maximum density range of *M. incognita* was recorded in Sirsa (425-940) followed by Jind (310-930), Fatehabad (280-820) and Hisar district (98-710). Among four districts, the highest density range of *Helicotylenchus* sp. (80-425) was in Jind, *Rotylenchulus reniformis* (35-230) was in Fatehabad and *Pratylenchus* sp. (60-280) was observed in Sirsa district.

Data collected (Table 2) during survey depicts information regarding guava cultivars, age of orchard, method of irrigation, gall index, soil population, root population, per cent incidence of guava decline and pathogens associated with it. Root-knot nematode species, *M. incognita* was predominant and more pathogenic to guava plants than *M. javanica* and this *M. javanica* was mostly found in guava orchards having intercropping with vegetables. The maximum range of gall index was recorded in Sirsa (4-5) while 2-5 gall index range was recorded in Hisar, Jind and Fatehabad districts. The mean soil population of root-knot nematode (j_2/200cc soil) was highest in Jind (424.0) followed by Sirsa (389.3) and least was recorded in Hisar district (204.5). The mean root population of *M. incognita* was highest in Jind (298.7) and least was recorded in Hisar district (151.5). Method of irrigation practice also influenced damage potential of nematodes, reproduction and multiplication. The highest soil

and root population of *M. incognita* and compound galls were observed in orchards having drip irrigation than flood irrigated orchards.

Among four districts surveyed, mean of guava decline incidence was maximum in Jind (51.6%) followed by Sirsa (49.4%), Hisar (40.4%) and least disease was in observed in Fatehabad district (36.6%). The young orchards were less infected by guava decline when compared to old orchards. The field observations and survey results revealed that guava decline severity and incidence was prominent where guava plants were infected by *M. incognita* and *F. oxysporum.* The young orchards are less infected by guava decline when compared to old orchards.

Table 2: Occurrence of guava decline in four districts of western Haryana

District	Varieties	Age of plants (years)	Method of irrigation	Species of Root-knot nematode	Species of Fungus identified	Range of Gall Index	Mean Soil population of RKN j_2/200cc soil	Mean Root population of RKN /5g root	Mean Per cent incidence of guava decline
Hisar	Hisar Safeda Allahabad Safeda	3-10	Flood Irrigation	*M. incognita M. javanica*	*F. oxysporum M. phaseoli R. solani*	2-5	204.5	151.5	40.4
Jind	L-49 VNR-One Kg Hisar Safeda Allahabad Safeda	4-8	Flood Irrigation Drip Irrigation	*M. incognita M. javanica*	*F. oxysporum M. phaseoli R. solani*	3-5	424.0	298.7	51.6
Fatehabad	Hisar Safeda Allahabad Safeda L-49	4-10	Flood Irrigation Drip Irrigation	*M. incognita M.javanica*	*F. oxysporum M. phaseoli R. solani*	3-5	341.1	219.2	36.6
Sirsa	Hisar Surka Allahabad Safeda L-49	2-12	Flood Irrigation	*M. incognita*	*F. oxysporum M. phaseoli R. solani*	4-5	389.3	285.7	49.4

The individual and concomitant presence and incidence of *M. incognita* and *F. oxysporum* was observed on guava plants during survey (Fig.1). Disease incidence and severity was low in orchards infected by *F. oxysporum* and *M. incognita* individually, whereas more disease severity was observed in orchards infected with both *M. incognita* and *F. oxysporum*. Among four districts, the incidence of *F. oxysporum* varies from 23.0 to 42.6 and *M. incognita* incidence varies from 33 to 57.7 per cent. The combined infection by *M. incognita* and *F. oxysporum* was recorded maximum in Sirsa (67.7%) followed by Jind (61.4%), Hisar (54.1%) and 49.1 per cent in Fatehabad district.

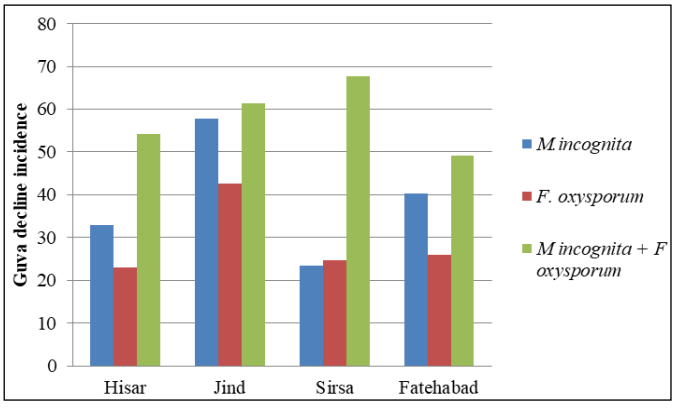

Fig 1: Incidence of *M. incognita*, *F. oxysporum* individually and in combined association in westren Haryana

Discussion

The survey results revealed that *M. incognita, Rotylenchulus reniformis, Helicotylenchus* spp., *Hoplolaimus* spp., and *Pratylenchus* spp. were predominant and having parasitic association with guava orchards showing symptoms of guava decline. The similar results were reported by Khan *et al.*, (2007) [10] from guava orchards of West Bengal. Among plant parasitic nematodes, *M. incognita* caused much damage and was highly pathogenic to guava orchards (Ansari and Khan, 2012) [1] as are the findings of the present studies. Fungal pathogens, *Fusarium oxysporum*, *Macrophomina phaseoli* and *Rhizoctonia solani* were isolated from infected guava orchards from few districts of Haryana. Dwivedi *et al.*, (1990) [5] also reported that *F. oxysporum*, *F. solani* were primary colonizers followed by *Macrophomina phaseoli* and *Rhizoctonia solani* from wilted guava orchards. The density range of *M. incognita* was observed maximum in Sirsa (425-940) followed by Jind (310-930), Fatehabad (280-820) and Hisar district (98-710). Among four districts, the highest density range of *Helicotylenchus* spp. (80-425) in Jind, *Rotylenchulus reniformis* (35-230) in Fatehabad and *Pratylenchus* spp. (60-280) observed in Sirsa district. Khan *et al.*, (2007) [10] reported the high population density of *M. incognita*, *R. reniformis* and *Helicotylenchus* from guava rhizosphere of West Bengal. Willers and Grech (1986) [15] proved the pathogenicity of *Helicotylenchus dihystera* on guava.

The maximum guava decline incidence and severity was observed in orchards infected by *M. incoginta* and *Fusarium oxysporum.* The presence of *Meloidogyne* spp. in the rhizosphere acts as co-factor by directly or indirectly involvement in the disease established phenomenon (Khan *et al.*, 2001) [9]. Suarez *et al.*, (1999) [14] reported that simultaneous presence of *M. incognita*, *F. oxysporum* and *M. phaseolina* caused a greater detrimental effect than that of each pathogen alone on guava plants. Gomes *et al.*, (2014) [7] reported that guava decline occurs in orchards infested with *M. enterolobii* with the associated presence of *F. solani.* Parasitisation by *M. enterolobii*, breakdown the resistance of guava plants to *F. solani*, makes morpho-physiological changes in the root system of guava plants and galled tissues, the modified chemical composition of root exudates by nematode infestation favors colonization by the fungus. The incidence and severity of guava decline was more in old orchards than young orchards. Misra and shukla (2002) [11]

also reported that guava plants above age of five year were more susceptible to the disease. Method of irrigation practice also influences the nematode damage potential, reproduction and multiplication. The continuous availability of moisture in soil by drip irrigation method favors the nematode multiplication and reproduction than flood irrigation method. The gap of 15-20 days between two irrigations in flood irrigation and also irrigation at specific time interval affect the nematode population (Poornima and Walia, 2017) [13]. Such studies would lead us to pave the way for management strategies taking into account nematicides and fungicides and their proper dosages and application time.

Conclusion

Guava decline, a complex disease syndrome and it is serious threat to guava production in India. The disease incidence was more in all four surveyed districts of western Haryana. Disease incidence and severity was low in orchards infected by *F. oxysporum* and *M. incognita* individually, whereas more disease severity was observed in orchards infected with both *M. incognita* and *F. oxysporum.* The combined infection by the pathogens *viz*., *M. incognita* and *F. oxysporum* and their interaction is predominant causes the sudden and severe decline of guava plants in western Haryana districts.

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