J. Cotton Res. Dev. **38**(2), 213-218 (July, 2024) **ISSN No. 0972-8619**

Abundance and Diversity of Plant Parasitic Nematodes in Major cotton growing Districts of Haryana

VINOD KUMAR AND RUBAL KAMBOJ*
Department of Nematology, CCS Haryana Agricultural University, Hisar-125004
**Email : *rubalkamboj36@gmail.com*

Abstract : A survey was conducted to assess the frequency and abundance of plant parasitic nematodes (PPNs) associated with cotton (*Gossypium hirsutum* L.) in Haryana (Sonipat, Bhiwani, Jind, Hisar, Sirsa and Fatehabad districts) during 2020-2023. Survey of Sonipat, Fatehabad and Hisar districts was conducted for the presence of root-knot nematode, *Meloidogyne incognita* during 2020-2021. A total of 31 soil and root samples of cotton crop were collected from Sonipat, Fatehabad and Hisar districts. Out of 31 samples, 13 were found infected with *M. incognita* (Race 4) with 41.9 per cent frequency of occurrence. During 2021-2022, a total of 58 samples of cotton crop were collected from Jind, Bhiwani and Sirsa districts. Out of 58 samples, 30 were found infested with *M. incognita* with 51.7 per cent frequency of occurrence. During 2022-2023, a total of 58 samples of cotton crop were collected from Sirsa and Fatehabad districts. Out of 58 samples, 21 were found infested with *M. incognita* with 36.2 per cent frequency of occurrence. Overall, out of 147 samples were collected from cotton fields in different districts of Haryana, 64 samples were found infested with *M. incognita* with 43.5 per cent frequency of occurrence and having density range of 40-890 j2/200 cc soil during 2020-2023. Based on the incidence, population density and associated damage in affected cotton crops, *M. incognita* was considered to be the most important PPN in major cotton growing districts of Haryana. Other PPNs found associated with the cotton belonged to genera *Rotylenchus reniformis, Hoplolaimus* spp., *Helicotylenchus* sp. and *Tylenchorhynchus* spp.

Key words: Cotton, *Meloidogyne incognita*, plant parasitic nematodes, *Rotylenchulus reniformis*, survey

Cotton, scientifically known as *Gossypium* spp., is indeed a crucial source of natural textile fiber and is extensively cultivated worldwide. When it comes to cotton cultivation, various factors can influence crop growth and productivity (Kumar *et al.*, 2020). Biotic factors, such as plant parasitic nematodes (PPNs) impact on cotton production (Kamboj *et al.*, 2023). In India, one of the major challenges in cotton cultivation is posed by economically important PPNs, particularly root-knot nematodes (RKNs), *Meloidogyne incognita* and *M. javanica* (Abad *et al.*, 2008). These are soil-dwelling parasites that infect the roots of cotton plants, causing damage and negatively affect crop yield (Dabbert *et al.*, 2014). Above ground symptoms are often misattributed to drought stress, poor soil fertility or other causes. Severely damaged plants may show interveinal chlorosis on leaves. Root galling is a diagnostic symptom that occurs on all infected plants and is the only symptom caused solely by RKNs. Compared to many crops; the galls caused by *M. incognita* on cotton are relatively small.

A global survey estimated annual economic crop yield losses due to PPNs at USD 173 billion, emphasizing the widespread and substantial economic burden imposed by these pests (Elling, 2013). Specifically, in the context of cotton, it is reported worldwide, that the annual yield loss due to damage done by PPNs is estimated to be 10.7 per cent. In India, the annual cotton yield loss due to damage done by RKNs is estimated to be even higher, at 20.5 per cent (Kumar *et al.*, 2020). To manage and mitigate the impact of PPNs on cotton crop, various strategies are employed. These may include the use of nematode resistant cotton varieties, crop rotation with non-host crops, soil amendments, and the application of

nematicides. Integrated pest management (IPM) approaches are often recommended, combining various control methods to create a more sustainable and effective nematode management strategy. It is crucial for farmers and researchers to stay vigilant in monitoring and addressing the presence of PPNs to ensure sustainable cotton production and minimize economic losses. Research and development efforts continue to explore new and innovative approaches to tackle nematode related challenges in cotton cultivation. Hence, in the present study an attempt has been made to assess the frequency and abundance of PPNs associated with cotton crop in different districts of Haryana.

MATERIALS AND METHODS

Soil sample collection

Survey of Sonipat, Jind, Hisar, Bhiwani, Sirsa and Fatehabad districts was conducted for the presence of PPNs during 2020-2023. The villages were purposefully selected based on their potential for cotton production. The samples were collected from individual fields from villages within tehsils (administrative subunit of a district) of each of the districts. Soil and root samples of plants exhibiting nematode symptoms were collected and analyzed for plant parasitic nematodes. When the samples were being collected, the farmers and villagers were interviewed to collect data on previous crop history, cropping pattern, fertilizer, irrigation and pesticide inputs. A total of 147 soil and root samples of cotton were collected from different districts of Haryana. The samples were analyzed as per the standard protocol for identification of PPNs. The extraction and identification of nematodes from soil samples involve several steps, and the methods employed play a crucial role in obtaining accurate results.

Extraction method

Cobb's decanting and sieving are commonly used methods to extract nematodes from soil. These techniques involve separating nematodes from soil particles based on their movement in water. The Modified Baermann's funnel technique, as described by Schindler in 1961, is likely used to isolate nematodes further. This technique utilizes the nematodes' tendency to move away from light, allowing them to be concentrated in a collection container.

Killing and fixing of nematodes

Boiling fixative, in this case, 8 per cent formalin is added to the nematode suspension. Formalin is a commonly used fixative that preserves the nematodes for subsequent analysis.

Processing of nematode suspension

The nematode suspension is further processed using the glycerol ethanol method. This method likely involves dehydration and clearing of nematodes, making them suitable for microscopic examination.

Identification of root knot nematode

To identify root-knot nematode species, root samples infected with nematodes were washed in running tap water to eliminate soil particles. The infected roots were then cut into 2 cm bits and boiled in a 0.1 per cent acid fuchsin lactophenol stain for 2 to 3 minutes. After washing away excess stain and overnight soaking in plain lactophenol, matured females were dissected from root galls using a stereo binocular microscope. The posterior part of the female was cut, and its contents were cleaned. Subsequently, the trimmed posterior portion, displaying the perennial pattern, was mounted on a glass slide in lactophenol. A cover slip was placed and sealed with nail polish. The species confirmation was done as described by followed Chitwood. The RKN population was estimated by microscopic counting.

RESULTS AD DISCUSSION

Survey of Sonipat, Fatehabad and Hisar districts was conducted for the presence of *M.*

incognita during 2020-2021. Soil samples were collected from different locations along with roots. The samples were analyzed as per the standard protocol. A total of 31 soil and root samples of cotton were collected from Sonipat, Fatehabad and Hisar districts. Out of 31 samples, 13 were found infested with *M. incognita* (Race 4) with 41.9 per cent frequency of occurrence. The results revealed that out of 8 samples from Sonipat district, 4 were found infested with *M. incognita* with 50.0 per cent frequency of occurrence and density range of 70-670 j2/200 cc soil. In Fatehabad district, this nematode had 33.3 per cent frequency of occurrence (2 out of 6 samples) with density range of 115-410 j2/200 cc soil while in Hisar district, 7 out of 17 samples were found infested with root-knot nematode with 41.2 per cent frequency of occurrence and density range of 40-295 j2/200 cc soil (Table 1).

The survey was conducted during 2021-2022, a total of 58 samples of cotton crop were collected from Jind, Bhiwani and Sirsa districts. Out of 58 samples, 30 were found infested with *M. incognita* (Race 4) with 51.7 per cent frequency of occurrence (Table 2). The results revealed that out of 17 samples (Jind), seven were found infested with *M. incognita* with 41.2 per cent frequency of occurrence and density range of 90-245 j2/200 cc soil. In Bhiwani district, this nematode had 42.3 per cent frequency of occurrence (11 out of 26 samples) with density range of 110-395 j2/200 cc soil. Out of 15 samples from Sirsa district, 12 were found infested with *M. incognita* with 80.0 per cent frequency of occurrence.

Survey of Sirsa and Fatehabad districts were conducted for the presence of *M. incognita* in cotton crop during 2022-2023 (Table 3). Results revealed that a total of 58 samples of cotton were collected from Sirsa and Fatehabad districts. Out of 58 samples, 21 were found infested with *M. incognita* (Race 4) with 36.2 per cent frequency of occurrence. The results revealed that out of 33 samples from Sirsa district, 15 were found infested with *M. incognita* with 45.4 per cent frequency of occurrence and having density range of 125-345 j2/200 cc soil.

Table 1. Diversity for economically important plant parasitic nematodes infecting cotton crop (2020-2021)

District	Incidence		GPS Location	Frequency of occurrence (%)	Density range (J2/200 cc soil)	RKI
	No. of cultivation units					
	Surveyed	Infected				
Sonipat	8	4	N 28'554-28'555 E 77'527-77'529	50.0	70-670	2.0-3.0
Fatehabad	6	2	N 29'368-29'572 E 75'346-75'518	33.3	115-410	2.0-5.0
Hisar	17	7	N 29'428-29'558 E 76'157-76'202	41.2	40-295	2.0-4.0
Total	31	13	-	41.9	40-670	2.0-5.0

Table 2. Diversity for economically important plant parasitic nematodes infecting cotton crop (2021-2022)

District	Incidence		GPS Location	Frequency of occurrence (%)	Density range (J2/200 cc soil)	RKI
	No. of cultivation units					
	Surveyed	Infected				
Jind	17	7	N 29'169-29'442 E 75'705-76'413	41.2	90-245	2.0-3.0
Bhiwani	26	11	N 28'851-29'124 E 75'703-76'099	42.3	110-395	2.0-3.0
Sirsa	15	12	N 29'316-29'641 E 74'862-75'219	80.0	240-890	2.0-5.0
Total	58	30	-	51.7	90-890	2.0-5.0

Table 3. Diversity for economically important plant parasitic nematodes infecting cotton crop (2022-2023)

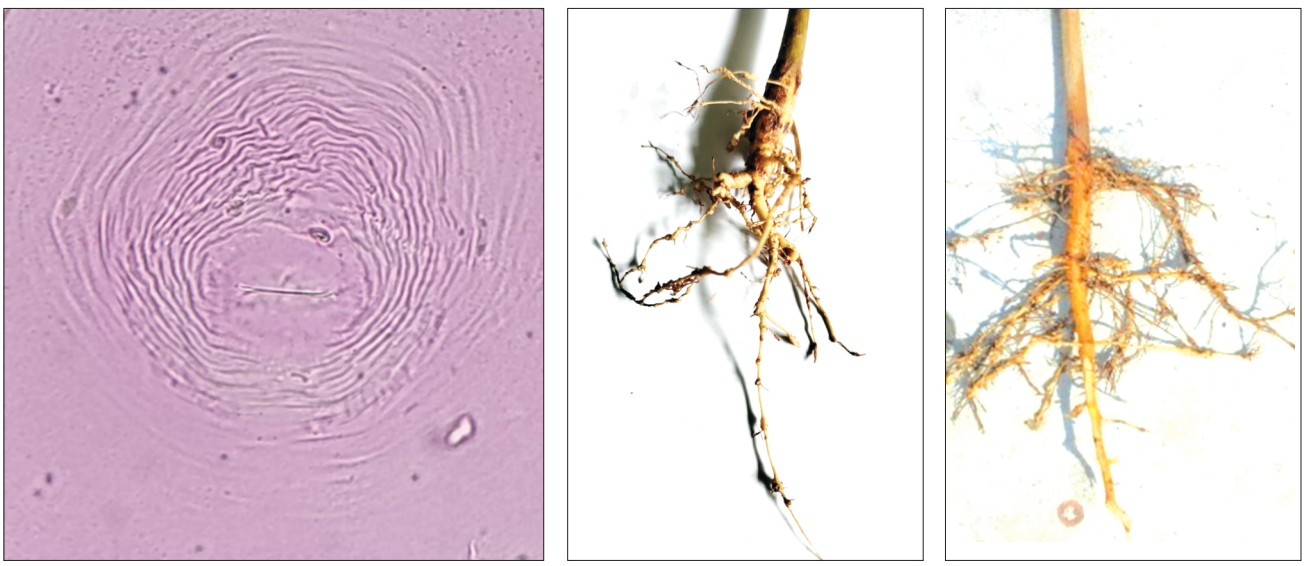
District	Incidence		GPS Location	Frequency of occurrence (%)	Density range (J2/200 cc soil)	RKI
	No. of cultivation units					
	Surveyed	Infested				
Sirsa	33	15	N 29'379-29'486 E 75'092-75'553	45.4	125-345	2.0-5.0
Fatehabad	25	6	N 29'368-29'572 E 75'346-75'518	24.0	150-450	2.0-4.0
Total	58	21	-	36.2	125-450	2.0-5.0
Grand total (2020-2023)	**147**	**64**	**-**	**43.5**	**40-890**	**2.0-5.0**

Table 4. Diversity for economically important plant parasitic nematodes infecting cotton crop (2020-2023)

District	Surveyed	Infested	Frequency of occurrence (%)	Major nematode identified	Other PPNs associated with crop
Sonipat, Jind, Bhiwani, Sirsa and Fatehabad	147	64	43.5	*M. incognita*	*Rotylenchus reniformis* *Hoplolaimus* sp. *Helicotylenchus* sp. *Tylenchorhynchus* sp.

In Fatehabad district, this nematode had 24.0 per cent frequency of occurrence (6 out of 25 samples) with density range of 150-450 j2/200 cc soil. Overall, out of 147 samples were collected from cotton fields in different districts of Haryana, 64 samples were found infested with *M. incognita* with 43.5 per cent frequency of occurrence and having density range of 40-890 j2/200 cc soil during 2020-23 (Table 4). Based on incidence, population density and associated damage on affected crops, *M. incognita* was considered to be the most important PPN damming crop (Plate 1). Other PPNs found associated with the cotton belonged to genera *Rotylenchus reniformis, Hoplolaimus* sp., *Helicotylenchus* sp. and *Tylenchorhynchus* sp.

In the surveyed areas, *M. incognita* and *R. reniformis* are identified as economically damaging nematodes to cotton. *M. incognita* is found to be the most predominant species in the



(A) **(B)** **(C)**

Plate 1. A- Perineal pattern of Meloidogyne incognita (Race 4); B & C- Infected cotton roots collected during the survey

soil samples collected from cotton rhizospheres in different locations. Other nematode species detected include *Hoplolaimus* sp., *Helicotylenchus* sp. and *Tylenchorhynchus* sp. *R. reniformis* is reported to be prevalent in higher densities in cotton-growing areas of Punjab, Haryana, and Uttar Pradesh in India; Verma and Jain, 1999). A survey in the *Bt* cotton-growing districts of northern Karnataka revealed the presence of *R. reniformis*, *Pratylenchus* sp. and some Dorylaimid pathogens in cotton rhizosphere soil and root samples (Lingaraju, 2012). In Florida, *M. incognita* was found in 61 per cent of cotton fields, and in Georgia, 38 per cent of the fields were infested (Kinloch and Sprenkel, 1994; Baird *et al.*, 1996). Cotton fields in Florida and Georgia often have sandy soils. *M. incognita* was also identified in cotton fields in Missouri (30%), Arkansas (20 to 30%), and Mississippi (10%); (Bateman *et al.*, 2000; Robinson *et al.*, 2006).

The findings of the survey in the cotton production regions of Haryana indicate the widespread presence of economically important nematodes, specifically *M. incognita* and *R. reniformis*. Given the prevalence of these nematodes, it is suggested that their presence could be contributing to the observed widespread yield suppression in the area. Addressing nematode related issues is crucial for improving cotton yield in the region. Implementing effective nematode management strategies is essential to mitigate the impact of these parasites on crop productivity (Kamboj *et al.*, 2023). Given the economic importance of cotton in the region and the observed nematode induced yield suppression, a holistic and integrated approach to nematode management is recommended.

CONCLUSION

The results of the present investigation highlight the significant threat posed by various plant-parasitic nematodes, including *M. incognita*, *R. reniformis*, *Helicotylenchus* sp., *Hoplolaimus* spp. and *Tylenchorhychus* spp to cotton crops in Bhiwani, Sirsa and Fatehabad districts of Haryana. The presence of these nematode species is associated with the potential for severe economic yield losses if proper management practices are not implemented to control nematode populations. The recommendation to adopt an INM schedule is a proactive and comprehensive approach to address the nematode-related challenges. Integrated Nematode Management involves combining various strategies to effectively manage nematode populations while promoting sustainable and economically viable cotton production. Collaboration with agricultural extension services, research institutions, and government agencies can facilitate the implementation and dissemination of effective nematode management practices among cotton farmers in the region.

REFERENCES

Abad, P., Gouzy, J., Aury, J.M., Castagnone-Sereno, P., Danchin, E.G.J. and Deleury, E. 2008. Genome sequence of the metazoan plant-parasitic nematode, *Meloidogyne incognita*. *Nat. Biotechnol.* **26**: 909–15.

Baird, R.E., Davis, R.F., Alt, P.J., Mullinix, B.G. and Padgett. G.B. 1996. Frequency and geographical distribution of plant-parasitic nematodes on cotton in Georgia. *Supp. J. Nematol.* **28**: 661-67.

Bateman, R.J., Kirkpatrick, T.L., Robbins, R.T. and Lorenz, G. 2000. The distribution of root-knot and vermiform nematodes in Arkansas, 1990–1999. in P. Duggar and D. A. Richter, eds. *Proceedings of the Beltwide cotton conferences*, San Antonio. pp. 171.

Bucker Abu, A.H. and Seshadri, A.R. 1968. Report on a survey of nematode parasites associated with cotton in Madras State. *Indian J. Agric. Sci.* **38**: 470-76.

Dabbert, T.A. and Gore, M.A. 2014. Challenges and perspectives on improving heat and drought stress resilience in cotton. *J. Cotton Sci.* **18**: 393-409

Das, D.K. and Gaur, H.S. 2009. Distribution and abundance of *Rotylenchulus reniformis* in cotton growing areas of North India. *Indian J. Nematol.* **39**: 98-103.

Elling, A.A. 2013. Major emerging problems with minor *Meloidogyne* species. *Phytopathology*. **103**:1092–1102.

Kamboj, R., Kumar, V. and Mann, S.S 2023. Sustainable approaches to the management of plant pararsitic nematode in cotton crop. *J. Cotton Res. Dev*. **37**: 247-58.

Kinloch, R.A. and Sprenkel, R.K. 1994. Plant parasitic nematodes associated with cotton in Florida. *Supp. J. Nematol.* **26**: 749-52.

Kumar, V., Banakar, P., Kumar, A. and Duggal, P. 2020. Survey of plant-parasitic nematodes associated with cotton in Nuh and Palwal districts of Haryana. *Int. J. Econ. Plants*. **7**: 44-48.

Kumar, V., Khan, M.R. and Walia, R.K. 2020. Crop loss estimations due to plant-parasitic nematodes in major crops in India. Natl. Acad. Sci. Lett. https://doi.org/10.1007/s40009-020-00895-2.

Lingaraju, S., Sonavane, P., Jamadar, M.M., Harlapur, S., Bhat, I.R.S. and Udikeri, S.S. 2012. Plant parasitic nematodes associated with *Bt* cotton. *Curr. Sci.* **103**: 926-32.

Robinson, A.F., Akridge, J.R., Bradford, J.B., Cook, C.C., Gazaway, W.S., McGawley, E.C., Starr, J.L. and Young, L.D. 2006. Suppression of *Rotylenchulus reniformis* 122 cm deep endorses resistance introgression in *Gossypium*. *J. Nematol.* **38**:195-209.

Schindler, A.F. 1961. A simple substitute for a Baermanann funnel. *Plant Disease Reporter*, **45**: 747-48.

Verma, K.K. and Jain, R.K. 1999. Prevalence and distribution of phytoparasitic nematodes associated with cotton in Haryana. *Indian J. Nematol.* **37**: 23-26.

Received for publication : November 10, 2023
Accepted for publication : January 14, 2024