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Survey of Major Bitter Gourd Growing Areas of Punjab to Determine the Incidence and Prevalence of Root-Knot Nematode

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ABSTRACT: In the present study, with the aim to study the nematode infestation and to identify the prevalent root-knot species, major bitter gourd growing areas were surveyed in different districts of Punjab. From these fields one hundred eighteen samples were collected out of which sixty six samples were found to be infested showing overall 57.27 percent mean disease incidence. District wise maximum disease incidence (75%) was recorded in Ferozepur followed by Sangrur (65%) and the minimum disease incidence was seen in Ludhiana (44.16%). The observation recorded on cropping sequence followed by the farmers revealed that fields where crops like brinjal, okra, chilli and cucumber were grown in rotation with the bitter gourd crop, nematode infestation was higher as compared to the fields where marigold, garlic and onion crops were included in the crop rotation. Further, it was noticed that fields with wild bitter gourd/*Jhaar Karela* (*Momordica balsamina*) grown areas showed less soil nematode population as compared with fields where cultivated bitter gourd (*M. charantia*) was grown. Morphological identification using perineal pattern studies and the PCR based detection using species specific primers revealed *Meloidogyne incognita* as the most prevalent species.

Keywords: *Momordica charantia, M. balsamina*, *Meloidogyne* spp., survey.

Bitter gourd is one of the most popular cucurbitaceous vegetable grown in India. Among cucurbits, bitter gourd has the highest nutritive value and is good source of proteins, minerals, vitamins and carbohydrates. It possesses various antioxidant, anti-hepatotoxic, antimicrobial and antiviral properties and have ability to lower blood sugar. The genus *Momordica* consists of 60 species (Schaefer and Renner, 2011); out of which seven species are known to occur in India but only *Momordica balsamina* (commonly known as *Jhaar Karela*), *M. diocia* (spine gourd), *M. cochinchinensis* (sweet gourd of Assam) and *M. charantia* (cultivated bitter gourd) are major cultivated species available in India (Yadav *et al.,* 2004) with *M. charantia* as most widely grown specie. At country level crop is being cultivated over an area of 95 thousand ha with production of 1030 metric tons/ ha (Anonymous, 2016-17)

In the Punjab state, farmers are taking two bitter gourd crops; one is sown in Feb.- March and crop remains in the field up to the month of July and second crop is sown in June-July and continues till the month of October. Therefore, bitter gourd crop remains in the field for about 8-9 months. Like other cucurbitacious vegetable crops, bitter gourd is susceptible to several pathogens including viruses, fungal pathogens and plant parasitic nematodes. Among the plant parasitic nematodes, root-knot nematodes *Meloidogyne* spp. are considered as first among the ten most important genera of plant parasitic nematodes causing economic losses to the crops throughout the world (Kayani *et al.,* 2013).

For any management strategy to be successful, correct identification of the pathogen is a pre-requisite. The information on prevalence of root knot nematode species associated with bitter gourd crop under Punjab conditions was lacking. Therefore, in order to quantify and document the occurrence and prevalence of root knot nematode species associated with bitter gourd crop survey of the major bitter gourd growing areas in different districts was conducted during the crop season. Identification of the *Meloidogyne* species associated with the bitter gourd crop was done on the basis of morphology and morphometric study of the mature

females and confirmation was done using species specific molecular markers.

MATERIAL AND METHODS

Prevalence, incidence and severity of root knot nematodes

Surveys were conducted in major bitter gourd growing areas falling in different districts of Punjab in order to assess the incidence and prevalence of root knot nematode in the year 2015-16. Soil and root samples of plants were taken from rhizospheric region showing nematode symptoms were brought to the laboratory for further nematode examination and population estimation. The root gall index as given by (Bridge and Page, 1980) was used for the determination of severity of root-knot nematodes. The percent root-knot nematode incidence of individual bitter gourd fields was determined as follows:

$$\text{Percent incidence} = \frac{\text{Number of infected sites}}{\text{Total number of sites surveyed}} \times 100$$

Soil samples were analyzed for the estimation of root knot nematode population using Cobb's sieving and decanting technique (Cobb, 1918; Schnidler, 1961).

Morphological identification of root knot nematode species

Morphological identification of prevailing *Meloidogyne* species associated with bitter gourd was done on the basis of perineal pattern studies of the mature females extracted from the infested root samples collected from the farmers' fields. Identification of root knot nematode specie(s) was done on the basis of female perineal patterns as described by Taylor and Netschler (1974). Further, morphometric observations of perineal patterns were also recorded to study the variation among the populations collected from different districts. Perineal pattern of ten females was studied per population and distribution of each *Meloidogyne* species was calculated in each district.

Confirmation of root knot nematode species using molecular markers

For further confirmation of most prevalent root knot nematode species associated with bitter gourd in Punjab, total DNA was extracted from root knot nematode population collected from the farmer's fields using a Proteinase K enzyme method (Williamson *et al.,* 1997). About 50 egg masses were transferred to a 1.5-ml micro tube containing 50 µl nematode lysis buffer (1X PCR buffer with 100 ìg/ml proteinase K), and crushed using a conical micro pestle before freezing at -20°C for 1 hr. Incubation was performed at 60°C for 1 hr, followed by inactivation of proteinase K by incubation at 94°C for 10 min. Centrifugation was done at 13,000 rpm for 2 min. and supernatants (DNA extracts) were taken in separate sterile tubes. Two volumes of cold absolute ethanol (-20°C) were added to the supernatants and left in the freezer at -20°C for 1 hr. The precipitated DNA was pelleted by centrifugation at 14,000 rpm for 3 min., washed with 70% ethanol, air dried for 2 hours at room temperature (25°C). The DNA pellet was re-suspended in 50 µl of tris extraction (1xTE) buffer and spectrophotometer (Thermo Scientific NanoDrop™ 1000) was used for quantitative and qualitative assessment of nucleic acid. primers specific to three main *Meloidogyne* species, *M. incognita, M. javanica,* and *M. arenaria.* The amplified PCR product was run in 1 percent agarose gel. It was then visualized and documented using UV trans-illumination (Alphaimager, USA). The results were verified against DNA marker.

RESULTS

Survey of fields for prevalence of root knot nematode in Punjab

Samples were collected from five districts; Ludhiana, Patiala, Sangrur, Ferozepur and Gurdaspur of Punjab state for prevalence and incidence of root knot nematode infecting bitter gourd crop during the year 2015-2016. A total of 118 samples were collected from the different fields (Table 1). In Ludhiana district, total thirty samples were collected out of which, fifteen samples were found to be infested with root knot nematode showing overall 44.16 percent mean incidence. Maximum nematode

Table 1. Prevalence and incidence of root knot nematode in bitter-gourd growing areas in different districts of Punjab State

S. No.	District/Villages	Crop rotation followed by the farmer	Percent incidence (%)	Mean soil population/ 250cc soil sample	RGI (0-10) scale	RKN spp. prevalent
	District Ludhiana					
1.	Punjab Agricultural University	Brinjal-Bitter-gourd- Tomato	80.00	390	6.5-8.0	*M. incognita* & *M javanica*
2.	Abbuwal	Chilli-Bitter-gourd-Okra	60.00	265	4.0-6.5	*M. incognita*
3.	Abbuwal	Cucumber-Bitter-gourd-Tomato	80.00	307	5.0-6.5	*M. incognita*
4.	Boparai kalan	Brinjal-Bitter-gourd-Onion	33.33	62	50-75	*M. incognita*
5.	Boparai kalan	Chilli-Bitter-gourd-Okra	66.66	295	5.6-6.0	*M. incognita*
6.	Boparai kalan	Okra-Bitter-gourd-Aloevera	33.33	115	2.0-4.0	*M. incognita*
7.	Dhatt	Onion-Bitter-gourd-Chilli	nil	nil	nil	
8.	Dhatt	Garlic-Bitter-gourd-Aloevera	nil	nil	nil	
		Overall mean	44.16	179		
	District Sangrur					
9.	Malerkotla	Bitter-gourd-Okra	80.00	375	7.0-8.0	*M. incognita* & *M. javanica*
10.	Malerkotla	Bitter-gourd-Chilli	80.00	385	6.0-8.0	*M. incognita*
11.	Sandhaur	Brinjal-Bitter-gourd-Tomato	100.00	525	8.0-8.5	*M. incognita*
12.	Bhani kalan	Brinjal-Jhaarkarela-Onion	nil	Nil	nil	
		Mean	65	321		
	Gurdaspur					
13.	Kot(dhar)	Bitter-gourd-Chilli	80.00	345	6.0-7.0	*M. incognita*
14.	Kot(dhar)	Bitter-gourd- Okra	60.00	262	5.0-6.0	*M. incognita*
15.	Khanpur	Bitter-gourd-Cucumber	100.00	405	7.0-8.0	*M. incognita*
16.	Khanpur	Bitter-gourd-Marigold	nil	nil	nil	
17.	Azampur	Brinjal-JhaarKarela-Tomato	33.33	82	2.0	*M. incognita*
18.	Baheri	Chilli- JhaarKarela-Onion	nil	Nil	nil	
		Overall mean	45.55	182		
	Ferozepur					
19.	Behak	Bitter-gourd-Chilli	100.00	420	6.0-8.0	*M. incognita*
20.	Behak	Bitter-gourd-Okra	100.00	395	7.0-8.0	*M. incognita*
21.	Ittanwali	Potato-Bitter-gourd	80.00	405	7.0-8.0	*M. incognita*
22.	Ittanwali	Bitter-gourd-Onion	20.00	72	2.0	*M. incognita*
		Overall mean	75		323	
	Patiala					
23.	Pattran	Bitter-gourd-Cucumber	75.00	317	6.0-7.0	*M. incognita*
24.	Pattran	Bitter-gourd-Chilli-Onion	25.00	65	2.0	*M. incognita*
25.	Amarpur	Bitter gourd-Marigold	nil	nil	nil	
26.	Amarpur	Bottle gourd-Bitter-gourd-Chilli	80.00	395	7.0-7.5	*M. incognita*
27.	Sanaur	Bitter-gourd-Brinjal-Chilli	80.00	352	6.0-7.0	*M. incognita*
28.	Banaur	Chilli-Potato-Bitter-gourd	80.00	346	6.0-7.0	*M. incognita*
		Overall mean	56.66	246		

infestation was observed in the samples collected from the Vegetable Research Farm, PAU, Ludhiana in terms of percent disease incidence (80%), soil population (360-420 J_2/250cc soil) and root gall index (RGI) (6.5-8.0) followed by samples collected from the village Abbuwal with percent disease incidence 80 percent, soil population 170-375 J_2/250cc soil and RGI ranging from 4.0-6.5. In the village Boparai kalan infested fields showed percent disease incidence ranging from 33.3 to 66.6 percent, soil nematode population ranging from 50-310 J_2/250cc soil and RGI from 2.0-6.0. Negligible root-knot nematode incidence was observed in the samples collected from village Dhatt, Ludhiana. Both the fields were found free from nematode infestation. This might be due to the cropping sequence as in both these fields garlic and onion crop was a succeeding crop to bitter gourd.

In Sangrur district, total eighteen samples were collected and twelve samples were found to be infested with root knot nematode showing mean disease incidence of 65.00 percent. Location wise maximum nematode incidence was recorded in Sandhaur village showing 100 percent disease incidence, soil population (480-570 J_2/ 250cc soil) and root gall index (RGI) (6.0-8.0). This was followed by Malerkotla, with disease incidence 80 percent, soil population ranging from 300-470 J_2/250cc soil and RGI from 8.0-8.5. In the village Bhani kalan, all the samples collected were found to be free from nematode attack. The less infestation may be due to the cultivation of wild bitter gourd, *M. balsamina i.e. Jhaar karela* in these fields which is reported to possess resistance against root knot nematode.

Total twenty six samples were collected from Gurdaspur district, out of which thirteen samples were found to be infested with nematode showing 45.55 percent mean disease incidence in this district. Maximum disease incidence was seen in village Khanpur, with soil population (370-440 J_2/250cc soil) and RGI from 7.0-8.0 followed by samples collected from the village Kot , Dhar with 70 percent disease incidence, soil population ranging from 230-365 J_2/250cc soil) and RGI ranging from 5.0-7.0. In the village Azampur all the fields surveyed were found to be infested however percent disease incidence recorded was 33.3 percent, soil nematode population ranged from 75-90 J_2/250cc soil with RGI (2.0). Negligible root knot nematode attack was recorded in the samples from village Baheri, Gurdaspur. The less infestation in both (Azampur and Baheri) villages might be due to the cultivation of *Jhaar karela* (*M. balsamina*) as a crop rotation with chilli, tomato, brinjal and onion.

In Ferozepur district out of twenty samples taken, fifteen samples were found to be infested showing 75 percent mean incidence. The village Behak showed maximum nematode incidence with percent disease incidence 80 percent, soil population (340-500 J_2/250cc soil) and RGI from 6.0-8.0 followed by samples collected from Ittanwali with percent disease incidence ranging from 20-80 percent, soil population from 60-440 J_2/250cc soil and RGI ranging from 2.0-8.0.

Fifteen soil samples were found to be infested with root knot nematode out of twenty eight samples collected from Patiala district showing overall 56.66 percent incidence in the district. Village Amarpur showed maximum infestation in terms of percent disease incidence (80%), soil population (380-410 J_2/250cc soil) and root gall index (RGI) (7.0-7.5) followed by village Sanaur with 80 percent disease incidence, soil population 320-385 J_2/250cc soil and RGI 6.0-7.0. In the village Pattran, lowest nematode attack was recorded showing percent disease incidence (25 to 75 percent), soil nematode population (55-345J_2/250cc soil) and RGI from 2.0-7.0. Low root knot nematode infestation was observed in the fields where Marigold crop was taken in the cropping sequence by the farmers.

Samples collected from Ludhiana (Vegetable Farm of PAU) and Sangrur (Malerkotla) showed the presence of both *M. javanica* and *M. incognita*. In all other soil samples *M. incognita* was found to be most prevalent root knot nematode species associated with bitter gourd. However, the samples collected from different districts during the survey showed variation regarding morphometric characters of perineal patterns (LVS= Length of vulval slit, AVS= Anus to vulval slit, ATT= Anus to tail terminus) (Table 2). LVS was recorded to be highest (18.79µm) in Patiala population followed by Ferozepur population (16.18 µm) whereas, Gurdaspur populations showed minimum LVS (14.10 µm). The length of anus to vulval slit (AVS) was maximum (19.79µm) in Ludhiana population followed by Ferozepur

Table 2: Morphometric characters of perineal pattern of populations of *M. incognita* collected during the survey (Mean±SD; range) (µm)

Characters	Ludhiana	Patiala	Ferozepur	Sangrur	Gurdaspur
LVS (µm)	14.21±1.09 (14.10-16.45)	18.79±1.87 (15.98-20.83)	16.18±1.56 (14.74-18.46)	15.69±1.98 (12.45-17.46)	14.10±1.24 (13.46-16.46)
AVS (µm)	19.79±1.38 (17.45-21.05)	15.11±3.21 (10.87-19.73)	17.54±3.09 (13.45-20.67)	12.16±2.26 (10.46-15.84)	13.66±2.52 (10.57-16.47)
ATT (µm)	14.83±1.19 (12.65-15.76)	18.18±2.43 (14.64-20.65)	18.44±1.61 (16.46-20.89)	16.37±3.03 (12.46-20.75)	11.83±1.13 (10.57-13.58)

Character ranking (CR): LVS= Length of vulval slit, AVS= Anus to vulval slit, ATT= Anus to tail terminus

population (17.54µm). The value of ATT was maximum (18.44µm) in Ferozepur population followed by Patiala population (18.18µm) while minimum (11.83µm) in Gurdaspur population.

Molecular identification

Confirmation of morphologically characterized most prevalent root knot nematode sp. was done by PCR amplification of DNA with SCAR (sequence characterized amplified regions) primers Finc/Rinc specific to *M. incognita* (Zijlstra *et al.,* 2000). The DNA extracted from *M. incognita* populations collected during survey was subjected to PCR amplification with *M. incognita* specific primer pairs Finc/Rinc along with negative control. An expected size amplicon of ~1200bp with Finc/Rinc primer was observed from all the samples while no amplification was seen in negative control (Fig. 1) which further confirmed the prevalence and association of *M. incognita* sp. with bitter gourd crop in Punjab.

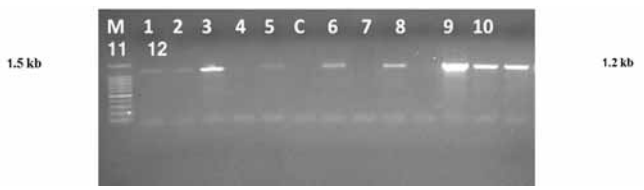

Fig. 1. 1% agarose gel showing amplicon of 1.2kb with SCAR Finc/Rinc primer pair from Samples: 1-2 (Sangrur); 3-5 (Patiala); 6-7 (Ludhiana); 8-9 (Gurdaspur); 10-11 (Ferozepur), C- control, M-marker (100bp)

DISCUSSION

Results showed that out of total twenty eight bitter gourd fields surveyed from five different districts of Punjab state, nineteen fields were found to be infested with root knot nematode. From the total one hundred eighteen samples collected, sixty six samples were found to be infested showing overall 57.27 percent mean disease incidence. About 60 percent fields were found to be highly infested with root knot nematode showing soil population >250J_2/250cc soil sample. District wise, Ferozepur showed maximum mean percent disease incidence (75.0%) with 323 J_2/ 250 cc soil followed by Sangrur (65.00%) with 321 J_2/250 cc soil, Patiala (56.66%) with 246 J_2/250 cc soil, Gurdaspur (45.55%) with 194 J_2/250 cc soil and Ludhiana (44.16%) with 171 J_2/ 250 cc soil.

Further, it was observed that more nematode infestation was recorded in the fields where susceptible vegetables like cucumber, okra, chilli and brinjal *etc*. were grown in rotation with the bitter gourd crop as compared to the fields where crops like marigold, garlic and onion crops were included in the cropping sequence. It was also observed that *Jhaar Karela* (*M. balsamina*) cultivated areas showed less nematode attack as compared with areas where cultivated bitter gourd (*M. charantia*) was grown. *Jhaar Karela* is reported to acquire resistance against root knot nematode (Pofu *et al.,* 2010). Pofu *et al.,* (2015) also revealed that *M.*

balsamina cultivated areas showed less nematode population and incidence. So this might be the reason for less nematode incidence in field where *M. balsamina* was cultivated. Marigold (*Tagetus* spp.) is well known to produce compounds such as á-terthienyl which are reported to have allelopathic properties against different species of plant parasitic nematodes including root knot nematodes (Siddiqui *et al.,* 1988; Natarajan *et al.,* 2006). Recently, Xie *et al.,* (2017) reported that for the control of root knot nematode in angelica (*Angelica sinensis*) both crop rotation and intercropping with marigold are effective however, crop rotation was more effective than intercropping. Garlic (*Allium sativum*) is known to produce allicin which have both antimicrobial and nematicidal properties (Gupta and Sharma, 2008; El-Nagdi and Youssef, 2013).

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