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Survey and Identification of Root-Knot Nematodes Associated with Brinjal Crops in Fatehabad, Agra

BINDHYA CHAL YADAV[1]* and SANOJ KUMAR[2]

[1]Department of Botany, Government Post Graduate College Fatehabad, Agra India.
[2]Department of Life Science, Mewar University, Chittorgarh India.

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ABSTRACT

A survey was conducted over a period of one and half year to estimate the occurrence of root-knot nematode disease on brinjal crops in five selected localities in and around Fatehabad, Agra. Our survey indicates that brinjal crops grown in the selected localities were heavily infested with root-knot nematodes. Highest frequency of disease occurrence in which almost all the roots have knot-like appearance (>85%) was reported from Firozabad Road area. Other localities were also having the significant infestations and these were reported as 80%, 78%, 65%,62% respectively. To access the damage caused by root-knot nematodes in brinjal crop, *Meloidogyne* gall index and its egg-mass index were calculated and these were found in the range of 2-5. An increased gall index and egg-mass index point to heavy infection on selected crops. Sampled crop showed the significant presence of *Meloidogyne javanica*. However, there were other species of root-knot nematode as mixed population was also reported. Other species of the root-knot nematodes which were reported in this survey was *M. incognita*. This survey indicates that the *M. javanica* is a frequently occurring population in brinjal field infested with root-knot disease.

Keywords: Root-Knot Nematodes, *M. incognita, M. javanica,* Firozabad Area.

INTRODUCTION

Root-knot nematode, *Meloidogyne sp.* is an important group of plant-parasitic nematode[1]. *Meloidogyne* is an endo-parasitic nematode dwells in almost all crops worldwide[5, 16]. These animals can be found wherever a plant can grow[14]. There are several species of root-knot nematodes reported from the different parts of the world, but the major damage to crops are mainly attributed to its four species *M. incognita, M. javanica, M. arenaria* & *M. hapla*[14,15,21]. Due to their unique ability to survive on several plants and apomictic type of reproduction, root-knot nematodes have a wide host range[12]. Brinjal (*Solanum melongena*) is an important vegetable crop grown throughout the world. Brinjal is reported to be infected by various plant pathogen including root-knot nematodes[3,19].

Root knot nematodes are reported from all terrains of all ecosystems[14,15]. These nematodes are reported from all the places in our planet earth wherever the average temperature goes above 3°C. Root-knot nematodes are serious pathogens of the crops particularly vegetable crops. The damage caused by these worms primarily goes unnoticed as these nematodes infects the underground parts of the plants and also quite some time the damage was being attributed by other pathogens too. The damage caused by these nematodes has been estimated to be around $157 billion worldwide[20]. We have only a handful method to contain the damage caused by them. Our inability to design novel control methods and limit yield loses due to root-knot nematodes is primarily due to our poor understanding of the biology of this parasite[6]. Nevertheless, in recent past the field of plant nematology was in focus with the genome sequences of more than half a dozen of nematodes associated with plants are either sequenced or several ESTs are sequenced leading to significant genetic information from these nematodes[7]. We and others in Uttar Pradesh have

conducted surveys of vegetable fields infested by root-knot nematodes on brinjal crops from various regions of Uttar Pradesh. In this paper we are presenting the results of the survey conducted over one and half years in cropping season of brinjal crops in Fatehabad, Agra. In our study we have examined more than 535 root samples to examine the nematode prevalence in a particular area. The purpose of this study was to estimate the distribution of root-knot nematodes and their incidence on brinjal crop.

MATERIALS AND METHODS

For this study, a field survey was conducted. We have made extensive field visit to five selected locations in and around Fatehabad, Agra and uprooted 535 brinjal plants to access the damage caused by the nematode. To access the damage caused by the nematodes, brinjal plants were uprooted from the selected localitities. Root samples were collected in polythene bags (HIMEDIA) and neatly labeled. These were then brought to laboratory for examination. Upon arrival in laboratory, root samples were examined for overall root structure following the infection, presence of galls on roots. Numbers of gall in each root were counted.

In order to count the number of egg masses on root surface, roots were washed clean in running tap water for 10-15 minutes, these were then immersed in aqueous solution of acid fuschin (875 ml of lactic acid, 63 ml glycerol, 62 ml of water and 0.1gm of acid fuschin) for 30 minutes and then washed with tap water to examine the stained egg masses. Initially number of egg masses in each root were counted, gall index and egg mass index was determined by the scale described by [18]. These are as follows 0=0, 1=1-2, 2=3-10, 3=11-30, 4=31-100 and 5 if the number of galls or egg mass is more than 100 per root.

Table 1: Frequency of root-knot nematode on brinjal plants in selected experimental fields

S.No	Place	No of plants observed	Galling Index	Average no of knot/plant	Average number of ne matode/ knot dissected	Plant physiognomy
1.	Fatehabad	125	4	630 (50)	12 (34)	Stunted
2.	Shamsabad Road	90	4	488 (47)	08 (27)	Healthy looking
3.	Salempur	110	5	876 (51)	11 (23)	Yellowish
4.	Madayna	105	5	965 (65)	18 (32)	Yellowish
5.	Firozabad Road	105	5	1057 (48)	21 (10)	Stunted

Number in parentheses indicates the number of plant and number of knot dissected from the studied plant materials.

Table 2: Species of root-knot nematode reported from the sampled field

S. No	Name of the Experimental Field	Species Reported
1.	Fatehabad	Meloidogyne javanica
2.	Shamsabad Road	Meloidogyne incognita and Meloidogyne javanica
3.	Salempur	Meloidogyne javanica
4.	Madayna	Meloidogyne incognita and Meloidogyne javanica
5.	Firozabad Road	Meloidogyne javanica

Maintenance of Nematodes

Plant nematodes collected from the root samples from each locality was maintained on the nematodes susceptible variety of tomato

(*Lycopersicon esculentum* var. Pusa Ruby). To maintain the pure culture of the nematode population, single egg mass were picked up from the infected root and hatched separately in distilled water at room temperature (egg mass were picked from the infected root using the forceps (sigma) and then were put on kimwipes paper that was laid over a wire gauge and a petridish with water. This setup was covered with another petriplate to avoid water evaporation. The setup was then placed in an incubator at 28^{0}C for 16 hours. Next day the water in lower petriplate was examined using microscope. Water from each petriplate containing hatched active juveniles was poured near the root surface of the freshly planted tomato plants and then maintained for subsequent experiments. The infection was allowed to go for 45 days and then these were sub-cultured to raise enough number of nematodes for subsequent studies.

Identification of Nematode Species

Identification of the root-knot nematode that was maintained on tomato root-stocks was done by cutting the perineal pattern as described[8]. Mature females were dissected out from the nematode galls on roots under simple microscope and collected in water. Approximately 15-20 mature females were collected from each plant stock and their perineal pattern was prepared and examined under microscope to study various characteristics. Nematode species were identified on the basis of the perineal pattern characteristics as described8

RESULTS

This survey was conducted to access the damage caused by root-knot nematode on brinjal crop. The survey was design to access the damage at five selected locations in and around Fatehabad, Agra. This study finds that the brinjal plant sampled in selected location had significant amount of infection. Based on the observations made during the survey it can be concluded that the incidence of the root-knot nematode infection was above 85% on selected sites.

In general the rate of infection by the root-knot nematodes was predominantly high in selected sites, but there were some variations among the selected sites. Highest frequency of the infection was observed at Firozabad Road which was similar to Madayana. In both the location the rate of incidence of nematode infection was around 80%. Rate of incidence of nematode infection in Salempur area was around 78%. In Fatehabad the infection rate was calculated to be 65% and in Shamsabad road it was estimated to be 62% (Table-1).

Studies focused on number of gall and egg mass indicate that in general the rate of incidence of root-knot nematode infection was quite high. However, area wise variation in terms of infection was also noticed. The bigger gall and egg masses were reported from Firozabad Road area which was in line with the rate of nematode infection in Madayana area. The reported gall and the egg mass index were 05 in Firozabad Road area. The gall and egg mass index were taken together in rate of infection on brinjal crop was highest in Firozabad Road area which was followed by Madayana, Salempur,Fatehabad and Shamsabad Road area (Table-1). As described earlier, the nematodes were identified using the perineal pattern as described by[8]. Based on perineal pattern the population of the nematode was identified as *Meloidogyne incognita* and *Meloidogyne javanica*. These two species of root-knot nematodes were found in all the sampled site and these were occurring as mixed population in these fields. *M. javanica* was more frequently occurring species in sampled localities. In certain sampled sites both of these species was occurring as mixed species in the locality (Table-2).

This survey was conducted with aim to access the damage caused by root-knot nematode in brinjal. It also reveals that while the *M. javanica* is quite a prevalent species in the sampled field, other root-knot nematode species, *M. incognita* was also frequently observed in this survey. The incidence of the disease in general was quite high in all sampled locations. The intensity of the disease in the sampled location was in tandem with the increase gall index and egg mass index. This survey indicates that brinjal crop grown in the selected areas had significant impact by root-knot nematode infections.

Since root-knot nematode infestation on vegetable crops has already been reported from various parts of India, this is the first study from Fatehabad, Agra in Uttar Pradesh. Root-knot nematodes have been reported from all terrains of all

ecosystems. These animals have been reported from various places on earth. Although *M. incognita* and *M. javanica* is quite common in hotter areas, the other species of *Meloidogyne, M. arenaria* is quite suited to cooler climatic conditions. High incidence of the root-knot disease in brinjal crops is partly due to its survival on collateral host in seasons when the main crop is not available in the field. Since the inoculum of the disease is available all throughout the year, the rate of infection is quite high in vegetable crop surveyed. *M. javanica* can successfully proliferate on weeds in absence of the main crops and this could be another potential reason for high incidence of the disease in sampled fields.

Other workers have conducted the similar survey with tomato crops in adjoining areas of Agra [10]. These workers have reported the high incidence of rot-knot nematode disease in fields wherever the tomato crop is grown. They have reported the presence of *M. javanica* in their survey report. This survey also finds that even in case of brinjal in the selected sampled field, *M. javanica* was the prevalent species. Perineal pattern of the isolated nematode confirms that the *Meloidogyne javanica* is the common and most prevalent species in the area.

DISCUSSIONS

Result of this survey indicates that brinjal is very susceptible to root-not nematode infection. As seen in table-1 wherever the brinjal crop is grown in Fatehabad, Agra, root-knot nematode infects this crop. The galling index indicates that the extent of damage is really a serious problem for brinjal growers. While dissecting the knots from these plants, we found that there are more than 10 females in each of the knot dissected which further adds that brinjal crops is particularly susceptible to root-knot nematode infection.

In case of tomato crops it has already been observed that tomato crops (not all) have potential and dominant gene for nematode resistance. This gene is named as *Mi* gene indicating the name of pathogen against which this gene confers the resistance, *M. incognita*[13,17]. The biggest problem with this gene is that it does not work at temperatures beyond 28° C. We have not yet confirmed whether the plant observed had the *Mi* gene in them or not. Work in author's laboratory is in progress to determine the presence of *Mi* gene in these plants using standard molecular techniques. Results of this survey can also be used as an advisory to farmers who intent to take only a particular variety of crops in their filed but they are unaware of the damage that is happening underground. Another important point that this survey revealed that the soil type have a significant influence on extent of damage caused by the root-knot nematode. In Fatehabad area the soil is sandy loam type which favors the damage caused by the root-knot nematode. Another reason which we found for this damage is the use of Pusa variety of the brinjal by most the growers. This variety of brinjal although high yielding but is very susceptible to root-knot nematode infection.

Brinjal crops grown in various parts of the county are particularly susceptible to root-knot nematode infection. Root-knot nematode causes significant damage to brinjal crops. Among various group of plant-parasitic nematodes, root-knot nematode are the leading nematodes in terms of the damage caused to crop plants. As discussed earlier in this paper as well as several other workers, these nematode have a wide host range. Several option of plant-disease management like crop rotation[11] is of not good values as nematode can make the available crop as a host plant. As we begin to understand the complex nature of this parasite and associated damage caused by the root-knot nematode, a combination of strategies must be put on use to contain the damage caused by this tiny worm. Several tools and strategies needs to be combined such as crop-rotation, cover crops, planting resistant varieties of the intended crop, and destroying the rouge materials carefully, using nematode resistant germplasm etc to name a few. Recent developments in field of molecular plant nematology have opened totally new vistas in field of plant nematology. Now we are able to genetically engineer the plants at will and also the efforts are underway to transform

the nematode like *C. elegans* in an aim to get more information from this nematode[2]. Biotechnology has offered an unparallel approach to conventional methods in nematode control[4]. Recent demonstration of RNAi with plant-parasitic nematodes particularly root-knot nematodes have shown the great promise that this technique hold for future[9,20]. In summary a baggage of many techniques will be required to understand the biology of *M. incognita* and will help us in designing the novel control strategies.

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