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Research Article

ISOLATION AND IDENTIFICATION OF ROOT KNOT NEMATODES ASSOCIATED WITH *SORGHUM BICOLOUR* (L.) MOENCH CROP

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ABSTRACT

A study was performed over a time of one year to assess the incidence of root-knot nematode disease on sorghum crops in five selected localities of Telangana region. Our study shows that sorghum crops grown in the selected localities were moderately infested with root-knot nematodes. Maximum frequency of disease occurrence in which almost all the roots have knot-like appearance (≤45%) was reported from Ranagareddy area. Other localities were also having the significant infestations. To access the damage caused by root-knot nematodes in sorghum crop, Nematode gall index or its egg-mass index were calculated and these were found in the range of 1-4. Sampled crop showed the considerable presence of mixed population of root knot nematode. This study indicates that the root knot nematodes (*Melioidogyne spp)* are a frequently occurring population in sorghum field infested with root-knot disease.

INTRODUCTION

Nematodes or roundworms are one of the most widespread phyla of animals, with over 20,000 diverse described species (Lorenzen, 1994), omnipresent in freshwater, marine, and terrestrial environments. They have significant life-styles both in free-living and parasitic variants, having the capacity to adjust to difficult environments or to occupy numerous hosts, respectively. Economic losses caused by parasites to agriculture (domesticated animals and crops) have a major impact on farm productivity, exacerbating global food shortage situations e.g., plant parasitic nematodes are responsible for $80 billion in annual crop damage (Barker *et al*., 1994). Recognizing the importance of root-knot nematodes in agricultural economy, an international project called International *Melioidogyne* Project (IMP) has studied the occurrence and distribution of species, their relative importance and differentiation (Taylor and Sasser, 1978**).** Although, there are about 60 illustrated species of root-knot nematodes, most taxonomic interest has focused on less than the dozen that are typically associated with diseases of agronomically important plant species. The genus, *Meloidogyne* is an endoparasite that has evolved very tight interactions with its plant host. The success of parasitism depends for a large part on the capacity of the infective larva to penetrate the root tip and to migrate intercellularly towards the vascular cylinder. *Meloidogyne incognita, M. javanica, M. arenaria,* and *M. hapla* account for the majority of crop losses caused by root-knot nematodes. An extensive study of about 1,300 *Meloidogyne* populations from over 70 countries signifying the primary food production regions of the world found at least one of these four species in 95% of the samples (Carter and Sasser, 1982). The four species are universal in distribution, but the first three are usually limited by temperature to a region between 40°N and 33°S latitude. The root-knot nematodes *(Meloidogyne* spp) are serious and endemic problems on many crops grown in India, particularly in Telangana region due to its variable climatic conditions.

Sorghum bicolour (L) Moench grows in a wide range of temperature, high altitudes, toxic soils and can recover growth after some drought. The pertinence of *S. bicolor* as a host of *Meloidogyne* spp. has been pontificated by several authors (Birchfield, 1983; Rodrlguez-Kabana and Touchton. 1984). Information about host range and host specificity is also included in the original descriptions of some species. In Louisiana 9 of 10 sorghum cultivars are highly susceptible to

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M. incognita and one is moderately resistant (Birchfield, 1983). Others showed sorghum was a poor host for *M. incognita,* although some reproduction occurred (Orr and Morey, 1978). Variation in the host suitability of other graminaceous crops to populations of *Meloidogyne* spp. suggests that the reaction of sorghum to populations *of Meloidogyne* spp. may vary by location (Baldwin and Barker, 1970). Taxonomic studies on nematode, by and large, engage counting and measuring of diverse characters and examining them under different types of microscopes. In practice, identification of the most common and the most agriculturally important species is often attempted by microscopic inspection of perineal patterns of adult females and by conducting differential host tests. Species characterization is based primarily on morphological features of second-stage juveniles, males, and adult females. Recognition of juveniles typically involves a combination of painstaking examination of morphological characters (Eisenback *et al*., 1981) and time-consuming reproduction on a set of differential host plants. In recent years, species-specific DNA hybridization probes have been sought as a replacement or complement for these procedures (Burrows, 1990; Powers, 1992). Although many of these probes appear promising, it is unlikely that any hybridization method involving radioactive isotopes will be accepted as a general diagnostic protocol. Non isotopic labelling methods are available, but their lower sensitivity may fail to detect low numbers of eggs or second-stage juveniles. Many studies have been designed to study *Meloidogyne* species, with respect to different hosts and varied geographical regions. Most of these protocols require long time and cost to attain specific objectives and frequently result in a low rate of detection. According to our knowledge no attempt has been made to study sorghum crop in Telangana region. In the present study, we report the collection of samples for root knot nematodes and related information about soil and crop and microscopic analysis.

MATERIALS AND METHODS

Collection and preparation of soil samples: The protocol was applied to root knot nematodes, isolated from 10 districts of Telangana region, India. A survey was conducted in different localities in and around Hyderabad, Warangal, Rangareddy, Karimnagar, Khammam districts to assess the incidence of nematode disease on sorghum crop (Sample collection performa for root knot nematodes studies was given in Performa 1). Five pre-selected fields in Telangana region were sampled to a depth of 15 cm at two month intervals for 1 year during the month of December and January. Eight such composite samples were collected from each field at two month intervals. During the survey of sorghum crop in each locality samples of the sorghum plant parts such as roots were collected randomly. Plant samples kept in polythene bags and properly labelled were brought to the laboratory and thoroughly examined for the presence of galls. Each sample was sieved through a screen with 6-mm openings and then thoroughly mixed by passing it through a sample divider four times. Samples were processed within 24 hr after collection. Numbers of galls per plant part, if present, were counted. Plant parts were cleaned by washing and then immersed in an aqueous solution of phloxin B (0.15 g/lit) for 15 minutes and then washed with tap water to stain egg masses. Number of egg masses per plant part was then counted. Gall index (GI) and egg mass index (EMI) were determined on the following scale: 0=0, 1=1-2, 2=3-10, 3=11-30, 4= 31-100 and 5=greater than 100 galls or egg masses per plant part (Taylor and Sasser, 1978). The frequency of occurrence (percentage) of the disease in each locality was calculated by the following formula:

$$\text{Frequency of occurrence} = \frac{\text{Number of fields with root knot nematode infection}}{\text{Number of fields surveyed}} \quad 100$$

Extraction of Nematodes from plant and soil samples: An aliquot from each of the eight composite soil samples was processed by each of three extraction methods: Root dissecting method, Baermann funnel technique and Cobb's sieving or Gravitation technique (Decanting and sieving method). Detailed procedures followed for each method of extraction were given below. All counts were converted to numbers of nematodes per 500 cc of soil.

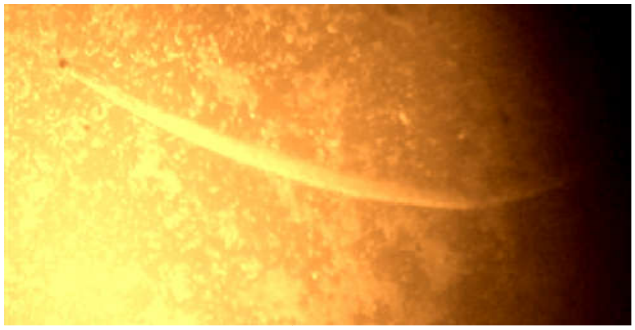

Figure 1 Whitish curve shaped flat nematode along with intestine was observed on light microscope (40X)

Root dissecting method*:* Plant materials were washed carefully and placed in water filled petri dishes. Dissect the sample with dissecting needles and forceps, using a 15-50x magnification. The emerging nematodes, egg masses, etc. were picked from the suspension with a handling needle or painting brush. Infected plant sections were analysed under a microscope for the presence of nematodes (Figure 1).

Baermann Funnel technique*:* Infected plant material was placed in water nematodes crawl out of the material and sink (Baermann, 1917). After 48 hours, extraction of active nematodes from plant material and soil was achieved.

Cobb's sieving or Gravitation technique (Cobb, 1918): The method uses different properties such as size, shape, sedimentation rate and nematode mobility of nematodes and soil particles for extraction of active nematodes from soil and sediments.

RESULTS

This study was conducted to access an injury caused by root-knot nematode on sorghum crop. Eight samples from five districts (Warangal, Khammam, Rangareddy, Hyderabad and Karimnagar) were collected after complete survey using sample collection performa for root-knot nematodes study. Samples were processed by standard protocols routinely used in our lab. This study finds that the sorghum plant sampled in selected location had significant amount of infection.

Table 1 Frequency of root-knot nematode on sorghum plants

Place	No of field	No of plants	No of fields with roots	Egg masse index	Average no of knot/ plant	Average no of nematodes/ knot	Symptom of the plant
Rangareddy	4	20	4	4	8 (4)	2 (4)	Skeletal, Stunted
Hyderabad	1	10	0	0	2 (1)	0 (1)	Wilted
Karimnagar	1	10	1	1	3 (1)	1 (1)	Skeletal
Khammam	1	10	1	1	1 (1)	0 (1)	Healthy
Warangal	2	10	2	2	4 (2)	1 (1)	Skeletal, Yellowish
Total	9	60	8	8	18(9)	4(8)	STDEV is
STDEV	1.304	4.472136	1.5165751	1.5165751	2.70185122	0.83666003	calculated Excel function argument

Based on the observations made during the study it can be concluded that frequency of occurrence of the root knot nematode infection was below 45% on selected sites. Occurrence of disease in locality was calculated based on the data given and using the formula given in above section. 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 Rangareddy which was different from other sites. Rate of incidence of nematode infection at different places were summarised in Table-1. Studies focused on number of gall or 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 or egg masses were reported from Rangareddy area. Overall incidences of the disease caused to the sorghum plant by nematodes were determined. Locality-wise variations in the incidence of the disease were, however, found. The different structures such as stylet, basal knobs, and vulva and tail etc, were observed. The intensity of the disease on sorghum plant in these localities based on average gall and egg mass indicate was variable. On the basis of perineal pattern characteristics, *Meloidogyne spp*, of root-knot nematodes were identified to infect sorghum in different areas included in the study. Examination of the roots of infected plants revealed the presence of root-knot nematodes in large numbers. Mixed populations of both species in sorghum crop were also common. This survey indicates that sorghum crop grown in the selected areas had significant impact by root-knot nematode infections. Since root-knot nematode infestation on various crops has already been reported from various parts of India, this is the first study from Telangana. Root-knot nematodes have been reported from all terrains of all ecosystems. These animals have been reported from various places on earth. Among the species of *Meloidogyne* recorded in association with crops of agricultural importance in subtropical and tropical regions, *M .incognita* and *M. javanica* were considered as common and wild–spread (Sasser, 1979). 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 sorghum crops is partly due to its survival on collateral host in seasons when the main crop is not available in the field. Other workers have conducted the similar study with tomato and brinjal crops in adjoining areas of Fatehabad and Agra (Khan *et al*., 1984; Yadav and Kumar, 2016). These workers have reported the high incidence of root-knot nematode disease in fields wherever the tomato and brinjal crop is grown. They have reported the presence of *M. javanica* in their investigation report.

This study also finds that even in case of sorghum in the selected sampled field, *M. javanica* was the prevalent species. Perineal pattern of the isolated nematode confirms that the *M. javanica* is the common and most prevalent species in the area. Yield losses caused by the root-knot nematode, *M. incognita*, were assessed in gherkin (cucumber) fields in Kolar and Bagepalli, Karnataka, India. Root-knot nematode incidence and severity were high at both locations. Root-knot index ranged from 3.2 to 4.5 on a 0-5 scale with number of egg masses ranging from 69 to 98/g root (Nagesh *et al.,* 2005).

DISCUSSION

Root-knot infested sorghum plants have been reported to be stunted, to bloom later, and to have suppressed yields. Root systems are small, and galls are observed on lateral roots of infested plants. Small galls contained only fragments of nematodes; others contained no detectable traces of developing larvae; and druses are formed in galls of both susceptible and resistant plant roots, but not in healthy tissue. Among the histopathological changes that contribute to formation of galls are hypertrophy of cortex, xylem parenchyma, and metaxylem; hyperplasia of the pericycle and xylem parenchyma; and production of giant cells (Siddiqui and Taylor, 1970). Many workers have noted that the size and general appearance of galls depend not only on the number and species of the nematode, but also on the host plant species (Dropkin, 1955). Galls resulting from infection with *M. hapla* Chitwood can often be distinguished from those of other root-knot species by the matted appearance of roots formed by a combination of relatively small galls and numerous lateral roots growing out of each gall (Sasser, 1954). Krusberg and Nielsen (1958) also noted that nematode feeding stimulated the formation of several atypical tissues: giant cells, "abnormal xylem," hyperplastic parenchyma, and cork. Cells some distances away from the nematode's head are often affected (Christie, 1936). Christie in 1936 reported instances in which certain walls of xylem elements near giant cells became greatly thickened or swollen. Currently there are several varieties of *Sorghum bicolor* (L.) Moench and the nematodes of the *Meloidogyne* sp. are not a threat at all, and some varieties are resistant to these nematodes. This study on the prevalence of *Meloidogyne* sp. with well characterised, locally available variety (Gundu Jonna) is more relevant to semi-urban farmers. Results of this study can 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 study revealed that the soil type have a significant influence on extent of damage caused by the root-knot nematode. In Telangana area the soil is rocky black-red

dust type which favours the damage caused by the root-knot nematode. Another reason which we found for this damage is the use of *S bicolour* (Gundu Jonna) variety of the sorghum as a feed for dairy cattle by most the growers. This variety of sorghum although high yielding but is very susceptible to root-knot nematode infection. Among various group of plant-parasitic nematodes, root-knot nematode are the leading nematodes in terms of the damage caused to crop plants. This nematode has a wide host range. Several option of plant-disease management like crop rotation 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. However, methods used in this study are easily available and needs no skill. Therefore this can benefit nematology, entomology or farming**.** Recent developments in field of molecular plant nematology have opened totally new vistas in field of plant nematology. Genetic engineering and Biotechnology has offered an unparallel approach to conventional methods in nematode control. Recent demonstration of RNAi with plant-parasitic nematodes particularly root-knot nematodes have shown the great promise that this technique hold for future (Yadav *et al*., 2006; Huang *et al*., 2006b). In summary a baggage of many techniques will be required to understand the biology of *Meloidogyne* spp and will help us in designing the novel control strategies (Yadav and Kumar, 2016).

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