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Population Density and Distribution of Root lesion and Knot Nematodes in Sugarcane fields of Agra regions

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Nematodes are tiny, worm-like, multicellular animals adapted to living in water. The number of nematode species is estimated at half a million, many of which are free-living types found in the oceans, in freshwater habitats, and in soils. Parasitic species form a smaller group. Nematodes are common in soils all over the world. As a commentator in the early twentieth century wrote: "If all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable, and if, as disembodied spirits, we could investigate it, we should find its mountains, hills, vales, rivers, lakes and oceans represented by a thin film of nematodes." An important part of the soil fauna, nematodes live in the maze of interconnected channels called pores that are formed by soil processes. They move in the films of water that cling to soil particles [Ahuja, S., and Arora, J.S. (1980 Dropkin, V.H. (1980). 1-2]. Many genera and species have particular soil and climatic requirements. For example, certain species do best in sandy soils, while others favor clay soils. Nematode populations are generally denser and more prevalent in the world warmer regions, where longer growing seasons extend feeding periods and increase reproductive rates. Direct feeding by nematodes can drastically decrease a plants uptake of nutrients and water. Nematodes have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination. Nematode feeding also creates open wounds that provide entry to a wide variety of plant-pathogenic fungi and bacteria. These microbial infections are often more economically damaging than the direct effects of nematode feeding. Root-lesion or meadow nematodes cause internal browning in potato tubers and in the roots of corn, lettuce, peas, carrots, tomatoes, and brassicas, while Root-knot nematodes form galls on injured plant tissue. The galls block water and nutrient flow to the plant, stunting growth, impairing fruit production, and causing foliage to yellow and wilt. Roots become rough and pimpled and susceptible to cracking.The environmental conditions of India are ideal for maximizing nematode damage. Nematodes thrive at the temperatures under which most crops and landscape plants are grown in India, especially where frequent rainfall or irrigation keeps the soil moist [3].

MATERIALS AND METHODS

An extensive survey was carried out during February 2011 – February 2012 for the isolation of plant parasitic nematodes from Sugarcane fields of Agra regions. Over 50 soil and root samples were collected from Sugarcane fields.

Soil samples were taken from the rhizospere of plants by using a hand shovel from 5 different points on the field while moving in a zigzag pattern as outlined by Barker [4] [Fig-1] and a hole near the base of the roots 15- 30 Cm deep depending upon the plant size. Soil and root samples were collected randomly from plants showing retarted growth, with reduced leaf size and chlorosis of foliage or wilting of plants. One kg soil samples was taken from each plant and kept in polythene bags. Plant and root samples were placed in separate polythene bags, sealed tightly and labeled with details of host, locality and date of collection. Samples were stored at 5-10^{0}C.nematodes were processed by the following procedure given by [5, 6].

For extraction of nematodes from the soil, modified cobb's sieving and Baermann funnel technique [7] was followed by using a series of sieves (250, 45, 37 mm pore size). Each soil sample was thoroughly mixed and

200cc of Soil was drawn from the homogenous mixtures for processing. The representative soil sample was placed in a plastic bucket and soaked in a litre of water. The soil lumps were broken to enable them to go into a suspension. The soil suspension was stirred well and allowed to stand for 5 seconds for the heavy soil particles to settle at the bottom of the bucket. This muddy suspension was then pored through 20 mesh sieve to discard stone and other coarse materials. The suspension from previous bucket was collected in a second bucket and was allowed to settle or about 15-30 seconds and

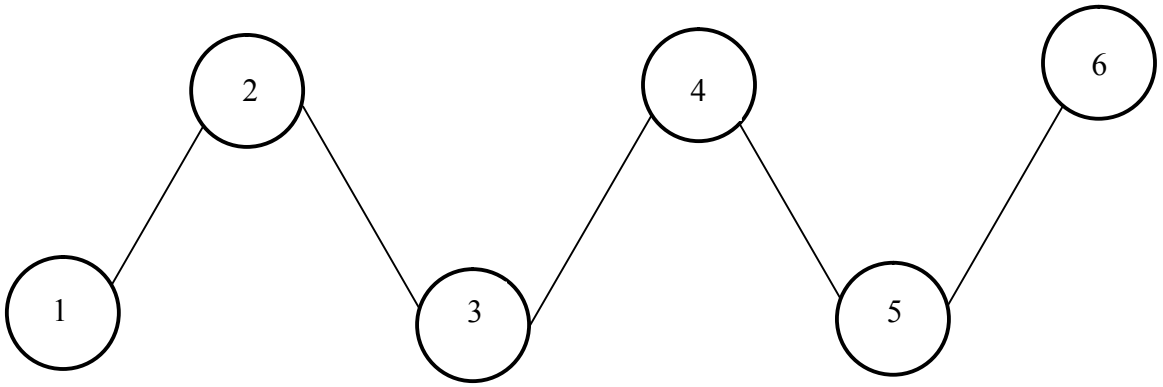

Figure 1: Sketch of sampling route on a rectangular field

passed through a 60 mesh (45 mm pore) leaving the heavy particles in bucket. The washing from the 60 mesh sieve were collected in a beaker and were examined. The whole aliquot obtained from 60 mesh sieve was then passed through 350 mesh (250 mm pore) sieve after allowing the soil suspension to settle for some time. Then, the quantity of water was minimized by decantation after allowing the nematodes to settle at bottom. The suspension containing nematodes was then poured gently over a double layered tissue paper placed over an aluminum mesh support placed as such in a petridish containing filtered water. After 48 hrs the nematode suspension was collected and examined under a stereoscopic binocular microscope. The nematode genera were tentatively identified by comparing the characters given by Mai and Lyon [8]. They were confirmed by making semipermanent and permanent slides.

Root samples were washed in cold tap water and blotted dry before being finely chopped with clean scissors. No attempt was made to standardise the amount of roots used. Nematodes were extracted by placing the chopped roots into a single-ply paper tissue supported by a plastic mesh in an open ended funnel suspended over a glass boiling tube. These were placed in a Seinhorst mistifier chamber (5 s on : 4 min 55 s off mist cycle) for 48-72 h [6]. The funnel was not sealed onto the boiling tube so that excess water overflowed the tube allowing the nematodes to accumulate undisturbed in the bottom of the tube. The lower 5 ml of this extraction was transferred to a smaller tube where the nematodes could be condensed to a 1 ml volume. From this point a procedure similar to that described for the soil samples was followed.

The permanent slides were prepared by killing and fixing method. The nematodes were killed in a beaker by heating it for 3-4 mins. in boiling water at 60^0C and they were allowed to settle at the bottom for 2-3 hrs. Then suspension was minimized, to remove excess water and to have an aliquot having nematodes settled to the bottom and added with the equal volume of double-strength fixative (FA 4:1), prepared using 40 per cent formaldehye (10 ml) + glacial acetic acid (1 ml) + distilled water to make the solution of 100 ml and nematodes from double strength fixatives added beaker were transferred to small cavity block in which 0.5 ml of Solution-I which was prepared by mixing 96 per cent ethanol (20 ml)+ glycerol (1 ml) + distilled water (79 ml) was added before, and cavity block containing Seinhorst Solution-I and nematodes were placed in the in desiccator containing about 1/10 of its volume, 96 per cent ethanol in the desiccator, was kept in oven which was set for 35^0C for 12 hrs after keeping it for 12 hrs. Then it was removed and added with Seinhorst Solution- II which was prepared by mixing 96 per cent ethanol (95 ml) + glycerol (5 ml) Again, cavity block was placed in a desiccator (petridish) which was partially covered, to remove the excess

ethanol by evaporation for three hours. After completing all the above procedures, nematodes were transferred from cavity block to a clean glass slide on which a drop of glycerol was placed. By means of a nematode pick, the specimens were neatly arranged in the center of the glycerol drop (placed in the center of slide) so that their heads all pointed in the same direction and pushed to the bottom of glycerol drop. Three small pieces of glasswool of about similar diameter of the specimens were cut into small bits of the same size and were placed radially in the glycerol drop to prevent the pressure of the cover slip on the specimen. A clean round cover slip (of 18 mm) was heated gently and placed carefully over the drop to avoid any air bubble entering the glycerol drop. Excess of glycerol was removed with small pieces of blotting paper. Then the cover slip was sealed with glycerol. The details of nematode species and host were written on slide.

For quantitative analysis 5ml nematode suspension was poured in a counting chamber and nematodes were counted under a stereomicroscope. Al least three readings were taken to calculate the average number of nematodes in 100ml of suspension. The nematode population per 200 gm of soil was thus determined.

For quantitative analysis the nematodes suspension was allowed to settle for the sometime. The excess supernatant water was poured off, the remaining concentrated contents were transferred into a cavity block for examination under stereomicroscope.

Percentage frequency was determined using the formula n/N x 100. Where n = the number of times an individual nematode occurred in all the samples and N is the sample size

RESULTS AND DISCUSSION

The intensification of agriculture has led to continuous change and lack of stability in the ecosystem, making conditions favorable for certain species of plant parasitic nematodes while exerting immense selection pressure upon others. The sustainability of these intensified sugarcane based systems is threatened by buildup of soil borne constraints, particularly plant parasitic nematodes. Plant parasitic nematodes have been reported to constitute serious impediments to sugarcane production in various parts of the world. Eight genera of plant parasitic nematodes were encountered in soil and root samples collected from five regions of Agra respectively. In the soil and root, plant parasitic nematodes identified were *Pratylenchus* spp., *Meloidogyne* spp., *Rotylenchus* spp., *Radopholus* spp., *Tylenchrorhynchus* spp., *Rotylenchulus* spp., *Rotylenchulus spp., Radopholus spp., Heterodera spp., and Helicotylenchus spp.*

The frequently occurring species which was followed by *Meloidogyne* species with 75 % (Sugarcane), while *Pratylenchus* species had frequency rating of 67% (Sugarcane), Rotylenchulus spp were found in frequency rating as 50% and a population of *Tylenchrorhynchus spp. Radopholus* spp., *Rotylenchus* spp., *Heterodera* had the lowest frequency rating associated with significantly population rating higher of *Tylenchrorhynchus spp.*, *Rotylenchus* spp., *Heterodera* in sugarcane field. [Table – 1, 2]. Root Knot (*Meloidogyne spp*) and root lesion nematodes (*Pratylenchus* species) was the most frequently encountered genus indicated in data.

Plant-parasitic nematodes damage is an important factor in tuber quality reduction and yield loss in sugarcanes both in the field and in storage. Sugarcanes are vulnerable to nematode damage as they reduce the yield and quality of the tubers as a result of root gallings, root lesions, dry and soft rots depending on the type of plant parasitic nematodes present. The results indicated a significant population of plant parasitic nematodes on the different soil texture. The highest population of nematodes was found in areas having sandy soil and lowest population was found in clay soils. In this survey soil and root sampling from sugarcane field showed a significant differences in plant parasitic nematodes population, highest being at Etmaadpur, Jaleasar, Ladukhera and Fatehabad areas.

However, the presence of nematode population density beyond the threshold level warrants initiation of immediate corrective measures. Ideally, such alternative approaches should not only be effective in suppressing nematode population but also offer other agronomic benefits about which farmers are already convinced. Otherwise, it would be difficult to get these approaches adopted by the farmers.

Table :1- Distribution of plant parasitic nematodes associated with Sugarcane fields in AGRA

Host Association	Nematodes	Distribution
SUGARCANE	*Helicotylenchus dihystera*	ET,JAL, FBD,LK
	Helicotylenchus digonicus	LK, ET, JAL
	Heterodera mothi	SA, ET
	Meloidogyne incognita	ET, JAL,LK,FBD
	M. javanica	ET, JAL,LK,FBD
	M. arenaria	ET, JAL,LK,FBD, SA
	Pratylenchus thornei	ET, JAL,LK,FBD
	P.vulnus	ET, JAL,LK,FBD
	P.pratensis	ET, JAL,LK,FBD
	P.zeae	ET, JAL,LK,FBD
	P.penetrans	ET, FBD, LK, SA
	Radopholus similis	ET, SA
	Rotylenchulus reniformis	ET, FBD
	Rotylenchus capsicum	JAL,LK,FBD
	Tylenchrorhynchus annulatus	SA, JAL

Abbreviation: LK= Ladukhera, SA= Saiyan, ET= Etmaadpur, JAL= Jalesar, FBD= Fatehabad

Table 2: Frequency % of the occurrence of nematodes in Ornamentals, vegetables plants and Sugarcane field in Agra regions

Host Association	Frequency % in plants
	Sugarcane
Meloidogyne	75
Pratylenchus	67
Rotylenchulus	50
Tylenchrorhynchus	20
Helicotylenchus	9
Heterodera	18.7
Radopholus	10
Rotylenchus	24

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