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Studies of plant Parasitic nematodes in sugarcane plants in Meerut district (Uttar Pradesh)

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Abstract: phytonematodes (PPN) are microscopic round worms that feed on roots and soil, endoparasitic nematodes enter into the roots feed and reproduce in the roots. Ectoparasitic phytonematodes live in the soil and feed outside the roots. Ectoparasitic nematodes (*heterodera spp*.) and endoparasitic nematodes (*Meloidogyne spp*) are serious pest on the sugarcane crops.

Key words: PPN, Ecto-phytonematodes & Endo-parasite, sugarcane etc.

Introduction

Nematodes are microscopic worms that cause billions of dollars in crop losses annually, and all crops in the world are susceptible to at least one species of nematode parasites (Bozbuga et al., 2018). Nematodes are among the most important and abundant animals in the animal kingdom and are able to survive in any environment (Aleuy and Kutz, 2020,)

Sugarcane (*Saccharum officinerum*) is a major agricultural crop in Meerut district, India. Sugarcane is the World's major cash crops providing about 75% of the sugar harvested for human consumption (FAO 2004). In India, Uttar Pradesh is the major sugarcane growing state, contributing about 48% of the area and 40% of the production. Sugar mills of Uttar Pradesh have produced 70.80Lac tons produced by these mills in 2013-14 on the corresponding period.

A major global challenge in the need to sustainably increase agricultural productivity in line with increasing demand more pertinent than in resource poor areas of the India. There are

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about 6000 known species of plant parasitic nematodes belonging to 197 genera. Being obligate parasites, they must draw their nutrition from plant hosts and in the process, must debilitate the plant. A handful of soil from around the roots of any plant would yield hundreds of plant parasitic nematodes belonging to at least 4-5 genera.

All India Coordinated Research Project (AICRP) on Nematodes over the years, a critical analysis has been made on losses in different crops in India. Overall, phytonematodes cause 21.3% crop losses amounting to Rs. 102,039.79 million annually; the losses in 19 horticultural crops were assessed at Rs. 50,224.98 million, while for eleven field crops it was estimated at Rs. 51,814.81 million. Rice root-knot nematode, *Meloidogyne graminicola* (RKN) was economically most important causing yield loss of Rs. 23,272.32 million in rice. Citrus (Rs. 9828.22 million), banana (Rs. 9710.46 million) among fruit crops; and tomato (Rs. 6035.2 million), brinjal (Rs. 3499.12 million) and okra (2480.86 million) among the vegetable crops suffered comparatively more losses.

Plant parasitic nematodes are obligate parasites and mainly attack the underground parts of the plants such as roots, bulbs, corms; rhizomes etc and incite hindrance with water uptake and transport. Some Plant Parasitic Nematodes can also infect aboveground parts such as leaves, buds, flowers etc. Plant nematodes not only have negative impact on plant growth but also interfere in the nodulation, nitrogen fixation and suppress the overall yield of plants. In addition to direct harm, nematodes also affect the plant health indirectly.

The infections of other plant pathogens like bacteria, viruses and fungi are on the rise and further weaken the plant. Nematodes make the plant vulnerable to damage from pathogens which are otherwise weak. Some nematodes are vectors of soil borne pathogenic viruses. Symptoms of nematode damage to the plants**.** The symptoms vary with the types of nematodes, plant age, initial population density and ecological factors.

Some symptoms of damage crops are as follows:

1. Patches of stunted plants.

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2. General decline followed by reduction in crop yield.

3. Field symptoms are of generalized nature giving an impression that the crop is suffering from shortage of water and fertilizers even though both of these are in adequate amounts. Decline or die-back of fruits is an outstanding example of nematode infection. Predominantly are the slow and spreading declines of citrus, banana, other fruits and vegetables.

4. Stunted growth of plants is seen in patches with low and poor quality of fruits, cotton, citrus etc.

5. Chlorosis, yellowing, discoloration and drying of the leaves. Such symptoms can be readily observed in coffee seedlings, potato and cherries. Premature yellowing of the leaves is seen in flowering, ornamentals, groundnut, cotton etc. Severely infected plants show drying of the leaf margins, burning of leaf tips and brittling of leaves that are eventually shed-off. The auxiliary buds develop into tiny leaves only to drop- off. These symptoms are also indicative of poor nutrient absorption due to root damage.

6. Wilting - Wilting, defoliation and consequent death may be associated with root knots in hot weather in spite of abundant water. Combination of nematodes and fungi are prevalent in wilts of many plants.

Plant Parasitic Nematodes are obligate parasites. During feeding they are penetrate the plant cells causing mechanical injury. This is evident in case of a large number of plant nematodes infecting the crops. These phytonematodes also injects digestive juices in the plant cells causing hydrolysis of the host components altering the host metabolism. Feeding nematodes deprive the host off nutrients and water necessary for plant growth.

The identification of new or potentially harmful species of plant parasitic nematodes is important to the success of agriculture and aids in the development and evaluation of quarantine or regulatory procedures to minimize their spread.

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Although chemical nematicides are the most reliable of controlling phytonematodes but they are increasingly being withdrawn owing to their toxicity to humans and environment.

Root-knot nematode (*Meloidogyne* spp.) ranks first as far as damage to crops at global level is concerned because of their World-wide distribution, extremely wide host range, destructive nature of the diseases caused by this plant nematode and their role in many destructive disease complexes.

Although over 4,100 species of Plant Parasitic Nematodes have been identified, new species are continually being described while others, they can be managed by using different methods like; sanitation coming years will be to ensure food security and to feed the increasing human population. Nowhere will be the of fields and equipment like pots potting soil, bio-control agents, periodic rotation, further research has to be done to evaluate the efficacy of some promising botanicals for the management of some Plant Parasitic Nematodes.

Biological control is difficult in soil, because it is a complex environment. Many of the possible organisms that could provide biological control lack specificity and therefore will not focus on a particular organism and may even interfere with beneficial. Therefore biological control of plant nematodes is achieved mainly by conservation of existing biological control; meaning that the soil environment is modified to aid the survival and reproduction of nematode natural enemies that are already present. Organic amendments (manure) such as neem extracts cotton seed manure can improve the soil environment to aid biological control, benefit general plant health by helping with water retention and providing additional nutrients, and affect plant parasitic nematodes directly and negatively through detrimental decomposition products. The impact of organic amendments on plant parasitic nematodes is often inconsistent and unpredictable. In most cases when organic amendments are applied, they are helpful mainly as a plant nutrient source and do not directly aid in plant parasitic nematode management.

Plant parasitic nematodes are major agricultural pests worldwide and are responsible for start-up investment, public awareness and acceptance, technology dissemination and proper

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implementation and national policies on bio-safety and intellectual property issues are critical and those areas have to be strengthened to obtain real results in the farmer's field.

Material methods:

1. **Collection of Soil Sample** Generally the soil samples and roots samples are collected from the root zones of sugarcane plants and 1-4 feet away from the collar region of large perennial tree and bushes, close to the feeder roots, where plant parasitic nematodes concentration is expected to be relatively high Random sample (5-6) are taken, variation in sampling depth depends on the presence of moisture in the soil. it should be noted that in rainy and winter season the moisture level in the soil is fairly high and that most of the phytonematodes could be obtained from soil 4-6 inches below the surface ,whereas in summer time when top soil become dry.

Plant Parasitic nematodes may migrate to depth greater than one foot. In view of this in dry periods soil samples are collected from the lower depths. (J. Van Bezooijen (2006)

2. **Equipment for Soil Sampling:**

Different types of soil samples of varying lengths are available. Normally soil augers are used. In the absence of a proper soil sample ordinary khurpi (khurpa) or a trowel can be used. Polyethylene bags of suitable size are best for collecting and storing the soil samples and roots samples of sugarcane plants for the purpose of recording information about the collected soil sample and roots sample suitable labels and forms should be prepared in advance and kept ready. These should contain detailed information about the location of sugarcane field, the crop sampled, check the symptoms observed, the cropping history of at least the past two to three years together with approximate losses if any or cash returns, approximate size of the area sampled, the name and address of the farmer and other details.

3. **Collection of Root Sample:** For collection of sugarcane plant roots, the whole plant may be uprooted in a manner that would enable the removal of finer roots intact from the

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soil with the aid of a khurpi. In case of shrubs and trees the finer roots with soil may be dug out from varying depths with a shovel or auger. The soil adhering to the roots may be tapped off and roots subjected to treatment for desired purpose.

4. **Baermann Funnel Method:** This method is useful for collecting small plant parasitic nematodes from the soil or endoparasitic phytonematodes from infected sugarcane plant tissue; it is based on the principle that plant parasitic nematodes have a tendency to move downward when the soil or plant material is placed in a funnel. Only active and motile phytonematodes can be collected by this method (Kavitha Govindasamy, jimmy R.Rich Maria. L.Mendes) The Baermann Funnel is a regular glass or plastic funnel, about 7.5 to 15 cm in diameter with a piece of rubber tubing attached to the stem and closed with a clamp or pinchcock. A molded wire gauge is placed in the funnel. The funnel is filled with fresh tap water. Care should be taken to ensure that there are no air bubbles in the funnel or rubber tubing. The wire gauge is covered with a layer of muslin cloth and then with double thickness of tissue paper. The edge of the tissue paper should not protrude out of the funnel otherwise water will flow out.

The material for extraction is placed on the muslin cloth – tissue paper lined wire gauge. The soil sample taken 500-1000Gms and small roots cut into 3 cm pieces. Soil mixed with infested roots, washing obtained and from 325 mesh sieve. A modification of the method that is followed in many laboratories is to take the sample in a cup matching the inner diameter of the funnel. A piece of cloth is placed over the cup and secured with a rubber band. The cup is inverted rapidly in the funnel filled with water. The entire setup is kept undisturbed for several hours to two to three days at room temperature. Fresh water is added daily to the funnel to compensate for loss by evaporation. The funnel may be covered with a Petridis. In no case the material should become dry. Do not pour water through the sample, add through the sides. The plant parasitic nematodes being active migrate to the bottom of the rubber tubing through the cloth tissue paper. At the end of the waiting period 5 to 10 ml of the water containing the nematodes can be collected for counting and examination of the plant parasitic nematodes.

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5. **Extraction root sample:** The infested sugarcane plant roots is washed gently with water and placed in a shallow dish or watch glass with little water. The dish is placed under a phase contrast microscope. The material is carefully teased apart with the help of two dissecting needles. If plant parasitic nematodes are present they come out in the water.

Result and Discussion:

The present study confirms that nematodes *Rotylenchus, Hoplolaimus* and *Helicotylenchus* can potentially cause great damage to sugarcane crop indicating the need to pay more attention to these plant parasitic nematodes in order to increase the productivity of most sugarcane crops grown in Meerut (U.P). During the study on sugarcane crops grown in field, 11 plant parasitic nematode genera were they include: *Hoplolaimus spp., Rotylenchus reniformis, Helicotylenchus dihystera, Pratylenchus zea, Tylenchorhynchus nudus, Longidorus elongates, Meloidogyne incognita, Xiphinema attenuatus, Scutellonema brachyurus and Tylenchus arcuatus. Hoplolaimus spp.* and *Rotylenchus reniformis* was the most ubiquitous occurring at 100% frequency rating in all the soil samples.

Of these, *Hoplolaimus spp.* and *Rotylenchus reniformis* turned out to be the most common one with the average population density of 35 plant parasitic nematodes per gram of soil, followed by *Helicotylenchus* (5 nematodes per gram), *Pratylenchus* (4 nematodes per gram) and *Scutellonema* (2 nematodes per gram) etc. Several important plant-parasitic nematodes are responsible for crop damage. Some of them important mostly found in sugarcane crops these are following:

1. *Helicotylenchus* (Spiral nematodes): Small to medium sized nematodes (0.4-1.2mm), usually in spiral shape. Ectoparasitic, semi-endoparasitc or endoparasitic nematodes of roots. The most damaging species is *H. multicinctus.* Major species: *H. multicinctus, H. mucronatus, H. dihystera, H. pseudorobustus.*

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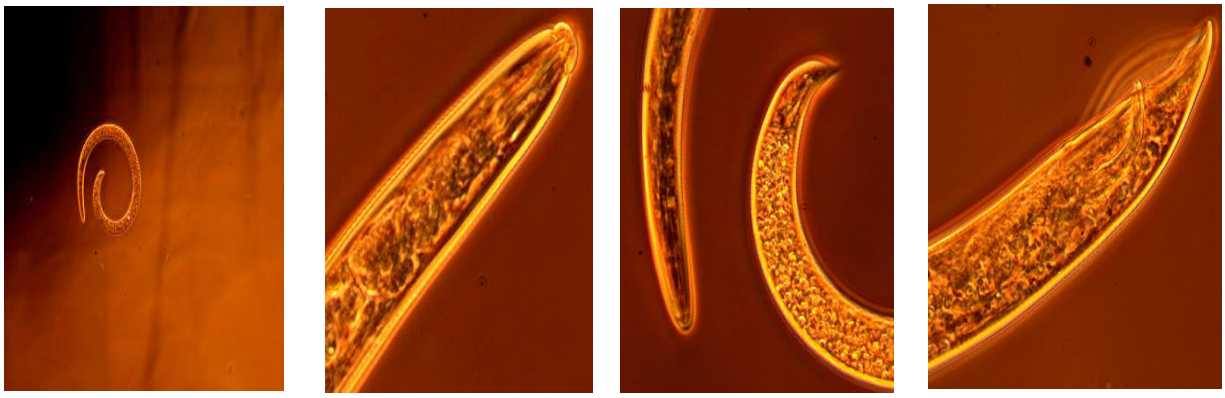

Plate -1

A. Microphotographs of Holotype entire body of *Helicotylenchus dihystera* (10X10)

B. Microphotographs of anterior region of *Helicotylenchus dihystera* (10X40)

C. Microphotographs of anterior region of juvenile *Helicotylenchus dihystera* (10X40)

D. Microphotographs of tail region of *Helicotylenchus dihystera* (10X40)

2. *Rotylenchulus* (Reniform nematodes): Immature females establish permanent feeding sites in roots, become semi-swollen, and protrude from roots. They are 0.23-0.64 mm long and have a kidney shaped body. Males are vermiform. Eggs are laid in gelatinous matrix. The major species is: *R. reniformis* which found in both tropical and warm temperate soils.

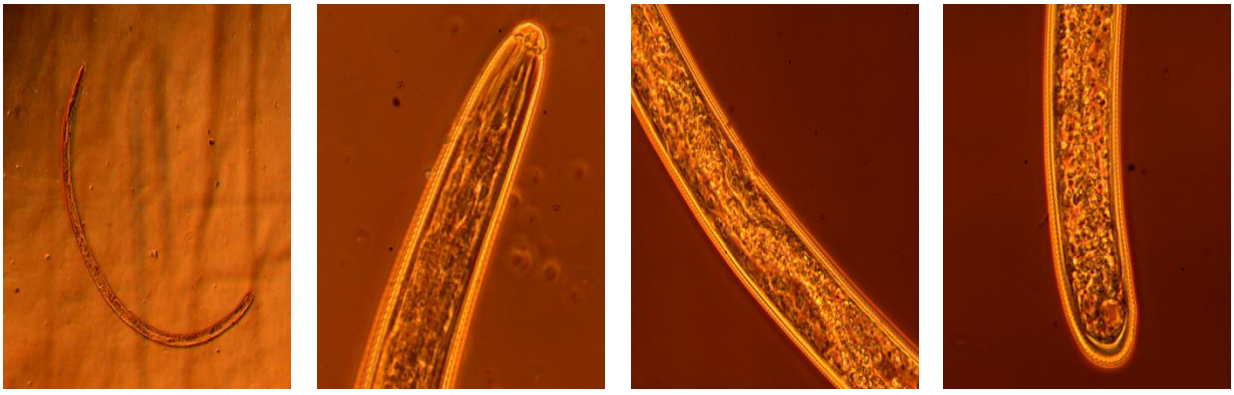

Plate -2

A. Microphotographs of Holotype entire body of *Rotylenchus reniformis* (10X10)

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B. B-Microphotographs of anterior region of *Rotylenchus reniformis* (10X40)

C. Microphotographs of vulva region of *Rotylenchus reniformis* (10X40)

D. Microphotographs of tail region of *Rotylenchus reniformis* (10X40)

3. Longidorus, Trichodorus & *Paratrichodorus* (Dagger, needle and stubby root nematodes): Slender, virus transmitting nematodes 0.8-5mm long. Ecto-parasites on roots. Major species: *X. americanum, X. elongatum, L. africanus, P. minor.*


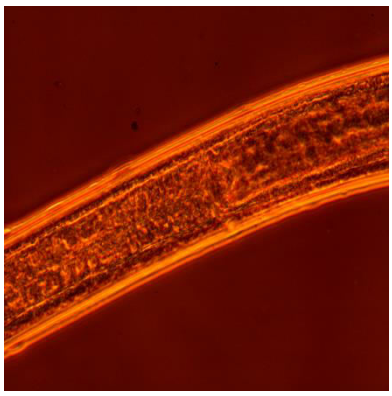
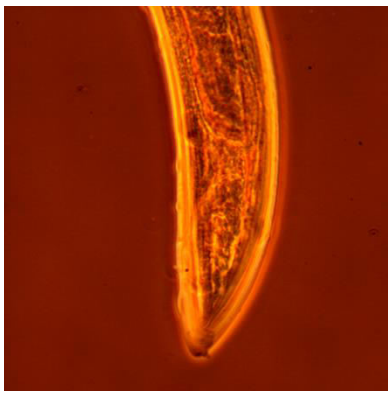

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Plate-3

A. Microphotographs of anterior region of *Longidorus elongatus* (10X4)

B. Microphotographs of vulva region of *Longidorus elongatus* (10X40)

C. Microphotographs of tail region of *Longidorus elongatus* (10X40)

4. Hoplolaimus (Lance nematodes): Are an important group of basically migratory ectoparasites which feed on roots of many kinds of fruits and other economic plants world-wide. Medium length (1-2mm). Major species: *H. columbus, H. seinhorsti, H. indicus.*

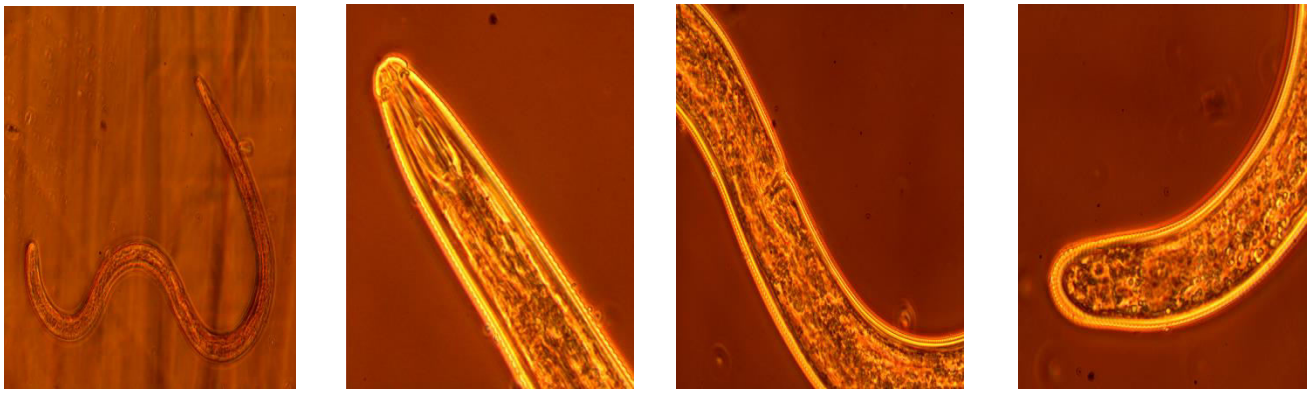

Plate -4

A. Microphotographs of Holotype entire body of *Hoplolaimus indicus* (10X4)

B. Microphotographs of anterior region of *Hoplolaimus indicus* (10X40)

C. Microphotographs of vulva region of *Hoplolaimus indicus* (10X40)

D. Microphotographs of tail region of *Hoplolaimus indicus* (10X40)

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5. *Tylenchorhynchus nudus:*

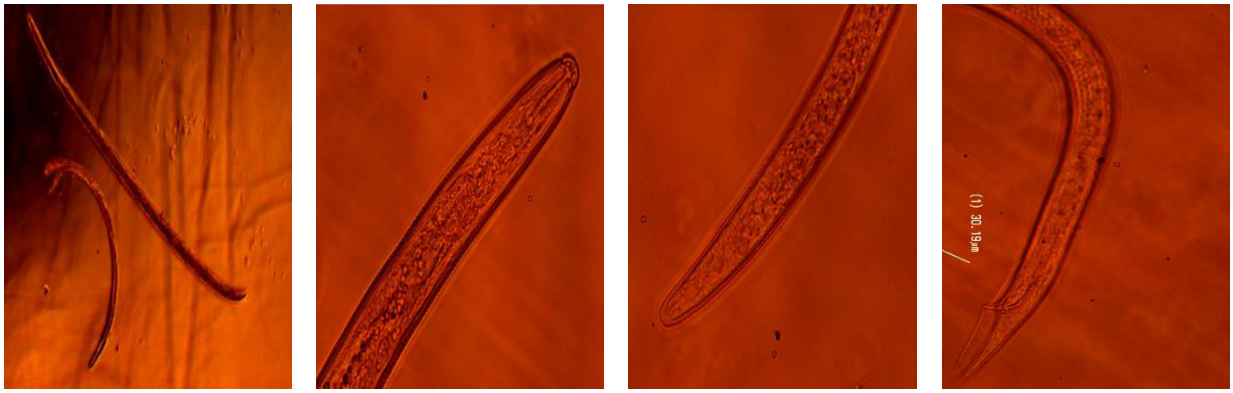

Plate -5

A. Microphotographs of Holotype entire body of *Tylenchorhynchus nudus* (10X10)

B. B-Microphotographs of anterior region of *Tylenchorhynchus nudus* (10X40)

C. Microphotographs of tail region of *Tylenchorhynchus nudus* (10X40)

D. Microphotographs of tail region of *Tylenchorhynchus nudus* (10X40)

Conclusion:

Phytonematodes are by far the most abundant organisms on earth (Van Den Hoogen et al., 2019) and a dominant component of the soil (Bardgett and Van Der Putten, 2014). Plant-parasitic nematodes are a great threat to agriculture, causing an estimated annual yield loss of over $100 billion worldwide (Abad et al., 2003; Thoden et al., 2011). Among Plant Parasitic Nematodes, the most yield-limiting group is root-knot nematodes (*Meloidogyne* spp.). Root Knot Nematodes are obligate sedentary endo-parasites which can easily reproduce in roots of over 3,000 plant species (Abad et al., 2003). They are widespread all over the world (Jones et al., 2013), and their population in the soil increases easily under appropriate conditions (Calderón-Urrea et al., 2016; Hajihassani et al., 2018). Application of nematicide has remained the most common short-term management strategy against Root Knot Nematodes (Hajihassani et al., 2019a; Medina-Canales et al., 2019);

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Farmer's awareness and skills are equally important in minimizing nematode infestation and yield losses, to sustain the agriculture production. the major plant parasitic nematodes of economic importance, the importance of nematode identifications to agriculture, the nematode problems and diseases on major crops, the plant parasitic nematode disease symptoms and diagnosis, the examination and collection of soil and plant material, and the extraction of these plant parasitic nematodes from soil, in order to assist and make them aware of what they should be looking for while dealing with any phytonematodes interceptions of quarantine importance and the kind of samples they should be receiving or sending for identification purposes.

Screening of seed plants and regular plant quarantine services should be employed prior to the introduction of new verities to sugarcane farmers and also effective phytonematodes management strategies should be considered in order to improve yield in sugarcane plantations.

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