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PLANT PARASITIC NEMATODES OF COTTON-FARMER'S HIDDEN ENEMY

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PREFACE

Ever since the dawn of civilization, cotton has played a major role in weaving social, economic and political fabric of our country and still occupies place of pride in Indian economy. Unfortunately, despite having maximum area under cotton cultivation, productivity of cotton in India at 319 Kg lint / ha lags far behind as against productivity of 1709 Kg lint / ha in Israel. One of the ways to increase cotton productivity so as to meet the projected demand of 190- 200 lakh bales by next decade and make Indian cotton competitive in world market is to optimize environment in which crop is grown and neutralize damaging biotic factors so that inherent yield potential comes close to realization. Plant parasitic nematodes in last few decades have been recognized as important limiting factors for crop production, particularly, in the tropics. Several species of plant parasitic nematodes have been reported to cause serious losses in this high value commercial crop. However, non-specificity of symptoms often leads to nematode problems being diagnosed as due to nutritional or soil factors. Nematode diseases can be said to be 'Life Style Diseases' of crop plants and these have been accentuated due to replacement of traditional agriculture with modem farming practices. The present bulletin attempts to present a brief diagnosis of nematode problems and their management practices that could be followed.

Authors

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INTRODUCTION

Plant parasitic nematodes in last two decades have been recognized as important limiting factor for crop production particularly in the tropics. Several species of plant parasitic nematodes have been reported to cause serious losses in cotton, a high value commercial crop.

ABOUT NEMATODES

Plant parasitic nematodes are tiny microscopic worms and occur abundantly in soil. When seen under microscope, plant parasitic nematodes are slender, unsegmented usually shorter than 2 mm in length with serpentine mode of locomotion. Rarely is any crop free from attack of these tiny microscopic organisms. At present 24 genera of plant parasitic nematodes include species that are economically important as crop pests. It has been estimated that about 10% of world agricultural production is lost due to nematode damage. Plant parasitic nematodes are ubiquitous and yet their presence is generally not felt till faced with the problem of continuous decline in yield in spite of best agronomic practices. The losses incurred as a result of nematode attack do not consist of yield reductions alone but other aspects like lesser ability of infected roots to utilize fully the available nutrients from soil, or the necessity to grow uneconomical rotational crops in an effort to control the nematodes are some of the effects that follow as a result of nematode infestation. However, not all nematodes attack crop plants. Some attack insects, some feed on bacteria while others feed on fungi. Still some other nematodes attack and eat other nematodes. These nematodes are being exploited for biological control of other crop pests and pathogens as insects, fungi and weeds. Only those nematodes, which feed on plants, have been dealt here.

HABITAT

Most plant parasitic nematode species feed on roots and underground plant parts. All plant nematodes have to pass atleast a part of life cycle in the soil as eggs, juveniles or adults. The nematodes may feed ecto (feed from out side), semi-endo (feed partially inside) or endo (feed internally) parasitically. Plant parasitic nematodes feed with a narrow mouth spear called stylet. Most plant parasitic nematodes belonging to Tylenchida are characterized by possession of a hollow, feeding stylet with basal knobs. Enzymes produced in oesophagus and pumped by the muscular median bulb bring about extra corporeal digestion. Predigested cell contents are withdrawn into the oesophagus.

NEMATODE SURVIVAL

Plant parasitic nematodes survive adverse conditions in several ways. In most cases eggs can survive drought better than juveniles or adults. Some juvenile stages may be more tolerant than others. Eggs may be retained in female body, which becomes thickened and becomes a cyst or eggs may be laid in gelatinous egg sac, which offers to the eggs protection. Thus the nematodes having very wide plant host range get better protection from hostile environment. Migration into deep soil may protect some vulnerable species from desiccation in the topsoil.

Nematodes on their own cannot traverse much but depend on other agencies like man. animals, farm implements, wind and water for their dispersal.

RECOGNIZING PLANT DAMAGE CAUSED BY NEMATODES

In cotton crop, plant parasitic nematodes feed only on roots. Mere presence of nematodes in soil is not indicative of nematode disease, as nematode damage is population density dependent. Damage to plants gets manifested when the nematode population crosses the threshold level of damage. In nutshell, nematodes increase demand on plant energy resources while reducing the supply. They can prevent plants from getting enough water and plant food. Thirsty plants wilt (loss of turgidity) easily while nutrient deficient plants look sickly. Symptoms of nematode injury on cotton can get expressed on above ground plant parts as weakened plant condition, leaf chlorosis, less ability to tolerate adverse conditions, reduced boll size and reduced lint percentage. On roots, nematode feeding may result in symptoms as root galling, root rot, root lesion, discoloration, necrosis and excessive root branching.

As a cumulative effect, yields get reduced. For diagnosis of nematode disease, it is important to know which nematode species are present and what is their population. The best way to diagnose nematode damage is to have an expert examine the soil. If no expert is available, followings are some of the clues to ascertain involvement of nematodes in crop damage.

CLUES FOR DIAGNOSIS OF NEMATODE DISEASE

1. Check for presence of patches of yellowing, unthrifty plants in the field. If weak and unthrifty plants occur in same patch every year and if this patch increases gradually in size each year, then it could be a nematode problem. Nematodes cannot spread rapidly over an entire field. Nematodes can spread by farm tools and machinery, movement of man and animals, and with water flow.

2. Check if unhealthy plants are shorter or lighter in colour than healthy plants. Do the plants wilt more readily than healthy looking ones?

3. At the edge of area where plants are sickly, root system of these plants must be examined. Nematode disease is indicated, if roots are unusually small, less in number, have small brown spots or have knots. Knots or galls that are formed by nematodes are different from bacterial nodules seen in roots of leguminous plants as soybean, gram, pea etc. Nodules formed by bacteria can be easily separated and plucked from root. However, irregularly shaped knots or galls fom1ed due to nematodes cannot be separated easily from roots which is indicative of the presence of nematodes. In some plants, tiny lemon shaped glistening bodies (Cyst nematode) protruding from root also indicate nematode problem.

SAMPLING THE FIELD FOR DETECTION OF NEMATODES

If nematode disease is suspected in a field, then best way to confirm presence of

nematodes is to get soil checked for presence of nematodes by a Nematologist. Sampling from depth of 8-12 inches is recommended during crop season. However, deeper sampling is required for fallow soil. Twenty to forty samples need to be collected from any location in the sample area by following W -shaped path. After thorough mixing of soil (coning and quartering), the sub-samples of 250 cc are taken for analysis. Most migratory nematodes are found near plant roots and hence, rhizosphere samples are preferable.

It is better to take wide rather than narrow cores (soil sample) and treat the samples gently as rough handling was found to be deleterious for recovery of Longidorid and Trichodorid nematodes. Brief history of crop and disease, including information about crop details, stage of crop growth and extent of damage with sick plants should accompany these soil samples. This information should preferably be written by pencil and should not be put inside the bag as moisture content in soil may erase the information.

STORAGE OF SOIL SAMPLES

Soil samples and plant material should be kept moist and stored in a polythene bag at 13°C. Most categories of nematodes store very well at this temperature. Plant material should be stored separately after wrapping in moist blotting sheet and then kept in polythene bag at 13°C. Refrigeration at very low temperatures can adversely affect the recovery of nematodes in tropical areas. However, storage of samples at 30°C for 5 days was found to improve the recovery of Reniform nematodes. The higher recovery under such cases may be due to increased egg hatch. Samples in bags should not be exposed to direct sunlight as this can overheat the bag resulting in nematode mortality.

DETECTION AND ESTIMATION OF NEMATODE POPULATIONS IN SOIL

Nematode population density in soil can be estimated qualitatively and quantitatively by extraction of nematodes from soil. For this purpose, sieving and decanting technique developed by Dr. N.A.Cobb, is most commonly followed.

Equipment required: Two plastic pan, 5 sieves with pore sizes of 0.84 mm (BSS 18 mesh) (1), 250 µ (BSS 60 mesh) (2), 150 µ (BSS 100 mesh) (3),45 µ (BSS 350 mesh) (4) and 37 µ (BSS 400 mesh) 250ml beakers, aluminium wire mesh molded in a shape which can be placed on Petri-plate, facial tissue paper.

Procedure: First of all mix soil thoroughly removing big stones etc. Using 250cc beaker, 250cc soil is taken in plastic pan and mixed with 500cc quantity of water. For clayey cotton soil (vertisols), soil should be allowed to stand for about 10 min. so as to dissolve lumps. Before use, the sieves must be cleaned so that no soil from previous washings cling to the sieve.

Sieves are also wetted with water before use so that a thin water film covers the sieve holes. The surface tension due to water film prevents loss of nematodes through sieve holes and thus the efficiency of nematode extraction is improved.

Stir the suspension and allow the soil to settle for about 5 seconds. Pour out the suspension in pan No.1 through coarse (0.84 mesh) sieve into second pan. Since very long sized nematodes do not pass through this sieve very quickly, this sieve is placed for a short time in suspension in pan no. 2. After removing the sieve, the soil suspension is again thoroughly stirred and after waiting for 5 seconds poured over sieve No.2 placed over now cleaned Pan 1. As far as possible the sieve should be held in inclined position at 45° angle. Debris collected over finer sieve No.2 is collected in a beaker using a gentle stream of water. This operation is repeated with other sieves and debris is collected separately for each sieve. The filtered suspension is allowed to stand for 10-15 minutes so that debris and nematodes get settled. Supernatent water should be poured-off gently without disturbing the settled residue. Aluminium supports are prepared which can be fitted on the lid of Petri-plate. Single or double layer of facial tissue paper depending on paper quality is placed over aluminium support and gently wetted with water so that no air pockets are formed and tissue paper does not get torn. Sieved soil suspension from each sieve is poured over this. The support is then placed on a Petri-plate filled with water so that bottom of aluminium support remains in touch with water. In hot weather, to prevent drying of water, the aluminium support is covered with a Petriplate. Nematodes will migrate across tissue paper and will get collected in the water in the Petri-plate in 24 hrs. The resulting suspension need to be observed under microscope and the nematode population is estimated both qualitatively and quantitatively.

NEMATODE DETECTION IN PLANT TISSUES

Extraction: Cobb's sieving and decanting technique is suitable only for extraction of mobile vermiform stages of nematodes from soil. Extraction of motile stages from roots can be done by chopping of roots and placing chopped root pieces on tissue paper supported on aluminium support as described earlier. This support is to be placed on a Petri-plate full of water so that water wets bottom of support. In 24 h, the motile stages present in root will come-out in water suspension.

Direct examination: Plant material can be directly examined for presence of nematode infection. Roots are first washed gently to remove as much soil, as possible. Plant material can be teased with a pair of stout needles. It is better to examine the suspension after 1 to 2 h as nematodes tend to migrate from damaged tissues. To recover the endoparasites as Reniform and Root-knot nematodes, roots are examined under stereo- binocular microscope and nematodes are gently teased away. Dissection in 0.9% NaCl (common salt solution) helps to avoid bursting of females.

Maceration: This method is quicker. About 5 g of roots are cut into 1 cm pieces, placed in about 100 ml water and chopped in a blender. Chopping time need to be regulated as it should be just enough to permit nematodes to escape from plant tissue but not to damage them. Running ordinary blender for about 5 seconds (s) to reach full speed, 5 s at full speed and 5 s to stop the blender has been found to be sufficient for plant tissue maceration. The resulting suspension can be poured over tissue paper supported on aluminium support as described earlier and placed on Petriplate full of water for extraction of mobile stages. Immobile stages of root-knot and reniform females can be directly observed in suspension under stereo-binocular microscope.

Staining of plant tissue

For extraction and estimation of sedentary stages of endoparasitic nematodes, it is necessary to stain nematodes in plant tissues. Infected roots are washed free of soil. After removal of excess water, the root is wrapped in muslin cloth and plunged in warm Acid fuchsin (0.1 %) and Lacto-phenol solution and left as such for 2- 3 minutes. The root is taken-out and after cooling, washed gently in water. Adding drop of HCI (Hydrochloric acid) to this water, clears the tissue faster. The root is then transferred to plain lacto phenol to clear the plant tissue.

As the phenol fumes are harmful to inhale, Lacto-glycerol (equal volume of glycerol, lactic acid and distilled water with 0.05% acid fuchsin or 0.05% methyl blue stain) can be used. After staining, roots can be cleared by immersion in equal volumes of glycerol and distilled water acidified with few drops of lactic acid. Differentiation may take from several hours to 2-3 days depending on the type of material so that nematodes will be stained strongly while the plant tissue remaining largely unstained.

Handling of nematodes

For handling and picking nematodes individually, distal end of feather of a big bird such as crow or eagle sharpened to a very fine point and fixed in a needle holder can serve as the most convenient accessary. Nematodes for temporary examination can be mounted in water and covered with zero number slide-mounting coverglass. Three glass wool support are also kept to prevent nematodes from getting crushed by coverglass. To prevent evaporation, the edges of coverglass can be sealed with nail polish.

Alternatively, on the glass slide, a thin ring of paraffin is applied with a metal wire dipped in molten paraffin. This paraffin supports the coverglass and prevents evaporation. If the mount is thicker and thus cannot be examined under high power microscope or when the nematodes float inside the mount, the coverglass can be lowered by heating the glass slide slightly.

These temporary mounts help in differentiation of plant parasitic nematodes from free-living saprophytic ones which are not harmful to crop plants and thus assist in taking total counts of only plant parasitic nematodes.

Plant parasitic nematodes can be easily differentiated from freeliving saprophytic nematodes because of the presence of stylet in head region which looks like needle like structure black in colour in plant parastic nematodes. For taxonomic identification, the suspension needs to be killed and then fixed for long term preservation. Equal quantity of boiling water is added to nematode suspension to kill the living nematodes. Most commonly used fixative is double strength 8-10% Formalin, the equal quantity of which is added to nematode suspension. This suspension can be stored and further passed on to Nematologist for proper identification.

DISTRIBUTION OF COTTON NEMATODES IN INDIA

About 19 Genera of plant parasitic nematodes have been recorded for their association with cotton. Of these the most important generic species in Indian context are *Rotylenchulus reniformis*, which is commonly known as Reniform nematode, *Meloidogyne incognita* (Root-knot nematode), *Hoplolaimus sp.* (Lance nematode) and *Pratylenchus spp.* (Lesion nematode). The Reniform nematode (*R. reniformis*) has been recorded to be the key nematode species on cotton in Central and Southern India while in Northern cotton-growing areas, the Root knot nematode (*M.incognita*) is important.

Table 1 Nematode species associated with cotton

Nematode species	Associated with
Aphelenchoides indicus, A.teres	*Gossypium arboreum* Race *indicum*
Criconemoides morgomorgum	*G.hirsutum*
Ecphydophora quadrata	*G.herbaceum*
Helicotylenchus retusus	*Gossypium spp.*
Hirschmaniella oryzae	*Gossypium spp.*
Hoplolaimus indicus, H.seinhorstii	*Gossypium spp.*
Longidorus elongatus,. L. sylphus	*Gossypium spp.*
Meloidogyne incognita, M. javanica	*Gossypium spp.*
Paralongidorus citri	*Gossypium spp.*
Pratylenchus pratensis, P.sudanensis, P.coffeae	*Gossypium spp.*
Rotylenchulus reniformis	*G.anomalum, G.arboreum, G.armourianum, G.barbadense, G.davidsoni, G. hirsutum, G. raimondii, G.thurberi*
Telotylenchus housii, T.vulgaris	*G.hirsutum, G.herbaceum*
Tylenchorhynchus zeae, T.brassicae,	*Gossypium hirsutum.*
T.brevidens, T.mashoodi	*G.herbaceum*
Xiphinema basiri, X.americanum	*Gossypium spp.*

RENIFORM NEMATODE

Reniform nematode (*R. reniformis*), first described from Hawaii, USA is widespread in the tropics and subtropics. As the name indicates, reniform nematode female is characterized by typical kidney shaped female. The Reniform nematode has a wide host range spanning 115 plant species in 4 families. There also exist two races i.e. A and B for Reniform nematode. However, only race' A' attacks cotton.

Losses estimates:

In India, crop loss due to Reniform nematode (*R. reniformis*) on cotton has been put at 14.7%. Damages to cotton by *R. reniformis* has been studied in USA and estimated at 5.6% due to direct reduction in yield, lint percentage and reduced fiber elongation. The nematode also

causes delay in boll maturity, as well as the reduction in boll size and lint quantity. It is also reported to cause an increase in Wilt disease development in wilt susceptible varieties. Work done at CICR, Nagpur indicated increase in yield by 8 to 10% in nematicide treated plots over the untreated control. Field trials on avoidable yield losses conducted at CICR regional Station, Coimbatore showed yield increase by 9.5 to 17.4% when the nematicide Metham sodium (Vapam, Sistan) was applied.

Symptoms:

Symptoms of nematode damage on cotton are highly nonspecific and resemble symptoms of nutritional imbalance. Non specificity of symptoms often result in nematode problems being diagnosed as nutritional malady. Plants in nematode infection rarely succumb to disease. Only vitality of plant gets sapped gradually over the time and thus escaping notice.

On cotton, apart from general non-specific symptoms of nematode infection in field, few small and less dark green leaves are noticeable in nematode stressed plants. As early as third leaf stage, seedlings may be stunted and have a pale green appearance. Infected cotton plants remain stunted with less vigour and chlorosis of leaves. Leaf margins develop a purple tinge when infestation is heavy. Many of seedlings may succumb resulting in poor crop stand. Symptoms of nematode injury resemble those caused by nutritional deficiency, salt injury, root diseases or adverse soil factors. The cultivated field with old history of infection shows uniform crop damage that may go unnoticed while new infection shows up as patches of poor growth. Severe infection often results in shortening of roots.

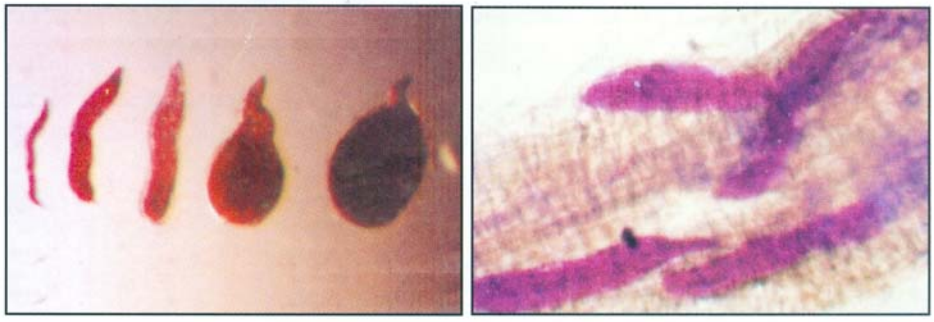
Population dynamics and damage threshold

R. reniformis has been noticed as the most frequent and dominant species in cotton growing areas of Central India. Populations of *R. reniformis* showed two peaks, a low during summer and high in autumn. Work done at CICR, Nagpur showed 20 nematodes/ 250cc soil as threshold level for root damage. Involvement of *R. reniformis* as causal agent of Para wilt of cotton was initially suspected. However, only the association of *R.reniformis* was recorded in parawilted crop, and thus its role in development of Parawilt was ruled out.

In Reniform nemotode infection, the growth of cotton roots and shoots was reduced at level of more than 8 and 1 nematode per g soil, respectively. In the field, cotton responded to nematicide use at populations of 1 to 2.4 nematodes/g soil. It has been shown that cotton yields were negatively correlated with population density at planting time. Yield loss due to reniform nematode depends on soil conditions, especially the soil moisture, cultivar tolerance and type of population involved.

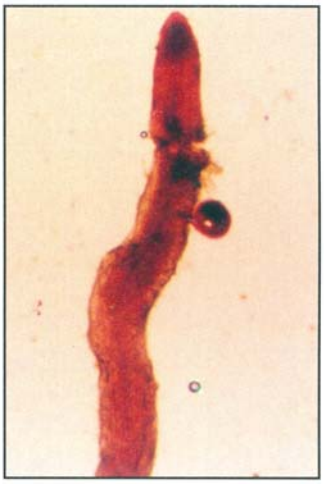
Root-knot Nematode infection in cotton field

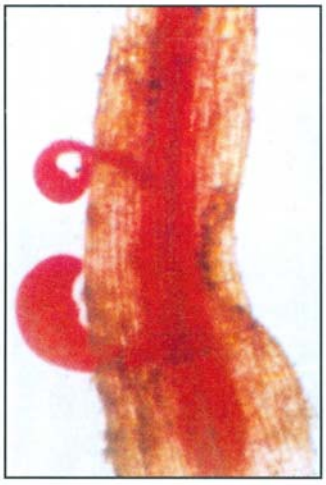
Root-knot Nematode infection showing galls on roots



Root-knot Nematode Developmental Stages

Typical patchy Field showing Nematode Infestation

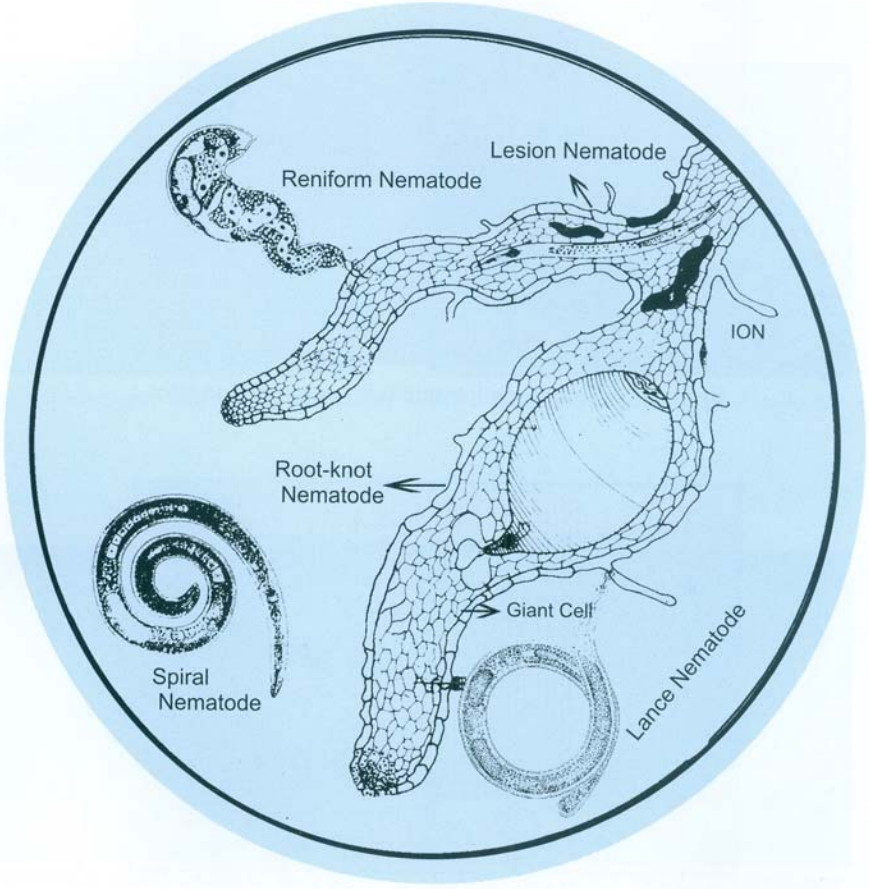




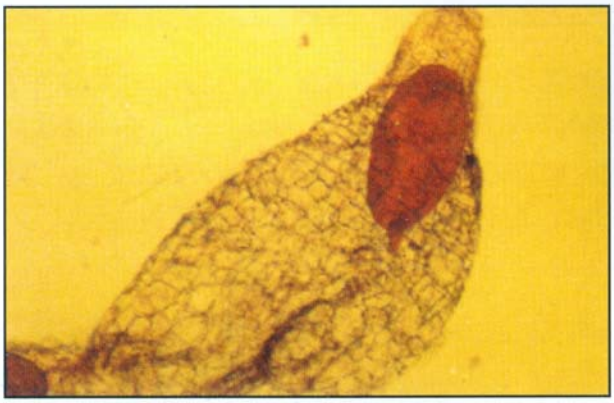
Reniform Nematode Females on cotton roots

Reniform Nematode infected cotton roots

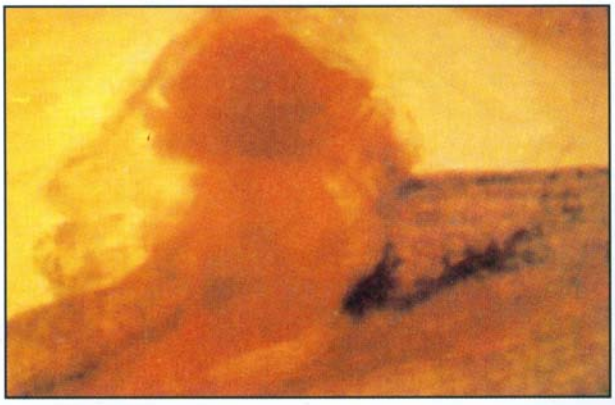
Soyabean crop also benefits from previous Nematicide Treatment



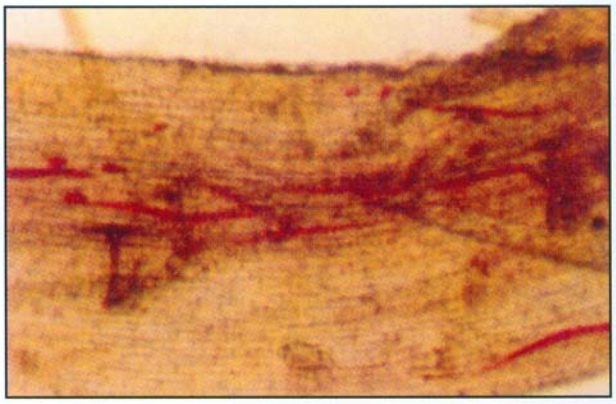
Schematic Representation of Nematodes associated with cotton
(After Sasser, 1971)



Developing Root-knot Nematode Female

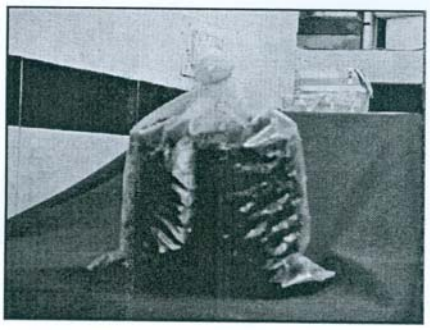


Root-knot Nematode Female with egg mass on root

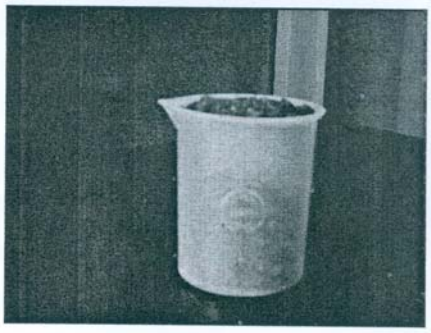


Lesion Nematode Developmental Stages in root

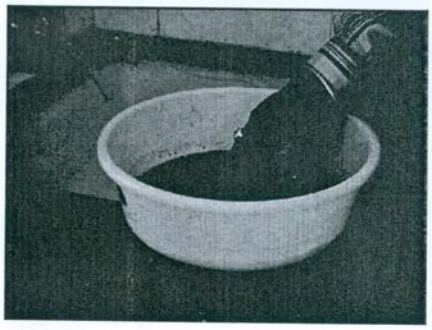
Nematode extraction by Sieving and Decanting Technique



Soil samples are stored in polythene bags



250 ml soil is taken for processing



Soil is mixed thoroughly with water to make slurry

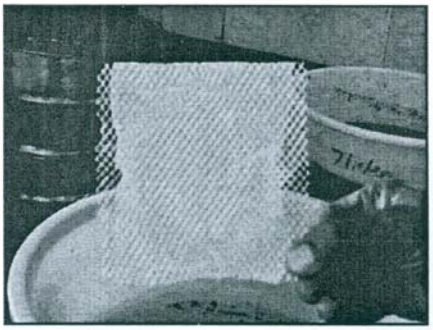
Soil suspension is passed through 18mesh sieve

Soil suspension is passed through 60 mesh sieve

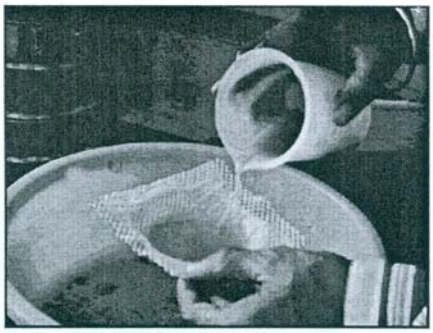
Suspension is passed through 100 mesh sieve

Suspension is passed through 350 mesh sieve

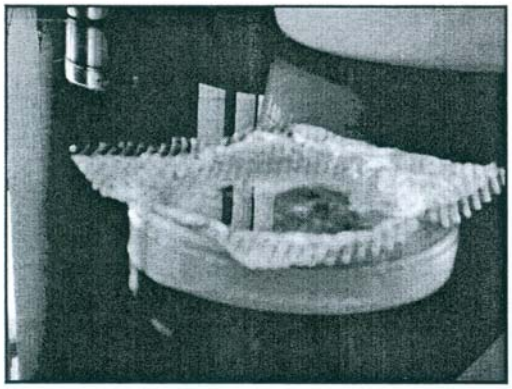
Suspension is passed through 400 mesh sieve



Tissue paper is wetted over aluminium mesh support



Suspension from sieves is poured



The Aluminium support is placed over a Petri-plate full of water

Biology:

Mature females of Reniform nematode are sedentary, semiendoparasite of roots which feed on cortical parenchyrna, the pericycle or phloem. Males do not feed and have poorly developed stylet. Second stage larvae hatch out of eggs. Second stage larvae transform into immature females as well as males through three superimposed moults without feeding. Infective and feeding stages are confined to immature females only. *R. reniformis* has characteristic kidney shaped females and vermiform larvae and males. Reproduction is normally amphimictic (sexual) and rarely parthenogenetic (asexual). Up to about 70 eggs depending on host are laid by the female in a gelatinous matrix. This egg sac is often concealed by adhering soil particles.

Life cycle gets completed in 17-30 days and with each female laying average of 66 eggs per egg mass, there is a rapid population build-up in crop season. The duration of development stages and fecundity are influenced by environmental factors. It has been observed that diffusates produced by live seeds probably account for the attraction of *R. reniformis* to germinating seeds. No specific root region is preferred and the nematode penetration occurs all along the root except the root tip.

Evidence has been mounting that there exist more races of Reniform nematode than thought of earlier.

Optimal soil moisture for reproduction ranges between 25- 30% and *R. reniformis* can survive without host for more than 25 months. Even in air dried soil (3.3 % moisture) survival for 7 months at 20- 30°C is reported. Soil pH is also an important factor affecting reproduction of the nematode. Generally, it thrives best in slightly acidic soils with optimum pH between 4.8 and 5.3. Even under saline conditions, the nematode has been observed to reduce plant growth. Perhaps limited development of nematode is due to poor root growth to support enough population rather than any adverse effect on the nematode itself.

Work done at CICR, Nagpur also indicates that Individuals of population pool of reniform nematode did not show simultaneous infectivity but exhibited phenomenon of staggered infectivity. This has significance that in case of invading juveniles not completing their life cycle, a small percentage of population always remains to ensure survival and perpetuation of the species.

ROOT KNOT NEMATODE, *MELOIDOGYNE* SPP. Goeldi

Besides reniform nematode, the Root-knot nematode, *Meloidogyne* species are wide spread throughout India. Only race 3 and race 4 of *M. incognita* and the *M.acronea* are known to parasitize cotton. *M.acronea* is known to occur on cotton only in South Africa and Malawi and has not been reported from India so far. Most distinctive symptom of Root knot nematode infection is appearance of knot like galls on the roots. In India, *M. incognita* is widespread in Punjab and Haryana on *G. hirsutum* (American) and *G. arboreum* (Desi) cottons, with galling being most extensive on *G. hirsutum.* Cotton fields showing characteristic patchy growth and poorly growing plants can be seen in areas around Sirsa and Hisar in Haryana. In Tamil Nadu,

root knot infestation has been recorded 45 days after sowing of Egyptian cotton variety Suvin (*G.barbadense*) and inter-specific hybrid Varalakshmi (*G. hirsutum* X *G. barbadense*). In Gujarat cotton is attacked by both *M. incognita* and *M. javanica*, wherein highest population of 1456 per 200 g soil has been recorded. A tolerance limit of 27 eggs /juveniles per 1000 g soil has been reported. Other studies have put tolerance limit at 100 eggs / juveniles per 100cm^3 of soil. At Hisar in Haryana State 17.7 to 19.9% reduction in yield with root knot index of 4.0 has been reported.

Symptoms

Root galls are the most distinctive symptom of root knot nematode infection. Galls on cotton can measure upto 1/4 inch in diameter which in multiple infection can coalesce to form bigger gall. However, cotton roots being woody, galls may not enlarge as much as in succulent roots. Infected plants have poorly developed tap root and shallow lateral roots. Each root gall can have several short roots arising from it giving it a whiskered appearance. Above ground symptoms are manifestation of slow debility of roots in its functions of water & nutrient uptake and translocation. Infected plants obviously contribute less energy than normal ones for producing fruits. As a result, relatively few bolls are set and these also tend to be small. Injury is greatest when plants are infected early in the season. Infected plants wilt early and are slow to recover from stresses. Classic symptom of nematode infection reflects poor growth of plants in patches which advance circumferentially every year.

Population dynamics & damage threshold

Root knot nematode population increase rapidly during cropping season. In North India, the population declines rapidly as crop maturity coincides with onset of winter. Majority of over wintering population consist of eggs and juveniles, a small proportion of which can survive for one year or more without a host. Relationship between number of root-knot nematode juveniles and yield loss has been worked out by the United States Department of Agriculture, USA.

Table 2: Relationship between number of root-knot juveniles per kg soil and yield loss in American Cotton (*G. hirsutum*).

Number of juveniles	Per cent loss per normal yield	Number of juveniles	Per cent loss per normal yield
0-55	0	350	13
100	2	400	15
150	5	450	17
200	7	500	19
250	9	550	20
300	11	600	22

Yield loss is due to decreased plant height and reduced number of fruiting bodies.

Biology

Root knot nematode being polyphagous has a very wide host range spanning over 232 plant species which include weeds commonly observed in cotton cultivation. The rate of development and fecundity may vary with populations/races. Second stage juveniles seek and infect roots. The juveniles can move as far as 2-3 ft attracted by chemicals released from growing roots. Penetration is usually just behind the root tip. Galls, the characteristic symptom, results from hypertrophy i.e. Enlargement in size of the individual plant cell and hyperplasia i.e. Multiplication of the host plant cells due to nematode feeding. Except for second stage of nematode and males, all other life stages remain in the root.

Temperature developmental threshold for the juveniles is about 10^0C. However, temperature above 15.5^0C is required for penetration. About 3-4 weeks are required for completion of one generation from egg to adult. Favorable conditions for nematode development closely resemble conditions in the field at the time of cotton planting. In a conducive crop season, nematode population build-up increases rapidly and reaches as high as 10, 000 eggs/ juveniles per 500cc soil.

LANCE NEMATODE

Hoplolaimus spp., commonly known as lance nematode is a serious pest or cotton seedlings and affect badly establishment of plants. A total number or six species of *Hoplolaimus* are known to parasitize cotton. Most commonly occurring species are *H. seinhorstii* and *H. indicus*. Hardly any work on this nematode in cotton crop has been done in India. Lance nematode species are essentially ectoparasites, occasionally becoming semi-endoparasites. The nematodes feed on phloem parenchyma and phloem elements. Because of the penetration of vascular region when root is young, the vascular elements are not differentiated which leads to abnormal division of phloem parenchyma, disorganization of cellular elements and eventually the cell death.

Severely involved tissues swell, becomes spongy and the outer few layers of cortex may appear brown. Large cavities in cortex may be formed which slough-off from central core of vascular element. Generally the nematode does not feed on xylem but its activities results in extensive damage to xylem vessels. Tylosis are known to occur in damaged xylem vessel leading to disruption of water uptake and translocation.

LESION NEMATODE

As the name suggest the most characteristic symptom of lesion nematode ***Pratylenchus*** spp. is the appearance of lesions on the roots which initially look as tiny elongated water-soaked spots. These spots soon turn brown and then almost black. The lesions enlarge gradually, coalesce, and ultimately girdle the root giving an appearance of constriction. The lesions are formed due to release of hydrolytic enzymes during feeding. The enzymes hydrolyze amagdylin to glucose, benzaldehyde, and HCN (Hydrogen cyanide), the last two formations are toxic that causes cell death and finally the necrosis.

In general, necrotic symptoms are limited to feeding site. Browning of roots also occurs due to the accumulation of phenolic compounds in areas of injury. Infected plants show moderately to severely stunted plants in discrete patches with yellowish to chlorotic leaves. Stunting of crop, general loss of vigour and gradual wilting are also associated in lesion nematode complex.

ASSOCIATION OF NEMATODES WITH OTHER PATHOGENS

Nematodes have long been suspected of playing a greater role in plant disease scenario in association with other fungal and bacterial pathogens rather than alone. Nematodes may act as wound maker, host substrate modifier, vector and rhizosphere modifier to make the environment more conducive for development of other pathogens. Combined attack of *R. reniformis* and Fusarium wilt (*Fusarium oxysporum f. sp. vasinfectum*) fungus results in greater damage to susceptible plants. In disease complexes involving nematodes and other pathogens, physiology of host plant is altered either to the advantage or detriment of pathogens. Nematode infection may also provide ready Avenue for entry of the pathogens. The non-virulent strain of the Rhizoctonia root rot fungus *Rhizoctonia bataticola* becomes virulent in presence of Reniform nematode.

Role of Root knot nematode as resistance breaker was reported for the first time in case of Fusarium Wilt of cotton. Post-emergence damping- off of cotton seedlings caused due to *Rhizoctonia solani* and *Pythium debarynum* is aggravated by *Meloidogyne* sp. Besides providing avenue for entry of pathogens, biochemical changes initiated in nematode infected plants and enriched nutritional status of giant cells also favour wilt causing fungi.

Severity of Verticillium wilt (*Verticillium dahliae*) is increased in presence of root knot, reniform and lesion nematodes. Non-virulent isolates of fungus, *Rhizoctonia bataticola* behaved as virulent in presence of Reniform nematode. Mortality of seedlings in root rot infection was preponed by about a week due to interaction of virulent fungus isolates with nematode.

MANAGEMENT STRATEGIES

The main objective of nematode management is to prevent the loss in yield and crop quality as well to keep nematode population below economic threshold. It is necessary to reduce nematode population density below sublethal level. The damage threshold density is relevant to the tolerance of the crop and environment in which it is grown and need continuous monitoring and detailed studies.

To prevent damage, the percentage of nematodes (K) that must be killed is given by Whitehead as below:

$K=100\ (Pi-Pt)/Pi$

Pi = Initial population, Pt = Damage threshold population

It is universally acknowledged that Integrated Pest Management (IPM) would be the best strategy for nematode management as no single method may be effective alone. The most recent' approaches have relied upon economic injury threshold rather than 'tolerance level' which is not of much use in relating to management cost. The object of the Integrated Nematode Management (INM) approach is to maintain the population densities wherein the loss caused is just equal to and not more than management cost necessary to accrete the loss. The objective of INM is to maximize profits while minimizing human health hazard. The components of program for nematode management on cotton include.

1. Survey for the damage due to nematodes (patchy growth)
2. Monitoring for the known nematode damage areas
3. Crop sanitation (including removal of weeds)
4. Summer ploughing
5. Cultivation of resistant cultivars
6. Crop rotation
7. Chemical treatment

Components of Strategy for Nematode Management will depend on whether application is intended for low input rainfed cotton production system or high input irrigated cotton production system.

MANAGEMENT STRATEGIES

MONITORING: Monitoring is an assessment of nematode population density in relation to crop development. For accurate diagnosis under INM, it is necessary to collect soil and root samples. Sample area should reflect differences in cropping pattern or soil texture. It is necessary to strike balance between accuracy of the sampling and economics. Smaller is the sample area, more accurate will be sampling. Sampling from depth of 8-12 inches is recommended during crop season with no special equipment required.

Weighted nematode rating: Weighted nematode rating (WNR) has been recommended for estimating root knot nematode infection. The technique is based on a subjective rating of the root galling and is particularly useful where cotton is grown again after cotton. Based on field size, cropping history and soil conditions, field is divided into several blocks and 15-20 plants are examined per block. Roots are rated as per rating given in Table 3.

Table 3: Weighted nematode rating (WNR) for assessment of root galling due to root-knot nematode.

Rating	Per cent roots galled	Weighted rating
0	0	0
1	1-25	1
2	26-50	3
3	51-75	5
4	76-100	7

Number of root systems in each rating is multiplied by the weighting factor and summed. This is divided by maximum weighting possible and then multiplied by 100.

$$WNR = \frac{\text{Number of root systems X weighting factor}}{\text{Maximum weighting factor}} \times 100$$

If WNR is greater than 10, then the chemical treatment is advised. If WNR is between 1 & 10 then soil solarization is recommended. WNR offers advantages of quick results and easy identification of problem areas.

Work done at CICR, Nagpur has shown that initial Reniform nematode population can be used to estimate crop loss.

Table 4: Relationship between initial population level of Reniform nematode and predicted crop loss.

Nematode Population per 250 cc soil	Per cent yield loss
10-70	-
71-200	
201-350	8
351-480	10

PHYSICAL MEASURES

Nematodes in soil may be killed by soil solarization. Soil is covered with one or two layers of polyethylene film and sun light is used for raising soil temperature. This is very effective for top soil in hot tropical summer months. Eggs and juveniles of reniform nematode get killed by exposure for 1-24 h at 41-47°C and repeated exposure to lethal temperature for sub lethal period has been observed to have cumulative lethal effect.

CULTURAL MANAGEMENT

Cultural management methods have the advantage of offering low cost options for nematode management with no toxicity or residue problems. On the flip side, however, they are not always applicable because of low efficiency and also interfere with normal cultural practices. Essential information for nematode management planning in hot tropical and sub-tropical cotton areas include the cropping history, cropping plans, soil texture and the rough estimate for nematode population reduction.

Sanitation

Age old practice of burning crop residues that lies on field surface should be promoted. It is suggested that root systems of susceptible crop should be removed and destroyed immediately after harvest.

Weed hosts usually found in cotton cultivation as *Amaranthus sp.*, *Trianthema monogyna* and *Convolvulus arvensis* are good hosts for Reniform nematode. Keeping fields weed free will help in keeping nematode populations in general under check.

Summer Ploughing and other Farm Operations

Hot summer months in India can be utilized profitably for summer ploughing as it is known to be a very effective farm operation in reducing nematode population. Four hoeings at weekly intervals are known to reduce the nematode population in soil by about 50% and thus it can be a most feasible option of nematode management.

The practice of deep tillage has been shown to increase yields in fields where nematodes are present. Deep tillage opens up soil for early cotton root development which is presumed to allow the roots to escape invasion by Root-knot nematode.

Crop Rotation

Population of Reniform nematode has been reported to get reduced by 80%, when chilli and other non-host crops are grown. In general, the inclusion of Marigold (*Tagetes patula*), Zinnia (*Zinnia elegans*), Sugarcane (*Saccharum officinalis*) and Maize (*Zea mays*) in cropping sequence reduces reniform nematode population.

Cropping sequence involving Mustard (*Brassica campestris*) Kulfa (*Portulaca oleracea*), Methi (*Trigonella foenum-graecum*), Zinnia (*Zinnia elegans*), Turnip (*Brassica rapa*), Mung (*Vigna mungo*), Wheat (*Triticum aestivum*) and Barley (*Hordeum vulgare*) reduced both reniform and root knot populations. Crops as Mustard (*Brassica spp*.), Sesamum (*Sesamum indicum*), Sannhemp (*Crotolaria spectabilis*), Asparagus and African marigold have antagonistic effect which suppresses root knot nematodes. African marigold, Custard apple and Bitter gourd were found to exert repelling effect on nematodes.

Cropping sequence involving Sorghum (*Sorghum vulgare*) resulted in reduced Reniform nematode population in Central India. However, wide host range of root knot and reniform nematode, both among cultivated and weed species, necessitates thorough weed control to ensure efficacy of rotation. Furthermore, the farmer is usually hesitant to adopt alternative crop as these are commercially less valuable compared to the main crop. In areas near urban cities, crops as African marigold can be planted with good monetary benefit.

Two- year crop rotation using green sorghum or resistant soybean in place of cotton has been found quite effective. Safflower (*Carthamus tinctorius*) was found to decrease reniform population by 96-100% 45 days after the sowing. Growing non-host red pepper for four months was found to decrease *R. reniformis* population by 80%.

Use of Trap Crop

Trap crop such as Sannhemp (*Crotolaria spectabilis*) which traps root-knot larvae can be grown and used as a green manure.

Host Resistance

Replacing the susceptible varieties with genetically resistant ones is a convenient option for nematode management. No variety in India is released resistant to nematodes. In USA, Nemax variety of cotton released is resistant to Root-knot nematode. LA 881 cotton has been registered for resistance to Reniform nematode. Growing resistant soybean cv. Pickett 71 for two years was found to reduce reniform population and increase the yields in subsequently grown susceptible cotton cultivar. However, in India work is restricted to evaluation of germplasm against Root-knot and Reniform nematodes. The resistant lines need to be utilized in breeding programme.

Cotton germplasm accessions reported resistant to root-knot and reniform nematodes.

Germplasm Lines Resistant to Root Knot Nematode: *G. hirsutum* -TX 1174, TS 1440, TX 2076 and TX 2107.

Germplasm Lines Resistant to Reniform Nematode: EC 13761779-4291, DP 503, IC-284, IC-9-1487, IC-137607, AKl/Acala 1517, AK361-51622, E792-134389, ICl27/Elvis 403-2, Lohit X Cernum, IC 35/H14, IC 948/9-3-081113, SI367/cpd 8-1, Si254/EC115936, Ze 1455/ B4-C02 (W-3), Auburn 56, Delfos 9169, Cario-2, Tamcot, SP21, Tamcot SP 37, 53-D-7, CB 2482, PK 1069, Ac 123/62, Acp 71, JK 119, Bar 12/B, Bar 12/18,1142, Lycmh PRS-7, 116 TLYC, Macha, 1039 kekichand, 25-36 1416, VPA (57) and TT Hairy.

Germplasm Lines with Low resistance to Reniform Nematode but High resistance to Root Knot Nematode: *G. hirsutum* N 2221-31 and 320-2-91.

These resistant accessions were found to restrict entry of nematodes. Nematodes penetrated roots of susceptible lines earlier by 2448 h compared to resistant ones. There was also found to be reduction in attraction and aggregation of nematodes around roots of resistant germplasm lines. Possibility of existence of qualitative difference in root exudates of susceptible and resistant germplasm lines may account for this phenomenon.

Biological Control

In recent years much attention has been paid to bio-entities or agents with potential against Root knot and Reniform nematodes. Recent experiments with fungus *Paecilomyces lilacinus* which parasitize the nematode eggs have shown good promise. Mycorrhizal fungus, *Glomus fasciculatum* was also found to reduce population of Reniform nematode. Penetration and reproduction of *Meloidogyne incognita* on cotton was affected by *Glomus intraradices*. In last decade, bacteria *Pasteuria penetrans* has been projected as a potentially important biological agent against Root-knot nematode. The biggest disadvantage is its obligate nature of relationship that makes it incapable of artificial culturing. Extracts of large number of cultivated and weed plants have been found effective against root-knot and reniform nematodes. Parts of Neem, Zinnia, Marigold and many essential oils are also effective in the nematode management. Soil amendment with non-edible cakes of Neem (*Azadirachta indica*), Karanj (*Pongamia glabra*), Mahua (*Madhuca latifolia)* etc. have also been observed effective against root-knot nematodes. Plant extracts toxic to *R. reniformis* include Marigold (*Tagetes erecta*), Custard apple (*Annona squamosa*), Korphad (*Aloe barbadensis*), Jonkh-mari (*Anagallis arvensis*), Bitter gourd (*Momordica charantia*), Double bean (*Phaseolus lunatus*), Snake gourd (*Trichosanthes anguina*) and Country-mallow (*Sida cordifolia*).

Improving Soil Health

Maintenance of soil health and incorporation of organic residues promotes plant growth and reduces nematode population. Large amount of organic waste, farm yard manure can control *R.reniformis.* Poultry dung added at 5-20 tonnes/ha was found lethal to nematodes. Soil amendment with material as Neem Sawdust enriched with ammonium sulphate, Groundnut cake or Neem seed cake at 2 tonnes per hectare were found to be effective against Reniform nematode. However, large amount of material must be applied to soil to get visible results. Furthermore, the results may vary as soil texture varies from place to place. Combination of 0.5 ton Neem or Karanj seedcake with 1 kg aldicarb plus 15 Kg N/ha was also found to be effective. Urea applied as seed dressing at 25 Kg N/ha reduces nematode symptoms and increases yield.

Chemical treatment/application: Few chemical control options exist for managing nematodes in cotton. Treatment with Aldicarb (Temik) at 1.7 to 2.2 Kg/ha was found to increase cotton yields by as much as 65% in infested soils. Incorporation of Aldicarb in a 15cm wide band over the rows was found better than the application in seed furrows. Aldicarb and Carbofuran offer good control of nematodes. Since the Bhopal gas tragedy (cyanide gas) and closing down of Union Carbide establishment, Carbofuran (Furadan) has become a very important subsitute. Carbofuran @6 g a.i. /W/W and Fenamiphos (Nemacur) at 100 to 1000 ppm have been found to be good for seed treatment in studies conducted at TNAU, Coimbatore and GAU, Anand. Around 20.2% increase in yield was recorded in trials at TNAU, Coimbatore because of seed treatment with Carbofuran (2% W/W) and soil application (l Kg a.i./ha). Carbofuran followed by Fenamiphos and Phorate at 2 Kg a.i./ha were found effective in reducing population and increasing yield by 4.6 to 39.9% at MPKV,Rahuri. Dressing of cotton seed with 1, 2 or 4 % (w /w) Carbofuran, Isofenphos or Benfuracarb reduced root-knot galling in cotton seedlings in pot culture. Carbofuran was found most effective in preventing gall formation. Seed dressing with

Carbosulfan 6.0% was recorded to reduce populations of root- knot nematode. Work done at TNAU, Coimbatore shows that growing cotton crop after treatment with 1 Kg a.i./ha carbofuran, phorate or aldicarb applied to the soil 15 days after sowing reduced nematode population by 99-100%.

MANAGEMENT STRATEGIES FOR LOW INPUT RAINFED COTTON PRODUCTION SYSTEM

All management strategies except chemical control can be employed for low input rainfed cotton production systems.

MANAGEMENT STRATEGIES FOR HIGH INPUT IRRIGATED COTTON PRODUCTION SYSTEM

Chemical management strategies along with all other strategies can be employed for high input irrigated cotton production systems.

DESIRABLE OBJECTIVES

Problem recognition

Recognizing nematode damage to crop is first step towards initiating management of nematodes. This is because of lack of expertise to estimate nematode density and correlate with declining crop yields.

Crop health

Nematode diseases can be said to be 'Life-style diseases of plants'. Mono cropping (same crop year after year), use of synthetic fertilizers and limited number of popular cultivars are some of the factors that may contribute towards nematode disease incidence. Maintenance of good cropping practices such as use of organic amendment, crop rotation and summer ploughing leading to good soil and crop health can ameliorate nematode damage to a larger extent.

Soil solarization

With abundance of sunshine available almost all round the year, this technique could be extensively used for management of nematodes in a hot tropical country like India Development of precision machinery to apply the polyethylene soil cover for fumigation and solarization could make the technique more feasible and less problematic. Development of rapidly degradable plastic cover that may add to organic matter or act as manure would solve the problem of cumbersome process of retrieving the polyethylene sheet after use.

Crop rotation

There may be more than one nematode species in the soil. Therefore, effect of crop

rotation on the nematode pest complex of soil needs to be assessed locally before specific rotations are introduced.

Crop resistance

There has not been much progress regarding use of resistance cultivars because poor yield and quality of resistant cultivars makes them unattractive proposition compared to susceptible cultivars. Also the narrow base of their resistance makes them more prone to selection of higher virulent biotypes or sibling species present in field population.

Ideal Nematicide

Discovery and development of less toxic, phloem mobile nematicide would be very useful in control of nematode pests.

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OTHER USEFUL PREVIOUS BULLETIN ON PHYTONEMATODES

Nematode Infected Seed and Planting Material : Denematization and Salvaging Techniques (2002). Nandini Gokte-Narkhedkar, P M. Mukewar and C. D. Mayee. Technical Bulletin No. 20, Central Institute for Cotton Research, Nagpur, 41 Pages.

Man plays a major role in dissemination of nematodes through movement of plant propagules and soil accompanying the seed and planting material. Globalization of agriculture and boom in tourism has led to acceleration in plant introduction business. The CICR Bulletin No. 20 covers symptoms of nematode infection as well as various salvaging techniques that could be employed for denematization especially in nursery and plant quarantine laboratories. Various disinfestation and denematization techniques have been compiled in this bulletin which can be employed for the total elimination of nematodes from seed and planting material. This can be a handy reference book for quarantine officials, germplasm explorers, seed and plant importers, Agriculture officials and orchard and nursery growers, particularly floriculturists and horticulturists.

Price of Bulletin is Rs. 50/-. The copies of this can be obtained from Director, Central Institute for Cotton Research, Post Bag No.2, Shankar Nagar P.O., Nagpur - 440 010 (M.S.) , India.

---- End of the reports ----

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