PLANT NEMATODES AND THEIR MANAGEMENT

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Nematodes are invertebrates, which, unlike insects, are unique in their numbers, varieties, microscopic size, high degree of internal organization and ubiquitous distribution. They are prevalent in almost every ecological habitat. Their attack on a large number of food crops and may result in death, debility, and in impairment and loss of food. The degree of damage to a particular crop is influenced by the crop and cultivar, nematode species, level of soil infestation and environment. Severe damage may result if high infestation levels occur in soil where susceptible crops are planted. These deleterious effects on plant growth result in low yields and poor quality. Nematodes often cause decline or death of crops, vegetables, ornamentals and turf. The role of nematodes, in general in the ecosystem is not fully understood. Many of them are believed to play beneficial roles in the food web and the biotic activity responsible for the nutrient cycling and environmental scavenging or decomposition. However, many others are predators of small organisms or parasites of animals and plants from the simplest to the most complex forms.

Nematodes are microscopic, bilaterally symmetrical roundworms that live in many habitats. At least 2500 species of plant parasitic nematodes have been described, characterized by the presence of stylet, which is used for penetration of host plant tissue. Most attack roots and underground parts of plants, but some are able to feed on leaves and flowers. There are numerous estimates of the economic importance of nematodes in crop production on a worldwide and individual country basis, but precise values cannot be determined. Extensive research in developed countries and the more than 70 developing countries leaves little doubt concerning the destructive nature of plant parasitic nematodes and the importance of their management for successful crop production.

Significance of phytonematodes in agriculture :

Plant parasitic nematodes are prime factors involved in affecting the normal growth and development of crops in following ways :

i) As direct pathogens : Nematodes act as direct parasites of cultivated plants, damage the plants and lead to the yield reduction. e.g. Root-knot Nematode, Meloidogyne sp. on a wide range of crops and cyst nematode Heterodera sp. on cereals.

ii) Involved in complex diseases: Due to the mode of their feeding, they act as " predisposing factors" and encourage the attack by other fungal and bacterial pathogens. The involvement of more than one pathogen can aggravate the disease causing huge loss to the crops e.g. Meloidogyne sp. with Fusarium wilt fungus in tomato.

iii) As virus vectors: Several phytonematodes, apart from acting as plant pathogens, help in transmission of plant viruses from plant to plant thus severing the virus infection. There are

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two groups of viruses NEPO and NETU transmitted by the nematodes.

iv) As resistance breakers: Phytonematodes are known to breakdown the resistance in a resistant cultivars against a fungal/ bacterial pathogen thus making it susceptible to the pathogens.

Classification based on Nematode feeding habit

Based on nematode feeding habits, plant parasitic nematodes can be classified into the following groups :

I. *Migratory ectoparasites* : These nematodes remain outside the root throughout their life cycle and feed on epidermal cells or on cells deeper in the root. They, however, retain the ability to move to new feeding sites e.g. *Longidorous, Xiphinema*, *Trichodorous, Paratylenchus*.

II. *Sedentary ectoparasites* : They remain outside the root throughout the lifecycle but feed from a modified nurse cells for an extended period. They do not move to new feeding sites e.g. *Hemicriconemoides, Hemicycliophora, Cacopaurus*.

III. *Migratory endoparasites* : These nematodes enters the root system and feed from cells and can move/ migrate freely within the tissues e.g. *Radopholus*. *Pratylenchus, Helicotylenchus, Hirschmaniella*.

IV. *Sedentary endoparasites* : They enters the root system and feed from highly modified cells. Loss the ability to move and most maintain an active feeding site e.g. *Meloidogyne, Heterodera, Globodera*.

V. *Semi-endoparasites* : Only the anterior portion of the nematode body can enter the root while the posterior portion remains outside i.e. on the root surface e.g. *Tylenchulus semipenetrans, Rotylenchulus reniformis.*

Examples for : (i) endoparasites of flower buds, bulbs, leaves and stems: *Anguina tritici*, *Aphelenchoides* spp., *Ditylenchus* spp.

(ii) Parasites of tree trunk: *Rhadinaphelenchus cocophilous, Bursaphelenchus xylophilous*.

Economically important phytonematodes/ diseases of crops

1. Root-knot nematodes (*Meloidogyne*): They are the major threat to a very wide range of crops including vegetables, fruits, pulses, oilseeds, cereals, crops grown under protected cultivation, plantation crops, medicinal and aromatic crops etc. which are susceptible to several species of *Meloidogyne*.
2. Wheat seed gall nematode (*Anguina tritici*) on wheat, barley, oats and rye.
3. Cereal cyst nematode (*Heterodera avenae*) on wheat and barley.
4. Potato cyst nematode (*Globodera rostochiensis*, *G. pallida*).
5. Rice Root-knot nematode (*Meloidogyne graminicola*).
6. White tip nematodes (*Aphelenchoides besseyi*) on rice and tube rose.
7. Burrowing nematode (*Radopholus similis*) on banana, coconut, areca nut, black pepper and citrus.
8. Citrus nematode (*Tylenchulus semipenetrance*) on citrus group of crops.
9. Reniform nematode (*Rotylenchulus reniformis*) on a wide range of food, fibre, pulses, oilseed, fruits and plantation crops.
10. Lesion nematodes (*Pratylenchus* spp.) on a very wide host range including wheat, maize, cotton, coffee, soybean, chickpea, potato, rice, banana, tea, vegetables, ornamentals and fruits.
11. Pigeon pea cyst nematode (*Heterodera cajani*).

The current status of development in Nematode diseases and their management has been reviewed extensively in books published recently (Trivedi 1998a,b,c,d; 2003a,b; 2011, 2015a,b)

Management of Plant Parasitic Nematodes

The pattern of crop protection for an agricultural ecosystem has been classified into five phases, which may be applicable in many crop situations.

(i) Subsistence phase : Here the crops are usually grown under non-irrigated conditions, crop yields are low. Crop protection is dependent on natural control.

(ii) Exploitation phase : The crop protection programmes were developed mainly through the use of chemicals. The crop yields are high thereby leading to excessive use of chemicals for managing pest populations and obtaining higher yields.

(iii) Crisis phase : The overdependence and excessive use of chemicals led to development of resistance in pest populations. Moreover, the occasional pests became serious primary pests. The combination of pesticide resistance , pest resurgence etc. increased the cost of crop production.

(iv) Disaster phase : The production cost increases so high due to the excessive and higher dose of chemicals, that crop production become profiteers. The environmental pollution and residue problems occur at high levels.

(v) Integrated pest control phase : Integrated pest control programmes include acceptance and utilization of ecological factors and compatibility, of other control of measures that result in favourable economic, ecologic and sociologic consequences.

In India, most of the pest control programmes are either in the exploitation phase or crisis phase and the integrated pest management concept need to be adopted at an early stage to avoid the crisis and disaster phase.

Integrated pest management strategies include:

1. Pest Avoidance
2. Pest Eradication
3. Pest Population Manipulation
4. No action, which may be considered because of the low population build-up of the pest population.

Plant parasitic nematodes present a formidable pest problems for the successful cultivation of economically important crops. The estimated overall annual yield loss of the world's major crops due to damage by phytoparasitic nematodes is 12.3 per cent (Sasser and Freckman 1987). Further, it has been observed that losses in developing countries were relatively higher (14.6 per cent) than the developed countries (8.8 per cent).

The monetary losses due to the nematodes on 21 crops, 15 of which are life sustaining were estimated at 77 million dollars. This figure may well exceed 100 million dollars, if all the crops are taken into account.

Nematode Management Strategies

In order to reduce and minimize the adverse effect of increasing nematode populations on crop plants and for economical crop production different control options has been tried. Once the nematode populations are established in a field, eradication is neither practical nor desired. So, we have to manage the nematode populations and learn to live with them. The direct and indirect benefits of nematode control include increased quantity and quality of produce, improved health of plants thereby reducing their chances of suffering from nematode diseases and increasing the plants ability to withstand adverse growing conditions. The selection of different nematode management options is dependent upon the biology of nematode species, the host crop, means of nematode spread, survival and ecological relationships.

(A) Physical Methods : The use of physical methods needs special facilities which are sometimes beyond the reach of common farmers under Indian conditions. The most documented physical control include use of hot water treatment. Soil/ seed treatment by means of solar heating or by electrical means, radiation and seed cleaning etc. Hot water treatment of seed tuber, bulbs and root cuttings is most helpful in reducing nematode spread and damage. Hot water treatment of rice seeds against root-knot nematodes, rooted cuttings of citrus against *Tylenchulus semipenetrans*, grapewine and roses cuttings against *Meloidogyne* spp. and *Rotylenchulus reniformis* and banana suckers for *Radopholus similis* are quite effective. Cleaning wheat seeds infested with earcockle nematode (*Anguina tritici*) or by water floatation or in 5-10 per cent brine solution is useful in separating cockles/ galls from wheat seed.

(B) Cultural Methods : Cultural methods of nematode control are normal practices carried out at little or no extra cost to reduce nematode population and crop losses. Cultural methods for nematode control are the attempts to adopt crop husbandry practices so as to minimize the crop losses due to nematodes. These can be achieved by following practices such as : (a) Crop rotation (b) Summer solarization and deep summer ploughing (c) flooding (d) phytotherapy and use of organic amendmends (e) growing Trap crops and antagonistic crops (g) destruction of infected plant residue (h) time of planting (i) manuring and irrigation etc. Trivedi and Barker (1986) reviewed the work done on management of nematodes by cultural practices.

In order to achieve successful nematode management the thorough knowledge of following aspects is needed.

(i) Racial composition of nematode community

(ii) Biology, host range, population dynamics, rate of decline in the absence of host

(iii) Host status of various adaptable crops

(iv) Prevailing agroclimatic conditions

(v) Economics

The efficacy of most cultural practices in reducing nematode population varies with

(i) Soil types

(ii) Local climatic conditions

(iii) Crop husbandry practices

(iv) Target nematode species or race involved

(C) Crop rotation and cropping sequence : It is well recognized that soil becomes sick if monoculture is practiced. The control of phytoparasitic nematodes by adopting cropping sequences is based on the fact that some nematode species are able to feed and multiply on certain crop plants but not on others. The success of crop rotation depends on proper selection of crops in the sequence. The length of rotation depends on the initial nematode population level and rate of population decline during rotation. Hence information on the nematode species, its biotypes, host range, biology, survival, multiplication, relationship between populations and yield losses must be obtained prior to adopting any crop rotation/ cropping sequence for nematode management.

The use of trap crops is expensive without any return. However, use of antagonistic crops like (*Poa pratensis* and *Poa trivialis* against pigeon pea cyst nematode, *Tagetes erecta* against *Pratylenchus penetrans* and root knot nematode etc. have been reported to be effective (Gommers 1981). Flooding can be employed to reduce the incidence of phytoparasitic nematodes if sufficient amount of water is available. Nematodes are killed due to aphysixation and production of chemicals lethal to nematodes such as butyric acid, propionic acid and production of hydrogen sulphide gas because of low pH as a result of decomposition of organic matter. This practice does not seem to be practical as it adversely affects soil structure, fertility status and pH of soil.

Under tropical climatic conditions, harnessing of solar energy by deep summer ploughings has been recognized as important method to control weeds, diseases, soil insects including nematodes. This practice involves exposure of soil to solar heat and dessication, killing substantial proportion of insect pest, pathogen and weeds and nematodes (Raghavan 1964). In order to further enhance the efficacy of solar heating for nematode management polyethylene mulching of moist soil during hottest period of the year is being advocated and termed as summer solarization (Sharma and Trivedi 1991). Summer ploughing of nematode infested field during May-June has been found to be effective in managing root-knot nematodes (Jain and Bhatti 1987, Jain and Gupta 1997).

The selection of nematode free healthy transplants and destruction of infected crop residue of previous crop needs to emphasis, since these if not cared can become major source of infection in successive crops. The use of organic amendments and phytotherapeutic substances implies the addition of various organic substances to soil to bring about physical, chemical or biological changes adverse to the survival and multiplication of nematodes. Due to intense microbial activity during decomposition of organic amendments, these amendments have proved very useful in nematode management in the tropical countries. There is a need for better understanding of the mechanisms involved in this type of control to guarantee its success.

(**D) Chemical Methods :** The chemical used for controlling nematodes are designated as nematicides. The use of soil fumigants, which are applied to the soil and diffuse through the soil as gas had great influence in the development of science of Nematology. The discovery of DD soil fumigant in1943 and EDB in 1945 played remarkable role in demonstrating the nematode damage and crop losses in field. DBCP was developed in 1954., which had greater advantage over earlier nematicides as it was available as liquid formulation and could be applied with irrigation water. The era of soil fumigants was followed by the advent of non-volatile nematicides such as fensulfothion, aldicarb, carbofuran, ethoprop etc. Several workers have reviewed the work on chemical control of nematodes (Castro and Thompson 1971, Van Gundy and McKenry 1977, Wright 1981, Sethi and Gaur 1986, Bhatti and Jain, 1984). The use of chemicals happens to be a costly proposition especially for Indian farmers but these methods cannot be totally dispensed away with. They are normally used when nematode population is too high to permit successful cultivation of crops. However, the concept of nematode control changed with more emphasis on nematode management practices, which are less expensive, ecofriendly, less hazardous and easy to apply. In recent past, many of the effective chemical nematicides like DBCP, MBr, aldicarb etc. has been banned for use because of toxicity hazards and environmental pollution. Application of nematicides for practical nematode control in India at Farmer's level is not very popular except in a few cases like in potato in Nilgiris, tobacco in Gujrat and Karnataka. The judicious use of chemicals as nursery treatment/ seed treatment in vegetables and pulses against root-knot nematode has also been advocated.

(E) Biological Control : There are several biological agents which parasitise on nematodes and reduce their populations. Some of these are fungi, bacteria, viruses, nematodes, insects and mites. Biocontrol of plant parasitic nematodes has been reviewed recently (Trivedi 2014).

(i) **Predacious Fungi** : There are more than 50 species of predacious fungi which capture and kill nematodes in soil. Few important ones are *Arthrobotrys, Trichothesium*, *Dactylaria*, and *Dactylella*. These belong to Hyphomycetes. Some species belong to Zoopagale. These fungi capture nematodes by traps, mechanical traps and constricting rings. Barrons (1977) considered only *Rhapalomyces elegans* as egg parasite. *Verticillium chlamydosporium* is a major pathogen of *H. schachtii* eggs.*V. chlamydosporium* parasititize *H. avenae* (Bhardwaj and Trivedi 1996, 2000; *H. cajani* (Bhardwaj and Trivedi 1998, 2000; Meena *et al.* 2009) *Paecilomyces lilacinus* has been reported by Jatala *et al.* (1979) infecting eggs of root-knot nematodes. This fungus also attacks eggs of *Globodera pallida*. Extensive work was carried out for managing root knot nematode using *P.lilacinus* in our laboratory (Sharma and Trivedi 1989, 1992, Sharma *et al.* 2004, Pandey and Trivedi 1992).

(ii) Bacteria : Work on bacteria in the management of plant parasitic nematodes has been reviewed recently. (Trivedi and Malhotra 2013). *Pasteuria penetrans* has been described as potent biological agent against nematodes, The ability of *P. penetrans* to prevent reproduction and eventually kill the root knot nematodes and several other species present one of the best opportunities for biological control of major nematode pest. In addition certain rhizospheric bacteria like *Azotobacter chroococcum*, *Azospirillum lipoferum* and *Pseudomonas* sp. has been found to be promising in reducing the population of *H. avenae* infecting wheat (Bansal *et al.* 1999). *P.flurorescence* have been investigated for its antagonism to nematodes more extensively than others and found effective against *Meloidogyne* spp. (Pandey *et al.* 2003)

(iii) Viruses : Root knot nematode larvae infected with virus were observed to exhibit sluggishness.

In addition to above bioagents, protozoans, tardigrades, mites, turbellarians etc. have been reported as important nematode enemies. Further, certain predacious nematodes such as *Mononchus*, Diplogastrids and *Seinura* etc. are known to feed on other nematodes. These genera has been found to be efficient under laboratory conditions. However, their efficacy under field conditions is doubtful. Although biological control offers a very attractive and promising area and many potential bioagents for nematode management are reported. But, one of the major constraints in their use is their mass production for field application.

(F) Host Plant Resistance : Growing of nematode resistant varieties in problem areas constitute an important approach for reducing nematode populations. It is an effective economical and environmentally safe means of reducing losses caused by phytoparasitic nematodes. Resistance in host plant is normally depended upon the physiological differences between varieties of host plants, which makes it difficult for the nematodes to meet its food requirements or in some cases inactivate the salivary secretions released by nematodes, which play important role in establishment of compatible host-parasite relationship. Once a resistant plant is developed, then it would normally be able to maintain its property of antibiosis against pathogen. Resistance breeding would continue to be one of the important methods of development of new crop varieties offering resistance to key nematode pest. The information on the mechanism of resistance to nematodes presented by Gommers (1981), Premchandran and Dasgupta (1983) helps in understanding the concept of resistance against nematodes. Advances in biotechnology, tissue culture, protoplast fusion technique and somaclonal variations etc. has opened up new dimensions in the area of development of resistant cultivars against plant pathogens including nematodes.

(G) Integrated Nematode Management: Integrated nematode management (INM) has been defined as a system approach to reduce nematode populations below economic threshold level through integration of various techniques such as :

(i) Use of biocontrol agents

(ii) Genetically resistant host cultivars

(iii) Modification of natural environment or cultural practices

(iv) Judicious use of nematicides in a cost effective manner etc.

Implementation of INM programme is based on the principles of nematode exclusion, population modifications and tolerance (Bird 1981). The aim of INM is

(a) To utilize several compatible control techniques in combination.

(b) To maximize natural environmental resistance to plant parasitic nematodes.

(c) To apply specific and drastic control measures only as and when necessary and

(d) To maximize profit of the grower with location and resource specific recommendations.

The requirement for integrated nematode management are the research, development, transfer of technology for implementation of combining different nematode management options in most effective manner (Bird 1981). Hence, the components of INM system includes (a) biological and environmental monitoring (b) agricultural production system (c) pest crop ecosystem models and system design and implementation (Bird 1981).

Newer approaches for Nematode Management

Application of biotechnology in plant nematology has been reviewed earlier (Trivedi 2000). The major and potential novel concepts in the nematode management includes manipulation of genes/ transgenic nematode management, nematode ecdysteroids, juvenile hormones, vertebrate steroid hormones, inhibition of steroid metabolism, biological activity and mode of action of avermectins, endotoxins of Bacillus thuriengiensis, 2,4-diacetylphloroglucinol (DAPG), phytoecdysteroids and others (Ravichandra 2014).

(i) Plant nematode resistance genes : One of the greatest uses of recombinant DNA technology is the production of large quantities of scarce and/ of novel proteins coded by the structural genes. The production command of these structural genes is with the "promoter" sequences which act as transcriptional control elements. With increasing restrictions on chemical pesticides, the role of host resistance for nematode control has grown in importance. A number of genes that mediate nematode resistance have now been, or soon will be, cloned from a variety of plant species. Various approaches are being developed to provide resistance to a range of nematode species, Natural, R gene-based resistance is currently exploited in traditional breeding programmes and research is going to characterize the molecular basis for the observed resistance phenotypes. A number of transgenic approaches hold promise, the best described being the expression of proteinase inhibitors to disrupt nematode digestion. The applicaton of plant delivered RNA interference (RNAi) to silence essential nematode genes has emerged as a potentially valuable resistance strategy (Victorial *et al.* 2008). A few have been cloned and a number of additional genes are likely to be cloned in the near future (Table-1).

(ii) Manipulation of natural resistance genes : Genetic host resistance in the host cost effective and environmentally sound method for management of plant parasitic nematodes. Resistance to the various species of root-knot nematodes and cyst in limited number of host species. Resistant cultivars on infection exhibit activation of number of inducible responses that are thought to be disease resistance mechanism. Hypersensitive response (HR) is one such response in which there is a ability of the nematode. It is known that many plant genes are up-regulated during a resistance reaction, including genes encoding proteins such as glucanases, chitinases and other enzymes commonly referred to as pathogenesis related proteins

(C) Application of recombinant DNA technology : Recombinant DNA technology utilizes the power of microbiological selection and screening procedures to allow investigators to isolate a gene that represents as little as 1 part in a million of the genetic material in an organism. Insertion of resistance genes into the host crops genome is made possible by recombinant DNA technology. The understanding of nematode –host interactions using the tools of comparative and functional genomics has provided avenues for engineering specific and broad resistance against plant parasitic nematodes. Engineering resistance can be achieved by transforming plants with proteinase inhibitors, lectins, toxic proteins and dsRNA targeted against the phytonematodes (Sirohi *et al.* 2010).

Broad Classes of Transgenic Nematode Control Strategies

Nematode Targets **:** Antifeedant, nematicidal proteins like enzyme inhibitors, etc., disrupt essential nematode gene product with mai, disrupt sensory function with mai/ peptide/ plantibody and nematicidal metabolite.

Nematode-plant Interface **:** Disrupt nematode pathogenecity factor, invasion, migration and nematode pathogenecity factor, feeding site induction and maintenance (with RNAi, plantibody, etc); stealth plant, repellant plant and conversion of plant to non-host.

Plant Response **:** Plant resistance gene / hypersensitive response is activated by nematode invasion. Feeding site specific promoter induces cell death or other site incompatibility and conversion of plant to tolerance.

Molecules for transgenic expression **:** A defense strategy that delivers a protein such as proteinase inhibitor with efficacy against nematodes has distinct advantages. Effect of molecules can be envisaged that are without phytotoxic effects. This enables promoters that provide expression in cells that are not attacked by nematodes to be of value. Also such a defense strategy may prove effective against a wide range of nematodes irrespective of their feeding type and this has a number of commercial advantages as most crops are parasitized by more than one economically important nematodes.

Plantibodies **:** A plantibody is an antibody produced by genetically modified crops. Antibodies are part of animal immune systems and are produced in plants by transforming them with antibody genes from animals. Although plants do not naturally make antibodies, plantibodies have been shown to function in the same way as normal antibodies. One of the most remarkable aspects of recombinant DNA technology is when a protein belonging to the exclusive realm of animals can be successfully used in plants to help them fight against pathogens that are difficult to control. The expression of nematode specific antibodies in planta and hence , the term 'Plantibody', is a promising new avenue for controlling plant pathogens, A strategy has been devised against major phytonematode, *Meloidogyne* spp.. In root-knot nematode a cellulose from *M. incognita*, and other *Meloidogyne* species, in addition to other stylet secreted proteins have been selected as targets for this strategy. Cellulases in plant parasitic nematodes are important in the initial steps of pathogenesis. The rationale of selecting cellulases as the target of plantibodies is that upon contact between the anti-cellulase plantibody and the nematode cellulose, the migration of the nematode inside the plant will be stopped or diminished. If successful, this strategy will allow farmers to avoid using highly toxic nematicides, soil sterilents or fumigants. Other useful targets under analysis are the proteins involved in the initiation of the cell cycle that leads to the generation of giant cells that support the feeding nematodes in infected roots.

Proteinase Inhibitors **:** Proteinase inhibitors (PIs) are an important element of natural plant defence strategies. Protein inhibitors of a range of proteinase classes are widely expressed in plants where they are often induced by wounding and herbivory. They are already consumed in many plant food stuff, such as rice seeds, potato tubers and cowpea. Toxicological studies have demonstrated the lack of harmful effects of serine PLs in mammalian systems.

The potential of plant PLs as anti-nematode effectors was first explored using the serine PI cowpea trypsin inhibitor (CpTI) (Victoria *et al.* 2008). The cowpea trypsin inhibitor, CpTI expressed in transgenic potato influences the sexual fate of newly established *G. pallida*, more towards becoming male, but doesn't reduce the fecundity of the females so formed. In contract, CpTI reduces fecundity of females of *M. incognita* without influencing their fate. Transgenic expression of the sweet potato serine PI, sporamin, inhibited growth and development of female *H. schachtii* parasitizing sugar beet hairy roots (Urwin *et al.* 2002). Here, the severity of the effect was clearly correlated to the level of trypsin-inhibitory activity detected in the transformed root line. Inhibitory activity of a potato serine PI (PIN2) expressed in transgenic wheat also showed a positive correlation with plant growth and yield following infestation with Heterodera avenae. The potential of cysteine proteinase inhibitors (Cystatins) to provide nematode resistance has been explored in more depth. Initially the efficacy of a rice cystatin (Oc-1) was enhanced by protein engineering, using crystallograhic data, to produce a PI with greater inhibitory activity.

Cystatins have also been used to protect other plant species against a range of nematodes with diverse feeding strategies. Rice plants expressing a low level of the Oc1D86 cystatin under the control of a short CaMV35S promoter reduced reproductive success of *M.incognita* by 55 %., A peptide that disrupts chemoreception of nematodes without a lethal effect was reported, which provided resistance to *G.pallida* in both in a containment and a field trial, when precisely targeted under control of a root tip specific promoter. DNA barcoding and quantitative PCR were combined to recognize nematode genera from soil samples without microscope–based observation and use the method for nematode faunal analysis.

Promoters **:** Well defined synthetic plant promoters that direct controlled local gene expression in response to pathogens are available. These promoters could be used to help define signaling pathways, to isolate novel mutants using " targeted genetics" and to engineer plants with increased disease resistance. The control regions of plant genes are modular and contain a number of cis-acting elements, each of which may contribute to one or more aspects of a complex expression profile. One strategy to overcome this complexity is to produce synthetic promoters containing only defined individual elements, thereby reducing expression profile complexity.

Nematode ecdysteroids **:** Ecdysteroids are polyhydroxylated AT-6-ketosteroids with complete side chains, i.e. polyhydroxylated derivatives of cholesterol containing a C-7 double bond and a keto-group at C-6 and are involved in hormonal regulation of molting and other developmental processes. Ecdysteroids regulate molting in insects and hence a good amount of work has been carried out on these compounds. Moulting in nematodes is regulated by ecdysteroids and juvenile hormones, the effort has been placed on isolation of these and similar compounds from the nematodes. Cholesterol is the major sterol of the few vertebrate-parasitic nematodes analyzed thus far. Significant amount of sitosterol, campesterol, cholestanol, stigmastanol and campestanol occur in *Ascaridia galli* and *Ascaris suum*. Presence of 24-alkylsterols in these digestive tract parasites is probably due to occurrence in the host diet. Effects of ecdysteroids on various activities of nematodes have been documented (Table-2).

Among phytoparasitic nematodes, the only sterols detected in *Ditylenchus triformis* and *D. dipsaci* were cholesterol and lathosterol (cholest-7-enol), except for traces of phytosterols in the latter species. Their host contained only 24-alkylsterols. Curiously, the sedentary plant parasites examined to date contain greater relative propositions of phytosterols than the migratory *Ditylenchus* spp.. It is not known whether the more highly evolved parasitism of the sedentary plant parasites has included an adaptation of these organisms to utilize or store substantial amounts of plant sterols.

Inhibition of sterol metabolism **:** Specific information about the hormone related events in nematode has been obtained through the basic studies of nematode sterol metabolism. Structurally sterols contain a tetracyclic ring system, a hydroxyl group at C-3, an aliphatic side chain.

Phytoecdysteroid **:** As nematodes are placed in a clade of molting metazoans, the *Ecdysozoa*, it is likely they all have similar hormonal regulation of ecdysis. Since ecdysteroids possess biological activities in free living and animal nematodes,

Table 1 : Some cloned phytonematodes 2 Effect of ecdysteroids on nematodes

Gene	Host plant	Nematode	Gene structure
Hs 1 *pro*-1	*Beta procumbens* (wild relative of surar beet *B. vulgaris*)	*Heterodera schachtii* Amino	-terminus leucine-rich region
*Gpa*2	*Solanum tuberosum*	*Globodera pallida* resistance to narrow range of pathotypes	LZ,NBS-LRR
*Gro*1-4	*S. tuberosum*	*G.rostochiensis* Pathotype Ro1 onl	TIR-NBS-LRR
Hero A	*S. pimpinellifolium (wild relative of S. lycopersicum*)	Resistance against *G.pallida & G. rostochiensis pathotypes)*	CC-NBS-LRR
Rhg 1 & *Rhg*4	*Glycine max*	*H.glycines*-resistance against pathotype 0	LRR,
Mi-1.2	*S. peruvianum*	*M. incognita, M.arenaria M.javanica*	CC-NBS-LRR

Table 2 : Effect of ecdysteroids on nematodes

Dose	Effect	Nematode
0.01 M	Supernumerary moulting	*Heterodera schachtii*
$3.5x10^{-4}$ M	egg hatch inhibited	*Heterodera glycines*
Precocene-II	Toxic	*Caenorhabdities elegans*
100µg/ml	toxic	*Steinemema feltiae*
50µg/ml	Inhibited development	*C.briggsae*
0.1 µg/ml	Retarded Growth	Free living nematode, *Cephalobus* sp
50µg/ml	Inhibited growth & reproduction	*C. elegans*
0.01 ng/ml	Stimulated moulting	*Nematospiroides dubius*
0.05 ng/ml	Increased length	Animal parasite*, Ascaris suum*
464 µg/ml	Inhibited development	*Trichostrongylid, Haemonchus*
15µg/ml	Decreased length, promoted moulting	*Trichinella spiralis*
480µg/ml	Inhibited development	*Haemonchus contortus*
0.05 ng/ml	Increased length	*Ascaris suum*
5 ng/ml	Promoted moulting	*Ascaris suum*

Table-3: Effect of juvenile hormone on nematodes

Dose	Effect	Nematode
0.01 M	Supernumerary moulting	*Heterodera schachtii*
0.0001 M	Inhibited Moulting	*Trichinella spiralis*
10^{-7} M	Retarded formation of male	*Trichinella spiralis*
	Copulatory organ	
50 µg/ml	Inhibited growth & reproduction	*C. elegans*
50µg/ml	Inhibit development	*C. briggsae*
100µg/ml	Toxic	*Steinernema feltiae*
Precocene-II	Toxic	*C. elegans*
$3.5x10^{-4}$ M	Egg hatch inhibited	*Heterodera glycines*

phytoecdysteroids may also provide an important plant defense against nematodes and thus, may serve as a basis for the development of resistant cultivars. The phytoecdysteroid, 20-hydroxyecdysone (20E), is a major moulting hormone of invertebrates, possibly including nematodes. As 20E is inducible in spinach, the defensive role against plant parasitic nematodes was investigated. The effects of direct application on nematodes was assessed by treating cereal cyst nematode, *Heterodera avenae*, juveniles with concentrations of 20E from 8.2×10.8 to 10.5 M before applying to *Triticum aestivum* growing in sand.

Juvenile hormone (JH) **:** The insect juvenile hormone, epoxyfernasoic acid methyl ester derivatives are known to possess many bio regulatory roles and also involved in inhibiting/ affecting various activities in nematodes (Table-3). The extracts of juveniles of *H. contortus* to contain JH activity. Compared to the ecdysteroids, a greater number of JH and analogs have been evaluated for bioactivity towards nematodes and have been found to inhibit nematode development to a greater extent than the ecdysteroids.

DAPG (2, 4-diacetylphloroglucinol) **:** The antibiotic 2, 4-diacetylphloroglucinol (DAPG) is produced by some isolates of the beneficial bacterium *Pseudomonas fluorescens*. DAPG is toxic to many organisms , and crop yield increases have been reported after application of DAPG- producing *P.fluorescens*. Production of the antibiotic DAPG contributes to biological control activity of many beneficial strains of the bacterium *P. fluorescens*. DAPG is not toxic to all nematodes and did not affect the tested species of beneficial bacterial feeding nematodes. Augmentation *Xiphinema americanum* of DAPG-producing *P. fluorescens* populations for nematode biocontrol could be targeted to specific nematodes species known to be affected by this compound and by other antibiotics produced by the bacteria, or these bacteria could be used for other possible effects, such as induced plant resistance. *Heterodera glycines, Meloidogyne incognita, Pratylenchus scribneri* and the bacterial feeding nematodes *Caenorhabditis elegans*, *Pristionchus pacificus* and *Rhabditis rainai* were immersed in concentrations ranging from 0 to 100 mg/ml DAPG. Egg hatch and viability of juveniles and adults were determined.

Avermectins **:** The avermectins are series 16-membered macrocyclic lactone derivatives with potent anthelmintic and insecticidal properties. These naturally occurring compounds are generated as fermentation products by *Streptomyces avermitilis*, a soil actinomycete. Eight different avermectins were isolated in 4 pairs of homologue compounds, with a major (a-component) and minor (b-component) component usually in ratios of 80:20 to 90:10. Other anthelmintics derived from the avermectins include ivermectin, selamectin, doramectin and abamectin. Ivermectin (22,23-dihydroavermectin B1a + 22,23-dihydroavermectin B1b) is a broad spectrum antiparasitic avermectin. Abamectin is a mixture of avermectins containing more than 80% avermectin B 1a and less than 20 avermectin B 1b. It is widely used as nematicide.

Lectins **:** Lectins simply are "All plant proteins possessing at least one –catalytic domain, which binds reversibly to a specific mono- or oligosaccharide". Lectins have been used mainly to characterize sugar moieties on nematode surfaces or in secretions via binding experiments. They have been shown to bind to several sedentary parasitic nematodes at the excretion pore, the cuticle, the amphids or the head region in general, as well as at the spicules and vulva. The behavior of *Radopholus similis* and *Pratylenchus coffeae* were affected by lectins or lectin related proteins viz. the egg hatching, the chemotaxis, infection and reproduction of adult females and the mobility of the nematodes at stages intermediate to these crucial steps.

Endotoxins of Bacillus thuringiensis (Bt) **:** The effect of *B. thuringiensis* as a nematicidal biocontrol agent has been investigated for free living nematodes, animal parasitic nematodes, insect parasitic nematodes and phytonematodes. The strains Bt. Israelensis, Bt. Kurstaki and Bt. morrisoni have shown considerable variability with respect to lethality for animal parasitic and free living nematodes. The crystal proteins encoded by cry genes have been classified as Cryl to CryVI depending on a host specificity and amino acid homology. The mode of action of bacterial toxin on second stage juveniles of *M. incognita* was due to bacterial endotoxin, the efficacy of endotoxin as a nematicidal effect based on the morphological structure of crystal toxin. The spherical crystal toxin gave the highest reduction in nematode population because they can easily pass through the nematode mouth part. The toxin produced by the four studied isolates of *B. thuringiensis* were classified into three groups based on the toxin structure, which was observed by light microscope.

Bio-fumigation **:** Bio-fumigation refers to the process by which soil-borne pests and pathogens are suppressed by naturally occurring biocides released in soil when tissues of Brassicaceous plants decompose in soil. Bio-fumigation is based on incorporating soil amendmends like fresh plant mass or manure into the soil, which will release chemical substances known as isothiocyanates (ITC's), able to suppress nematodes, apart from acting as a soil heater to enhance biological activities. Plant from Cruciferae family release large amount of these toxic to soil-borne pests and disease substances in the soil and are considered the best material for biofumigation. When tissues of crucifers or green manure crops decompose in soil, ITC's are released, when glucosinolates (GSL's) in the tissues are hydrolysed. Major commercially available bio-fumigant plants : Fodder radish (*Raphanus sativus*), Indian mustard (*Brassica juncea*), Turnip (*B.napus*), Rape (*B. campestris*), white mustard (*Sinapis alba*), Ehiopian mustard, Abyssinian mustard (*B. carinata*) and Garden Rocket, Roquette, arugula (*Eruca sativa*). Such crops are rich in organic substances called glucosinolates. Under certain conditions they become isothiocyanates during decomposition. Sorghums produce a cyanogenic glucoside compound called "Dhurrin" that breaks down to release toxic cyanide when the tissue is damaged.

Future Strategies and Recommendations :

The developments in Biotechnology, especially in the area of molecular techniques have shown their immense potential in fundamental and applied nematology. Some of these advances have been imbibed, although yet on a limited experimental stage, for characterization/ differentiation of otherwise difficult to distinguish nematode taxa and to incorporate resistance into plants against economically important parasitic nematodes. The embryo rescue technique has been used to incorporate resistance to wild plant species into cultivated species, such as from *L. peruvianum* to the cultivated *L. esculentum*. Similarly, recombinant DNA technology has found use in the isolation and incorporation of nematode resistance genes to produce transgenic plants. These techniques have the potential of providing environment friendly and economically desirable plant types to overcome the nuisance caused by nematodes and other pests/ pathogens. However, the research costs and possible risks of undesirable effects on humans and other non-target organisms, whosoever remote, are still the limitations that must be tackled.

If chemical treatment are unavailable or inappropriate, the management of plant parasitic nematodes will have to depend on alternative strategies. (Ravichandra 2015). Government supported research will be necessary, particularly for alternate management research is unlikely to be funded by manufacturing companies. New research will require multidisciplinary collaboration to enable the integration of traditional practices with new ideas created by the scientists. Through more research on nematode biology and the environment, nematologist must consider several possibilities like how nematode lifecycle may be interrupted; how microbial activity at the root-soil interface, which could lessen nematode invasion, can be promoted; identification of compounds that interfere with reception of stimuli or repel nematodes; the selection or breeding of resistant varieties, or varieties that tolerate nematode infestation, and that can produce acceptable yields; in conducting field experiments that evaluate the effect of cropping systems on nematode populations, scientist should plan to suit the needs of the farmer. Many research station trials are designed to produce results for ready analysis and presentation in journals; in consequence, treatments may be selected to suit the needs of the scientist. Most farmers grow short cycle, annual and perennial crops in close proximity. Such mixed cropping practices are rarely undertaken in field experimentation by nematologist. Any new cultural, microbial or management approaches that are developed must be within the capabilities of the farmer and meet the necessary environmental and economic requirements. New strategies will be considered as successful if they lower nematode damage thresholds. The farmer will be satisfied if these treatments are reliable, practicable and economically justified; his customer, the consumer, will be content if the product has the desired quality, contains no toxic residues and fairly priced.

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