RECENT TRENDS IN NEMATODE MANAGEMENT PRACTICES: THE INDIAN CONTEXT

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Abstract - *Human existence has depended heavily on the ability of plants to harness energy from sunlight and fix it into carbohydrates and to produce oxygen and organic matter. Today, agriculture is a global business and a necessity for the fulfillment of human needs of food, fiber, medicines and drugs, drinks etc. Plants dominate our lives and economy, just as they have in all civilizations. Humankind is not alone in need of plants for survival, microbes do exist which derive their nutrition from plants, either as saprophytes or as parasites. Plants are in a continuous battle to defend themselves from these pathogens and understanding these responses will ensure good agricultural outcomes. Of these pathogens, nematodes are emerging as a serious concern in agriculture, horticulture and forestry. Nematodes pose a serious threat to the production of economically important crops, vegetables and, a lesser extend to some tree species also. Some estimates suggest they cause 77 billion dollars of damage worldwide each year. In order to banish this limiting factor in agricultural production, it is essential to accurately identify the nematode infestation and to understand their biology. Although nematicides have emerged as an effective and economically feasible tool against nematode infestation in plants, but they have also raised environmental, resistance development and health concerns during the last decade. A gradual shift of interest towards developing more sustainable methods for nematode management has been noted in recent years. Current work summarizes the recently developed approaches for the control of nematode populations through genetic manipulations of host plants, design of cropping systems, integrated pest management, development of naturally occurring nematicides and employing biological control methods. Since these alternatives are more target specific and of biological origins, they will better respect the diversity and environment and hence, priorities must be urgently established for research and development in field of applied nematology.*

***Key Words*: Phytoparasities, Bionematicides, Resistance Genes, *RNAi*, Microbial Control Agents**

1. INTRODUCTION

Nematodes are un-segmented microscopic roundworms, resembling a tiny thread, the characteristic responsible for the origin of their groups name (Greek word, *nema* = thread and *tode* = form). Members of the phylum Nematoda (round worms) have been in existence for an estimated one billion years, making them one of the most ancient and diverse group of animals on earth [28]. Nematodes have evolved extensively to inhabit vast niches, from aquatic to marine and terrestrial, present on planet earth. Soil moisture and relative humidity directly affect nematode survival as they depend on moisture for locomotion and active life cycle. Nematodes are mostly free-living and feed on bacteria, fungi, protozoan's and other nematode (40% of the described species); many are parasities of animals (invertebrates and vertebrates, 44% of the described species) and plants (15% of the described species) [13].

Nematodes are well equipped to parasitize their host plants. All of them posses a strong, hollow, syringe like structure called stylet at their head region directly attached to pharynx. The nematode uses this stylet to puncture plant cells, to withdraw food, and also to secrete protein and metabolites that aid in parasitism. Stylet represents an important evolutionary adaptation in nematodes as they vary in shape and size according to the feeding habits.

All nematodes undergo four molts from the juvenile to the adult phase in their life cycle. In many nematodes the first molt usually occurs in the egg and it is the second-stage juvenile that hatches. Nematodes display a wide range of feeding behaviours (or trophisms). Many species of nematodes are phytophagous (obtaining nourishment directly from plants), some are microphagous (feeding on small microorganisms) and, saprophagous (feeding on dead and decaying organic matter), whilst others are predatory. Parasites of invertebrates and vertebrates are also prevalent. The plant parasitic nematodes, which are of considerable economic importance, can be majorly restricted into two classes, the endoparasitic nematodes and ectoparasitic nematodes. In endoparasitism, the entire nematode penetrates the root tissue. Migratory endoparasites, such as *Pratylenchus* and *Radopholus*, retain their mobility and have no fixed feeding site within the plant tissue, whereas the more advanced sedentary endoparasites have a fixed feeding site and induce a complex system of feeding cells (syncytia or giant cells). Ectoparasitic nematodes remain in soil and do not enter the plant tissues. They fed by their stylet to puncture plant cells- the longer the stylet, the deeper they can feed.

2. PHYTOPARASITIC NEMATODES AND AGRICULTURE

Phytoparasitic nematodes are able to exploit all the plant parts but the most economically important nematode species exclusively infect roots. Reliable figures of economic damage on crop yields, caused by nematodes cannot be produced. It is because of the fact that infection by nematodes do not results in any obvious symptoms and crop loss may be entirely due to secondary infections after wounding. Moreover, the most prevalent control measures such as soil fumigants target a range of pathogens, not nematodes alone; hence the net impact of nematodes on agriculture is difficult to estimate. Based on extensive surveys, worldwide crop losses certainly exceed $100 billion annually. Damage figures differ from crop to crop and between different climates also. It has been estimated that the overall yield loss averages over 10%, with this figure approaching 20% for some crops. In developing countries, having tropical or sub-tropical climates, nematode damage was estimated to be about 14.6% compared with 8.8% in developed countries. The degree of damage caused by phytoparasitic nematodes also depends on host genotype and its age, soil environment and climatic conditions.

A relatively small group of nematodes are most economically sound and able to attack vegetables, food, cereals, fiber, ornamental crops and some tree species. The principal genera includes the sedentary root knot (*Meloidogyne spp.*), and cyst endoparasities (*Globodera* and *Heterodera spp.*) nematodes, as well as several migratory nematodes (including *Pratylenchus* and *Radopholus spp.*).

Table 1: summarizing the nematode pest of major crops in India and the estimated damage.

Host crop	Nematode sp.	Yield loss (%)
Wheat	Cereal cyst nematode (Heterodera avenae) Wheat seed gall nematode (Anguina tritici) Lesion nematode (Pratylenchus thornei)	32.4-66.6%
Rice	White tip nematode (Aphelenchoides besseyi) Root-knot nematode (Meloidogyne graminicola) Rice root nematode (Hirschmanniella oryzae/H. gracilis/H. mucronata) Rice cyst nematode (Heterodera oryzicola)	10.54%
Maize	Maize cyst nematode (Heterodera zeae) Lesion nematode (Pratylenchus zeae)	17-29%
Pulses	Root-knot nematode (Meloidogyne spp.) Reniform nematode (Rotylenchulus reniformis) Pigeonpea cyst nematode (Heterodera cajani) Lesion nematode (Pratylenchus thornei)	Mungbean and Urdbean 8.90% ,Pigeonpea 12.62%, Cowpea 27.30% ,Chickpea 18.30%
Oilseed	Root-knot nematode (Meloidogyne spp.) Reniform nematode (Rotylenchulus reniformis) Cyst nematode (Heterodera cajani)	Groundnut 21.60% ,Castor 13.93%, Sesame 4.40%
Vegetables	Root-knot nematodes (Meloidogyne incognita and M. javanica) Reniform nematode (Rotylenchulus reniformis)	Tomato27.20% , Brinjal 16.62% , Okra 14.10% , Chilli 12.85%, Cucurbits 18.20% , Carrot 18.20%
Fibres	Root-knot nematode (Meloidogyne incognita) Root-knot nematode (Meloidogyne javanica) Reniform nematode (Rotylenchulus reniformis) Lesion nematode (Pratylenchus spp.) Stunt nematode (Tylenchorhynchus spp.) Spiral nematode (Helicotylenchus spp.)	Jute 12.0-54.4% , Cotton 18-32%
Banana	Burrowing nematode (Radopholus similis) Root-knot nematode (Meloidogyne incognita) Reniform nematode (Rotylenchulus reniformis) Spiral nematode (Helicotylenchus multicinctus) Lesion nematode (Pratylenchus spp.) Cyst nematode (Heterodera oryzicola)	7.9-34.6%
Citrus	Citrus nematode (Tylenchulus semipenetrans) Stunt nematode (Tylenchorhynchus brevilineatus) Reniform nematode (Rotylenchulus reniformis) Root-knot nematodes (Meloidogyne indica)	6.8-17.5%

3. EFFECTS OF NEMATODE INFECTION ON PLANT HEALTH

Nematodes have a characteristic impact on the roots of host plant they infect. Some of the symptoms of nematode infection are very conspicuous but others are hardly resolved. In some cases, the severity of visible symptoms (as measured by root gall index) can be proportionally related to reduction in yield. Root infesting nematodes results in loss of harvest quality in crops with edible tubers, rhizomes and taproots. Nematode infected plants behaves similar to the plants with deformed or damaged root system. Different genera infesting different plant parts show a range of variable symptoms. Apart from formation of root galls and cysts in the roots, the above ground symptoms of nematode infestation include:

- Stunted shoot growth and subsequent decrease in shoot-root ratio

- Chlorosis in foliage due to nutritional deficiencie
- Temporary wilting, due to water stress caused by root inefficiency to transport water
- Decrease in plant fresh weight

4. MANAGEMENT PRACTICES

Plant parasitic nematodes have specialized functional niche in the soil of crop fields. Therefore, by altering their natural habitat (i.e. the soil) we can control their population buildup and further spreading. The first and foremost thing to combat the disaster caused by parasitic nematodes in fields, is the use of principle that "Prevention is always better than cure". Precautionary practices are essential to get rid of high population densities of nematodes in fields.

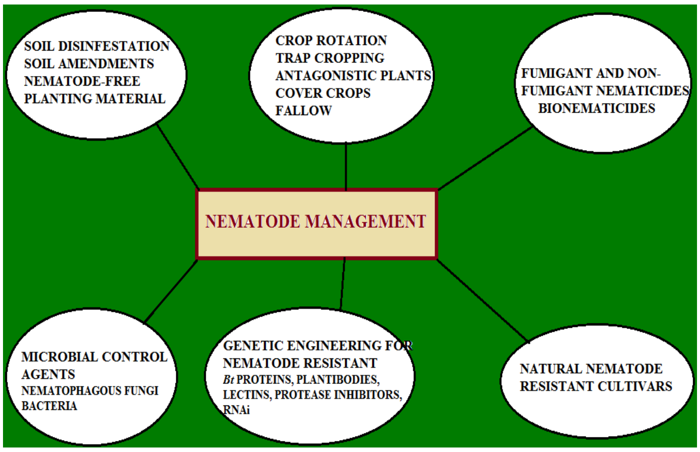

Fig-1: Illustrating all the possible methods of nematode management prevalent in India.

Soil should be thoroughly examined for nematode population densities before planting. They should be some orders less than the damage level threshold density; else they can destruct the whole crop in future. Method of treating the nematode infested soil should be according to the need and cost as that of the final crop value. Physical soil treatments includes dry heat, steam, solarization, flooding etc. and each one of them either modify soil temperature or moisture content for nematode control. Restricting movement of nematodes between fields through implements, irrigation or waste products is of utmost importance. If possible, seedlings should be grown in un-infested soil by the farmers themselves from healthy propagules and if the planting material is obtained from nursery in the form of seedlings or tubers they should be carefully examined for any galls or knots. Time of planting should be planned accordingly to avoid most active periods of nematodes for destruction. Diseased plantation areas should be burned and not to be mixed with manure or fed upon by animals.

4.1 Crop Rotation

Seasonal rotation of many different crops with different nutritional demands and different pests, on the same field has obvious benefits in pest management. The primary aim of crop rotation is to prevent the overlapping of most infective stages of nematodes with that of their susceptible host plants using a poor or non-host species. It ensures that nematode population densities decline below the threshold limit of damage before planting the susceptible crop. Seasonally rotating main crop with poor hosts, resistant or tolerant cultivars, nematode antagonistic plants, trap crop or cover crop have shown significant results. While crop rotation is an environmentally sound practice, it is not always convenient to perform. Nematodes with most damaging potential are polyphagus i.e. they have a broad host range which renders benefits of crop rotation useless. In the lieu of controlling a single nematode species, outbreak of an antagonistic species with more aggressive parasitism may result.

4.2 Nematicides

The pesticides used to control nematode populations, act either by directly killing them in lethal doses or as nematistatic in sub-lethal doses by hampering their reproduction. They are also known to limit transmission of nematode borne virus species also. Being cost effective and efficient for the control of a range of nematode species, they can be seen as a handy tool of farmers. They can be easily applied and works instantaneously. Based on their mode of application two classes of chemical nematicides, halogenated fumigants or non-fumigants are predominantly used globally. Once applied to soil, nematicides start degrading but they should be sufficiently persistent to reduce nematode populations. Losses by volatilization or through leaching and surface run-off may result in contamination of natural sources. Nematicides also affects non-target organism either through direct contact or ingestion or by altering their natural environment. With an increasing public awareness for pesticide dangers and after withdrawal of many routinely used nematicides, there is an urgent need of a safe and sustainable alternative. Biological nematicides can be an answer to this, but their efficacy remains to be proven. Since these are naturally occurring active compounds, they will better respect the environment and safety of other organisms including humans. There are also green manures and soil amendments that affect both nematode populations and plant growth [30]. Some green manures have been shown to have direct nematicidal activity, e.g. butyric acid formed by the decomposition of grasses timothy and rye, and isothiocyanate formed by decomposition of of oilseed rape and other brassicas [31].

Table-2: Showing the list of compounds proposed for development as commercial biological nematicides.

Structural class	Extracted from	Active substance	Active against
Polythienyls	Tagetes erectaplena	thiophene ®-terthienyl	Globodera rostochiensis Anguina tritici
Isothiocyanates and Glucosinolates	Brassica and Sinapsis spp	allyl isothiocyanate 2-phenylethyl isothiocyanate	G. rostochiensis
Cyanogenic Glycosides	Sorghum sudanense, Manihot esculenta	Dhurrin Linamarin	M. hapla
Polyacetylenes	Helenium sp. Rudbeckia hirta	tridec-1-en-3,5,7,9,11 pentayne thiarubrine C	M. incognita P. penetrans Pratylenchus coffeae
Alkaloids	Physostigma venenosum, Bocconia cordata Crotalaria spectabilis	Physostigmine, chelerythrine sanguinarine, and bocconine monocrotaline	D. dipsaci Rhabditis sp. Panagrolaimus sp. M. incognita
Terpenoids	Ocimum basilicum, O. sanctum; Mentha piperatum, Callistemon lanceolatus; and clove, Eugenia caryophyllata , Cymbopogon caesius,	linalool,eugenol , menthol, cineole, geraniol	A. tritici, M. javanica, T. semipenetrans, Heterodera cajani
Sesquiterpenoids	Gossypium hirsutum, Solanum tuberosum, Pinus massoniana	Hemigossypol, Solavetivone ®-humulene	M. incognita G. rostochiensis B. xylophilus
Diterpenoids	Daphne odora	odoracin	Aphelenchoides besseyi

4.3 Use of resistant cultivars & Biotechnological Approaches

Development of bioengineering strategies for raising transgenics resistant against nematode parasitism and disease is a durable method. The strategies come under three categories: (i) transfer of natural resistance genes from plants that have them to plants that do not, to mobilize the defense mechanisms in susceptible crops; (ii) interference with the biochemical signals that nematodes exchange with plants during parasitic interactions, especially those resulting in the formation of specialized feeding sites for the sedentary endoparasites- many nematode genes and many plant genes are potential targets for manipulation; and (iii) expression in plant cells of proteins toxic to nematodes [24]. Past many years have witnessed cloning and characterization of a wide range of plant R genes. These genes are often found in a cluster containing many homologues, including inactive copies. Perhaps these represent islands of potential genetic diversity that can be altered to create new R-genes under the selective pressure of new pathogen *avr* genes [26].

Table-3: Description of some R-genes for nematode resistance

Gene	Characteristics	Source	Target
Hs1 pro-1	N-terminal signal Sequence, leucine-rich domain, short positively charged C-terminal region [2]	Beta procumbns	Heterodera schachtii
Mi1.2	1257 amino acids, nucleotide-binding domain-LRR proteins, leucine zipper domain at N terminal [21]	Lycopersicon esculentum	Meloiodgyne incognita M. javanica M. arenaria Potato aphids
Gpa2	912 aa, leucine-zipper, nucleotide-binding site (NBS), LRR [27]	Solanum tuberosum	Globodera pallid
Hero	1283 aa (148 kDa) with no signal sequence, NBS-LRR type [6]	Tomato	95% resistance to Globodera rostochiensis and over 80% resistance to G. pallid

Recent technological advancements have relieved the task of transferring R genes to susceptible plants and raising resistant transgenics, even though it is not the most preferred method. It can because some success reports are not authenticate enough to implement this costly affair on broader scales. Too specific targeting of nematode species by resistant plants has resulted in establishment of other more aggressive nematode parasities on the same field. Plants can be engineered to directly target nematode.

The best known, insecticidal *Bacillus thuringiensis* Cry proteins, causes death of nematode juveniles. A four-fold reduction in the progeny of *M. incognita* was demonstrated by expressing Bt Cry6A in tomato hairy root culture [15]. Lectins, the glucan binding protein, are known to bind chitin present in nematode body walls or disrupt their sensory perception. Engineering potato for constitutive expression of snowdrop lectin (GNA) has provided partial resistance to *G. pallida* [1].

The most explored approach for engineering nematode resistance in plants is by expressing protease inhibitors in plant roots. Most of them are naturally occurring plant proteins and are detrimental to nematode feeding and growth. The potential use of PIs as anti-nematode effectors was first demonstrated using the serine PI cowpea trypsin inhibitor (CpTI). The rice cystatin Oc-I is the most favoured one. Expression of its engineered variant using CaMV35S promoter in tomato hairy roots resulted in much smaller G. pallida females after 6 weeks as compared to control roots [26]. These do not cause negative effects on humans or livestock, environment, other insects and soil microflora.

4.4 Microbial Control

When all the currently available practices for nematode control like crop rotation, nematicides, and resistant transgenics are proving ineffective in one or the other aspect, microbes as biocontrol agents for nematode control came to the rescue. Microbial biocontrol can be broadly described as the use of microbial inoculums or their derived metabolites to reduce the pest populations below threshold density or to combat its disease establishment on susceptible plants. Numerous species of nematophagous fungi and bacteria, and several species of algae are reported. These microorganism has evolved sophisticated ways for affecting particular stages of life cycle of phytoparasitic nematodes. Like, adhesing and digesting by secreting an array of degrading enzymes, trapping by forming trap rings or knots and directly digesting them. These pronounced effects on phytoparasitic nematodes, makes these soil residing microorganism ideal for use as biocontrol agents.

Currently, more than 700 species of nematophagous fungi have been described. These fungi belong to diverse phylogenetic groups, including Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota [11].

Table-4: Enlisting commercially available microbial-derived products for nematode control

Active antagonist	Products	Crop	Company or institution/country
Paecilomyces lilacinus	Biocon	Unspecified	Asiatic Technologies, Inc./Philippines
	Bioact	Vegetables	Prophyta/Philippines; Bayer CropScience/ United States
	PIPIus	Vegetables, citrus, banana	BCP/South Africa
	Yorker	Vegetables,trees	Agriland Biotech Limited/India
	PL Gold	Banana, tomato	BASF Worldwide/Germany
Myrothecium verrucaria	DiTera	Vegetables, almonds, fruits	Valent Biosciences Corp./Canada
Pochonia chlamydosporium	Xianchongbike	Tobacco, peanut, soybean, watermelon	Laboratory for Conservation and Utilization of Bio-resources, Yunnan University
	Klamic	Vegetables	Cuba
Bacillus firmus	Bio-Nemax	Vegetables	M.J. Exports/India
	BioNem-WP	Vegetables	Bayer/Germany
	BioSafe	Vegetables	AgroGreen/Israel
	VOTiVO	Vegetable, corn	
Pasteuria usgae	Econem	Vegetables, turf, soyabean	Pasteuria Bioscience/United States; Nematech/Japan; Syngenta/Switzerland
Pseudomonas fluorescens	Sudozome	Vegetables, fruit trees	Agriland Biotech Limited/India
Paenobacillus macerans	BioYield	Tomato, bell pepper, strawberry	

Distribution of these fungi in diverse phylogenetic groups suggests that they have evolved independently many times by convergent evolution [7]. Nematophagous fungi can be grouped into four different classes, on the basis of their mode of action, namely endoparasitic, nematode trapping, toxin producing and egg- parasitic fungi.

Nematode trapping fungi are known to produce characteristic structure called nematode traps, capable of capturing nematode and killing it. These fungi spent a considerable time as free living saprophytes in soil. Once they encounter a nematode in there vicinity, they shifts towards a predatory phase. Predation involves formation of nematode trapping structures like constricting or non-constricting rings, adhesive knobs, networks or columns. With the development of sequencing technology, the whole genomes of three nematode-trapping fungi (an adhesive networks-forming species, *Arthrobotrys oligospora*; an adhesive knobs-forming species, *Dactylellina haptotyla*; and a constricting rings-forming species, *Drechslerella stenobrocha*) have been sequenced [16]. There genomic data will greatly help in understanding the molecular mechanism involved in nematode trapping and further manipulation of which may resolve the present nematode crisis.

Endoparasitic fungi produce infective zoospores or conidia which penetrates nematodes directly by rapidly germinating and forming assimilative hyphae. This group lives as saprophyte for a very limited period and produces negligible mycelium in the soil, which have resulted in narrowing down of their use as biocontrols. *Drechmeria coniospora* is the most studied endoparasitic fungus. It is capable of producing as many as 10,000 adhesive conidia for infecting a single nematode. Its mature conidium produces adhesive buds to firmly attach to nematodes cuticle or to sensory organs in head or vulva region. After adhesion, an infection vesicle develops within the cuticle layers, and trophic hyphae are then produced inside the infected nematode. The fungal hyphae grow and digest the nematodes, typically within three days at which point conidiophores form new conidia and protrude from the nematode corpses [18].

Egg- parasitic fungi directly infect the nematode eggshells with the help of appressorium or lateral mycelia branches. Important species of this group, all belongs to the clavicipitaceous fungi in Ascomycota, are *Pochonia chlamydosporia*, *Paecilomyces lilacinus*, *Clonostachys rosea*, and *Lecanicillium psalliotae*. Genome of *P. chlamydosporia* has been sequenced recently.

A range of nematicidal compounds are produced by **toxin-producing fungi** which immobilizes nematode before their hyphae penetrates its cuticle. More than 200 compounds with nematicidal activities have been identified from approximately 280 fungal species in 150 genera of Ascomycota and Basidiomycota. These compounds belong to diverse chemical groups, including alkaloids, peptides, terpenoids, macrolides, oxygen heterocycle and benzo compounds, quinones, aliphatic compounds, simple aromatic compounds, and sterols [14]. Interestingly, in addition to producing toxins, two basidiomycete fungi, *Coprinus comatus* and *Stropharia rugosoannulata*, can produce special nematode-attacking devices, namely spiny ball and acanthocyte, respectively.

Nematophagous bacteria also constitute a major group of soil microorganisms that are capable of preventing infections from a wide range of nematode species including free living and parasities of plants and animals. Based on their mode of actions, nematophagous bacteria can be classified as obligate parasitic bacteria, opportunistic parasitic bacteria, rhizobacteria, parasporal Cry protein-forming bacteria, endophytic bacteria, and symbiotic bacteria [25]. In soil, *Bacillus*, *Pseudomonas*, and *Pasteuria* predominantly act as nematophagous bacteria.

Bacillus thuringiensis (*Bt*) the ideal biopesticide , known to specifically kill caterpillars and beetles is also reported to target nematode populations but do not affect vertebrates. For the first time in 1972 it was reported by Prasad *et a*l that populations of *Meloidogyne incognita* were significantly reduced by treatment with *B. thuringiensis* var. *thuringiensis.* Since then, a number reports are published on the successful use of *Bacillus thuringiensis* as an substitute of nematicides in organic agriculture systems. *Bacillus thuringiensis* (*Bt*) produces many different kinds of proteinaceous protoxin crystals (called crystal protein or Cry protein) during sporulation, which targets nematode larva. Currently, three families of Cry proteins have been found to exhibit potent activities against the larvae of nematodes (Cry5, Cry12, Cry13, Cry14, and Cry21 in the Cry5 family, Cry6 in the Cry6 family, and Cry55 in the Cry55 family) [14, 15, 29]. *Bt* strains are highly susceptible towards exposure to UV-radiation and other environmental chemicals, which restricts its use as an commercially available BCA in agriculture.

Bacillus nematocida (B16), using a Torjan horse mechanism lures nematodes to their death. It is reported to be highly nematicidal against the nematode *Panagrellus redivivus* [8]. This bacterium secrets benzaldehyde and 2-heptanone, as VOCs to attract nematodes. After the nematode has consumed it as food, it secretes a range of extracellular proteases which lyses the intestinal tissues, eventually killing it.
Pasteuria penetrans, an obligate, endospore-forming parasitic bacterium, can colonize more than 300 nematode species, including the majority of important PPNs and free-living nematodes. The obligate nature makes *P. penetrans* a promising agent for biological control of PPNs [11]. Many other *Pasteuria* species are known to infect nematodes like *Pasteuria thornei* infects *Pratylenchus* spp. (root lesion nematodes), *Pasteuria usgae* infects *Belonolaimus* spp., and *Pasteuria nishizawae* parasitizes *Heterodera* spp. and *Globodera* spp. [20]. Although the use of *P. penetrans* to control RKN is promising, its fastidious nature (the inability to grow outside its hosts and its host specificity) limits its commercial application as an effective BCA [3].

The primary obstacle in commercializing a BCA for usage by farmers is there inconsistent performance in the fields. The success rate is greatly affected in natural soil settings. Since, BCAs have to act in soil in presence of different factors like adaphic conditions, weather, and soil inhabiting microbes, which may have synergistic or antagonistic effect on their activity. No matter how well suited a nematode antagonist is to a target nematode in a laboratory test, rational management decision can be made only by analyzing the interactions naturally occurring among ''host plant–nematode target–soil–microbial control agent (MCA)–environment'' [4]. Potential advantages of biocontrol agents applied in combination include: (i) multiple modes of action against the target pathogen or nematode; (ii) ability to affect more than one stage of the life cycle of the target organism; (iii) activity of microbes during different times in the growing season; (iv) increased consistency in performance over a wider range of soil conditions, stemming from the different environmental niches of the applied microbes; and (v) potential to select organisms that affect more than one plant pathogen or pest, thus increasing the spectrum of uses for the product [17]. The negative interactions between different BCAs co-applied should be minimized in order to get better results out of there positive interactions. Understanding the ecological basis of the interactions among these co-applied biocontrols will greatly help in maximizing their performance. For commercial development of preparations of multiple biocontrols, quality control and shelf life are major difficulties encountered, which also adds to the cost. Currently, products containing more than one species of microbe are not sold specifically as biocontrol agents for plant-parasitic nematodes [17].

5. CONCLUSIONS

With the increase in documentation of nematode outbreaks and crop damage figures over the last few decades, the development of management strategies has also matured. Recent withdrawal of nematicides from market, one of the most efficient and cost effective method, have created an urgent need for development of a sustainable alternative. Nematologists have come up with bionematicides as an answer to it; they are as much effective as nematicides but better respect the environment being from plant origin. Although, nematicidal potential of phytoextracts from a large number of plant families has analysed, but there mode of action, concentrations within plants in natural settings and celllular or subcellular locations are not yet worked out. Cultural methods like crop rotation, organic soil amendments and use of resistant cultivars have gained much attention. Farmers are now aware of importance of preventionary measures and accepting more environmental friendly control measures. A large body of research is now involved in describing alternate host species for crop plants in crop rotation which have a significant market value. Trap crop and cover crop are recently introduced ideas which can overcome constrains imposed by crop rotation and are still effective enough. Increase in available genetics and molecular biology tools has resulted in an increase in identification, characterization and cloning of large number of nematode resistance genes naturally present in some cultivars of crop plants. Biotechnology and tissue culture have opened new possibilities for successfully engineering and regenerating transgenics with nematode resistance. Large variety of plants has developed and is being used commercially but some limitations do exist. There effect on environment and microbial diversity has not yet determined. With some advanced handy tools like *RNAi*, a shift in efforts towards describing nematode parasitism genes and there subsequent silencing, has been noted. They are yet far behind in commercial exploitation. Understanding of molecular mechanisms underlying complex interactions between nematophagous microbes and nematodes have progressed significantly. With the increase in –*omics* data, functional description of key genes which determines mode of action of BCAs have become convenient. In the near future, there targeted genetic manipulations should lead to improvements in efficiency and nematicidal potential of BCAs in PPN management. In the past, research in pathology has focused majorly on studying interactions between one host and one pathogen. It was not realized until recently that nematodes are a part of complex soil ecosystems and food webs and for any management approach to be sustainable, it must exploit these complex natural interactions. Strategies that use multiple microbial control agents with complementary and synergistic modes of action, and integrate biological control agents with other control methods, such as chemical nematicides, nematode-resistant cultivars, and crop rotations, could be highly effective in reducing pest nematode populations. Greater understanding of the molecular mechanisms of microbe-nematode interactions will provide further guidance from which to develop more effective strategies.

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