PART III

STUDIES ON PLANT PARASITIC NEMATODES

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INTRODUCTION

Sustainable agriculture aims at reducing the incidence of pests and diseases to such a degree that they do not seriously damage crops without upsetting nature’s balance. One of the aims of sustainable agriculture is to rediscover and develop strategies whose cost and ecological side-effects are minimal. The use of synthetic pesticides has resulted in an increased crop production. But the use of synthetic pesticides poses serious environmental and public health problems. Exposure to pesticides both occupationally and environmentally causes a wide range of health problems. It is estimated that nearly 10,000 deaths annually occur due to the use of chemical pesticides worldwide, with about three-fourths of these occurring in developing countries.

In India, crops are affected by over 200 major pests, 100 plant diseases, hundreds of weeds and other pests like nematodes, harmful birds, rodents etc. Approximately, 30% of Indian crop yield potential is being lost due to insects, disease and weeds which in terms of quantity it would mean 30 million tones of food grain. The value of total loss has been placed at Rs 50,000 million, representing about 18% of the gross national agriculture production.

3.1.1 Plant parasitic nematodes

Plant parasitic nematodes constitute an important group of pests in agro-ecosystems. They are members of a primitive group of animals called non segmented round
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worms and are obligate parasites ie, they can only feed on living plants. They are found in soils and plant roots, few species may attack above ground parts of the plant. Plant-parasitic nematodes occur in all sizes and shapes. The typical nematode shape is a long and slender worm-like animal, but often the adult animals are swollen and no longer even resemble worms. Most species are microscopic, length ranging from 300-4000µm and diameter of 10-35µm. Nematodes often look segmented because of the numerous annulations on the cuticle that allow the nematode to bend without kinking, but in fact nematodes are unsegmented and have no replication of body parts throughout the worm. Like most higher animals nematodes possess bilateral symmetry, but with a superimposed trilateral and hexalateral symmetry. Nematodes undergo four molts during their life cycle. Most nematodes may be observed easily under a dissection microscope at a magnification of 40X to 60X. Plant-parasitic nematodes range from 250 µm to 12 mm in length, averaging 1 mm, to about 15-35 µm in width. The time taken for plant parasitic nematodes to complete their life cycle varies from few weeks to more than a year depending on the nematode species, plant host status and ambient temperature.

Nematodes which feed on roots from outside are referred as ectoparasites and those feed from inside of the root is named as endoparasites. Free living nematodes (non-parasitic nematodes) feed on dead organic matter or microorganisms associated with organic matter. These nematodes recycle nutrients in soil and are an important component of ecosystems. Types and number of free living nematodes in soil samples form an indicator of the health of the ecosystem. Soil texture and structure are of primary importance in determining the number and types of plant parasitic nematodes found on suitable hosts.

Nematode problems are more prevalent in warmer, humid climates. Most plant parasitic nematodes do not produce any obvious or easily distinguishable symptoms on their host plant because these microscopic organisms feed from below the ground, many problems remain undiagnosed. Plant parasitic nematodes are typically found in the root zone of suitable host plants with most of the population occupying the top 15cm of soil. Above ground symptoms may include stunting, yellowing, and wilting. Root symptoms may include galls, lesions, stunting, stubby appearance and
excessive branching. Crop losses due to the nematodes range from 10-60% of yield depending on species, host status and environmental condition.

3.1.2 Root-knot nematodes

Root-knot nematodes (*Meloidogyne* species) are a wide spread and diverse group of plant parasitic nematodes. Although more than 100 species have been reported, four root-knot nematodes species *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, and *M. hapla* cause most of the damage in agricultural crop plants. Of these four, *Meloidogyne incognita* is the most common and has a wide host range. Root-knot nematodes are found all over the world, but are more common in warmer climates and in light sandy soils.

The taxonomic position of *Meloidogyne* may be given as:

- **Phylum**: Nematoda
- **Class**: Secernentia
- **Order**: Tylenchida
- **Family**: Meloidogynidae
- **Genus**: *Meloidogyne*

Root-knot nematodes female lay eggs in a gelatinous matrix and first stage juveniles undergo one molt while still in the egg. Worm-shaped or vermiform (length 400µm and diameter 15 µm) second stage juveniles hatch from the eggs using their stylet to break through the tough egg casing. Second stage juveniles are the only infective stage of root-knot nematodes. After penetrating suitable hosts at the root tips, the juveniles migrate to the developing vascular cylinder and begin feeding on several cells near the endodermis. These cells enlarge, become multinucleate, and serve as feeding cells for the rest of nematode life cycle. A gall rapidly begins to develop around the feeding juvenile as a result of cell division and cell enlargement. Once juveniles begin feeding, they undergo a series of three additional molting. The third- and fourth- stage juveniles are short lived stages and are slightly swollen to sausage shaped in appearance. Sexual dimorphism is found in root-knot nematodes. Females become enlarged and spherical (diameter 400 µm; length 700 µm) whereas males molt to a relatively large vermiform shape (diameter 30 µm; length1400 µm) and migrate from the root. Posterior end of the adult female usually protrudes from the surface of the root gall, where an egg mass containing 300-600 egg is produced.
Life cycle requires 21-50 days, depending on the species, plant host and environment (Figure 3.1.1). They survive intercrop periods primarily as eggs in the soil.

**Figure 3.1.1**: Life cycle of Root knot nematodes

Root-knot nematodes are essentially parasites of underground root, stem, etc affecting the nutrients and water uptake of the plants. The plants became dwarfed, yellowish with smaller foliage with poorer and fewer fruits. Infected plant may be stunted, chlorotic and wilted during the daylight hours (Plate II). Galls or knots on host roots due to nematode infection and are used for diagnosis (Plate III). Plant parasitic nematodes in addition to causing diseases complexes with bacteria, fungi or
viruses infecting common agricultural crops, are also known to act as vectors of the all the above pathogens causing diseases on a wide range of hosts.

Plate II: *Meloidogyne incognita* infected Tomato plant
Plate III: *Meloidogyne incognita* infected plant root with root galls
The effect of *Meloidogyne* on plant growth and yield vary. The damage caused by the nematodes on susceptible plants involves the removal of plant nutrients, changes in the nutrient flow patterns in plant tissues and retardation of root growth, all of which resulted in the suppression of yield. Adult female nematodes require large amount of nutrients for egg production and compete with the host for the pool of nutrients in the roots. The increased metabolic activity of giant cells stimulates mobilization of photosynthates from the shoots to roots for the utilization by the nematodes; this activity was maximum when the adult female commences egg laying. Disturbances caused by the *Meloidogyne* on plant have significant impact on the physiology of the whole plants, which ultimately affect the yield (Goswami and Mittal, 2004).

### 3.1.3: Mode of nematode feeding

Plant parasitic nematodes are characterized by the presence of hollow needle like structures called stylet present in the anterior end. Stylet is used to puncture cell wall by repeated thrusts. Once stylet puncture cell, the secretions from the oesophageal gland are passed into plant and then partially digested food is sucked back through stylet by the pumping action of the oesophagus. Ectoparasitic nematodes remain in the soil and feed from outside. They may rarely enter the plant tissue. Semiendoparasite burry their head deep inside the plant tissue whereas the remaining body develops outside root, while the endoparasite nematode enter into the plant tissue. Endoparasite may be sedentary i.e, develops to maturity at the site of penetration or may be migratory, when they keep on changing their feeding sites. Endoparasitic nematode may also enter through natural opening such as stomata or the point of emergence of secondary roots. Root-knot nematode or cyst nematodes also modify the host cells, cell around nematode head are large sized called giant cells, with large sized nucleus or nucleolus, dense cytoplasm and partially broken cell walls (syncytial cells). Root-knot nematode also induces excessive hyperplasia in the pericycle and cortical hypertrophy. Nematodes activity also induce increased synthesis of proteins, nucleoproteins, dehydrogenase and diphorase activity. Plant cells fed by nematodes are usually not killed.
3.1.4 Nematode Control

Based on extensive survey data (Sasser and Freekman, 1987; Koenning et al., 1999) it has been estimated that overall annual yield loss averages 12.3%; and this value may go up to 20% for the same crops at certain conditions. In monetary terms, the worldwide figure may exceed $ US 100 billions annually (Bird et al., 2003). The extent of crop damage (reduction in yield loss) by plant parasitic nematode is directly related to their populations. The minimal density that causes a measurable reduction on plant growth or yield is regarded as the damage threshold density, and when the cost of production and the value of a given crop are considered, the term economic threshold density is used. Economic thresholds can be defined as population density of pest at which the values of damage caused is equal to the cost of control. At densities up to the threshold there would be no economic incentive for pest control inputs since cost would exceeds crop loss. As nematode population rises above thresholds of economic loss, control become more difficult and more essential. The threshold density varies with nematode species, race, plant variety, environment and their reproduction potentials on the plant (Barker and Olthof, 1976). Economic losses start when the initial nematode population (Pi) reaches threshold level. In the absence of effective control it would lead to total crop failure.

The nematode infected plants show poor growth and become less productive because the damaged root systems are less efficient in absorbing water and nutrients. The nematodes feeding on roots cause injuries, which enable entry of other soil-borne pathogens. Whitehead (1998) suggested that 99.9% control is required in order to prevent the subsequent buildup of damaging populations because of the reproductive potential of *Meloidogyne* sp. The magnitude of damage caused by nematodes in combination with other soil-borne pathogens increases.

The nematode control aim to improve growth quality, and yield by keeping the nematode population below the economic threshold level. Control of nematodes and the damage they caused can be achieved by preventing the entry by nematodes, by suppressing their populations, by pacifying their effects or by a combination of these. The nematode control measures include physical, chemical and biological, cultural, host resistance and regulatory methods.
3.1.4.1 Physical methods

It is very easy to kill nematodes with heat, irradiation and by osmotic pressure. But this method is very difficult to adopt in the field. Hot water treatment of planting material is very successful in controlling nematode population.

3.1.4.2 Chemical methods

It involves the use of chemicals called nematicides. They are of two types, fumigants (halogenated hydrocarbon) and non fumigant (organophosphate and carbamate). Fumigants are highly volatile and when applied into the soil they turn to gaseous phase. These vapours diffuse through the pore space and are toxic to the nematodes. Eg. DD (1,3 Dichloropropene), MBr (methyl bromide), nemagom, DBCP(Dibromo chloropropane).

A number of organophosphate and oximecarbamate nematicides were developed in the 1960s, which had the advantage that application was relatively simple (Wright, 1981). These compounds are active at dosages of 2 to 10 kg (a.i)/ha which are smaller than the 200 to 300 litres/ha required for treatment with liquid fumigants. Most of the early formulations of these products were as granules that, when applied to the soil surface (or preferably incorporated in the top 10 cm of soil), release the active ingredient, which is spread through the soil by rainfall or irrigation. The efficacy of soil penetration depends on the amount of moisture, organic matter and soil structure. Heavy soils with relatively small pore spaces are more difficult to treat than sandy soils which have larger pore sizes. Some chemicals, particularly the organophosphates, are absorbed in organic matter, in which case efficacy may be impaired (Bromilow, 1980). Non fumigant includes organophosphate like Mocap, nemacur, phorate, phenamphos and carbamate like aldicarb, carbofuran, oxamyl.

3.1.4.3 Biological methods

Biological methods aim at increasing the parasitic, predator and pathogens of nematodes in the soil to increase the mortality of the plant parasitic nematodes. A fungus *Paecolimyces lilacinus* (oviparasite) and a bacterium *Pasteuria penetrans* (larval and female parasite) are now used as biological control agents. Other organisms that reduce nematode populations include various types of fungi, bacteria, viruses, protozoa and predatory nematodes. Biological control is more inconsistent, less effective and slower acting than control normally achieved with chemicals.
Success of biological control will depend on its integration with other control measures.

### 3.1.4.4 Cultural practices

Cultural control measures are agro economical practices employed to minimize nematode problem in the crop. It includes crop rotation, fallowing, soil solarization, flooding, removal or destruction of infested plants, organic amendments, trap cropping and planting of enemy plants. Nematodes are usually at their most vulnerable at the time when they are actively searching for host roots and when surviving unfavourable growing seasons. The survival of free-living stages in the absence of hosts can be longer than originally thought, and fallow periods of less than 12 months may be insufficient to control those species that reproduce rapidly. The main abiotic factors that influence free-living nematodes and eggs are heat, desiccation and anaerobiosis. Plant-parasitic nematodes will not survive long periods at temperatures above 40°C. These temperatures may be reached on bare soil in some countries during the hot season, but heat penetration may not extend below 10 cm.

The technique of increasing soil temperatures by solarization can be of value in controlling nematodes (FAO, 1991; Gaur and Perry, 1991b), but this is only possible where there are long periods of uninterrupted sunshine. Solarization is also practised for controlling soil-borne fungal pathogens (FAO, 1991). Nematodes are aquatic animals that inhabit the films of water that surround soil particles. As soils dry, the volume of soil water decreases and the ability of nematodes to move is impaired (Wallace, 1963), eventually free-living nematodes may die as the soil moisture decreases. The extent to which species have adaptations to survive desiccation is still a matter for further study. By regulating the metabolism in times of stress some species are capable of anhydrobiotic survival. The contrivance of bare fallows that deprive nematodes of a host may not always be a worthwhile control strategy especially as fallowing in hot, dry areas can deplete soil organic matter and the levels of beneficial soil biota. In such situations for specific nematode pests, a better alternative might be cover crops with non-host leguminous trees or shrubs such as *Crotalaria* spp.

The control of nematodes by flooding has been advocated in certain locations. When flooded all the soil pore spaces are filled and the oxygen supply for the soil microflora and fauna becomes limiting. Many plant-parasitic nematodes are
intolerant of oxygen starvation and soon die. Similar effects are observed when nematodes are stored in deep water in the laboratory, but often they can be revived by aeration. To be effective in the field therefore, the anaerobiosis has to be of sufficient duration to kill the nematodes. Flooding is not a technique that can be widely used for nematode control and whether it is equally efficacious against all plant-parasitic species is not known (Manazanilla lópez et al., 2004).

3.1.4.5 Regulatory methods
Legal enforcement of quarantine regulations has been employed in the case of several nematode diseases to check the introduction of pest disease into new areas or if already introduced to reduce the chance of their further spread.

3.1.4.6 Integrated control
Integrated control or more appropriately integrated nematode management (INM) seeks to stabilize pest nematode population below the damage no levels through the combination of different methods. Use of resistant varieties is also a part of integrated control.

3.1.5 Present work and objectives

Vegetables are important sources of proteins, vitamins and minerals in human diet and are considered next in importance to cereals and staple crops. Despite of the use of high yielding varieties and optimal inputs, the production of vegetables is hampered by the attack of plant parasitic nematodes in combination with other pests and diseases. In agricultural fields, nematode management usually relies on the application chemical pesticides-the nematicides- to the soil. Chemical control of root-knot nematode is very expensive, not sustainable and affects the agro ecosystem adversely. Use of nematicides for the management of plant parasitic nematodes is being restricted due to environmental and human health concerns. With the well-known problems facing the agrochemical industry, such as pest resistance to pesticides and the high costs of developing new products, there has been a renewed interest in biological control agents and naturally occurring pesticides. In addition, nematicides often do not provide long-term suppression of the pathogen. Therefore, there is a need to develop alternative, environmental friendly management strategies for root-knot nematodes, including use of
biocontrol agents and organic amendments. Several studies have been made on the use of organic amendments in controlling the population of plant parasitic nematodes in different crops. In this content, a few experiments were conducted in the present study in order to explore the possibility of using vermicompost, vermitea (an extract of vermicompost) for the management of *M. incognita* infecting vegetable crops. An extensive survey of literature revealed that the studies of the type described in this part using vermicompost have not been reported so far.

The general objective of the second part of the experiment is as follows:

- To study the impact of application of vermicomposts derived by using different substrates on the root knot nematode population in plants.
- To study the impact of application of vermitea derived from different vermicompost on the root knot nematode population in plants.

### 3.1.6: Organization of this part

This part of the thesis entitled ‘Plant parasitic nematode control’ is further divided into 5 chapters. The first and second chapters pertain to introduction and literature review respectively. The third chapter was on the control of root knot nematodes by using vermicompost. The fourth chapter discusses the control of plant parasitic nematode population infecting vegetable crops using vermicompost tea. Summary and conclusion has been included as chapter 5. The third and fourth chapters consist of a brief introduction, materials and methods, results and discussion with respect to that particular chapter.