Chapter 1
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The root-knot nematode, *Meloidogyne* spp. are major agricultural pests of a wide range of crops more than 3000 hosts species including monocots, dicots and several weed species (Abad et al., 2003). The root-knot nematode is sedentary endo-parasite. Numerous females of the root-knot nematodes are present in root galls. These survive in the soil and leaf debris. The primary infection is initiated by eggmass that persists in the soil and from which second stage juveniles (J2) hatch. The genus comprises of more than 90 described species with some species having several races of *Meloidogyne* but the four most common occurring species are *M. incognita* (Kofoid and White, 1919) Chitwood 1949, *M. javanica* (Treub, 1885) Chitwood 1949, *M. hapla* (Chitwood, 1949) and *M. arenaria* (Neal, 1889) Chitwood 1949 (Karsen, 2000; Hunt et al., 2005). These species are distributed worldwide (Karsen and Van Hoenselaar, 1998).

The root-knot nematode larvae infected plant roots, drain the plant’s photosynthate and nutrients (Ashworth, 1991). Infection caused by root-knot nematode in young plants may be lethal, while infection in mature plants cause decreased yield, but high level of root-knot nematode damage can lead to crop-loss. Symptoms of nematode damaged plants generally non-specific and are characterized by poor-growth, plant stunting, crop patchiness, wilting and chlorosis (yellowing and discoloration of leaves). Root-knot infected plants are not
weakened, but their root systems are more susceptible to secondary infection caused by fungi and bacteria.

Newly hatch juveniles have a short free-living stage in the soil in the rhizosphere of the host plant. They may reinvade the host plant of their parent or migrate through the soil to find a new host root. Second stage juveniles (J2) do not feed during the free living stage, but use lipids stored in the gut (Eisenback and Triantaphyllou, 1991). They invade in the root elongation region and migrate in the root until they became sedentary. Signals from the second stage juveniles promote parenchyma cells near the head of the juvenile to became multinucleate (Hussey and Grundler, 1998) to form feeding cells, generally known as “giant cells”, from which the second stage juveniles (J2) and later they adults feed (Sijmons et al., 1994). Concomitant with giant cell formation, the surrounding root tissue give rise to a gall in which the developing juveniles are embedded. Juveniles first feed from the giant cells about 24 hours after they become sedentary. After further feeding the J2 undergo morphological changes and become saccate. Without further feeding they moult three times and eventually become adults. In females, which are close to spherical, feeding become stopped and the reproductive system develops (Eisenback and Triantaphyllou, 1991). The life span of an adult female may extend to three months and many hundreds of eggs can be produced. M. javanica develops in a temperature range of 13-34°C with optimal temperature is about 29°C for the development of adult nematode (Dropkin, 1980).
The root-knot nematode *M. javanica* females lay eggs into a gelatinous matrix (GM) which is produced by six rectal glands and secreted before and during egg laying (Maggenti and Allen, 1960). The matrix, providing a barrier to water loss by maintaining a high moisture level around the eggs (Wallace, 1968). As the gelatinous matrix ages, it becomes tanned and shrunked turning from a sticky colorless jelly to an orange brown substance which appears layered.

The root-knot nematode *Meloidogyne* species cause most extensive damage on Solanaceous and Cucurbitaceous plants. Among the cucurbits, cucumber and bottle gourd were found severely infected crops. As parasites, nematodes affect plant growth and consequently productivity by disrupting physiological processes of the host plant.

**Host Description.**

*Lagenaria siceraria* (Molina) Standl. cv ‘Kanchan’ Family Cucurbitaceae, is popular vegetable grown almost all the year around, particularly in frost-free areas, it can be cultivated in all kinds of soil, but thrives best in heavily manured loams. It requires a warm humid climate or plenty of watering and sunshine when grown during dry weather. Seeds may be sown directly, 4-5 seeds together, in manured beds or kits 5-6 feet apart. They may be also sown in nursery beds and seedlings transplanted when they are in 2-3 leaves stage. The stem of bottle gourd is stout 5 angled and bifid tendrils found either wild or cultivated. Leaves are long petioled, 5 lobed, flowers large, white solitary, monoecious or dioecious vines are allowed to trail on the ground or trained over walls, tree or other support. Young
and tender fruits are eaten as vegetables, large up to 1.8m long usually bottle or
dumb-bell shaped, almost woody when ripe. The flesh is soft, spongy and
insipid when old seeds are numerous, long white, smooth 1.6-2.0 cm long
horizontally compressed with marginal groove. Analysis of the edible portion of
the fruit gave following value:

- Moisture = 96.3 %
- Protein = 0.2 %
- Fat = 0.1 %
- Carbohydrates = 2.9 %
- Mineral matters = 0.5 %
- Calcium = 0.02 %
- Phosphorus < 0.01 %.

The fruit is a good source of B vitamins and a fair source of Ascorbic acid. Edible
portion of fruit contains:

- Thiamine = 44 µg/100g
- Riboflavin = 23 µg /100g
- Niacin = 0.33 mg /100g
- Ascorbic acid = 13 mg /100 g

The pulp of the fruit is considered emetic, purgative, antioxidant,
immunomodulatory and cardiotonic agent. It works as coolant, diuretic, antibilious
Crop losses

The need for food is increasing continuously due to constantly expanding population; with currently more than 6.4 billion people worldwide (Food and Agriculture Statistics Global Outlook, Statistics division, June 2006). Based on the Food and Agriculture Organization (FAO) estimates, the world population will increase to 7.8 billion by 2025 and around 84% of these people will live in the developing countries.

A loss of yield and quality can occur without specific above ground symptoms. The crop losses are defined as the differences between the attainable yield and the actual yield (Chiarappa 1971). However, it is difficult to determine the attainable yield because it depends on many cultural, environment and pest factors. Actual yield is more precisely measured as that obtained without protection from any harm caused by various plant pathogens. Reducing yield losses caused by pathogens of tropical agricultural crops is one measure that can contribute to increased food production. Damage and yield caused by the pathogens are on average greater in tropical than in temperate regions because of greater pathogen diversity, more favorable environmental conditions for pathogen colonization, development, reproduction and dispersal, technical and financial resources.

It has been estimated that the global losses due to the root-knot nematode, *M. incognita* amounts to $78 billion (US dollar) (Chen et al, 2004). The production (tonnes) of pumpkins, squash and gourds is 35,00000 in India (FAO
United States Department of Agriculture (USDA) estimated worth US $372,335,000 loss per annum in 16 crops (Taylor, 1967). Earlier, Feldmesser et al., (1971) Hutchinson et al., (1960) and Cairns (1955) reported $ 500 million annual loss respectively. The annual loss on agriculture has been estimated as US $100 billion worldwide (Oka et al., 2000a) and recently Chitwood (2003) reported that plant parasitic nematodes are serious pathogens on most food and fiber crops. *M. incognita* alone accounts for 21.91% yield loss and *M. javanica* up to 40% yield loss (Jain et al., 1986).

The use of chemicals are effective in the management of nematodes but their hazardous effect on environment, ground water contamination and ill effects on human health create a necessity to search newer and harmless methods of nematode control (Chitwood, 2003).

**Management of Root-Knot Nematode**

Various methods are adopted for the control of nematodes but a system advocated nowadays i.e. integrated pest management (IPM). The Integrated Pest Management is a broad ecological approach which aims at keeping the pest population below economical threshold level by employing all available methods of pest control such as use of resistant/tolerant varieties of crops, cultural, mechanical, biological and chemical methods in a compatible manner. The IPM approach with a main goal of significantly reducing or eliminating the use of nematicides/pesticides while at the same time managing pest populations at an acceptable level.
Today integrated pest management is a combination of biological control with host plant resistant, appropriate farming practices and a minimal use of nematicides/pesticides is an important part of agriculture and horticulture worldwide and provide a basis for pest management in future (Hillocks, 2002; Phipps and Park, 2002; Feder et al., 2004).

The basis of sustainable approach to nematode management will integrate several tools including the use of cover crops, crop rotations, soil solarization, least toxic pesticides/nematicides, organic amendments and plant varieties resistant to nematode damage. When the soil environment is rich in diverse population of microorganisms then integrated management of nematodes works best. Some organisms that are associated with well managed crop soil e.g. rhizobacteria and mycorrhizae may induce systemic host resistance to nematode and some foliar diseases (Barker and Koenning, 1998).

To maintain healthy environment of soil, the routine application of organic matter is required. The addition of organic matter in the form of compost or manure will decrease nematode pest population and associated damage to crops (Akhtar and Alam, 1993 a,b; McSorely and Gallaher, 1995a,b). This could be a result of improved soil structure and fertility, alteration of the level of plant resistance, release of nemato-toxic or increased population of fungal and bacterial parasites and other nematode antagonistic agents (Bongers and Ferris 1999; Akhtar and Malik, 2000; Riegal and Noe, 2000).
Now many methods are applied for the management of nematode. That can be divided into five broad categories viz., chemical, regulatory, physical, cultural and biological methods.

**Chemical Method**

Nematicides are usually categorized as fumigants and non-fumigants, two basic chemicals which can be incorporated into soil during the chemical means of nematode control. Soil fumigation began to be used extensively since the discovery of DD mixture (Dichloro-propane-dichloropropene) in 1943 and EDB (Ethyl-di-bromide) and DBCP (Dibromo-chloro-propane) were formulated as efficient soil fumigant for nematode management in 1945 and 1954 respectively. Fumigants required water to activate toxicity in soil, which directly penetrate the cuticle and reacts with amino acids, oxidizes and nucleophilic sites on protein. As a result of this many enzymes or metabolic sites are inhibited simultaneously, thus stopping several vital processes and leading to a rapid death.

Non-fumigants are systemic in nature and grouped into organophosphates and carbamates. These nematicides directly penetrate nematodes and inhibit neuromuscular activity of nematodes which reduces their movement, inversion, feeding and rate of reproduction.

Nematicidal chemicals besides being expensive, non-available when it needed and photo-toxic (Jesse and Jada, 2004), also found to be unsafe, i.e., highly toxic aldicarb and methyl bromide used to control nematodes have been detected in ground water (Xaki et al., 1982; Thomas, 1996) having good
systemic activity in plants against nematode (Goswami et al., 2006). The incorporation of chemicals in soil lead to the elimination of soil micro-flora.

Now more emphasis put onto those chemicals which are bio-degradable, eco-friendly and easily available.

**Regulatory Methods**

Several attempts have been made to prevent introduction of nematodes into countries and provinces by means of plant quarantine. Plant quarantine is an effective and possible method to control plant parasitic nematodes. It is the process to prevent the introduction or spread of nematodes or disease. This is achieved by legal restrictions on the movement of commodities for the purpose of preventives or inhibiting the establishment of nematodes and diseases in areas where they are not known to occur. There are some other measures which can prove beneficial in preventing human assisted spread of nematode to uninfested areas like using certified planting material, cleaning soil from equipments / working tools before moving between fields, reducing animal movement from infested to uninfested fields and eliminating important weed host such as crabgrass and ragweed (Kodira and Wasterdahl, 1995).

**Physical methods**

In nematode management various methods adopted for eradicating or reducing the nematode population include heat treatment, steam sterilization, electrical soil heating, hot water treatment, radiation treatment, ultrasonics, osmotic pressure process and seed cleaning etc. (Alam and Jairajpuri, 1990). Soil
solarization is a method which entails laying clear plastic sheets over moist soil for approximately 6-8 weeks, can effectively suppress nematode population. The combination of various organic amendments (soybean oil cake, groundnut oil cake, cotton oil cake, Brassica residues), different plant parts with soil solarization were more effective than the amendment or soil solarization alone in reducing nematode population and gall indices of *M.incognita* and *M.javanica* in pot and greenhouse (Oka et al., 2007; Gamliel and Stapleton, 1993; Siddiqui et al., 1998; Siddiqui, 2005). Steam sterilization of soil is for killing nematode, in which autoclaved soil used. The suspension of nematode in steam sterilization is similar to soil solarization.

Though all these practices are effective for infected material like propagating tuber, shoot or root, but temperature regulation is quite difficult. Besides that there are some problems for large scale infestations.

**Cultural methods**

The various cultural measures applied for the management of root-knot nematode, include means like crop rotation, soil sanitation and flooding which had been considered as potential and cultural practices. However, they are adaptable in certain regions. Cultural practices implementation include prevention of spread, fallowing, selection of appropriate propagating material, field sanitation at the time of planting, harvesting, deep ploughing, nutrition general care of host crops (proper irrigation and timely weeding), cropping and manuring etc.

**Crop rotation**
Crop rotation involves growing a crop that is not a host for the nematode present in the field, before growing a crop i.e. susceptible. The non-host is a immune crop will cause nematode population to decline, giving the subsequent crop a chance to establish a good root system. The rotation must be selected carefully because some nematodes have a very wide host range. Also, crop rotation is a difficult to use with most perennial crops.

Flooding of the soil for 7-9 months kills nematode by reducing the amount of oxygen (O₂) available for respiration and increasing concentration of naturally occurring substances like organic acids, methane (CH₄) and hydrogen sulfide (H₂S) that are toxic to nematode (Mc.Guidwin, 1993). Flooding work best if soil and air temperature remain warm.

These cultural control practices have been found to be economically feasible in reducing disease losses. The growers should properly identify the disease that limits the production and then use a variety of most appropriate controls in combination.

**Biological methods**

According to Garrett (1965) biological control is “Any condition under which or practice whereby survival or activity of a pathogen is reduced through the agency of any other living organism (except by man himself), with the result that there is reduction in incidence of the disease caused by the pathogen”.

Biological control of nematode thus aims at increasing the natural enemies of nematode (parasite and predators of nematode) in the soil to increase the
mortality of nematode. Now a days biological method are most acceptable and widely used to control the nematode population.

Addition of organic amendments contribute to the biological activity in the soil and stimulate the activity of microbial population of actinomycetes, bacteria and fungi, elements of which might be antagonistic to nematode (Badra et al., 1979; Godoy et al., 1983, Sikora, 1992). These organic amendments also possess some nematicidal properties (Pandey, 2002; Saxena and Gangopadhyay, 2005; Jesse et. al., 2006). Some alternative methods are being sought to control the population of root-knot nematode by using non chemical methods which are safer and more compatible among which natural products are of first importance.

Therefore, present studies were under taken in in vitro and glass-house conditions to evaluate the potential of organic soil amendments and biocontrol agents, alone and in combination with least use of nematicides, to combat the harmful impact of chemicals in the effective and rational management of root-knot nematode *M. javanica* infesting bottle gourd cv. ‘Kanchan’.