Chapter-5

Discussion
DISCUSSION

India is basically an agriculture-based country, where agricultural technologies in practice are both traditional as well as modern. However, the former one being quite predominant. As far as the net output is concerned it is quite low to the advanced countries. The major constraints for the low productivity in food production of our crops are low rainfall, low fertilizer inputs, less availability of certified seeds, traditional agriculture systems in practice and the damage caused by different pests and pathogens including various plant-parasitic nematodes, where the root-knot nematodes (Meloidogyne spp.) occupy a paramount position. Their extensive host range, worldwide distribution and interaction (disease complexes) with bacteria, fungi and viruses make them potentially serious constraints and limiting factors to the crop produce. Majority of the plant species (particularly the members of Family Solanaceae and Cucurbitaceae) that account for the major world’s food supply are susceptible to attack from one or the other species of root-knot nematodes which are capable of causing sustainable economic losses in the quantity and quality of the crops. Some of the major vegetable crops grown throughout the world such as potato, tomato, chilli, okra, brinjal etc. being short duration crops, can be cultivated in succession on the same plot. In India, like many other developed countries, the entire families of the vegetable growers work in their small fields throughout the year. The monoculture practice is largely responsible for the prevalence, predominance and severity of root-knot diseases of these commercially important crops.

The extents of crop losses caused by the infestation of various plant-parasitic nematodes have been interpreted by several workers in economic
terms. Previously it was Sasser and Freckman (1987), who reported a loss of more than US $100 billion per annum due to nematodes. Recently, Chitwood (2003) reported that nematode pests cause significant crop damage with annual losses estimated at US $125 billion worldwide. This adverse situation faced by the farmers worldwide certainly justifies the adoption of proper management practices for nematode control. The efficient and most promising management of these unseen enemies of the farmers requires the carefully integrated combination of several methods and strategies and chemical control being one of them. However, the various setbacks of the use of these nematicidal chemicals like higher cost, reinfestation of the soil after harvest, contamination of groundwater and residues in fruits and vegetables etc. have put a serious question mark on their reliance for future utilization. Infact many of these chemicals still have great relevance particularly in integrated pest management (IPM) programme. A great deal of research has been carried out on nematode control which focus on either the elimination of the nematode pests or reducing the nematode numbers to levels below the damage threshold where the damage caused by them is of negligible economic value. For the effective management of plant-parasitic nematodes, the critical steps involved are i) the accurate diagnosis and ii) proper selection of the most effective control method.

The Indian farmers are mostly illiterate and commonly believe in the traditional agricultural practices and hence do not readily accept the use of modern technologies like the use of resistant cultivars and nematicidal chemicals etc. to combat nematode menace. The main hurdles in the use of nematicides are that they are very expensive, not sustainable and affect the agro ecosystem adversely besides having inherent difficulties in their handling (Noling and Becker, 1994; Jesse and Jada, 2004). Keeping these facts in view,
Researchers all over the world have diverted their attention to standardize the methods of bio-management of plant-parasitic nematodes. The addition of many organic soil amendments particularly those with high nitrogen contents, may be effective alternative control of root-knot nematode, *Meloidogyne* spp. and other plant-parasitic nematodes (Rodriguez-Kabana, 1986). The use of organic wastes, which are available in plenty particularly in the developing countries, for nematode control provides a new channel for their safe disposal. Mannion *et al.* (1994) suggested that despite of some inconsistencies in performance, municipal solid waste amendments may be suppressive to the root-knot nematode, *Meloidogyne incognita*. In Spain municipal compost residues had some activity against *M. javanica* (Marull *et al.*, 1997) and raw sewage sludge affected *Meloidogyne incognita* in greenhouse tests in Guadeloupe (Castagnone-Sereno and Kermarrec, 1991).

One of the cheapest and effective methods of altering the soil environment is its amendment with decomposable organic matter, which is considered as one of the methods of biological control of plant diseases. The materials used for soil amendment include dry or green crop residues, oil cakes and other organic wastes. These materials are allowed to decompose in the field itself where the target pathogens are supposed to be present. However, the efficacy of an organic amendment against plant-parasitic nematodes depends on many different factors including the nematode species present (McSorley and Gallaher, 1996), the chemical composition of the amendment (Mojtahedi *et al.*, 1993) including its C:N ratio (Ritzinger and McSorley, 1998), the length of time since application (McSorley and Gallaher, 1996), the other organisms present in the soil environment including those which feed on nematodes (Stirling, 1991), as well as other environmental factors and agricultural
practices. Therefore, it is extremely difficult to anticipate or generalize about the performance of specific organic amendments. However, organic materials with C:N ratios less than 20:1 have higher degradation rates and often nematicidal activities (McSorley and Gallaher, 1995a, b). The high decomposition rates have been associated with increased numbers of nematode antagonists and the release of nutrient elements (Muller and Gooch, 1982; Stirling, 1991).

Organic amendments are a source of plant nutrients which may improve plant performance and complicate the interpretation of nematode management experiments. The compost amendments and the fertilizer applications used, increased soil organic matter, pH and soil nutrient levels (McSorley and Gallaher 1997). The organic amendments also increase the water holding capacity of sandy soils (McSorley and Gallaher, 1995a). Further the various chemicals released during decomposition of organic amendments such as, phenols etc., may induce disease resistance in the roots of host plants.

In the current study, during a series of various *in vitro* and field experiments, the efficacy of different organic amendments viz., oil cakes of neem, castor, mustard, groundnut and leaves of different plant species alone and in combination with different nematicides, were evaluated against some of the potent and most commonly occurring plant-parasitic nematodes. The neem tree (*Azadirachta indica* A. Juss., Family Meliaceae) has become a topic of active scientific research in the last three decades in the West, although it is known in Asia for centuries. In India it is regarded as a miraculous tree. It has a variety of biologically active ingredients that have different modes of action. Considerable research covers uses of neem tree, as well as its effects on
organisms such as insects, viruses, bacteria, fungi, protozoa, vertebrates and nematodes (Saxena, 1989; Schmutterer, 1990). The presence of azadirachtins, phenols, and tannins at certain concentrations in neem products has effects on nematode mortality (Alam et al., 1979). The decomposition products of neem include organic chemicals that curb nematode populations. The mature seeds of neem synthesize more metabolic substances like azadirachtins and other closely related metabolites – vepaol, isovepaol and nimbidin. Such synthesized metabolites in mature seeds of neem accumulate in more concentrated form and are likely to be more lethal to the plant pathogen including nematodes allowing better plant growth. It is generally accepted that the tetranotriterpenoid azadirachtin is responsible for the majority of the biological effects observed in insects exposed to neem compounds and that one key function of azadirachtin is its impact on the insect hormonal system. Azadirachtin inhibits the release of prothoracicotropic hormones, allatotropins and allatoinhins and this is of special relevance for immature development of insect pests (Rembold, 2002). The bioactive principles in crude extracts of neem seed have also been reported to inhibit the penetration, hatchability and development of nematodes (Mojumder, 1995). Castor (Ricinus communis L., Family Asteraceae) is grown for its invaluable oil that is used in industry as well as in medicine. The leaves of both these plants are not used for cattle feed or for any other purpose and therefore the fallen leaves usually go as wastes. Their seed cakes are non-edible, hence usually used as manures. Soil amendment with different plant parts/products of neem and castor besides providing nutrients to the soil, does not pose any pollution or toxic hazards to the soil environment. Recently extensive work has been carried out with respect to their insecticidal/nematicidal properties for the management of
various plant-parasitic nematodes but most researches are still inconclusive and need further investigation.

During the current study, three broad aspects were assessed for the management of various plant-parasitic nematodes: organic soil amendments, mixed-cropping practices with various antagonistic crops, and integrated nematode management. Cost effective bare-root dip treatment with various plant parts/products and a newer approach of urea coating with ‘Nimin’ and a commercial neem based product, neem-gold, have also been attempted. The three test plants used herein (viz., tomato, chilli and okra) are most widely used vegetables and the antagonistic crops (viz., berseem, kasni and rizka) represent important fodder crops. The results obtained from different in vitro and field experiments conducted during the present study are discussed here under.

Rizka/lucerne, alfalfa (*Medicago sativa*), berseem/Egyptian clover (*Trifolium alexandrinum*) and kasni/chicory (*Cichorium intybus*) are the three important and major fodder crops cultivated throughout the Northern India and used as cattle feed for dairy animals. Berseem and rizka being members of Family Leguminosae, also aids in the biological nitrogen fixation. However, various plant-parasitic nematodes are supposed to be the limiting factors in their production. Kasni/chicory, a member of Family Asteraceae, considered to be a weed until recently, has now been considered and is gaining importance as a fodder crop. When grown intermixed with berseem and rizka, the results obtained are often more beneficial, however, preliminary studies revealed that the fields having kasni as a sole crop or intermixed with other different crops, harboured low population of different plant-parasitic nematodes. Keeping in view the importance of above mentioned fodder crops, a thorough study was
undertaken under field conditions naturally infested with different plant-parasitic nematodes, to evaluate the mixed-cropping practice of kasni with berseem and rizka against these plant pathogens.

The results obtained from the current study revealed that the populations of *Meloidogyne incognita*, *Tylenchorhynchus brassicae*, *Rotylenchulus reniformis* and *Hoplolaimus indicus* multiplied freely on berseem in both normal and deep ploughed fields, showing that this crop is a good host for these nematode species, whereas *Helicotylenchus indicus* reproduced only slightly and *Tylenchus filiformis* was not supported by the crop and deep ploughed field being more suppressive. Rizka appears to be a good host for *M. incognita*, *T. brassicae* and *Hoplolaimus indicus*, whereas it can be regarded as a poor host for *Helicotylenchus indicus* and *R. reniformis* and a non-host for *T. filiformis*. In case of kasni the multiplication rate of all the nematodes was greatly reduced in both normal as well as deep ploughed beds, however, *M. incognita* showed a little bit of free multiplication. The nematode suppressant effects of kasni were also noted even when it was grown as a mixed crop with other two fodder crops viz., berseem and rizka, under both deep as well as normal ploughed fields, indicating the antagonistic nature of this crop towards various plant-parasitic nematodes.

Kasni/chicory is a member of Family Compositae (Asteraceae). Gommers (1973) observed several nematicidal principles while he was surveying the various members of Asteraceae for locating nematicidal factors present if any. However, much work has been carried out on the antagonistic nature of marigold (*Tagetes* spp.), which is an important member of Asteraceae. The first report of the resistance of marigolds to nematodes was by Goff (1936), who
noted that the French marigolds (*Tagetes patula*) and African types (*T. erecta*) were two of seven plant species devoid of root-knot nematode (*Meloidogyne* spp.) infection during trails of 80 different ornamental annuals. Since then many researchers have reported that the cultivation of marigold as a cover crop, rotation crop, green manure, or source of nematode-antagonistic extracts, significantly suppressed the population build-up of many noxious nematode species (Slootweg, 1956; Hackney and Dickerson, 1975). Reynolds *et al.* (2000) reported that the rotations of *Tagetes patula* or *T. erecta* in fields can provide economic control of *Pratylenchus penetrans* on tobacco (*Nicotiana tabacum*) for two successive years. Wallace (1963) suggested that the effects of marigold manifest in the soil apparently by release of the nematicidal chemicals from the roots. Several workers reported that the decrease in the nematode population by intercropping marigold with many other plant species, may be attributed to the toxic root-exudates like α-terthienyl produced by marigold which also acts like trap crop (Kyo *et al.*, 1990; Yen *et al.*, 1998; Dhanger *et al.*, 2002; Uma Shankar *et al.*, 2005). Sundararaju (2005) suggested the possible use of marigold (*Tagetes erecta*) as an intercrop in banana field to enhance the production and productivity of banana as well as for the control of plant-parasitic nematodes. However, the reports about the possible use of kasni as an antagonistic crop as well as its use in the mixed-cropping sequences for the suppression of plant-parasitic nematodes are lacking in the literature. In all the possibilities, the nematicidal chemicals of kasni are released along with its root-exudates as has been shown in case of marigold (Alam *et al.*, 1975).

The perusal of the results indicated that there was significant reduction in various species of plant-parasitic nematodes in both normal as well as deep ploughed fields, however, the deep ploughing was more effective. The results
were in confirmation with those obtained by various other workers (Jain and Bhatti, 1985; Mathur et al., 1991; Siddiqui, 2003; Anver, 2006) who reported that depth of ploughing played an important role in reducing the population of plant-parasitic nematodes as the nematodes are exposed to solar heat. Mathur et al. (1987) observed that 1-5 deep summer ploughing in May-June resulted in reduction in population of cereal cyst nematodes and increased yield of wheat crops. Most probably the deep ploughing disturbs the ecological set up of nematodes and exposes the lower strata of soil containing a large number of nematodes which are exposed to the external unfavourable environmental factors like solar heat and desiccation, thus adversely affecting the nematode populations (Khan and Saxena, 1980; Lopez-Fando and Bello, 1995).

The nematode control efficiency of growing kasni with berseem and rizka was greatly enhanced when the different plots were treated with oil cakes of neem, castor and groundnut and chopped leaves of neem and castor and two nematicides, carbofuran and phorate. The combined application of the nematicides with inorganic fertilizer was found to be most effective. The other treatments were effective in the order of efficiency as neem cake, castor cake, groundnut cake, neem leaves, castor leaves, inorganic fertilizer and compost respectively. The results obtained here with respect to the efficiency of oilcakes are in conformity with those obtained by several other workers (Anver and Alam. 2000; Gopinatha et al., 2002; Srivastava, 2002; Pathak and Keshari, 2003; Ravindra et al., 2003; Hussain and Bora, 2006). The action of organic amendments showing increased frequency is attributed to increased microbial activity in the soil. The microbial activity in the amended soil may lead to release of a wide variety of chemically different substances, which may be directly toxic to phytonematodes. Orion et al. (1980) pointed out that higher
concentration of ammonia liberated out during the decomposition of organic additives inhibited the formation of syncytium which is essential for development of nematode, since syncytium is the feeding site for the nematode. In the present experiments, beneficial effects of oil cakes and other organic amendments persisted for longer duration as they remained effective against plant-parasitic nematodes even in the subsequent crop, when a highly susceptible crop tomato cv. 'K-25' was grown in the same plots which only received the normal and deep ploughing treatment as in the preceding crops. In the present investigation though initially the oil cakes did not give good response, their performance was significantly enhanced during the later phase of the plant growth when the residual effect of these organic amendments was observed on tomato cv. 'K-25' plants. This could be due the delay in nitrification (release of nitrogen from oil cakes), while during the earlier phase of plant growth, when the nitrification was not complete, partial decomposition may have released excess amount of fatty acids and aldehydes which are considered not favourable for plant growth and may also cause phytotoxicity (Singh and Sitaramaiah, 1973). The longer persisting nematicidal effects of different oil cakes seems to be because of the fact that these oil cakes are made up of complex organic substances which decompose rather slowly (Daji and Iyengar, 1971), and thus release nematotoxic-substances for longer durations. The least proliferation of nematodes in the plots treated with neem cake may be due to liberation of ammonia (Khan et al., 1974) and phenols, aldehydes, amino acids and fatty acids (Reddy et al., 1997) during decomposition which are detrimental for the nematode development. Apart from this, neem cake itself contained formaldehyde (0.25%), which may be another factor responsible for nematode control (Sitaramaiah and Singh, 1978). The
decomposition of neem leaves leads to the increase of compost temperature that might enhance the growth of thermophilic microorganisms and actinomycetes. Neem leaves also release allelochemicals like limonoids, nimbidic acid, meliacin etc. which are directly toxic to nematodes (Grewal, 1989).

The different nematode management strategies including different chemical, regulatory, physical and biological practices have their own limitations, though to varying extent. Oostenbrink (1972) pointed out that there is a scope of combining different control methods in a complementary manner. Thomson et al. (1983) suggested that integrated pest management (IPM) would be the best strategy for nematode management. The IPM is the philosophy of using our growing knowledge of pest biology and ecology to prevent pests from causing economic damage. It involves two components i) collecting samples to establish the pattern of pest distribution in a field, determining levels of both the pest and its natural enemies and ii) using a number of control practices in a compatible manner to maintain pest populations at acceptable levels. In more economically developed countries like Germany, USA, UK etc. spray treatments with commercial neem formulations are commonly used in IPM, especially in protected cultivation of vegetables and ornamental plants (Isman, 1997; Immaraju, 1998; Stadler and Staucke, 2002). In India also various integrated nematode management strategies studies have been attempted (Krishnappa and Reddy, 1993; Goswami and Mishra, 1994; Sarmah, 1995; Subhadra et al., 1998; Das and Sinha, 2005; Nageshwari and Mishra, 2005; Pandey et al., 2005; Shreenivasa et al., 2005). The mixed-cropping/intercropping in combination with different biological and chemical treatments, is an integrated approach which is found to be highly promising and
beneficial not only in controlling the nematode assault but also for improvement of crop yields (Somasekhar and Mehta, 1998; Haider et al., 2001b).

The mechanism(s) of action of organic soil amendments leading to the control of various plant diseases caused by various plant pathogens including plant-parasitic nematodes is not fully understood. The complex nature and structure of soil and soil environment makes it difficult to assess the possible course of activities occurring in the soil and probably the disease control in the amended soil is result of multiple factors and mechanisms occurring in the soil which may affect/reduce the disease severity through their effect on soil, host and the pathogen. The modification of physical, chemical and biotic environment of soil through decomposable organic matter has been found to influence the incidence of many plant diseases. The various cultural practices like soil solarization, flooding, ploughing etc., employed in combination with biological control, encourage the development of such microorganisms in the soil which either destroy or suppress pathogen through antagonism (antibiosis, competition, parasitism and predation etc.). The metabolites of these organisms or decomposition products of organic matter attached by them may induce physiological resistance in the plant.

Many theories have been put forth by different workers from time to time to explain the possible mechanism of action of nematode control by the application of various oil cakes amended with soil and so far various oil-seed cakes have shown great promise in the nematode management strategies (Siddiqui and Alam, 1997; Kumar and Khanna, 2006). Alam et al. (1979) and Alam (1990) reported that the oil-seed cakes undergo decomposition after
irrigation and release many compounds like phenols, aldehydes, and different gases including ammonia. Under *in vitro* conditions all these chemicals were found highly effective in killing plant-parasitic nematodes (Alam *et al.*, 1982), organic oils (Miller, 1979), neem chemicals (Dash and Padhi, 1990; Siddiqui and Alam, 1990; Akhtar and Mahmood, 1997) and organic nitrogen in the form of activated sludge were found highly deleterious to plant-parasitic nematodes. In case of oil cake amended soil, the microbial activity in the soil leads to release of a wide variety of chemically different substances which may be directly toxic to the nematodes or reduce the capacity of nematodes to penetrate the root system of the host plants, thereby reducing the inoculum density or inoculum capacity. The decomposing oil cakes may reduce the proneness of the hosts by loss of attraction or change in root physiology under the influence of various organic acids and other chemicals released during decomposition. The efficacy of neem cake over other oil cakes (castor, groundnut and mustard), could be attributed to the nature and amount of different chemicals released as they vary with the type of oil cakes, besides the prevailing microenvironment. Among the several chemical constituents present in neem kernels, the limonoids which are compounds belonging to B-furano-triterpenoids alone have been found to be nematotoxic. The water extracts of various oil cakes viz., neem, castor, groundnut, mustard, mahau etc., have been found to be toxic to a variety of plant-parasitic nematodes (Siataramaiah and Singh, 1977; Hussain *et al.*, 1992). They also inhibit juvenile hatching probably because they contain varying amounts of phenols, aldehydes, fatty acids and some other chemicals of unknown composition (Mishra *et al.*, 1989; Singh *et al.*, 2001). Alam *et al.* (1982) reported that the water soluble fractions of various oil cakes became progressively more toxic to nematodes and inhibitory to larval hatching of the
root-knot nematode during course of decomposition. They proposed that more toxic principles are liberated during decomposition of oil cakes and form solutions in water and occupy the soil pore spaces where most of the populations of noxious nematode species occur and thus bring out significant reductions in the inoculum density and keep the nematode populations below the economic threshold levels. It has also been reported that because of the high solubility of the oil cakes their toxic fractions can reach into the soil much beyond the rhizosphere region of plants and there they kill or reduce the mobility of nematodes which are left in the field from the preceding crop and therefore like other nematicidal chemicals, these oil cakes also play a preventive role. Bhatnagar and Goswami (1987) found a significant improvement of plant growth after neem cake treatment when compared to the synthetic nematicide, aldicarb.

The decomposition of oil cakes in the soil takes place due to the increased activity of soil microorganisms including bacteria, fungi, algae etc., whose microbial activity is stimulated due to the addition of organic matter to the soil (Sayre, 1980; Rodriguez-Kabana et al., 1987). The metabolites of these microbes have also shown varying degree of toxicity towards different nematodes. Various bio-control agents including different species of fungi like *Trichoderma viridae*, *T. harzianum*, *Paecilomyces lilacinus*, *Glomus fasciculatum*, *G. mosseae*, *Fusarium oxysporum*, *F. solani* etc. either alone or in combination with other organic amendments, have been found to be highly deleterious to plant-parasitic nematodes (Goswami, 1993; Rao *et al.*, 1997; Fazal *et al.*, 1998; Devi and Sharma, 2002; Goswami and Singh, 2004; Verma *et al.*, 2005). These fungi are known to produce toxins and antibodies, malformin, hedacidine, lilacin, leucinostatin, gliotoxin, viridin etc. Recently
Kantharaju et al. (2005) reported that the effective indigenous isolates of *Glomus fasciculatum* might have colonized faster than indigenous virulent isolates from root-knot nematode, *Meloidogyne incognita* and resulted in increasing the biochemicals imparting resistance and reduced the nematode development and reproduction (Singh et al., 1990; Kantharaju et al., 2002; Borah and Phukan, 2006). There are several reports about bacteria acting as potent bio-control agents by producing various nematicidal metabolites. Johnson (1959) reported toxicity of culture filtrates of *Clostridium butyricum* containing formic, acetic, propionic and butyric acids to reduce populations of *Tylenchorhynchus martini*. So far several mechanisms have been suggested by various scientists which are attributed to the suppression of phytonematodes by endophytic bacteria (*Bacillus subtilis*) which include systemic resistance due to enhanced activity of defense enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (PAL), production of antagonistic compounds and alteration of specific root exudates such as polysaccharides and aminoacids (Hallman et al., 1997; Kloepper et al., 1999; Jonathan and Umamaheshwari, 2006). The oxidative enzymes play a vital role in plant resistance to biotic stress. They are considered to be scavengers of hydrogen peroxide and involved in several plant defense responses including lignification, cross linking of cell wall proteins, wound healing and production of antimicrobial radicals (Lamb and Dixon, 1997). Peroxidase is a key enzyme in the biosynthesis of lignin (Bruce and West, 1989). Phenylalanine ammonia lyase is the first enzyme in phenyl propanoid metabolism and in the production of phenolics and phytoalexins that prevent establishment of pathogen (Daayf et al., 1997). These enzymes play a major role in the induction of systemic resistance in plants against phytoparasitic nematodes. Viswanathan and
Samiyappan (1999) reported that fluorescent pseudomonads promoting plant growth and possessing antagonistic activity would certainly be promising biocontrol agents. The increase in the plant growth might be associated with secretion of auxins, gibberellins and cytokinins (Ramamoorthy et al., 2001). Reduction in the nematode population in roots might be due to premature egg hatching and reduction in viability and mobility of juveniles induced by secondary metabolites such as 2,4-diacetyl phloroglucinol (PHL) and lytic enzymes produced by *Pseudomonas fluorescens* (Elsherif and Grossman, 1996; Dunne et al., 1998). A new group of macro-cyclic lactones, called avermectins, have been reported to possess excellent nematicidal and insecticidal properties (Ostlind et al., 1981; Stapley and Woodruff, 1982). These metabolites are produced by *Streptomyces avermitilis*, which is commonly occurring soil borne actinomycete. The crude avermectin isolated from *Streptomyces avermitilis* had some nematicidal principle, which could induce larval mortality, prevent egg hatching and inhibit larval penetration thus hindering the process of host-parasite relationship and resulted in low nematode disease incidence (Jansson and Rabatin, 1998; Chubachi et al., 1999; Jayakumar et al., 2005).

The organic additives release nutrients, which accelerate rapid root development and overall plant growth thus helping the plants to escape nematode attack. This theory has been substantiated by the results obtained from various pot experiments where the organic amendments have improved the plant length and plant weight by several folds.

The two systemic nematicides, carbofuran (3G) and phorate (10G) were also included in the present study for comparing the efficacy of various organic amendments particularly that of oil cakes and it was observed that the test oil
cakes were in no way inferior to the nematicides. These nematicides could have protected the plants by killing the nematodes in soil before their invasion in roots by control action or by accumulating in the roots as metabolites and killing the larvae which gained access inside the root system. The results obtained here suggest that the application of a specific organic amendment could be exploited to keep densities of nematodes at a safe threshold level, where they may not cause any harm to the economically important crops.

The principle underlying the efficacy of organic amendments is that the decomposable organic matter should be allowed to decompose in the field in such a way and for a particular period of time, so that the process of decomposition and its associated activities suppress or destroy the pathogen and that it should not interfere with normal cultural practices and after planting the crop, there is no harmful effect on the plants. Simultaneously, the disease susceptibility of the plants is also reduced. Since decomposition products of some of the organic matter may harm plant roots, such treatments should be applied before planting the crops, hence a waiting period is necessary.

The plants are important sources of many naturally occurring phytochemicals antagonistic towards plant-parasitic nematodes and other plant pests. Higher plants have yielded a large number of active compounds, including alkaloids, cyanogenic glycosides, terpenoids, diterpenoids, triterpenoids, lipids, fatty acids, steroids, polyacetylenes, quassinoids, glucosinolates, isothiocyanates, flavonoids, simple and complex phenolics, and several other classes (Blum, 1996; Chitwood, 2002). However, the agricultural utilization of these phytochemicals although currently uneconomic in many situations, offers tremendous potential in combating the menace caused by
different plant-parasitic nematodes and other plant pathogens. Therefore, a study was conducted to evaluate the nematicidal efficacy of chopped leaves of eight plant species viz., Azadirachta indica, Eucalyptus citriodora, Melia azedarach, Ricinus communis, Callistemon lanceolatus, Tagetes patula, Clerodendrum inerme and Thuja orientalis under glass house conditions, using two potential doses (50 and 100 g/pot). All the treatments significantly reduced the population of various plant-parasitic nematodes under test and the root-knot development of Meloidogyne incognita resulting in corresponding increase in plant growth of tomato, chilli and okra. The higher doses were found to be comparatively more effective and the highest reduction in nematode populations as well as root-knot development, and increase in plant growth was found in pots treated with A. indica. The effectiveness of different treatments varied from nematode to nematode. The reduction in the nematode development and enhancement in plant growth characters did not differ much between the plants treated with Thuja orientalis and untreated control, demonstrating a lack of phytotoxicity and a lack of fertilizer effect in the test crops at applied rates. Here the different plant species differed with respect to nematicidal activity suggesting that these plant species possess nematicidal principle (s) that varied qualitatively and quantitatively. Since the plant extracts that showed nematicidal activity, were prepared in water, the active compound (s) seems to be polar in nature. However, whether the inhibition of egg hatch and the nematicidal activity found here was due to a single compound or a number of compounds cannot be stated with certainty and further investigations are needed in this regard. Nevertheless, some similar results have been reported by several other workers about the effectiveness of A. indica (Akhtar et al.,

The leaf extracts of the above mentioned plants also showed high nematicidal potential *in vitro*, highest being with *A. indica* and lowest with *T. orientalis*, thereby supporting the above results. Similar results have also been reported by other workers with respect to *A. indica* (Mukhtar *et al.*, 1994; Aziz *et al.*, 1995a; Jain *et al.*, 1998; Singh and Dabur, 2004; Rajendran and Saritha, 2005). Neem (*Azadirachta indica*) is known to contain 34 different chemicals belonging to diterpenoid, triterpenoid and flavonoid groups (Rao and Parmar, 1984). Some companies have started to market several neem-based insecticides with different trade names as Limonol, Neemark, Neemgourd, Nimbicidin, Wellgro, Agricef, Neoconeem, 'Nimin', Suneem, Neemgold, Achook and Jawan. These products have been used by incorporating into the soil, seedling root-dips and seed treatments. The application of such products with or without urea in agricultural soil, exhibited highly effective suppression of plant-parasitic nematodes, whereas populations of non-target nematodes such as the predatory nematode *Monochus aquaticus* Coetzee. were not significantly affected (Akhtar, 1999a, b). Neem oil and Nimin (containing neem triterpenes) as urea coating agents, and root-dip or seed treatment with neem extracts, have also been found to be nematicidal against species of parasitic nematodes in vegetables. However, soil amendment with neem seems to be most practical method for nematode control (Alam, 1993). The wide range of pesticidal actions: nematicidal, antifeedant, repellant, growth disruption, juvenile toxicant and ovicidal potentials of neem parts/products could be linked to the multiple active compounds of the plant, the most active ingredients being the azadirachtins. Azadirachtins find application in neem-based pesticide
formulations which are safe, biodegradable and manageable by the farmers and environmental friendly, unlike synthetic pesticides some of which leave residues polluting air, water and soil. Similarly, nematicidal properties were also reported in the leaf and seed extracts of *Melia azedarach* (Lee, 1987; Ram and Baheti, 2003).

Thus from the series of these *in vitro* and *in vivo* experiments it may be concluded that the soil amendment with various plant species reduced the nematode population densities and nematode reproductive potential in tomato, chilli and okra, compared to the controls. These differences with reference to control could either be due to the changed nutrient status of the soil following amendments with plant material or because of the allelochemicals that were added to the soil either directly through the plant material or through their products of microbial degradation.

Urea is the major source of nitrogenous fertilizer in India. It is estimated that out of the total quantity of urea applied to crops, 50-70% is lost in various forms, thereby reducing the availability of nitrogen to the crop. Urea blended with neem cake as well as with deoiled neem seeds and used as manure increased yields of rice, sugarcane, cabbage, cauliflower, etc. (Siddiqui *et al.*, 1976). The Indian farmers have an age-old practice of blending neem cake with urea. The scientific explanation of this traditional practice is that neem seed contains chemicals called triterpenes, which have specific bacteriostatic properties. These chemicals retard the activity of 'nitrifying bacteria' which helps in regulating the nitrogen supply to crops at different stages of their growth. Urea is hydrolyzed by urease present in the soil and then nitrogen, in the form of urea amide which is rapidly converted into ammonical nitrogen and
subsequently to nitrates and nitrites. This form of nitrogen, besides being absorbed by plants, is also rapidly lost from the soil due to leaching, run-off, volatilization and denitrification. When neem products are mixed with urea and incorporated into the soil, the triterpenes retard the growth and multiplication of nitrifying bacteria resulting in delayed transformation of ammonical nitrogen into nitrate nitrogen (Akhtar and Alam, 1993a).

Recently, Godrej Agrovet Ltd., India has launched a neem-based commercial product ‘Nimin’ and has recommended it for urea coating to prevent loss of nitrogen by leaching. During present study, it was found that the recommended dose of ‘Nimin’ is additionally beneficial for the control of plant-parasitic nematodes occurring in naturally infested soils, including root-knot nematode, *Meloidogyne incognita* on tomato, chilli and okra. The nematode controlling efficiency of ‘Nimin’ and neem-gold is understandable because neem is already known to be rich in nematode toxic azadirachtin and other compounds. The improved plant growth is partly due to the availability of nitrogen from urea in the presence of neem products. The results presented herein were in confirmation with those reported by various other workers (Wani and Alam, 1999; Mojumder et al., 2004).

The bare-root dip treatment of plant seedlings (tomato and chilli), both pre-infected and post-infected with *Meloidogyne incognita*, with extracts of neem and castor and a nematicide (carbofuran), provided protection against root-knot disease. Thus the extracts exhibited prophylactic and therapeutic effects against the nematode on tomato and chilli plants (Akhtar and Mahmood, 1994, Aziz et al., 1995b). Inhibition of root-knot development was greater in pre-infected seedlings compared with those inoculated with the nematode after
root-dip treatment. This indicates that the chemicals absorbed by the roots have directly acted against the nematode present in the roots, whereas, when the inoculation was made after dip treatment, the potentiality of chemicals might have reduced to some extent by the time the nematodes had established an effective host-parasite relationship. The present findings also indicate that the water extracts of these organic amendments particularly that of oil cakes, rendered the roots of susceptible plants highly unfavourable to *Meloidogyne incognita*. Siddiqui and Alam (1988) concluded that leaf extracts tested induced some resistance in tomato and eggplant against *M. incognita* and *R. reniformis*. The poor root-knot development could be attributed to poor penetration and later retardation of feeding and/or reproduction of the second stage juveniles. Similar results were also reported by some other workers (Vats and Nandal, 1995; Tariq and Siddiqui, 2005).

The reduction in nematode population from these treatments was attributed to leached chemicals, either coating the seeds or being absorbed by the roots during bare-root dip into the rhizosphere which repelled or killed juveniles that attacked the host root.

These findings with respect to the protective action and the direct toxicity of these organic amendments (neem and castor) would go a long way to help in developing some potential and promising plant based nematicidal products.