INVESTIGATION ON NEMATODES ASSOCIATED WITH MEDICINAL AND AROMATIC PLANTS IN BHUBANESWAR

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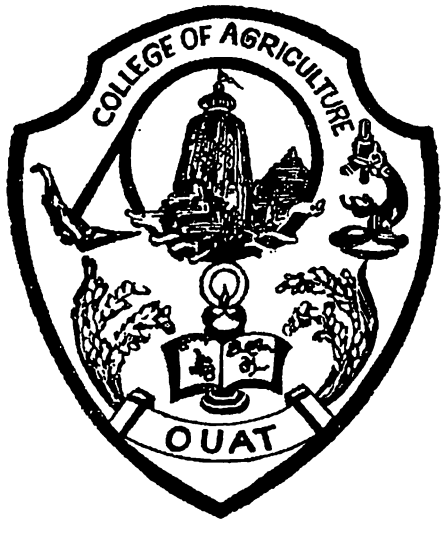
FOR THE DEGREE OF
MASTER OF SCIENCE IN AGRICULTURE
(NEMATOLOGY)

By

Biswadev Behera



DEPARTMENT OF NEMATOLOGY
COLLEGE OF AGRICULTURE
ORISSA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
BHUBANESWAR – 751003, ORISSA
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THESIS ADVISOR DR. N.K. SAHOO

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DEDICATED
TO
MY BELOVED PARENTS

Dr. N.K. Sahoo, Ph.D.
Associate Professor
Department of Nematology
College of Agriculture
Orissa University of Agriculture & Technology
Bhubaneswar-751003

C E R T I F I C A T E - I

This is to certify that the thesis entitled "**INVESTIGATION ON NEMATODES ASSOCIATED WITH MEDICINAL AND AROMATIC PLANTS IN BHUBANESWAR"** submitted by **BISWADEV BEHERA** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (NEMATOLOGY)** is a faithful record of *bona fide* research work carried out under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma. It is further certified that the help or information availed of during the course of investigation has been duly acknowledged by him.

Bhubaneswar
Dated, The 5th September, 2009

N. K. SAHOO
Chairman
Advisory Committee

CERTIFICATE - II

This is to certify that the thesis entitled "**INVESTIGATION ON NEMATODES ASSOCIATED WITH MEDICINAL AND AROMATIC PLANTS IN BHUBANESWAR"** submitted by **BISWADEV BEHERA** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirement for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (NEMATOLOGY)** has been approved by the student's Advisory Committee after oral examination on the same in collaboration with an External Examiner.

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CHAIRMAN:

Dr. N.K. Sahoo, Ph.D
Associate Professor
Department of Nematology
College of Agriculture
O.U.A.T., Bhubaneswar

24/9/09

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Professor and Head
Department of Nematology
College of Agriculture
O.U.A.T., Bhubaneswar

22.9.09

2. **Dr. A. K. Dash, Ph.D**
Professor
Department of Horticulture
College of Agriculture
O.U.A.T., Bhubaneswar

22/09/09

EXTERNAL EXAMINER 22.09.09

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Biswadev Behera.

Biswadev Behera

CONTENTS

LIST OF TABLES

LIST OF FIGURE

LIST OF FIGURE

23. *Plumbago zeylanica*

24. *Piper nigrum*

25. *Piper longum*

26. *Lawsonia alba*

27. *Abrus precatorius*

28. *Centella aciatica*

29. *Bacopa monnieri*

30. *Asparagus racemosus*

31. *Acoros calamus*

32. *Clitoria ternatea*

33. *Allium porum*

34. *Gymnema sylvestre*

35. *Curcuma zedoaria*

36. *Coleus aromaticus*

37. *Pogostemm patchordi*

38. *Bauhinia variegata*

39. Infected root knot root material of *Bauhinia variegata*

40. *Coleus forskholi*

41. *Curcuma amada*

Name of the Student	:	**Biswadev Behera**
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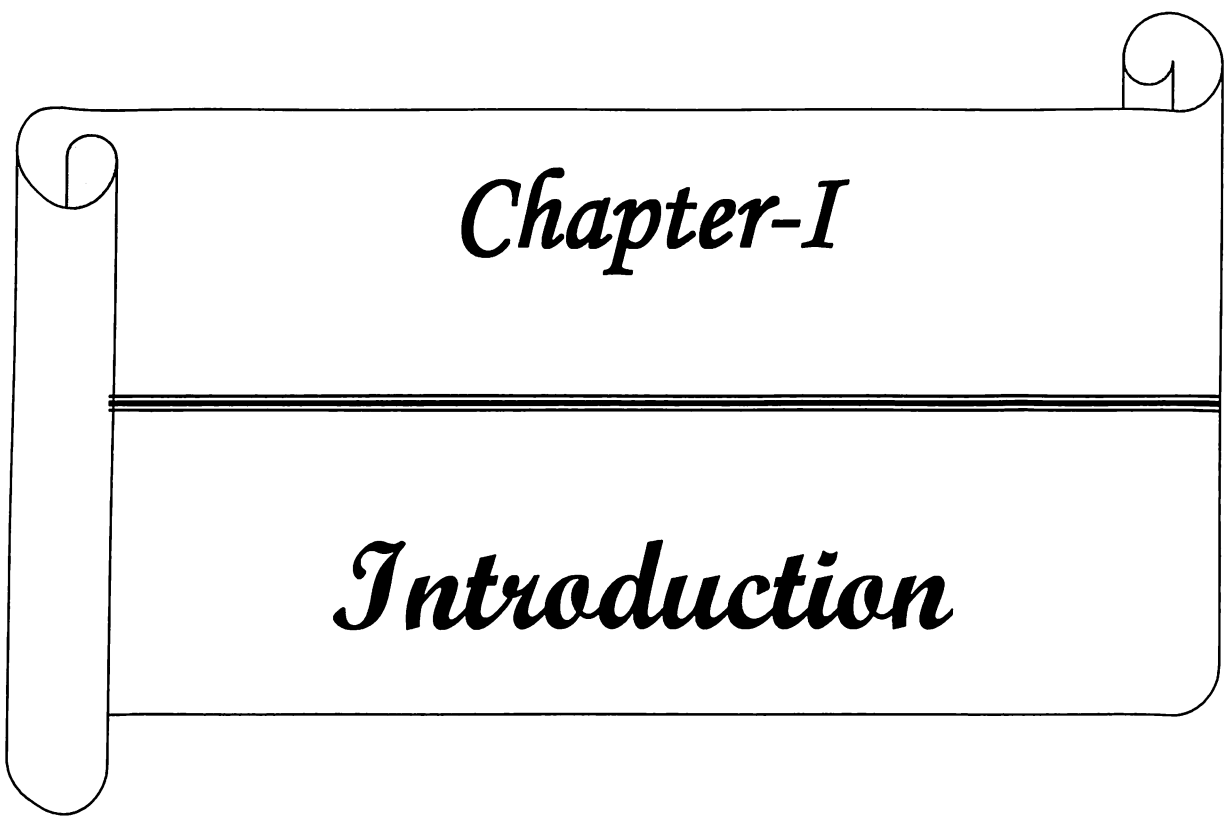
Advisor	:	**Dr. N.K. Sahoo, Ph.D** Associate Professor Department of Nematology College of Agriculture OUAT, Bhubaneswar-751003

ABSTRACT

Examination of rhizosphere soil samples obtained from economically important medicinal and aromatic plants growing in localities of Bhubaneswar revealed interesting pattern of nematode distribution, their population densities and possible plant nematode associations. It was observed that thirteen plant parasitic nematode genera were associated with 35 medicinal plants examined. The important nematode genera in decreasing order of their frequency in occurrence were *Helicotylenchus dihystera, Hoplolaimus indicus, Xiphinema insigne, Rotylenchulus reniformis, Meloidogyne* sp., *Aphelenchus avenae, Cephalenchus* sp., *Tylenchorhynchus coffeae, Criconemella ornata, Caloosia exilis, Hemicriconemoides cocophilus, Pratylenchus coffeae,* and *Tylenchus* sp. Similarly on examination of 5 aromatic plants, eight plant parasitic nematode genera were found. The important nematode genera in descending order of their prevalence were *Meloidogyne* sp., *Rotylenchulus reniformis, Helicotylenchus dihystera, Xiphinema insigne, Tylenchorhynchus coffeae, Hoplolaimus indicus, Criconemella ornata* and *Aphelenchus avenae.* Among free living nematodes Rhabditids were of common occurrence in both medicinal and aromatic plants in most of the samples followed by Mononchids and Dorylaimids.



Chapter-I

Introduction

Medicinal and aromatic plants (MAPs) as natural source of raw materials for industrial products offer a great scope to achieve net higher returns with ever increasing demand of natural products in food, pharmaceutical, perfumery, flavor and cosmetic industries. Cultivation of MAPs has now become popular and economically viable proposition. The global demand for MAPs is growing @7% per annum. It is estimated that total number of MAPs in international trade is around 2500 species world wide (Schippmann *et al,* 2002) .Global trade of medicinal and aromatic plants is valued at US $60 billion per year. By 2050, it is projected to grow up to US $800 billion per year. The present Indian flavours and medicinal market is valued at US $225 million. India ranks third in volume and second in value of world trade in aromatic oils and plants. (Pushpangadan and George 2008). The Indian systems of medicine Ayurved, Sidha and Unani entirely depended on plant materials or their derivatives for treating human ailments (Prajapati *et al*, 2003). About 25% of drugs of modern pharmacopoeia are derived from medicinal plants (Phytomedicines) and many other are synthetic analogues built on prototype compounds isolated from plants. India is endowed with about 8000 species of medicinal plants (Bahadur *et al,* 2007), which is 12.5% of the 4,22,000 plant species documented world wide to have medicinal value. The proportion of medicinal plants to the total documented species in different countries varies from 4.4% to 20% (Schippmann *et al*, 2002). Thus, considering the current market size and future growth, there has been large scale cultivation of medicinal & aromatic plants in India. In Orissa, various institutes, non government organizations and elite farmers are cultivating medicinal & aromatic plants in large scale. Use of promising cultivars coupled with balanced use of fertilizers undoubtedly boost the plant growth but continuous and intensive monoculture practice being a common feature for growing these MAPs in nurseries and unbunded uplands, prone to attack by several pests and diseases. Among various pests and diseases, the role of phytonematodes cannot be ruled out.

Nematodes are discrete group of biological entity which have immense potentiality to sustain any adverse environment. As soil is the common abode for both plant and nematode parasites, there is ample opportunity for competition for food leading to parasitism. Several nematode species have become obligate parasites on plants and due to their universal distribution, no living plant is likely to be free from their attack. Most of the cultivated as well as the uncultivated plant species are usually known to be associated with one or more species of phytonematodes. Although plant parasitic nematodes have been the real cause of stunted and poor growth of plants, such condition are also ascribed to poor soil, moisture, stress and other factors. So very often the problem of nematodes in the crop field go unnoticed by the farmers as well as field workers. There are reports of plant parasitic nematodes posing problem in medicinal and aromatic plants. *Meloidogyne incognita* suppressed plant growth and reduced root development in *Ammi visnaga, Cosfus speciosus* and *Solanum indicum* (Pandey and Haseeb, 1997). This species was also treated as the number one plant parasitic nematode affecting *Mentha arvensis* (Singh and Kumar, 1998).

So keeping in view the limiting factors of plant parasitic nematodes in reducing the growth of medicinal and aromatic plants and the present thrust for augmenting the yield potential in MAPs, it was imperative "Investigation on nematodes associated with medicinal and aromatic plants in Bhubaneswar" as part of preliminary study, so that the information may be useful for better understanding of nematode problem in MAPs for future line of work.

Chapter-II

Review of Literature

Review of Literature is the over view of past works on a topic which enlightens a beginner to get an idea about the work done and enthuses to march ahead for refinement of the work .In this context the information and literature on nematodes associated with medicinal and Aromatic plants are cited as follows.

Goffart (1931) studied the association of nematodes in *Ocimum Sp.* and reported that *Aphelenchoides ritzemabosi* was found associated with ocissum spp. Subsequently Buhrer (1938) found Meloidogyne sp. in *Ocimum* sp.

Edward *et. al.* (1964) reported *Paralongidorus fici* in the rhizosphere of *Ficus carica* L. from U.P., India, *Hemicriconemoides birchfieldi* from Allahbad (1965) in rhizosphere of *Phyllanthus emblica*, *Mangiferae indica* L., *Artocarpus ntegrifolia* L. and *Citrus rediculata* Balnco. And *Pratylenchus micoletzkyi* (1967) from the rhizosphere of *Citrus sinensis* and *Rosa barboniana.*

Liosetskaya (1968) studied the plant nematodes fauna of *Mentha piperita.* A list of 57 nematodes species from 24 genera were found on piper-mint in Moldavia, USSR. *Aphelenchoides fragariae and Aphelenchoides sps.* and *Pratylenchus hamatus* were abundant in all plant organs as well as in the soil.

Zhivkov and **Baicheva** (1973) made studied on nematode fauna of *Mentha piperita* and *Lavandula vera* in the Karlovo area of Bolgaria. Twenty six species of *Phytohelminths* were found, and *Tylenchorhynchus macrurus* was found abundantly.

Sharma and **Loof**, (1974) Examination of soil and root samples collected from Cocoa regions of Bahia, Brazil around *Piper nigrum* and *Eugenia caryophyllata* revealed 9 species of plant parasitic nematodes. Species

common to both hosts were *helicotylenchus dihystera, Meloidogyne javanica, Rotylenchulus reniformis, Dolichodorus sp. and Trichodorus sps.*

Alam *et. al.* (1974)found that *Meloidogyne incognita* was associated with all the varieties of pepper in India.

Koshy and **Sosamma** (1975) and **Vilsoni** *et al.* (1976) reported *Radopholus similis* on turmeric from south India.

Shah and **Raju** (1977) observed that *Meloidogyne* sp. infected all subterranean organs of *Zingiber officinale.*

The burrowing nematode *Radopholus similis* was widely distributed in Kerala and found infecting pepper vines (*piper nigrum*) for which **Venkitesan** and **Setty** (1977) suggested control measures.

Cheng and **Tu** (1979) found that second stage larvae of *Meloidogyne incognita* infected ginger (*Zingiber officinale)* rhizome in China.

Sunderaraju *et. al.* (1979) conducted a survey on plant-paracitic nematodes associated in Kerala, India. Thirty five nematode species were reported on *Piper nigrum*, 14 on *Zingiber officinale*, 12 on *Elettaria cardamom*, 6 on *Curcum longa* and 6 on *Cinnamomum zeylancicum. Helicotylenchus* sp. *and Meloidogyne spp.* affected all crops.

Meloidogyne incognita was reported to be parasitic on *Piper nigrum* (**Jacob** and **Kurian**,1979, **Lopes** and **Lordello**,1979).

Routray and **Das** (1982) conducted a survey of plant parasitic nematodes associated with some medicinal plants in silviculture farm at Kalinga, Phulbani District (Orissa), India and found the following nematodes on different plants: *Helicotylenchus abunamai:-Achyranthes aspera, Acorus calamus, Adhatoda vasica, Aloe indica, Asparagus racemosus, Cinnamomum*

zeylanicum, C. temela, Curcuma longa, ,Piper longum, P. nigrum , Plumbargo zeylanicum, Punica granatum, Rauwolfia serpentina, and Withania somnifera .

Tylenchorhynchus mashhoodi :Adhatoda vasica, Aloe indica, Eucalyptus cytrodora, Plumbago zeylanica, Santalum album.

Hoplolaimus indicus : Curcuma longa , Eucalyptus cytrodora.

Caloosia exilis : Asparagus racemosus.

Pratylenchus coffeae : Asparagus racemosus, Piper nigrum, , Punica granatum.

Longidorus elongatus : Achyranthes aspera ,Cinnamomum zeylanicum, Sida cordifolia , Vitis quadrangularis.

Siddigia Citri: Cinnamomum temela & Sida cordifolia.

Xiphinema insigne : Cinnamomum zeylanicum, Cinnamomum temela, Santalum album.

Aphelenchus avenae : Punica granatum, Sida cordifolia.

Interestingly, they also found endoparasitic lesion nematode, *Pratylenchus coffeae* of *Asparagus racemosus*, *Piper nigrum, Punica granatum* in varying densities.

Routray and **Das** (1982) conducted an experiment on association of plant parasitic and free living nematodes with some medicinal plants in Phulbani district of Orissa and found no parasitic nematode was found associated with two medicinal plants, *Barleria dichotoma* and *Nectanthes arbortristis,* where as *Helicotylenchus abonaamai* was the most species prevalent at the root zone of a number of plant types grown in the firm. *Pratylenchus coffeae* was present in the soil samples of *Aspargus racemosus*, *piper nigrum* and *Punica granatum* in varying densities. *Helicotylenchus sps.*, *Pratylenchus sps.* n. and quite a large no. of other plant feeding nematodes sps. have been reported on *piper nigrum* and *Corcoma longa.*

Routray and **Sahoo** (1983-1985). During random survey of ginger and turmeric growing areas of Phulbani and Koraput district of Orissa, *Rotylenchulus reniformis, Meloidogyne incognita, Helicotylenchus abumamai, Macroposthonia oronata* and *Xiphinema insigne* (per 200 cc soil) were found to be of common occurrence on both the crops, where as *Caloosia exalis* was only found on ginger, *Hoplolaimus culumbus* and *H.indicus* on turmeric.

Prasad and **Reddy** (1984) conducted an experiment of pathogenecity and analysis of crop losses in Patchouli due to *Meloidogyne incognita* and found the pathogenic effect of root knot nematode, *Meloidogyne incognita* to Patchouli, *Pogostemom cablin* were investigated at inoculums levels of 100, 1000 and 10000 nematodes per plant under pot culture condition . significant reduction in root and top weights were recorded. As high as 15.9 and 27.9% reduction in top and root weights respectively was recorded with an initial inoculums level of 10000 nematodes per plant over a period of 6 months. Loss in top weight and shade dried leaf yield due to *Meloidogyne incognita* was found to be 47 and 86.7% respectively under field conditions when the root knot index was 2.75 in 1-4 scale.

Azmi and **Singh** (1984) conducted an experiment on pathogenecity of *Tylenchorhynchus vulgaris* on Anjan grass ,*Cenchrus ciliaris cv.* Igfri-s-3108 and found that the Anjan Grass is most affected by the nematode stunt nematode (*Tylenchorhynchus vulgaris).*

Ray and **Das** (1985) surveyed thirty eight medicinal plant species from Orissa, out of which 29 plant species were associated with more than two nematode species and seven plant species had harbored a single nematode species. The following nematode species such as *Basirolaimus indicus, Helicotylenchus dihystera, Siddiqia citra, Xiphinema insigne* and *Tylenchorhynchus mashhoodi* were found to be of common in occurrence.

Routray ***et. al.*** (1987) experimented nemic association of ginger and turmeric in Orissa and found *Rotylenchulus reniformis, Meloidogyne incognita, Helichotylenchus abunanaamai, Macroposthania oranata* and *Xiphinema insigne* were common species recorded from both the crops. *Caloosia exilis* was found only on ginger and *Hoplolaimus columbus, Hoplymus indicus* only on turmeric. With ginger, maximum number of nematode population recorded was that of *R. reniformis*(106) and *M. incognita*(105), while on turmeric, *R. reniformis* and *H. indicus* predominated. *Heterodora abunaamai* (78.60) with ginger and *Heterodora indicus* (100%) with turmeric than other nematode populations.

Kaur ***et. al.*** (1989) observed on occurrence of *Pratylenchus zeae,* Graham, 1951 on ginger, *Zingiber officinale* Rosc. in Himachal Pradesh and found that *Pratylenchus coffeae* and *Meloidogyne incognita* were the two main nematode species associated with this crop. M. luqman khan and Arun k. makhnontra (1998) experimented on occurrence and distribution of plant nematodes in ginger in Himachal Pradesh and found that *Meloidogyne incognita* was most widely distributed with 90.25% frequency of occurance in the Sirmour district where as same species was recorded in 100% localities of Solan district. *Helicotylenchus dihystera* was the second largest nematode found in almost 100% localities. *Pratylenchus coffeae* and *P. zeae,* both species were found either simultaneously or alone in different samples. *Tylenchorhynchus mashoodi* was quite prevalent with 87% frequency of occurance and maximum population of 450 specimens were found in Sarahan in Sirmour district. The other nematode species found in low population were *Hoplymus indicus, Xiphinema basiri, Longidorus elongatum, Tylenchus devanae* and *Macroposthonia xenoplax.*

Pradhan and **Das** (1990) Studied the further observations on Nematodes associated with high altitude plants, forests and medicinal of Phulbani District of Orissa, and the results are presented and discussed of an analysis of the nematodes present in soil sample (about 1Kg) collected from the

rhizosphere of 52 medicinal and forest plants. Seventeen forest trees had 25 species of parasitic nematodes present in their rhizosphere. Associations of 22 plant parasitic nematodes were found on 35 medicinal plant species.

Kindelan *et. al.* (1991) conducted an experiment on Phytonematodes associated with medicinal plants in the Estacion experimental de plantas medicinales "Dr. Juan Tomas Roig" of the Havana province and they got result. The occurrence of 15 species of nematodes associated with medicinal plants in the Estacion Experimental de Plantas Medicinales "Dr. Juan Tomas Roig" was noted. *Rauvolfia serpentina* and *Orthosiphon stamineus* were damaged by *Meloidogyne incognita.* In *Artemisis abrotamum* and *Pluchea carolinensis* pathogenic association with *Meloidogyne arenaria* and *Rotylenchus reniformis* were observed. *Scutellonema clathricaudatum* was found in *Aloe barbadensis* causing severe damage.

Romabati *et. al.* (1992) conducted an experiment on Community analysis of nematodes associated with some medicinal plants of Imphal district and found that Fifty-four soil samples from the rhizosphere of 18 medicinal plants from the Imphal district of Manipur revealed the presence of 15 plant parasitic nematode species belonging to 12 genera. *Helicotylenchus* and *Tylenchorhynchus* were the predominant species followed by *Cephalenchus, Iotonchus, Aglenchus, Axonchium, Aphelenchus* and *Macroposthonia.* Hosts of the nematodes found are listed.

Khan and **Sharma** (1996) conducted an experiment on Plant-parasitic nematodes associated with medicinal plants in Himachal Pradesh and found Surveys carried out on nematode species associated with medicinal plants in Himachal Pradesh revealed the prevalence of 6 genera namely, *Meloidogyne, Pratylenchus, Helicotylenchus, Tylenchorhynchus, Hoplolaimus and Rotylenchus. Aphelenchus avenae* was also recorded in a few samples. *Salvia officinalis* was the most susceptible to *M. incognita* (up to 6700 juveniles/200 cc soil), followed by *Glaucium flavum* (up to 4000 juveniles/200 cc soil) and

Melissa *officinalis* (up to 1300 juveniles/200 cc soil). *Mentha spicata* was the most susceptible to *Pratylenchus spp.* and up to 600 juveniles/200 cc soil were record from its rhizosphere.

Pandey and **Haseeb** (1997) experimented on Plant parasitic nematodes associated with three medicinal plants and the pathogenicity of root-knot nematode .A survey of plant parasitic nematodes carried out in the rhizosphere of *Ammi visnaga, Costus speciosus* and *solanum indicum* grown in experimental fields, revealed the occurrence of *Tylenchus sp., T. ylenchorhynchus vulgaries, Hoplolaimus sp., Heliocotylenchus sp., Pratylenchus thornei, Rotylenchulus reniformis, Longidorus pisi* and J2 of *Meloididogyne* species. Pathogenicity tests undertaken in a glass house with *Meloidogyne incognita*, with different initial levels revealed significantly low plant growth and suppressed root development in all the three medicinal plants. Damage varied proportionally to the nematodes initial density and nematode reproduction was inversely proportional to initial density.

Shivakumar and **Vadivelu** (1997) experimented on parasitic nematodes associated with medicinal and aromatic plants. Forty six medicinal and aromatic plants were surveyed in the Nilagiris, Tamil nadu, India, for the presence of phytoparasitic nematodes. *Meloidogyne hapla* was the predominant nematode species, followed by *Helicotylenchus incises, Pratylenchus coffeae ,Tylenchornhynchus maritini, Xiphinema americanum, Scutellonema conicephalum* and *Hemicriconemoides mangifere. H. incises* was the most frequently occurring species. *Solanum khazianum, Rawolfia serpentine, Gaultheria frgrntissma , Cinchonna Officinalis , Ammi majus* and *Eucalyptus citriodora* were the important species of medicinal and aromatic plants.

Singh and **Kumar** (1998) experimented on the life cycle of *Meloidogyne incognita* on Japanese mini(*Mentha arvensis*) and found that the second stage juveniles started invading the young mint roots 48 hours after inoculation. The *Meloidogyne incognita* was no.1 plant parasitic nematode on

Japanese mint (*Mentha arvensis*) during the month of March and April at the ambient temperature 13-37 degree centigrade.

Joymati ***et. al.*** (1999) experimented distribution and host range of *Meloidogyne incognita* on medicinal plants of Manipur – part1 and concluded the results showed that the plants *Meriandra bengalensis*, *Psophocarpus tetragonolobus, Hibiscus cannabinus, Ciprus haspan, Persicaria posumbo, Ocenanthe javanica, Viola pilosa* and *Cannavalia ensiformis* were highly susceptible to the nematode *M. incognita* compared to other listed plants. The other plants showed galling of roots system except in *Alocasia indica. Eryngium foetidium, Persicaria posumbu, Ocenanthe javanica, Fagopoirum dibotrys, Meriandra bengalensis, ciprus haspan, Houttuynia cordata, Viola pilosa* and *Cannavalia ensiformis* are new host of *M. incognita*.

Dhanachand (2000) conducted an experiment on Nematodes of medicinal plants in Manipur II: the species of the genus *Coslenchus* and found that the the plant parasitic nematodes associated with medicinal plants, carried out in different localities of Manipur revealed the occurrence of a large number of known *Tylenchids*. In this survey, six known species belonging to the genus *Coslenchus* were recorded. Of these, *C. bisexualis* is being reported for the first time from India.

Dhanachand, (2000) conducted an experiment on Nematodes of medicinal plants in Manipur III: on the species of the genus *Scutellonema* and found that A survey of the plant parasitic nematodes associated with the medicinal plants carried out during 1987-89 in different localities of Manipur, India, revealed the occurrence of a large number of *Hoplolaimids*; nine known species belonging to the genus *Scutellonema* were recorded. Detailed measurements, habitat and localities of these species are provided. Of the nine known species, reported so far, *Scutellonema aberrans, S. africanum, S. clathricaudatum, S. commune* and *S. truncatum* are reported for the first time

from India. *S. clathricaudatum, S. commune and S. truncatum* are re described, and *S. aberrans, S. africanum* and *S. sheri* are illustrated.

Pande *et. al.* (2001) experimented the Nematicidal activity in flowers of some medicinal and aromatic plants and concluded that total mortality of *Meloidogyne incognita* occurred in the flower extracts of *Bauhinia variegata* , Ixora parviflora .Extracts of *Moringa oleifera ,Tagetes erecta ,Alostonia scholaris, Caesalpinia pucherrima,Argemone Mexicana,Brassica Campestris* were also highly toxic . Mild toxicity was recorded in *Lippea alba* and *cymbopogon flexuosus*, while the least activity was reported in the flowers of *calleandra* spp.. *Hibiscus rosasinensis ,vetiveria zizanoides ,cyperus rotundus ,Tabenaemontane divaricata. Thevetia nerifolia* and *Abelmoschus moschatus* flowers .Highest inhibition in egg hatching was found in *Ixora parviflora , Bauhinia variegate, Moringa oleifera Brassica Campestris, calotropis procera* after 120 hours of expousure period. The flowers of *B.variegata, I.parviflora, T.erecta, A.scholaris, C.pulcherrima ,A.mexicana,M.oleifera* and *B. campestris* appear to have nematicidial principles and these can be, therefore, exploited for isolation of nematicidial compound.

Ismail et. al. (2002) conducted an experiment on Plant parasitic nematodes associated with Chamomile (*Matricaria chamomilla L.*) in Egypt and concluded Chamomile [*Chamomilla recutita*] is one of the most important medicinal plants. An extensive survey conducted during the period from November 2001 until May 2002 for the occurrence and distribution of plant parasitic nematodes in major chamomile growing governorates of Assiut, Beni-Suief, Fayoum, Giza, Menia and Sharkia in Egypt revealed the presence of fourteen genera of plant parasitic and other nematodes. Absolute (APD) and relative population densities (RPD), absolute (AFO) and relative frequencies of occurrence (RFO) and prominence values (PV) were calculated for each nematode genus. The stunt nematode, *Tylenchorhynchus sp., fungivorous nematode, Tylenchus sp.*, root lesion nematode, *Pratylenchus sp.*, and root-knot nematode, *Meloidogyne sp.* are the major nematode pests on chamomile

showing an infection of 81.9, 63.3, 56 and 45.5%, respectively. The APD, RPD, AFO, RFO and PV values of these four nematodes were consistently higher than those of the other nematodes. The presence of these genera in relative abundance suggests the may be of potential significance as pests of chamomile. Occurrence and densities of the surveyed nematodes associated with chamomile plant are recorded in relation to different localities.

Rathour *et. al.* (2004) experimented on phytonematode communities associated with perennial ornamental and medicinal plants in Bareilly District, Uttar Pradesh and found that the A survey of Bareilly district of Uttar Pradesh, India was conducted to record the plant parasitic nematodes associated with some perennial ornamental and medicinal plants. The analysis of nematode communities revealed the presence of several plant parasitic nematodes, with *Hoplolaimus indicus [Basirolaimus indicus], Helicotylenchus dihystera, Meloidogyne incognita, M. javanica and Rotylenchulus reniformis* being the most dominant. *Hemicriconemoides cocophilus* was associated with *Hibiscus rosa-sinensis*, *Rosa* indica [*R. chinensis*] and *Nerium indicum [N. oleander*], but not with *Thevetia peruviana. B. spectabilis. Xiphinema* insigne and *Longidorus elongatus* were confined to only *T. peruviana*. The highest prominence value was recorded for *Helicotylenchus* (73.8) followed by *Rotylenchulus* (65) and *Hoplolaimus* (61.8), due to their high frequencies and densities. The maximum relative biomass was recorded for *Longidorus* (44.7), followed by *Xiphinema* (18) and *Hoplolaimus* (12.4). *L. elongatus* recorded the highest importance Value (48.8) followed by *Hoplolaimus* (42.7), *Helicotylenchus* (36.7) and *Rotylenchulus* (32.7). These 4 genera were the top-tanking parasitic nematodes associated with the crops surveyed.

Chapter-III

Materials and Methods

Experimental Site

Medicinal gardens clustered in three locations of Bhubaneswar at Patrapada, OUAT proper and Regional Research Laboratory campus area were selected for collection of soil samples around root zones of 35 medicinal and 5 aromatic plants during September-October 2008.

MATERIALS USED

3.1. Glasswares and Equipments

The following laboratory appliances and other materials required during the course of investigations are as under,

a. Khurpi
b. Spade
c. Polythene Bags
d. Label Cards
e. Rubber bands
f. Glass beaker(250cc)
g. Aluminium pan
h. Petridish
i. Tissue Paper
j. Aluminium wire gauge
k. A set of 20, 60 and 350 mesh sieves
l. Counting dish
m. Capillary micro-pipette
n. Wet collection bottles
o. Measuring cylinder
p. Hand tally counter
q. Cavity block with lid

r. Glass slides
s. Glass wool
t. Round cover slips
u. Glyceel
v. Desiccators
w. Hot air oven
x. Binocular stereoscopic microscope
y. Olympus research microscope
z. Nylon bristle

3.2. Chemicals Used

a. 96% Ethanol
b. Glycerol
c. Formaldehyde
d. Calcium chloride
e. Lactic acid
f. Phenol
g. Acid fuchsin stain

METHODS ADOPTED

3.3. Collection of Soil Samples

Soil samples each of 500gm from the rhizosphere of shallow rooted plants of 35 medicinal plants and 5 aromatic plants from 3 locations of Bhubaneswar were scooped up with the help of khurpi/spade. Each soil sample was filled into polythene bag tied with a rubber band and tagged with a label card indicating on it the name of the host, locality and date of collection.

3.4. Technique for Extraction of nematodes from Soil

Nematodes were wet screened from the soil samples by a combination of cobb's sieving and decanting technique (Cobb 1918) and improved Baermann funnel technique (Schindler,1961). First the soil from the polythene

bag was spread on the table, mixed well and most of the derbis as well as roots were removed .Then by cone and quarter method, about 250cc of soil sample was taken into aluminium pan. Sufficient amount of water was added and stirred well in order to prepare a soil suspension. The remaining clods were broken by hands while removing more of plant debris and gravels. The suspension was allowed to stand for about 10 seconds so that sand and heavier particles quickly settled down. The muddy soil suspension was passed through an assembly of phosphoro-bronze wire-netted sieves of 20, 60 and 350mesh sieves. Residues of 20 mesh sieve were washed out while the contents of 60 mesh was separately collected in a glass beaker, by backwashing of sieve with gentle stream of water and the suspension was directly examined under stereoscopic microscope, for presence of any cyst nematodes, if present Contents of 350 mesh sieve was collected into a 250cc beaker by backwashing the sieve with gentle stream of water from the tap. Each soil sample was wet screened in the same manner to collect most of the nematodes. The whole suspension collected in the beaker was poured onto a double layer tissue paper resting on a supporting wire-gauge matching the petridish. The wire gauge assembly was then placed on petridish containing sufficient water such that the bottom of the wire gauge tissue paper assembly was slightly submerged in water. The assembly was covered by lid to prevent loss of water due to evaporation and left as such for 24hours, so as to allow maximum number of nematodes to wriggle out through the tissue paper into the bottom of petridish. Next day, the wire gauge was removed. Petridishes were put on the stage of the stereoscopic microscope to examine the presence of nematodes.

In this manner all total 40 samples screened were processed following improved Baermann funnel technique to get clear nematode suspension.

3.4. Counting the Nematodes

For counting the nematodes present in petridishes a counting dish 7 cm x 7cm size was chosen. Its bottom was marked into squares. The nematode suspension present in petridish was transferred to this counting dish and

examined under stereoscopic microscope. Observation was started from one corner square of the counting dish and gradually by sliding the dish all the squares were completed. By means of a hand tally counter, the number of nematodes in each square was recorded, and finally summed up to asses number of nematodes present in the counting dish for each soil sample (250cc) collected from an individual host. This process was repeated for all samples and the no. of nematodes present was recorded genera wise from all samples belonging to different host plants.

3.5. Killing And Fixing Nematodes

The nematode suspension collected in petridishes were transferred into wet collection bottles. Nematodes were then killed, fixed and preserved in wet collection bottles for identification in the following manner.

The nematode suspension collected in a glass beaker was allowed to rest for at least one hour, so that the nematodes would settled down at the bottom. The aluminium pan containing water was put over the heater for heating simultaneously. The supernatant suspension was siphoned out through capillary micropipette so as to concentrate the nematode suspension reducing volume of suspension to approximately 10ml. The beaker containing nematode suspension was stirred uniformly on hot water bath for about 2 minutes for killing the nematodes. The nematode suspension was cooled down and poured into the wet collection bottle. Equal volume of freshly prepared double strength formalin glycerol fixative (Formalin 8ml, Glycerin 2ml, water 45ml) was added into the wet collection bottle for fixing the nematodes. Then the wet collection bottle was screw capped and put in the wooden cabinet.

3.6. Processing of Nematodes

Seinhorst solution-I was freshly prepared by mixing 96% ethanol-20ml, glycerol-1ml and distilled water-79 ml.0.5 ml of Seinhorst solution was pipetted out into a cavity block. Fixed nematodes were transferred with the help of bamboo splinter into the cavity block. Then lid of cavity block was partially opened after transferring the nematodes from fixed suspension

through bamboo splinter and cavity block containing nematode was put inside one desiccator containing 96% ethanol about 1/10th of its volume .The entire assembly was put inside an incubator at 35^{0C}-40^{0C} temperature for 12 hours. After 12 hours the partially processed nematodes in cavity block was taken out and the saturated alcohol was allowed to evaporate in the open air, so that minimum quantity of solution was finally left in cavity block .One ml of Seinhorst II solution (96%ethanol -95ml &glycerol 5 ml) was transferred into cavity block and the cavity block was put inside another desiccator containing $CaCl_2$. Similarly lid of cavity block was partially opened , so that quantity of alcohol and traces of water present inside cavity block was absorbed by $CaCl_2$ and finally nematodes were retained in glycerol.

3.7. Mounting of Nematodes

A plain glass (clean) slide was put on stage of the Stereoscopic microscope. A small drop of anhydrous glycerol was put in the centre of slide. 5-10 processed nematodes were transferred into glycerol mount through bamboo splinter. Nematodes were arranged in glycerol in such a way that all their heads pointed to one direction and nematodes were allowed to rest at the bottom of glycerol mount without floating on the surface. Three glass wools were separately placed radially in the glycerol mount. A round cover slip was warmed up and put carefully over the glycerol mount, so that glycerol is uniformly spread without disturbing nematodes. Periphery of glass slide was sealed through glyceel. Finally the prepared slide was observed under microscope.

3.8. Identification of Nematodes

Nematode specimens mounted on slides were examined under Olympus Research microscope for identification of various plant parasitic (*Tylenchids* and *Dorylaimids*) and free living (*Mononchids* and *Rhabditids*) nematodes associated with different medicinal and aromatic plants.

3.9. Staining of root knot infected root material

The infected root knot root material was washed free from soil. The infected root material was made air dry and plunged in to freshly prepared lacto phenol solution(lactic acid, phenol, glycerol and water @ 1:1:2:1 proportion) stained with 0.1% acid fuchsin contained in a glass beaker. Boiling was continued for about 3 minutes. After boiling, the root material was removed and washed off excess stain in running water and put in another beaker containing plain lacto phenol and preserved.

3.10. Isolation of root knot adult female from stained root

A small piece of stained root knot infected material was transferred in to a watch glass containing lacto phenol. The root knot infected portion was carefully teased apart with the help of a pair of needles to isolate five adult root knot females.

3.11. Cutting of perineal pattern of adult females of root knot nematod

One glass cavity slide containing lacto phenol was put on the stage of the stereoscopic microscope. Five root knot females were transferred in to the cavity slide. The swollen adult female was speared with a needle and holding it so, the posterior portion of the female was cut with the help of a fine blade. The posterior portion, thus, cut was further trimmed with a blade and carefully the inner tissues were removed with a nylon bristle. After cleaning, the perineum was picked with a bamboo splinter and put in to a drop of lacto phenol mounted at the centre of a plain glass slide. Likewise five perineums after trimming in a same manner were transferred in to glass slide. These perineums were arranged in such a way that the outside of the perineums were facing upward towards objective of the microscope. A round cover slip was warmed up and applied on lacto phenol and sealed. The slide was put on the stage of the Olympus research microscope and perineal patterns were observed at a magnification of about 800x. It was found that dorsal arch was very high. Striae on the dorsal arch were wavy and discontinues. Striae formed whorl at the tail tip. Lateral lines were very faint, striae of the ventral arch formed "Y" shaped fork at the lateral line. All the perineal patterns exhibited the same morphological characters confirming the species as Meloidogyne incognita.

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Chapter-IV

Results

The results of a survey conducted at 3 locations (Patrapada, OUAT Proper and Regional Research Laboratory) of Bhubaneswar to study the nematode associations on medicinal and aromatic plants showed some interesting pattern of their occurrence and distribution. The population densities of important groups of plant parasites and free living nematodes have also been indicated.

4.1. Nematodes associated with Medicinal plants

In the present study, 35 medicinal plants were chosen and the type of plant parasitic as well as free living nematodes occurring in the rhizosphere have been identified, indexed and their density of population have been recorded and presented below in detail after each plant type examined.

1. Stevia(*Stevia rebudiana*)

Locality	:	OUAT Proper
Date of collection	:	08. 09. 2008
Nematodes observed and density of population per 250 cc soil noted within Parenthesis	:	Rotylenchulus reniformis (425) *Hoplolaimus indicus* (05) *Helicotylenchus dihystera*(27) *Tylenchorhynchus coffeae* (1) *Xiphinema insigne* (12) *Aphelenchus avenae* (05) *Rhabditids* (65) *Dorylaimid* (4)

2. **Podina (*Mentha arvensis*)**

Locality	:	OUAT Proper
Date of collection	:	08.09 2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (376) *Calosia exilis* (252) *Helicotylenchus dihystera* (16) *Xiphinema insigne* (8) *Mylonchulus* sp. (12) *Dorylaimid* (08)

3. **Bhrungaraj (*Eclipta prostrata*)**

Locality	:	OUAT Proper
Date of collection	:	08.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (224) *Helicotylenchus dihystera* (423) *Rhabditids* (85) *Dorylaimid* (05)

4. **Akarakara (*Spilanthes calva*)**

Locality	:	OUAT Proper
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (27) *Xiphinema insigne* (23) *Cephalenchus sp.* (12) *Hoplolaimus indicus* (04) *Helicotylenchus dihystera* (09) *Aphelenchus avenae* (04) *Rhabditids* (65) *Mononchids* (12) *Dorylaimids* (07)

5 Hadabhanga (*Vitis quadrangularis*)

Locality	:	OUAT Proper
Date of collection	:	15.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (252) *Helicotylenchus dihystera* (22) *Xiphinema insigne* (54) *Criconemalla ornata* (10) *Hoplolaimus indicus* (10) *Aphelenchus avenae* (8) *Pratylenchus coffeae* (5) *Rhabditids* (34)

6 Gheekuanri (*Aloe vera*)

Locality	:	OUAT Proper
Date of collection	:	15.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (64) *Helicotylenchus dihystera* (92) *Meloidogyne incognita* (330) *Xiphinema insigne* (25) *Aphelenchus avenae* (15) *Rhabditids* (85) *Mononchid* (14)

7. Gandha Sunthi (*Kaempferia galanga*)

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Meloidogyne incognita* (27) *Helicotylenchus dihystera* (8) *Xiphinema insigne* (15) *Hoplolaimus indicus* (6) *Aphelenchus avenae* (3) *Cephalenchus* sp. (2) *Dorylaimid* (4)

8. **Ayapana (*Eupatorium triplinerve*)**

Locality	:	Regional Research Laboratory
Date of collection	:	18.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (33) *Helicotylenchus dihystera* (528) *Hoplolaimus indicus* (12) *Rhabditids* (22)

9. **Kalmegh (*Andrographis paniculata*)**

Locality	:	OUAT Proper
Date of collection	:	15.09 2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Meloidogyne* sp. (45) *Hoplolaimus indicus* (07) *Helicotylenchus dihystera* (22) *Xiphinema insigne* (89) *Tylenchorhynchus coffeae* (03) *Dorylaimids* (11) *Rhabditids* (25)

10. **Safed musli (*Chlorophytum barivilianum*)**

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Xiphinema insigne* (7) *Aphelenchus avenae* (6) *Cephalenchus* sp. (3) *Hoplolaimus indicus* (04) *Meloidogyne* sp (02) *Rhabditids* (106) *Dorylaimid* (15) *Mylonchulus* sp. (3)

11. Aswagandha (*Withania somnifera*)

Locality	:	Regional Research Laboratory
Date of collection	:	18.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Hoplolaimus indicus* (47) *Aphelenchus avenae* (27) *Xiphinema insigne* (22) *Rhabditiid* (105)

12. Antamula (*Tylophora asthmatics*)

Locality	:	Regional Research Laboratory
Date of collection	:	18.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rhabditids* (157) *Dorylaimids* (72) *Mononchids* (05)

13. Pasaruni (*Paedria foetida*)

Locality	:	Regional Research Laboratory
Date of collection	:	18.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Hemicriconemoides cocophilus*(12) *Helicotylenchus dihystera* (24) *Hoplolaimus indicus* (05) *Mononchids* (22)

14. Patalagaruda (*Rauwolfia tetraphilas*)

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Xiphinema insigne* (38) *Helicotylenchus dihystera* (175) *Mononchids* (06)

15. **Hemakedar (*Kalanchoe pinnata*)**

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (24) *Rhabditiid* (68) *Dorylaimids* (12) *Mononchids* (06)

16. **Patalagaruda (*Rauwolfia serpentina*)**

Locality	:	OUAT Proper
Date of collection	:	15.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (08) *Helicotylenchus dihystera* (62) *Meloidogyne* sp.(03) *Aphelenchus avenae* (08) *Mononchids* (04) *Rhabditids* (02)

17. **Davana (*Artimesia pallens*)**

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (25) *Dorylaimids* (12) *Mononchids* (09)

18. Senna (*Cassia angustifolia*)

Locality	:	OUAT Proper
Date of collection	:	15.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (25) *Rotylenchulus reniformis* (62) *Xiphinema insigne* (08) *Mononchids* (12)

19. Tulsi (*Ocimum sanctum*)

Locality	:	Regional Research Laboratory
Date of collection	:	18.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dih*ystera (125) *Xiphinema insigne* (26) *Rhabditids* (45)

20. Pomegranate (*Punica granatum*)

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Cephalenchus* sp. (36) *Rhabditids* (52) *Mononchids* (18)

21. Nalichita (*Plumbago rosea*)

Locality	:	OUAT Proper
Date of collection	:	08.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (31) *Hoplolaimus indicus* (25) *Criconemella ornata* (10) *Aphelenchus avenae* (08) *Meloidogyne* sp. (06) *Dorylaimid* (72)

22. Kanta siju (*Euphorbia nerifolia*)

Locality	:	OUAT Proper
Date of collection	:	08.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (32) *Rotylenchulus reniformis* (25) *Aphelenchus avenae* (10) *Dorylaimids* (51) *Rhabditids* (35)

23. Dhalachita (*Plumbago zeylanica*)

Locality	:	OUAT Proper
Date of collection	:	15.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Aphelenchus avenae* (12) *Hoplolaimus indicus* (08) *Dorylaimids* (68) *Rhabditids* (18)

24. Black pepper (*Piper nigrum*)

Locality	:	OUAT Proper
Date of collection	:	22.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (93) *Tylenchorhynchus coffeae* (20) *Meloidogyne* sp.(06) *Rhabditids* (34)

25. Pipla (*Piper longum*)

Locality	:	OUAT Proper
Date of collection	:	22.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (564) *Meloidogyne* sp. (38) *Xiphinema insigne* (08) *Mononchids* (12) *Rhabditids* (23)

26. Manjuati (*Lawsonia alba*)

Locality	:	Patrapada
Date of collection	:	10.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (95) *Meloidogyne* sp. (12) *Mononchids* (25)

27. Gunja (*Abrus precatorius*)

Locality	:	OUAT Proper
Date of collection	:	22.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (65) *Xiphinema insigne* (72) *Hoplolaimus indicus* (18) *Mononchids* (24)

28. Thalkudi (*Centella aciatica*)

Locality	:	OUAT Proper
Date of collection	:	22.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (51) *Rotylenchulus reniformis* (125) *Hoplolaimus indicus* (48) *Meloidogyne* sp. (35) *Rhabditids* (62)

29. Brahmi (*Bacopa monnieri*)

Locality	:	OUAT Proper
Date of collection	:	22.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis*(1228) *Meloidogyne* sp. (76) *Hoplolaimus indicus* (51) *Xiphinema insigne* (32) *Rhabditids* (63) *Mononchids* (27)

30. **Satavari (*Asparagus racemosus*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis		*Helicotylenchus dihystera* (25) *Hoplolaimus indicus* (33) *Criconemella ornata* (10) *Xiphinema insigne* (04) *Rhabditids* (78) *Dorylaimids* (25)

31. **Sweety flag (*Acoros calamus*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (51) *Hoplolaimus indicus* (112) *Rhabditid* (65) *Dorylaimids* (27) *Mononchids* (13)

32. **Aparajita (*Clitoria ternatea*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (82) *Hoplolaimus indicus* (22) *Xiphinema insigne* (15) *Rhabditids* (44) Dorylaimids (27)

33. **Wild Garlic (*Allium porum*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Meloidogyne* sp. (227) *Hoplolaimus indicus* (15) *Rhabditids* (88) *Dorylaimids* (32)

34. **Gudamari (*Gymnema sylvestre*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Meloidogyne* sp. (57) *Rotylenchulus reniformis* (39) *Helicotylenchus dihystera* (12) *Dorylaimids* (87) *Mononchids* (40)

35. **Palua (*Curcuma zedoaria*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (107) *Tylenchus* sp. (34) *Rhabditid* (58) *Dorylaimids* (27) *Mononchids* (12)

4.2 Nematodes associated with Aromatic plants:

During this survey, soil samples from five aromatic plants have been examined for studying association of plant parasitic as well as free living nematodes in different aromatic plants and their numbers host wise have been recorded and presented as follows.

1. **Rukuna Hatapochha (*Coleus aromaticus*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Xiphinema insigne* (122) *Meloidogyne* sp. (15) *Aphelenchus avenae* (08) *Rhabditids* (46)

2. **Patcholi (*Pogostemm patchordi*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (27) *Tylenchorhynchus coffeae* 125) *Rhabditids* (15)

3. **Kanchan (*Bauhinia variegata*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Helicotylenchus dihystera* (348) *Meloidogyne incognita* (33) *Criconemella ornata* (264) *Xiphinema* (32)

4. **Coleus (*Coleus forskholi*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Rotylenchulus reniformis* (329) *Criconemella ornata* (25) *Rhabditids* (74) *Dorylaimids* (47) *Mononchids* (21)

5. **Amba Ada (*Curcuma amada*)**

Locality	:	OUAT Proper
Date of collection	:	25.09.2008
Nematodes observed and density of nematode population per 250cc soil noted within parenthesis	:	*Meloidogyne sp.* (142) *Rotylenchulus reniformis* (25) *Aphelenchus avenae* (12) *Hoplolaimus indicus* (10)

4.3 Table No. 1: Nematode index and medicinal plant associations

Sl. No.	Name of the Nematode in Descending order of frequency	Name of the associated host plants
4.3.1	**Plant parasitic nematodes**	
1.	***Helicotylenchus dihystera***	*Stevia rebudiane*
		Mentha arvensis
		Eclipta prostrata
		Spilanthes calva
		Vitis quadrangularis
		Aloe vera
		Kaempferia galangal
		Eupatorium triplinerve
		Andrographis paniculata
		Paedria foetida
		Rauwolfia tetraphilas
		Kalanchoe pinnata
		Rauwolfia serpentine
		Artimesia pallens
		Cassia angustifolia
		Ocimum sanctum
		Plumbago rosea
		Euphorbia nerifolia
		Piper nigrum
		Piper longum
		Lawsonia alba
		Abrus precatorius
		Centella aciatica
		Asparagus racemosus
		Acoros calamus
		Gymnema syvestina

2.	***Hoplolaimus indicus***	*Stevia rebudiana*
		Spilanthes calva
		Vitis quadrangularis
		Kaempferia galangal
		Eupatorium triplinerve
		Andrographis paniculata
		Chlorophytum barivilianum
		Withania somnifera
		Paedria foetida
		Plumbago rosea
		Plumbago zeylanica
		Abrus precatorius
		Centella aciatica
		Bacopa monnieri
		Asparagus racemosus
		Acoros calamus
		Clitoria ternatea
		Allium porum
3.	***Xiphinema insigne***	*Stevia rebudiana*
		Mentha arvensis
		Spilanthes calva
		Vitis quadrangularis
		Aloe vera
		Kaempferia galangal
		Andrographis paniculata
		Chlorophytum barivilianum
		Withania somnifera
		Rauwolfia tetraphilas
		Cassia angustifolia
		Ocimum sanctum
		Piper longum
		Arbus precatorius
		Bocopa monnieri
		Asparagus racemosus

		Clitoria ternatea
4.	***Rotylenchulus reniformis***	*Stevia rebudiana*
		Mentha arvensis
		Eclipta prostrate
		Spilanthes calva
		Vitis quadrangularis
		Aloe vera
		Eupatorium triplinerve
		Rauwolfia serpentine
		Cassia angustifolia
		Euphorbia nerifolia
		Centella aciatica
		Bacopa monnieri
		Clitoria ternatea
		Gymnema sylvstre
		Curcuma zedoaria
5.	***Meloidogyne incognita***	*Aloe vera*
	Meloidogyne sp.	*Kaemferia galangal*
		Andrographis paniculata
		Chlorophytum barivilianum
		Rauwolfia serpentine
		Plumbago rosea
		Piper nigrum
		Piper longum
		Lawasonia alba
		Centella aciatica
		Bacopa monnieri
		Allium porum
		Gymnema sylvestre

6.	***Aphelenchus avenae***	*Stevia rebudiana*
		Spilanthes calva
		Vitis quadrangularis
		Aloe vera
		Kaempferia galanga
		Chlorophytum barivilianum
		Withania somnifera
		Rauwolfia serpentine
		Plumbago rosea
		Euphorbia nerifolia
		Plumbago zeylanica
7.	***Cephalenchus* sp.**	*Spilanthes calva*
		Kaempferia galangal
		Chlorophytum barivilianum
		Punica granatum
8.	***Tylenchorhynchus coffeae***	*Stevia rebudiana*
		Andrographis paniculata
		Piper nigrum
9.	***Criconemella ornate***	*Plumbago rosea*
		Asparagus racemosus
		Vitis quadrangularis
10.	***Pratylenchus coffeae***	*Vitis quadrangularis*
11.	***Caloosia exilis***	*Mentha arvensis*
12.	***Hemicriconemoides cocophilus***	*Paedria foetida*
13.	***Tylenchus* sp.**	*Curcuma zedoaria*

4.3.2. Free living Nematodes

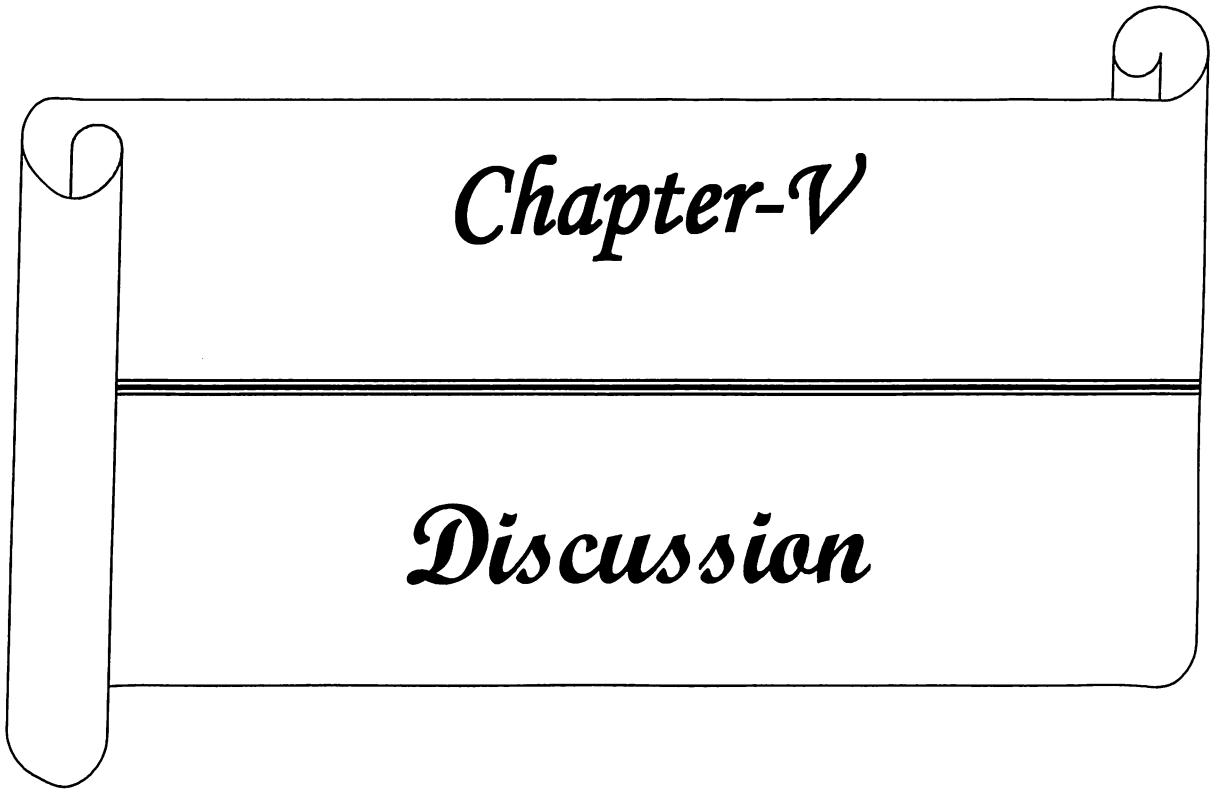
1.	***Rhabditids***	*Stevia rebudiana*
		Eclipta prostrate
		Spilanthes calva

		Vitis quadrangularis
		Aloe vera
		Eupatorium triplinerve
		Andrographis paniculata
		Chlorophytum barivilianum
		Withania somnifera
		Tylophora asthmatics
		Kalanchoe pinnata
		Rouwolfia serpentina
		Ocimum sanctum
		Punica granatum
		Euphorbia nerifolia
		Plumbago zeylanica
		Piper nigrum
		Piper longum
		Centella aciatica
		Bacopa monnieri
		Asparagus racemosus
		Acorus calamus
		Allium porum
		Curcuma zedoaria
		Clitoria ternatea
2.	***Mononchids***	*Mentha arvensis*
		Eclipta prostrata
		Spilanthes calva
		Aloe vera
		Chlorophytum barivilianum
		Tylophora asthmatica
		Paedria foetida
		Rauwolfia tetraphilas
		Kalanchoe pinnata

		Rauwolfia serpentina
		Artimesia pallens
		Cassia angustifolia
		Punica granatum
		Piper longum
		Lawasonia alba
		Abrus precatorius
		Bacopa monnieri
		Acoros calamus
		Gymnema sylvestre
		Curcuma zedoaria
3.	***Dorylaimids***	*Stevia rebudiane*
		Mentha arvensis
		Eclipta prostrata
		Spilanthes calva
		Kaempferia galanga
		Andrographis paniculata
		Chlorophytum barivilianum
		Tylophora asthmatica
		Kalanchoe pinnata
		Artimesia pallens
		Plumbago rosea
		Euphorbia nerifolia
		Plumbago zeylanica
		Asparagus racemosus
		Acoros calamus
		Clitoria tennatea
		Allium porum
		Gymnema sylvestre
		Curcuma zedoaria

4.4. Table-II Nematode index and Aromatic plant Association

Sl. No.	Name of the nematode with descending order of frequency	Host
I.	**Plant parasitic Nematodes**	
1.	***Meloidogyne incognita***	*Bauhinia variegate*
	Meloidogyne sp.	*Curcuma amada*
		Coleus aromaticus
2.	***Rotylenchulus reniformis***	*Coleus forskholi*
		Corcuma amaada
3.	***Helicotylenchus dihystera***	*Pogostemm patchordi*
		Bauhinia variegate
4.	***Xiphinema insigne***	*Coleus aromaticus*
		Bauhinia variegate
5.	***Tylenchorhynchus coffeae***	*Pogostemm patchordi*
6.	***Hoplolaimus indicus***	*Corcuma amaad*
7.	***Criconemella ornata***	*Coleus forskholi*
8.	***Aphelenchus avenae***	*Corcuma amaada*
4.4.2.	**Free living Nematodes**	
1.	***Rhabditids***	*Coleus aromaticus*
		Pogostemm patchordi
		Coleus forskholi
		Curcuma amada
2.	***Mononchids***	*coleus forskholi*
		Curcuma amada
3.	***Dorylaimids***	*Coleus forskholi*



Chapter-V

Discussion

Over the past few years, Medicinal and Aromatic plants have regained a wide recognition due to an escalating faith in herbal products in view of its lesser side effects compared to synthetic products in addition to the necessity of meeting the requirement of medicines and cosmetics for an increasing human population. Moreover, there had been a problem of obtaining right kind of medicinal and aromatic plant material in desired quantity from the wild sources growing in natural forest ecosystems as indiscriminate destructive collections resulted short supply of some important herbs resulting adulteration / substitution. So systematic cultivation of MAPs involving high yielding varieties producing quality plant materials maintaining the regular supply of raw material of desired quality with an ultimate aim of achieving green technologies for better health and life, oriented the industrialists, government and non government organizations to insist cultivation of medicinal and aromatic plants in massive scale. As a result of intensive cultivation of MAPs following recommended package of practices and also the monoculture practice of growing MAPs culminated upsurge of pest and disease problem. Since plant parasitic nematodes have wider adaptability to any adverse inclement weather condition, they are the limiting factors for low production of MAPs as Sharma and Pandey (2009) reported that Ashwagandha (*withania somnifera*) was highly susceptible to *Meloidogyne incognita* resulting root galling, stunted growth of the plant and low productivity. Also Singh and Kumar (1998) indicated that *Meloidogyne incognita* was the no.1 species affecting *Mentha arvensis*.

So with increasing realization of the economic importance and pathogenic role of nematodes in Agriculture and allied sectors, attempts are now being made to determine the nature of their parasitism with various types of beneficial and commercial plants like MAPs. So for a meaningful appreciation of namic problems in MAPs, it is necessary to study plant

nematode association to acquire more knowledge about the distribution pattern of different groups of phytonematodes, so that their population densities can be correlated with the possible logical implication.

Result of the present study involving analysis of soil samples of 40 MAPs obtained from 3 different localities in Bhubaneswar present a consolidated picture of the nature of nematode distribution and the plant nematode association. Out of 13 genera of plant parasitic nematodes encountered in 35 medicinal plants, the following genera on the basis of their population density and extent of host range cited in Table-1, appear to be important and are recorded in order of their frequency in occurrence as *Helicotylenchus dihystera, Hoplolaimus indicus, Xiphinema insigne, Rotylenchulus reniformis, Meloidogyne* sp. and *Aphelenchus avenae*. Where the species of *Helicotylenchus* is associated with 26 different hosts, *Hoplolaimus* (18), *Xiphinema* (17), *Rotylenchulus* (15), *Meloidogyne* (13) and *Aphelenchus* (11) hosts. While studying medicinal plants in Himachal Pradesh, Khan and Sharma (1996) reported that species of *Meloidogyne, Helicotylenchus, Pratylenchus, Tylenchorhynchus, Hoplolaimus* and *Aphelenchus* were frequently prevalent. Some difference in frequency of occurrence and also association of different type of nematode species might be due to prevalence of nematode in different agroclimatic zones. Also it is interesting to find, where *Rotylenchus reniformis* has been associated with 15 different medicinal plants, each having reasonably higher population density than other nematode species reaching to the peak population of 1228 per 250cc soil in *Bocopa monnieri* such higher density of population indicates those plant types to be good hosts of *Rotylenchulus reniformis*. But in case of species of *Meloidogyne*, though 13 different medicinal plants are hosts of this species, yet the density of population is quite low except *Aloe vera* where maximum of 330 J2 of *Meloidogyne* have been recorded per 250cc of soil. Moreover, *Aloe vera* is the only host where root galls were found and the adult female was identified as *Meloidogyne incognita* based on perennial pattern observation. Also it is

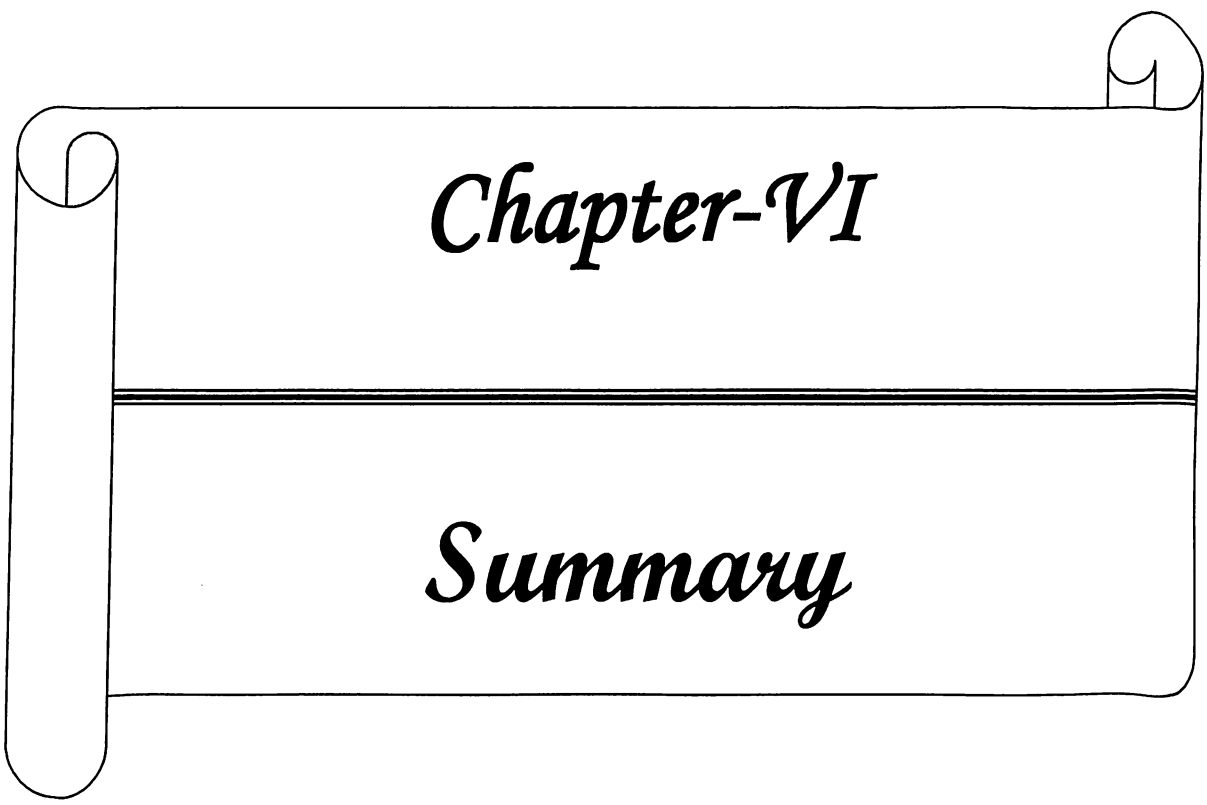
quite interesting that Antamula (*Tylophora asthmatica*) is the only medicinal plant surveyed, where no plant parasitic nematodes could be detected except free living nematodes. So it creates new avenue for future study on the plant types and its resistant property against phytonematodes.

In addition to parasitic nematodes, there is also record on the sizeable population of free living nematodes like Rhabditids and Mononchids, but few Dorylaimids. So heterogeneous nematode populations are found to be associated with those medicinal plants studies. This is in conformity with the study of parasitic and free living nematodes associated with medicinal plants in Phulbani districts (Routray and Das, 1982).

As regards to phytonematodes associated with five aromatic plants, the data summarized in table-II reveals that based on the order of frequency in occurrence, the predominant nematode genera are *Meloidogyne, Rotylenchulus, Helicotylenchus* and *Xiphinema*, though eight plant parasitic nematode genera are associated with the aromatic plants. This is an agreement with the report of Shivakumar and Vadivelu (1997) while studying association of plant parasitic nematodes in 46 medicinal and aromatic plants in Nilagiris. They reported seven plant parasitic nematode genera like *Meloidogyne, Helicotylenchus, Pratylenchus, Tylenchorhynchus, Xiphinema, Scutellonema* and *Hemicriconemoides* in order of their frequency in occurrence.

Bauhinia variegata is found harbouring population of 264 J2 of *Meloidogyne* and roots exhibited production of root knots. Extracting adult females from the roots and cutting perennial patterns in the species was confirmed as *Meloidogyne incognita*. But *Corluma amalca* and *Coleus aromaticus* exhibiting no root galls donot confirm its species identity. So these J2 are called *Meloidogyne* spp. Also free living nematodes particularly *Rhabditids* were the dominant populations in four aromatic plants followed by *Mononchid* in 2 hosts and *Dorylaimid* (one) host. So aromatic plants also harbour heterogeneous group of diverse type of nematode genera.

However the preliminary study on association of nematodes in medicinal and aromatic plants have been very interesting and encouraging. Further studies on a wider range of such plants will provide a wealth of information in evaluating the nature of host parasite relationship, their interaction and finally their management.



Chapter-VI

Summary

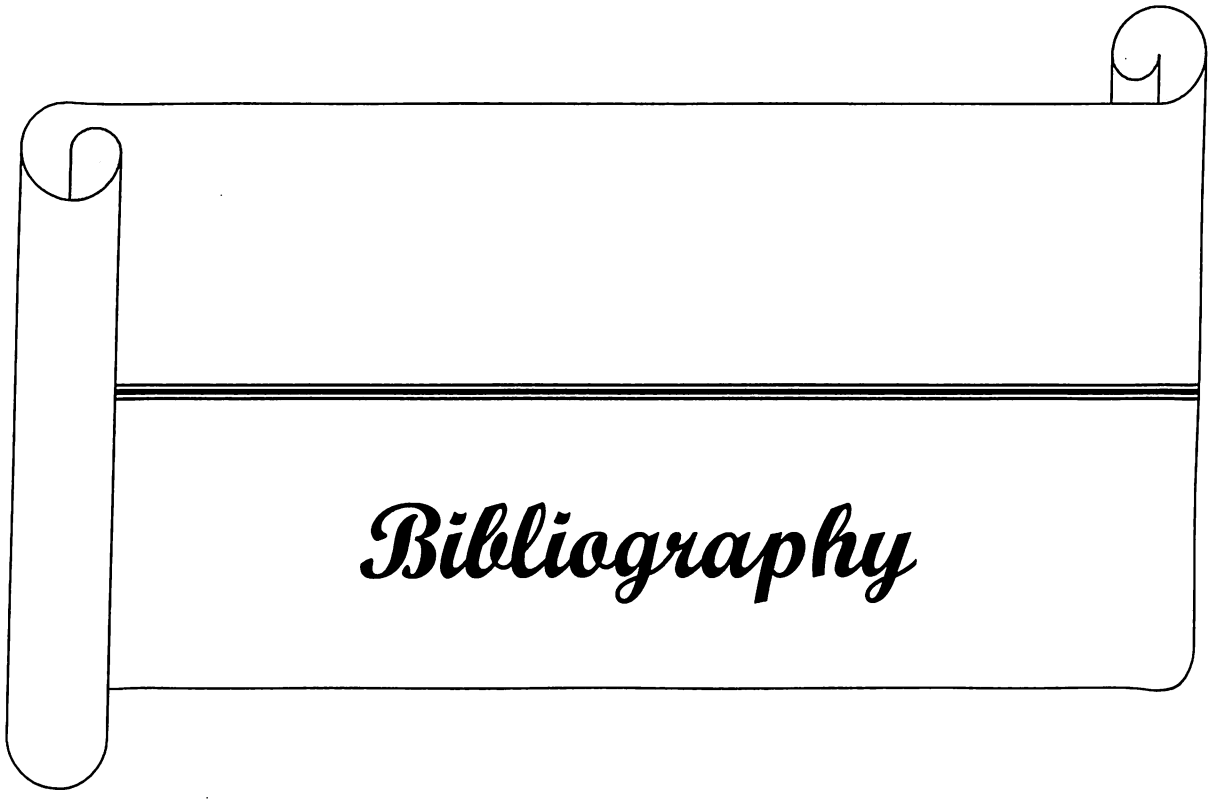
SUMMARY

Examination of rhizosphere soil samples obtained from economically important medicinal and aromatic plants growing in three localities of Bhubaneswar revealed interesting pattern of nematode distribution, their population densities and possible plant nematode associations.

It was observed that thirteen plant parasitic nematode genera were associated with 35 medicinal plants examined. The important nematode genera in decreasing order of their frequency in occurrence were *Helicotylenchus dihystera, Hoplolaimus indicus, Xiphinema insigne, Rotylenchulus reniformis, Meloidogyne* sp., *Aphelenchus avenae, Cephalenchus* sp., *Tylenchorhynchus coffeae, Criconemella ornata, Caloosia exilis, Hemicricinemoides cocophilus, Pratylenchus coffeae* and *Tylenchus* sp. .

Similarly on examination of 5 aromatic plants, eight plant parasitic nematode genera were found. The important nematode genera in descending order of their prevalence were *Meloidogyne* sp., *Rotylenchulus reniformis, Helicotylenchus dihystera, Xiphinema insigne, Tylenchorhynchus coffeae, Hoplolaimus indicus, Criconemella ornata* and *Aphelenchus avenae.*

Among free living nematodes, *Rhabditids* were of common occurrence in both medicinal and aromatic plants in most of the samples followed by *Mononchids* and *Dorylamids.*



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