NEMATODE DIVERSITY AND COMMUNITY COMPOSITION IN THE RHIZOSPHERE OF LEMON

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MASTER OF SCIENCE IN AGRICULTURE
(NEMATOLOGY)

BY

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C E R T I F I C A T E – I

This is to certify that the thesis entitled " **NEMATODE DIVERSITY AND COMMUNITY COMPOSITION IN THE RHIZOSPHERE OF LEMON**" submitted by **SRISHTI** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment of the requirement for the award of the degree of MASTER OF SCIENCE IN AGRICULTURE (NEMATOLOGY) is a faithful record of bonafide 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 her.

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Dated, 31.07.2014.

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C E R T I F I C A T E – II

This is to certify that the thesis entitled " **NEMATODE DIVERSITY AND COMMUNITY COMPOSITION IN THE RHIZOSPHERE OF LEMON**" submitted by **SRISHTI** to the Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfilment 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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Bhubaneswar

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ABSTRACT

Examination of rhizosphere soil samples obtained from economically important lemon (*Citrus limon*) plants growing in localities of Bhubaneswar revealed various pattern of nematode distribution and association, the population densities indicating existence of nematode diversity and differences in community composition. It was observed that 25 lemon plant rhizospheres contained maximum number of *Rotylenchulus reniformis* followed by *Helicotylenchus dihystera* and *Meloidogyne incognita,* respectively, among eight plant parasitic nematode recovered from the rhizospheres. Apart from these plant parasitic nematodes three groups of non-plant parasitic nematodes were also recorded such as Dorylaimids, Mononchids and Rhabditids with lesser population densities as compared to parasitic group of nematodes.

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Chapter 1

INTRODUCTION

INTRODUCTION

Citrus is grown in more than 125 countries in the belt within 35° latitude both North and South of the equator. Within the family Rutaceae the genera Citrus (oranges, mandarins, grapefruits, lemons, limes & citrons) and Poncirus (trifoliate oranges) contain the principal commercial species. (Swingle and Rese, 1967). This deep-rooted plant, a good source of vitamin C, is the third largest and the most important fruit crop industry in India. It occupies a place of considerable importance in the fruit economy of the country. This fruit is mainly grown as a subsistence crop in the backyards in Odisha.

The lemon (*Citrus limon*) is a small evergreen tree native to Asia. The religious ellipsoidal yellow fruit is used for both culinary and non-culinary purposes primarily for its juice. The juice of lemon contains about 5 to 6% citric acid, which gives lemon, a sour taste. The distinctive sour taste of lemon juice makes it a key ingredient in drinks and foods.

Lemon is supposed to be originated in North east India. India contributes about 16% of world production of lemon. Area under production is 286.3 thousand ha with production of total fruits 2429 thousand tonnes (FAO, 2013).

Lemon problems such as disorders, pest and disease damage, and nutritional deficiencies are numerous. A large number of diseases caused by viruses, bacteria, fungi and nematodes have been reported to occur in lemon.

Among various diseases the role of phytonematodes cannot be ruled out. Nematodes are discrete group of biological entity which have immense potentiality to sustain damage in each and every environment where lemon is grown. As soil is the common abode for both plant and nematode parasites, there is an ample opportunity for competition for food leading to parasitism. Several nematode species have become obligate parasites on lemon and due to their universal distribution, no lemon plant is likely to be free from the attack of plant parasitic nematodes.

Most of the lemon plants are known to be associated with one or more species of phytonematodes. Many nematode species are found to be associated with

the lemon rhizosphere (*Cohn,1972*). Work on nematode parasites on lemon in India was reviewed that 70 species within 37 genera of plant parasitic nematodes were associated (*Reddy and Singh,1979*). However , some of the plant parasitic nematodes have been shown to be of economic importance. Although plant parasitic nematodes have been the real cause of stunted and poor growth of plants, such conditions are also ascribed to poor soil, moisture stress and other related factors. Therefore, very often the problems of nematodes in this crop goes unnoticed by the farmers as well as field workers.

So, keeping this in view the economic loss caused by plant parasitic nematodes in reducing yield and quality of lemon, the present initiative for augmenting the yield potential in lemon was imperative. An Investigation to "nematode diversity and community composition in the rhizosphere of lemon," as part of preliminary study, so that the information generated may be useful for better understanding of nematode problems of lemon for future line of work.

Chapter II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Review of literature is the overview 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 related to nematode association and community composition associated with rhizosphere of lemon are cited as follows.

Nematode Association and Diversity:

Awareness of damage caused to economic plants by parasitic nematodes has been studied extensively during last few decades. There is a pressing demand for information on the existence of nematodes on plants and in agricultural soils. Citrus being a crop of worldwide importance , quite a good number of informations have been accumulated on nematode association , some of which have been established to be acclaimed parasites of the crop limiting its production.

O'Bannon *et. al,* (1972) observed in Florida that *Tylenchulus semipenetrans* on citrus has too high and too low population levels each year. High levels occur in April-May and November –December and low levels in February-March and August-September. Population increase occur about 4-5 weeks after the spring and fall flush of root growth. Populations of *Pratylenchus coffeae* on citrus varied widely, and were not related to season. Populations of *P. brachyurus* showed seasonal variation with a high in June- July and a low in March-May. Males of *T. semipenetrans* and *P. coffeae* were found throughout the year, whereas males of *P . brachyurus* were rare and were found only during November and December.

Parvatha Reddy & Singh (1979) reviewed work on nematode parasites of citrus in India from 1956 to 1977. Seventy species within 37 genera of plant parasitic nematodes in association with citrus roots have been reported from India. Work on four nematode species, *Tylenchulus semipenetrans*, *Pratylenchus coffeae, Hoplolaimus indicus* and *Meloidogyne* spp. For which proof of pathogenicity to citrus is extensively reviewed.

Khan *et. al,* (1979) described *Caloosia brevicaudata* n. sp. found in the soil around the roots of citrus has the shortest female tail.

Davis in 1984 studied the distribution of citrus nematode *Tylenchulus semipenetrans* over 18 months in a six year old orchard of grapefruit (*Citrus paradisi* cv. Ruby Red) and on sour orange (*Citrus aurantium*) rootstock. The 1.8 ha orchard was under chemical weed control, no tillage and flood irrigation. Highest numbers of nematodes were found in the top 15 cm of the soil profile. The nematode population peaked in April and declined to lowest levels in August and September. Numbers of nematodes were negatively correlated (r = -0.95) with soil temperatures above 29° C. Soil populations of nematodes were not correlated with soil moisture. The distribution of the nematode in the field was highly skewed and was described by a negative binomial.In this 1.8 ha block , five soil samples of 12 cores each would provide an estimate within 20% of the true nematode population mean with 95% confidence.

Mani *et. al,* in 1988 conducted a survey of citrus nurseries in Andhra Pradesh, India, 14.2%, 75.4% and 75.0% of those in Kadium, Kodur and panyam were infested with *T. semipenetrans* respectively. Nurseries in Palacole and Tirupati were uninfected. Percent infection of *Meloidogyne javanica* in Kadium, Kodur and Palacole nurseries was 60.7, 7.0 and 45.0 respectively. Nurseries in Panyam and Tirupati were not infected.

Ganguly(1988) observed during a survey at RamTek, Nagpur, during 1987. *Tylenchulus semipenetrans* was associated with almost all the varieties of *Citrus reticulata* (mandarin) surveyed in Katul while *Xiphinema basiri* was found in large numbers on some varieties. *Citrus limon* (lemon) var. Kagzi Gooti had moderate populations of *Rotylenchulus reniformis*.

Bridge(1988) studied nematode problems of the Pacific islands, observed citrus was having the problem of *Tylenchulus semipenetrans* along with *Rotylenchulus reniformis*, *Pratylenchus zeae, Pratylenchus brachyurus* and *Helicotylenchus* spp., *Meloidogyne* spp. etc.

Davide(1988) studied major nematode pests on most economic crops in the Phillipines, including citrus. *Tylenchulus semipenetrans* was a problem on citrus.

Teben'kova and Ivanova(1989) examined soil and root samples from fruit farms in the Gissarsk, Dangarinsk and Kolkhozobadsk lowland districts in the Tadzhik SSR(USSR) in 1984-1988, including greenhouse lemon and orange. 17 plant parasitic nematodes were

recorded, of which the most pathogenic were *Xiphinema index*, *X. pachtaicum*, *Helicotylenchus digonicus*, *H.vulgaris*, *Pratylenchus thornei* and *Criconemella xenoplax*.

Darekar *et. al*,(1990) collected soil and root samples from different fruit crops including citrus, and were examined for plant parasitic nematodes. Twenty two species were found associated with the crops and were listed.

Zhang *et. al*,(1990) described and illustrated *Meloidogyne citri* sp. nov. from the roots of *Citrus unshiu* collected in Shunchang, Fujian, China. The female is similar to that of *M. fujianensis* but has a slightly posterior protuberance; stylet cone is dorsally curved; perineal pattern is rounded with low, flattened arch and cheek like inner striae in ventro-lateral areas. The male stylet length is 25 micron, stylet knobs set off from the shaft; four incissures in the lateral field and aerolated. The male stylet length is 25 micron, stylet knobs set off from the shaft; four incisures in the lateral field and areolated. The body length is 465 micron; stylet length 11.5 micron; tail length 46.5 micron and hyaline tail terminus length 16.1 micron.

Anwar *et. al*,(1991) recorded damage caused by *Tylenchulus semipenetrans* during a survey of crops in Punjab area of Pakistan during 1987-89.

Zhu *et. al*,(1991) reported 16 species of plant parasitic nematodes, including three new species, viz. *Meloidogyne jianyangensis* sp.nov., *Ogma hechuanensis* sp.nov. and *Rotylenchoides cheni* sp.nov. have been identified from 78 mixed samples of soil and citrus rootlets taken from citrus orchards of 26 counties in Sichuan Province. The distribution of each species was recorded. *Tylenchulus semipenetrans* and *Meloidogyne jianyangensis* have been determined as pathogenic to citrus, but the relationship between the species and citrus has not yet been determined.

Kumar(1991) conducted a study on the population fluctuation of *Pratylenchus coffeae* on Coorg mandarin orange plants revealed that the fluctuation was season specific. The nematode was at a lower population level during the dry period between December and April and multiplied faster during rainy season reaching a peak in October on orange.

Chen (1991) observed *Pratylenchus penetrans* is the major pest in Jiangxi Province. Sandy soil and a pH of 5.8-7.4 are optimum for nematode growth and

development. The lack of organic matter in soil depresses citrus tree growth and reduces citrus tree resistance to the nematode.

Hu (1991) conducted a survey during 1986-1989, and collected 78 samples of soil around the rhizosphere of citrus trees from seven cities and 19 counties of Sichuan, China. Fifteen nematode species were identified, and were found *Tylenchulus semipenetrans, Meloidogyne jianyangensis* sp. Nov., *Ogma hechuanensis* sp. nov., *Tylenchorhyncus martine, T. nudus, Helicotylenchus belli, H. crenacauda, H. dihystera, H. exallus, Rotylenchus caudaphasmidius, R. devonensis, Scutellonema brachyurum, Xiphinema americanum, X. brevicolle* and *X. insigne.*

Viachopoulos (1991) made a survey during the period 1985-1989 in nurseries of various districts of Greece revealed presence of *Tylenchulus semipenetrans* in citrus. The species *Tylenchus davanei* was recorded for the first time in Greece, in the regions of Korinthos in citrus trees. Also *Hoplotylus femina* was found for the first time in Tirnavos, Larissa.

Khan (1991) surveyed lemon plantations in the Thatta district of Sindh, Pakistan revealed eight genera and 10 species of nematodes associated with roots. *Xiphinema basiri* was found in most soil samples followed by *Helicotylenchus dihystera* and *Bitylenchus brevilineatus* is reported for the first time from Pakistan.

Germani and Anderson (1991) described and illustrated *Hemicriconemoides scottolamassesei* n. sp. from lemon fields of New Caledonia. Primary diagnostic characters are females with a continuous head and three lip annules, the first annule being the smallest, stylet length of 65-75 µm, vulva without lateral flaps, and acute tail terminus; J4 has a smooth cuticle without scales or spines the male has a lateral field with two incisures. Emended measurements, descriptions, illustrations, and new records of distribution are given for *H. brachyurus*(Loos,1949) Chitwood & Birchfield, 1957, *H. mangiferae* Siddiqui,1961 and *H. Kanayaensis* Nakasono & Ichinoe,1961.

Zhang (1993) described and illustrated *Meloidogyne mingnanica* sp. nov. from the roots of *Citrus unshiu* collected in Hubei, Longhu and Jinjiang county in South Fujian. *M. mingnanica* is similar in certain characteristics to *M. Kongi* but the latter differs from the new species in perineal patterns with circular striae around the tail tip and anus, perivulval region with striae and phasmids large and rounded.

Mukherjee and Dasgupta (1993) recorded the seasonal variation in the population and vertical distribution of natural nematode populations (*Helicotylenchus abunaomai, Hemicriconemoides cocophilus, Hoplolaimus seinhorsti, Caloosia parlona and Xiphinema insigne*) were monitored in the rhizosphere of *Citrus limettoides* trees. The population density of different nematode species differed in the pattern of vertical distribution in relation to the state of decline in citrus trees. Population peaks were generally observed in August-September and in March-April. Moderately declined trees supported maximum populations of *H. abunaomai* and *X. insigne* suggesting their involvement with decline. But population levels of *H. cocophilus, H.seinhorsti* and *C. parlona* were maximum in healthy trees, suggesting their non-involvement with decline. The highest population density prevailed in the upper 20 cm soil depth and gradually the numbers declined in lower depths (20-40 and 40-60 cm).Rainfall pattern, air and soil temperatures, soil moisture and host root growth appeared to be more predominant in influencing the population of pathogenically significant nematode species.

Yin (1994) studied the most important nematodes on citrus in China were *Tylenchulus semipenetrans* was distributed very widely, while *Meloidogyne* spp. Were serious in sandy soils. Nematodes on citrus caused a yield decrease of 20-50%.

Sundaram and Vadivelu (1995) reported *Tylenchulus semipenetrans, Helicotylenchus spp. Rotylenchulus reniformis, Xiphinema basiri, Hemicriconemoides spp.*, and *Pratylenchus coffeae* were found associated with Mandarin Orange in the Nilgiri district of Tamil Nadu, India. Among them, *Tylenchulus semipenetrans* was the predominant nematode pest with an absolute frequency and relative frequency of 90.52 and 37.07% respectively in soil, and 75.78 and 33.02% respectively in root. Among the seven villages surveyed, Kengarai and Kolagiri villages in Kotagiri taluk recorded severe infestation. A high degree of decline and dieback symptoms were observed in the infested areas.

Ambrogioni and Tacconi (1995) observed *Radopholus citrophillus* is the agent of 'spreading decline' of citrus crops in the USA (Florida, Lousiana and Hawaii), Cuba, Dominican Republic and Guyana. In Brazil, it is also viewed that on citrus plant they do not produce symptoms of decline.

Al-Qasem and Abu-Gharbieh (1995) collected 162 composite soil samples during October 1990 and March 1991 from citrus groves in the Northern Jordan Valley,

Central Jordan Valley, Southern Jordan Valley, Sothern Ghors, Jerash and Wadi Shueib. Field sampling revealed that *Tylenchulus semipenetrans* was widely distributed in citrus groves in Jordan. Slow decline disease of citrus, caused by the citrus nematode, appeared to be of large magnitude in the older plantations in the Northern Jordan Valley. Second stage juvenile population densities at Dier Alla Station were found to increase in the autumn and spring seasons, but fell down to minimum levels in mid-summer. Nematode numbers were high in sandy-loam soils (30.8%-69.8% sand, 11.2%-33.2% clay and 19.0%-36.0% silt). Moreover, soils containing lower organic matter (0.77%-1.43%), sustained higher numbers of the citrus nematode.

Guidice (1995) observed from a survey in 1994 in the orange groves of eastern Sicily, the Province of Catania that 93.8% of 267 sampling areas were infested by *Tylenchulus semipenetrans*. Population densities were between one and 700 adult females per gram of roots in 90.6% of the cases and 701 to 1400 in 3.2%.

Philis (1995) collected soil and root samples over several years around Cyprus revealed the presence of 58 plant parasitic nematode species, the most economically important being *Globodera rostochiensis, Meloidogyne spp., Tylenchulus semipenetrans, Helicotylenchus multicinctus, Pratylenchus penetrans, Xiphinema index, Ditylenchus dipsaci* and *Heterodera latipons*. The crop loss caused by nematode attack to citrus and vegetables is estimated at 12% of their productive value.

Elekcioglu (1995) conducted a survey on plant parasitic nematodes in the East Mediterranean region of Turkey, 16 nematode species were found associated with citrus. Out of these, only four species, *Helicotylenchus pseudorobustus, Rotylenchulus macrodoratus, Tylenchorhynchus goffarti* and *Tylenchulus semipenetrans* are able to infect citrus. About 90% of the orchards sampled were infected with the citrus nematode, *T. semipenetrans* where 62.5% of the examined orchards revealed population densities above the economic threshold.

Inserra *et. al,* (1995) and Inserra and Duncan (1996) showed that rough lemon was not a suitable host for a population of *Rotylenchulus reniformis* from South Florida, USA.

Ferguson *et. al,* (1996) conducted a survey in five central, eastern coastal and southwest Florida counties. Citrus nematodes (*T. semipenetrans*) were found in 45 of 50

mature citrus groves. From a total of 1000 samples the citrus nematode was detected in 475 and 548 soil and root samples, respectively. Approximately half of the infested groves contained trees beneath which no citrus nematodes were found. Many infested groves contained a patchy distribution of infested trees, possibly from random inoculation events. Linear correlations existed between citrus nematode population densities and levels of soil K, Cu, and soluble salts in the Flatwoods counties. In the Central Ridge counties, nematode density was correlated with levels of soil P & K.

Vovlas and Inserra (1996) listed out *Meloidogyne* spp. detected on citrus in the western and eastern hemispheres their survey and detection also discussed.

Duncan *et. al,* (1996) initiated a survey to determine the incidence of *Belonolaimus* spp. (sting nematodes) in citrus orchards in the central ridge region of Florida, following widespread damage by these nematodes to young trees replanted after freezing weather in 1989-90.Sting nematodes were detected in 50% of 210 samples and in 64% of 84 orchards surveyed. More orchards in Polk County were infested with sting nematodes (82%) than in counties to the north (36%) or south (48%). Principal component analysis of morphometric data separated six of seven sting nematode populations in north eastern Polk County from six populations in adjacent regions. Stylet : tail ratio for nematodes in north eastern Polk County tend to be > 1.0 and were <1.0 for all other populations. Patchiness of nematodes within an orchard was associated with stunted trees (23% smaller), reduced root mass density (25% lower), and low fruit yield (57% reduction). Soil texture did not vary among trees of different size in the orchard, but soil water potential between irrigation events was highest beneath small trees with low root mass density. Results of the survey indicated that the incidence of sting nematodes in orchards on the central ridge is much higher than previously estimated and that sting nematodes can cause substantial damage in replanted orchards. Further research is needed to evaluate the significance of sting nematode population variability and its relationship to citrus crop loss in Florida.

Bridge *et. al,* (1996) conducted a series of surveys on plant nematode at 55 farm sites in six districts of Belize, Central America, during February, November and December, 1993. Soil and root samples were taken and a total of 17 plant parasitic nematode spp. were identified from different crops including citrus. *Tylenchulus semipenetrans* was prominent nematode on citrus, including some spiral nematodes

Helicotylenchus multicinctus and *H. mucronatus*. However, *T. semipenetrans* was specific on citrus.

Rahman *et. al,* (1996) reported *Criconemoides chamoliensis* sp. nov. from soil around roots of orange in Chamoli, UP, India is characterized by 54-65 ornamented body annules, absence of submedian lobes, enlarged pseudolips connected laterally, stylet 64-65 μm long and acutely conoid tail.

Machon and Bridge (1996) described and illustrated *Radopholus citri* sp. nov. from the roots of citrus seedlings and trees growing in sandy soils in East Java, Indonesia. The new species is characterized by the relatively strongly developed conus of the male stylet and less well developed, but distinct, knobs. It comes closest to *Radopholus vangundyi*, *R. neosimilis,* and *R. nativus*.

Khan & Khanna (1997) found out of 14 genera of plant parasitic nematodes *Tylenchulus semipenetrans* was the most prevalent and recorded in all the localities surveyed.

Bansa Singh (1997) observed in a 15-yr. old Citrus reticulate orchard near Nagpur, India, *Tylenchulus semipenetrans* populations in soil gradually decreased from May to October 1994 but increased from November, reaching a peak in January 1995. They then decreased until May 1995, gradually increasing from October to December 1995 reaching a maximum in February 1996 thereafter decreasing March 1996. Root populations showed a similar trend although numbers there increased slightly in June of both years.

McSorley (1997) observed population densities in soil of nematodes belonging to different genera were monitored for 3 years in a citrus grove in Florida, USA, to determine whether indices of community structure or densities of key genera were more useful in characterizing the soil nematode communities of these very different perennial agro-ecosystems. Population densities of *Acrobeles, Aphelenchus, Cervidellus, Ironus, Paratrichodorus* and *Zeldia* were consistently greater ($P \leq 0.10$) in the citrus site, where 64% to 83% of the nematodes found were bacterivores and only 1 to 16% herbivores.

Crozzoli *et. al,* (1997) carried out a nematode survey in 1995 (February-April) in the citrus growing areas of Valles Altos of Carobobo and Yaracuy States, *Tylenchulus semipenetrans* was present in 14.1% of 357 samples. The largest populations of the

nematode were in association with Citrus volkameriana and the smallest with Citrus reshni. *T. semipenetrans* did not occur in groves with Citrumelo swingle.

Gambhir *et. al,* (1998) extracted 450 soil samples collected from around the rhizosphere of 32 commonly growing fruit plants including citrus spp. In eight districts of Manipur yielded 19 genera of phytophagous tylenchid nematodes. *Helicotylenchus, Tylenhorhynchus, Aglenchus, Basiria, Psilenchus, Neopsilenchus, Scutellonema, Macroposthonia* and *Hemicriconemoides* were more abundant and widely distributed than the related genera *Imphalenchus, Hoplolaimus, Ditylenchus, Paratylenchus, Hemicycliophora, Discocriconemella, Caloosia* and *Hemicaloosia.*

Mor and Spiegel (1998) reported the presence of *Tylenchulus semipenetrans* in 13, and *Xiphinema brevicolle* in four of the 15 citrus nurseries during a survey in 1996 in the Negev area (southern Israel). It is suggested that these nematode species were brought to the plantation area, along with the citrus seedlings from the central region of Israel in 1993.

Willers (1998) discussed on economic losses caused by plant parasitic nematodes to subtropical crops in South Africa. On citrus the loss is R141 million.

Crozzoli *et. al,* (1998) carried a survey in the main citrus growing areas of Venezuela. A total of 1110 soil and root samples were collected and analysed. 34 species were identified : *Aorolaimus holdemani, A. macbethi, Criconema demani, Gracilacus aculenta, Helicotylenchus crenacauda, H. dihystera, H.erythrinae, H. multicinctus, Hemicriconemoides communis, H. mangiferae, Hoplolaimus seinhorsti, Meloidogyne exigua, M.incognita, Mesocriconema onoense, M. ornatum, M. sphaerocephalum, Monotrichodorus monohystera, Paratrichodorus minor, Paratylenchus elachystus, P. minutus, Pratylenchus brachyurus, P. zeae, Rotylenchus reniformis, R. caudaphasimidius, Scutellonema brachyurum, Tylenchorhynchus annulatus, T. capitatus, T. semipenetrans, Xiphinema brasiliense, X. brevicolle, X. krugi, X. peruvianum, X. simillimum* and *X. vulgare*. The most economically important and widespread species is *Tylenchulus semipenetrans*. Other species which may cause damage to citrus are *P. brachyurus, M. exigua, M. incognita, H. multicinctus, P. minor* and *X. brevicolle*. Brief descriptions and a key to the identification of genera and species reported from Venezuela are provided.

Souza *et. al,* (1999) studied the occurrence and distribution of nematode parasites of fruit crops including citrus spp. from 31 counties of states of Brazil. Plant parasitic nematode genera obtained with their percentage occurrence in relation to the total number of samples were : *Helicotylenchus* (84.4%), *Meloidogyne* (50.9%), *Criconemella* and *Tylenchulus* (24.9%), *Pratylenchus* (15.6%), *Aphelenchoides* (8%), *Radopholus*(6.9%), *Xiphinema* (6.4%) and *Rotylenchulus, Discocriconemella, Paratylenchus, Rotylenchus* and *Scutellonema* (0.6%). The most important species and their frequency of occurrence in relation to the total number of samples were: *Helicotylenchus dihystera* (54.3%), *H. multicinctus* (46.8%), *M. incognita* (26%), *T. semipenetrans* (24.9%), *M. javanica* (24.3%), *Pratylenchus brachyurus* (18.8%) and *Radopholus similis* (6.9%).

Pan *et. al,* (1999) observed the lowest *M. fujianensis* infection rate occurred in August (30%) and the highest in April of the following year (90%). The lowest average infectivity was recorded in August (1.8 females/ 2g roots) and the highest in February of the following year (24.2 females/plant). Infection occurred throughout the year with peaks of infection in september and October and again in March to April of the following year. The peak of oviposition occurred during December and January. The population density increased with increasing monthly average temperatures.The most active growth of *C. reticulata* roots coincided with the peaks of infection of nematode juveniles (September-october, march-april) which was associated with two main periods of disease symptomatology. The ratio of males decreased with increasing monthly rainfall.

Paz *et. al,* (1999) surveyed young citrus orchards which are affected by chlorosis, necrosis of branches and withering, all of which are caused by presence of pathogens in root system. Three types of nematodes were found *Tylenchulus* spp. (possibly *T. semipenetrans*) was found in 15.2% of soil sample analysed and in 10.2% of roots. Less frequent findings were *Rotylenchulus* spp., *Meloidogyne* sp., *Pratylenchus* sp. and *Xiphinema* sp. The nematode which presented the highest incidences of population was *Meloidogyne* sp. (350 and 125 individuals per 100cc and 1 g of dry roots) followed by *Tylenchulus* with 30 and 18 individuals in soil and roots, respectively.

Erdal *et. al,* (2001) tabulated plant parasitic nematodes (Tylenchida) inhabiting various crops including citrus. In Turkey in the mid 90's a total of 140 species belonging to 43 genera were determined. The most common species were *Filenchus*

filiformis, F. thornei, Boleodorus thylactus, Bitylenchus dubius, Bitylenchus parvus, Quinisulcius capitatus, Merlinus brevidens, M. nanus, Helicotylenchus digonicus, Pratylenchus penetrans, P. thornei, Zygotylenchus guevarai, Pratylenchoides alkani, Pratylenchoides conincki, M. arenaria, M. incognita, M. javanica, H. avenae, H. schachti, Ditylenchus destructor, D. dipsaci, D. myceliophagus, A. tritici and *T. semipenetrans*.

Mansoor A. Siddiqui (2005) studied two economically important species of citrus fruits viz. orange, *Citrus reticulata* and lemon, *C. aurantifolia*. These were selected for studying the changes in the population of plant parasitic nematodes around their roots.The nematode population of *Hoplolaimus indicus, Helicotylenchus indicus, Xiphinema americanum, P. coffeae, T. semipenetrans* and *Hemicriconemoides mangiferae* was observed at 10 cm (upper layer) followed by 20 cm (middle) and 40 cm (lower) depths. The population was higher at higher soil moisture level. Soil moisture and temperature directly affect the nematode population. The nematode population declined in drought conditions. The PH also indirectly affects the nematode population densities.

Mokbel *et. al,* (2006) collected 2100 rhizosphere soil samples from fruit trees. A total of 20 nematode genera were found in the samples. The citrus nematode, *Tylenchulus semipenetrans* was predominant in the citrus soil samples showing 82.1-87.5% frequency of occurrence.

Sorribas *et. al,* (2008) conducted a survey from April to June 2002 in 62 orchards of clementine mandarin grafted on Troyer or Carrizo citrange rootstock in Catalonia (north eastern Spain) to determine the relationship between physicochemical soil properties and *T. semipenetrans* population densities. Soil was analysed to determine texture,pH,electric conductivity,organic matter content, N, P, K, Mg, Calcium carbonate and calcium oxide. Also trials were conducted in 2003 and 2004 in three drip irrigated orchards of clementine mandarin cv. Clemenules (El Pla and Mariclaire).

Tanha Maafi and Damadzadeh (2008) conducted a survey of citrus orchards of northern Iran revealed that the citrus nematode, *Tylenchulus semipenetrans*, was present in 89% of soil and root samples collected. The number of juveniles and females averaged 6490/250ml soil and 214/g root, respectively, in infested samples, and the mean numbers of juveniles and females in soil and root samples of sweet orange and sour orange rootstocks were greater than the damage threshold level in most regions. Population patterns of *Citrus aurantium* and *Poncirus trifoliate*, rootstocks were

monitored for 2 successive years. Nematode population densities peaked once a year during July-August and declined to lower levels during autumn and winter on both rootstocks. The duration of the life cycle of *T. semipenetrans* was eight and nine weeks on sour orange *C. aurantium* and Troyer citrange, *C.sinensis* × *P.trifoliata*, seedlings, respectively, at 24±2℃. In glasshouse experiments, *T. semipenetrans* was controlled on sour orange seedlings by the granular nematicides, fenamiphos and cadusaphos, the doses 4 & 8 ppm having the greatest effect when applied simultaneously with nematodes, whereas the nematicides Vydate and fenamiphos at 8 ppm showed greatest effects on nematodes in roots when applied 6 weeks after nematodes had been added to the soil. The effects of granular fenamiphos and cadusaphos (10G) on *T. semipenetrans* population densities were also investigated in infested citrus orchards with rates 1.0 and 2.0 g a.s. of nematicides/m^2 for one year. Although the nematicide treatments reduced nematode population densities in soil and roots, fruit weight and size was not affected by these treatments.

Vyas *et. al,* (2008) surveyed Indira Gandji Nahar Project (IGNP) command area in Rajasthan. Eight important genera of plant parasitic nematodes, namely *M. incognita, Tylenchorhynchus mashoodi, Hoplolaimus indicus (Basirolaimus indicus), Helicotylenchus indicus, Xiphinema spp., Longidorus spp., Paralongidorus spp,* and *Paratrichodorus* porous were found from lemon (*citrus limon*) including 13 other crops.

Grandison *et. al,* (2009) carried out a survey of plant parasitic nematodes in New Caledonia and presented the economic importance of various species. The most commonly found nematodes are R.K.N.(*Meloidogyne* spp.), spiral nematode (*Helicotylenchus* spp.), citrus nematode (*T. semipenetrans*), burrowing nematode (*Radopholus similis*) and ring nematode (*Hemicriconemoides mangiferae*).

Dias-ariera *et. al,* (2010) carried out a survey in order to study the occurrence of plant parasitic nematodes in fruit crops in Northwest of Parana', from December 2007 to February 2009. A124 soil and roots samples were collected from 19 species of fruit crops in 15 different municipalities. Nematodes were extracted from the samples and identified under an optical microscope. Nine genera of plant parasitic nematode were found. In citrus, the most abundant species was *T. semipenetrans*, but it was recovered *Meloidogyne, Pratylenchus, Helicotylenchus, Xiphinema, Trichodorus, Mesocriconema* and *Dolichodorus*.

Pathak and Chandra (2011) made observations on seasonal fluctuations in the population of nematode species from two different localities viz. Mohebawala and Majri-Grant in Doon Valley between March, 2008 to February, 2009 and March, 2009 to February 2010 showed marked variations in the monthly collection i.e, mean value of count of nematodes per month in each locality and the population fluctuation trend was also found to be different from locality to locality. In both the localities, the population of *Tylenchulus semipenetrans* was high during April-May and July, and thereafter, remained low during other months. Again, it started increasing from December onwards, attained its first peak in April and second one in July, in both the years.

Zalpuri *et. al,* (2013) studied community analysis of plant nematodes which is an important criterion for assessment of their pathogenic potential in a particular region. This investigation involves a study of the community structure of phytonematodes associated with the citrus plants in the various districts of Jammu, J&K. The predominant nematode species were *Meloidogyne javanica, Hoplolaimus sp., Pratylenchus sp., Xiphinema sp.* and *Tylenchulus semipenetrans.*

Zalpuri *et. al,* (2013) conducted a survey and made assessment the frequency of occurrence of economically important plant parasitic nematodes associated with citrus crop. Samples were collected from roots and soil rhizosphere from 10 localities representing 3 districts namely Samba, Kathu and Rajouri for the study of nematodes infestations. The frequency of occurrence and populations varied from place to place which is simply indicative of the fact that the studied area is highly infested with different varieties of nematode species which ultimately affect the citrus plant species of high grade quality of citrus plantations.

Community Structure:

Conventionally, the simplest method of presenting data on nematode distributions has been to list the nematodes found in an area or a crop, frequently within specified political boundaries. Such a list provides no information concerning frequency, abundance or habitat relationships (except sometimes of associated plants) and thus has limited long term ecological merit. Such studies are not to be discredited, but information on habitats, soil frequency, density and other parameters of community analysis add much more meaning and have long term benefits.

Basing upon the presence or absence of different plant species in an area, **Jaccard** (1912) worked out the ratio of the number of species common to both areas to the number of all species found in both areas which later was termed as Index of similarity.

Beals (1960) gave an equation in order to relate density with frequency, while studying bird populations. He named it as prominence value (PV). The equation given by him, PV= density√Frequency and this equation applies to the study of other animals also.

Ferris and Ferris (1974) studied the ecological relationship between nematode and plant communities in both temperate and tropical areas and illustrated the change in nematode community structure that occurs when previously uncultured land was brought into agricultural production. They had also shown that knowledge of the structure and dynamic of nematode populations could be used in the formulation of strategies for the integrated control of phytopathogenic species.

Freckman, Mankan and Sher (1974) grouped the nematodes found in undisturbed desert soil in Nevada, USA, as microbial feeders, fungal feeders, omnivorus and predators and plant parasites. They also noticed that greatest biomass and number of individuals were distributed by Caphelobidae (microbial feeders) and Dorylaimina (omnivores and predators).

Wasilewaka and Paplinska (1975) analysed soil samples taken monthly over a 12 months period from a rye field in Poland. They found 1.0 to 8.5 million nematodes/m^2 and classified them as microbivores, fungivores, plant parasites and omnivores. They also noticed that the first two groups together consumed almost 7% of total food consumption by nematodes.

Ferris and McKeny (1976) studied the distribution of the nematode community in a vineyard over a 13 month period and found omnivorous and microbivorus nematodes with greatest densities occurring between the vine rows and near soil surface.

C.A. Callahan (1977) evaluated the community structure as a method for quantifying and interpreting ecological changes in water resources environments. He investigated the relationship of benthic nematode communities to water quality in two streams in Indiana, USA.

Phillipson *et. al,* (1977) estimated nematode numbers, and respiratory metabolism in a beach woodland in England. They also studied the seasonal fluctuation in nematodes numbers and estimated production ratio and population production efficiency.

Ferris, Ferris and Weischer (1978) made a comparison of nematode community structure in deciduous forests of Germany and USA. They found similarities in trophic structure between the two forest systems, despite differences in species and genera, in numbers of individuals and in species diversity.

Frechman, Duncan and Larson (1979) in a study of grassland in central California, USA, found that during the period January to May 1978 the nematode density was highest on grazed plots at all sampling periods except February. According to them, nematode density and community structure were considered to be controlled by soil moisture and temperature rather than grazing.

Wasilewska (1979) analysed the structure and function of soil nematode communities in natural ecosystems and agrocoenoses. He conducted the analysis on numbers, biomass, species, composition and trophic and functional structures including energy flow of nematode communities in grassland, forest and arable field ecosystems of Poland.

Popovici (1980) gave a description of the implications of biotype on the formation of soil nematodes communities.

Saly (1980) studied the dynamics of free living nematodes in rhizosphere of Alnus glutinosa. He also determined the densities and biomasses and found energetic values at monthly intervals.

Mukherjee and Dasgupta (1982) mae a community analysis of plant parasitic nematodes in tea plantations of West Bengal. He recorded 19 species of plant parasitic nematodes within 13 genera from 120 soil and root samples from five tea estates and plantations showing heavy decline.

Lavelle (1983) studied the soil fauna of tropical Savannas and the community structure.

Kotcon (1990) determined nematode population densities in soil and root samples collected from 205 peach orchard. Twenty eight sps. of plant parasitic nematodes were identified. Predaceous nematodes (Mononchidae) were observed in 71% of the samples. The most common plant parasitic genera were *Paratylenchus, Helicotylenchus*, *Pratylenchus,* and *Xiphinema*, occurring in 85, 84, 77 and 74% of the samples, respectively. Population densities of *Xiphinema, Pratylenchus, Meloidogyne, Hoplolaimus* and *Criconemella* were at potentially damaging levels in 74, 19, 13, 10 and 02% of the samples, respectively. Potentially damaging nematode densities were observed in 78% of orchard blocks surveyed, with 35% having two or more nematodes with densities high enough to warrant concern. Nematode densities differed among soil types and tree rootstocks and were correlated with tree mortality rates.

Ritz and Trudgill (1999) advocated soil nematode communities have the potential to provide unique insights into many aspects of soil processes. Since most nematodes are active in soil throughout the year, they can potentially provide a holistic measure of the biotic and functional status of soils. In contrast to other soil microbial groups, representative samples of soil nematode communities are relatively easy to obtain.

Baird *et. al,* (1996) conducted a survey to examine the geographical distribution of plant parasitic nematodes in Georgia cotton fields. Four nematode genera parasitic on cotton were found in this survey. *Meloidogyne* spp. *Rotylenchulus* sp., *Hoplolaimus sp., and Belonolaimus sp., Meloidogyne* spp. was present in 9% to 56% of the field, in individual counties. *Rotylenchulus* sp. was found in 10 counties, *Hoplolaimus* sp. was found in six counties, and *Belonolaimus* sp. was found in two counties. From all of the samples collected for this survey, *Meloidogyne* spp. were found in 31% of the samples, *Rotylenchulus* sp. was found in 14%, *Hoplolaimus* sp. was found in 7% and *Belonolaimus* sp. was found in 0.3%. Burke county had the greatest number of fields infested by at least one of these genera (67%) and the greatest number of fields above Georgia's action thresholds (38%). Laurns county had the fewest fields where these genera were present (13%), and only 3% of fields had nematode populations above threshold levels.

Sahu *et. al,* (2011) studied community analysis of plant nematodes which is an important criterion for assessment of their pathogenic potential in a

particular region and identification of hotspots of nematode attack. This investigation involves a study of community structure of phytonematodes associated with the vegetable crops in the district Durg of Chhattisgarh. The highest absolute density was of *Meloidogyne incognita* and *Meloidogyne* spp. followed by *M. javanica, M. arenaria,* and *Rotylenchulus reniformis* while, *Helicotylenchus* spp. and Tylenchidae had the lowest density. *T. indicus, R. reniformis* and *M. javanica* were most frequent while, *Meloidogyne* spp., *Helicotylenchus* spp. and *Tylenchidae* were the least frequent. Highest prominence value was recorded for *M. javanica,* followed by *M. incognita, Meloidogyne spp., R. reniformis* and *M. arenaria. Pratylenchus* spp., *Helicotylenchus* spp. and Tylenchidae were the least prominent nematodes. This is the first record of various species of phytonematodes associated with vegetable crops in this tribal state.

Rashid *et. al,* (2014) conducted a survey of ornamental plants in Rajouri district of J&K to record the nematode community structure. Nine plant parasitic nematodes viz; *Aphelenchoides sp., Helicotylenchus sp., Hoplolaimus sp., Longidorus sp., Meloidogyne sp., Rotylenchulus sp., Tylenchorhynchus sp., Tylenchus sp.,* and *Xiphinema* sp. were isolated and identified from 217 soil samples collected from the rhizosphere of ornamental plants. Out of these nematodes, the highest frequency of occurrence was recorded in *Meloidogyne* sp. (76.49%) followed by *Helicotylenchus* (54.83%), *Hoplolaimus* (37.32%), *Rotylenchulus* (42.39%), *Tylenchorhynchus* (25.03%), *Tylenchus* (19.81%), *Xiphinema* (16.58%), *Longidorus* (10.13%) and *Aphelenchoides* (8.75%).

Chapter 999

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental Site

Twenty five lemon plants were selected from different locations for collection of soil samples around rhizosphere during August, 2013 to May, 2014.

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(200cc)
g. Aluminium pan
h. Petridish
i. Tissue Paper
j. Aluminium wire gauge
k. A set of 20, 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

Methods Adopted :

3.3. Collection of Soil Samples

Soil samples each of 200 g from the rhizospheres of 25 lemon plants from various 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 debris as well as roots were removed . Then by cone and quarter method, about 200cc 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 and 350 mesh sieves . Residues of 20 mesh sieve were washed out. Contents of 350 mesh sieve was collected into a 200cc 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 gauze 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 24 hours, so as to allow maximum number of nematodes to wriggle out through the tissue paper into the bottom of petridish. Next day , the wire gauze was removed. Petridishes were put on the stage of the stereoscopic microscope to examine the presence of nematodes.

In this manner all total 100 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 7 cm 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 assess number of nematodes present in the counting dish for each soil sample (200cc) collected from an individual host. This process was repeated for all samples and the number of nematodes present was recorded genera wise from all samples belonging to different host plants.

3.5. Killing and Fixing of Nematodes

The nematode suspensions 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 settle 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 10 ml. 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 8 ml, Glycerine 2 ml, water 45 ml) 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- 20 ml, glycerol- 1 ml and distilled water - 79 ml. 0.5 ml of Seinhorst solution was pipette 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 desiccators containing 96% ethanol about $1/10^{th}$ of its volume . The entire assembly was put inside an incubator at 35°C - 40°C 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-95 ml & glycerol 5 ml) was transferred into cavity block and the cavity block was put inside another desiccators 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 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 rhizospheres of lemon plants.

Parameters used for community structure analysis :

The following parameters were used for analysis of community structures of nematodes in lemon rhizospheres.

1) Frequency: (a) Absolute frequency

$$= \frac{\text{Number of samples containing a species}}{\text{Number of samples collected}} \times 100$$

(b) Relative frequency

$$= \frac{\text{Frequency of species}}{\text{Sum of frequency of all species}} \times 100$$

2) Density: (a) Absolute density = Number of individuals per unit of soil.

(b)Relative density $= \frac{\text{Number of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times$ 100

3)Prominence value (PV) = Density√Frequency

Chapter IV

RESULTS

RESULTS

The results of the study conducted at various locations of Bhubaneswar to study the nematode diversity and community composition in the rhizospheres of lemon showed some pattern of their occurrence and distribution. The population densities including community composition of important plant parasitic nematodes and groups of saprophytic nematodes at four different periods of study have been indicated in this chapter.

Nematode diversity :

In the current study 25 numbers of plants were chosen. Genera of plant parasitic nematodes and groups of free living nematodes occurring in the rhizospheres have been identified and enumerated and their population were recorded during four different months such as August,2013; November,2013; February,2014 and May,2014 and are presented in Tables and Figures 1, 2, 3 and 4. The total number of nematodes encountered during the period of study are presented in Table and Figure 5. It is evident from the Table 1 *Rotylenchulus reniformis* was observed as the most prominent nematode followed by *Helicotylenchus dihystera* and *Meloidogyne incognita*. *Rotylenchulus reniformis* occurred in all the 25 sample plants with higher numbers as compared to other plant parasitic and saprophytic nematodes during August,2013. Similar trend of experimental findings were also observed during November,2013 ; February,2014 and May,2014 as reflected in the respective Tables.

Table 1. Nematode diversity of lemon plants during August,2013 .

Sample No.	Rr	Mi	Hd	Co	Hi	Xi	Pc	Tc	Dor	Mon	Rha
1	687	213	68	-	-	-	-	-	92	56	-
2	316	-	-	89	154	12	-	-	06	24	-
3	461	27	-	58	-	-	83	-	07	12	-
4	226	-	103	-	-	-	-	66	24	39	-
5	317	-	124	-	47	-	-	-	71	24	53
6	508	274	182	-	-	-	-	-	63	87	-
7	284	132	64	-	-	-	-	-	-	-	28
8	432	-	-	114	-	24	-	-	06	12	-
9	511	188	97	-	-	-	-	-	52	23	-
10	450	208	121	-	-	-	-	-	54	72	-
11	350	88	-	172	-	-	-	-	12	45	-
12	605	112	95	-	-	-	-	-	-	09	84
13	433	96	-	62	-	-	-	114	07	12	-
14	467	-	138	-	-	24	58	-	11	-	-
15	587	113	82	-	-	-	-	-	58	46	-
16	502	-	225	-	-	-	-	142	-	05	12
17	668	-	104	273	-	-	-	-	53	28	04
18	459	27	-	52	-	-	81	-	07	12	-
19	433	-	216	-	-	31	-	-	92	24	-
20	487	118	-	99	-	-	-	-	52	-	13
21	564	-	302	-	-	56	-	-	102	83	-
22	269	212	102	-	-	-	-	-	18	05	-
23	371	-	142	-	74	-	-	-	71	04	135
24	439	47	-	58	-	-	95	-	08	06	-
25	604	286	182	-	-	-	-	-	53	67	-

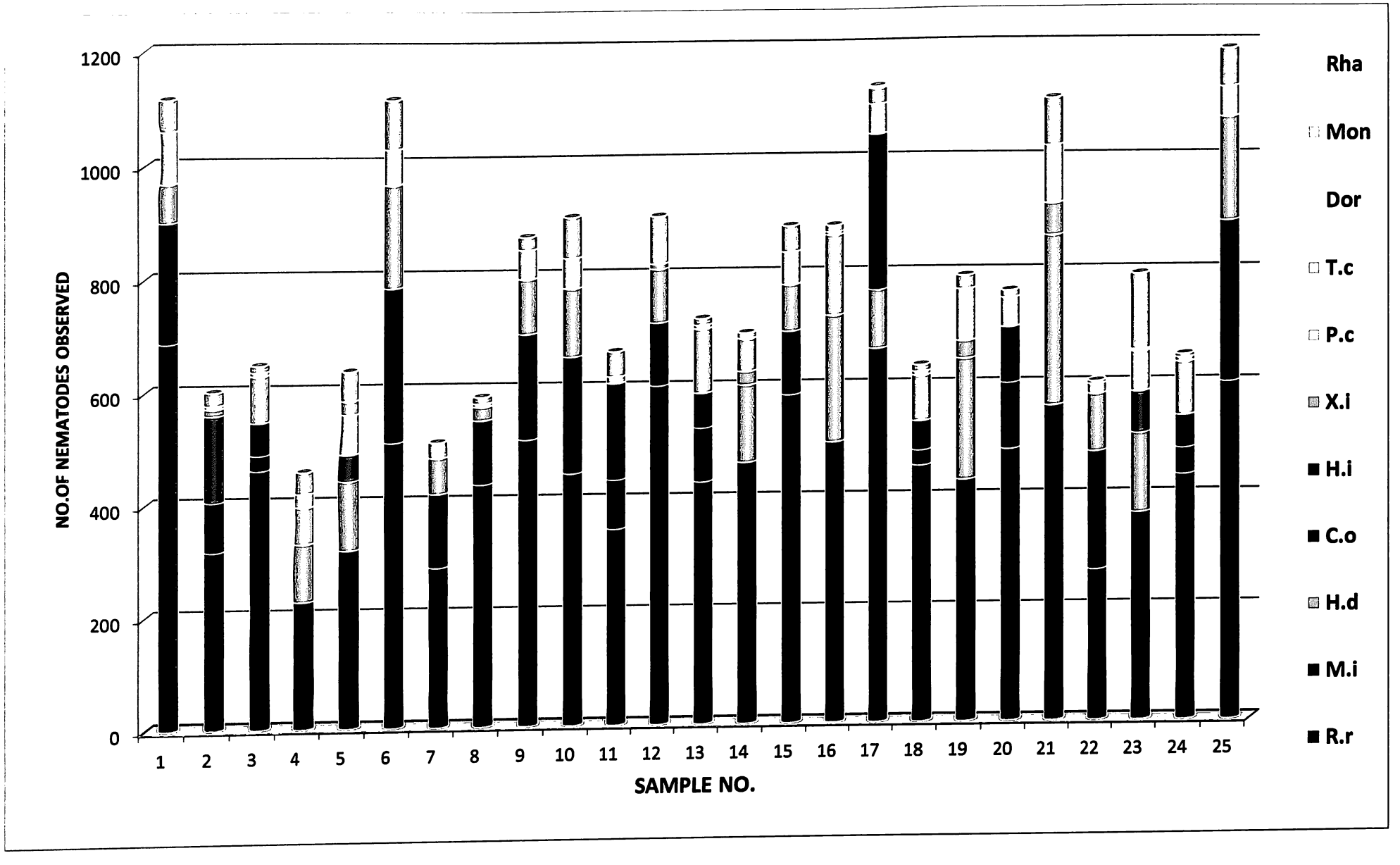

Figure 1- Nematode population observed in the month of august, 2013

Table 2: Nematode diversity of lemon plants during November,2013 .

Sample No.	Rr	Mi	Hd	Co	Hi	Xi	Pz	Tc	Dor	Mon	Rha
1	728	305	112	-	-	-	-	-	64	58	-
2	433	-	-	126	161	34	-	-	16	54	-
3	546	63	-	67	-	-	128	-	17	22	-
4	632	-	230	-	-	-	-	114	17	59	-
5	517	-	222	-	84	-	-	-	58	94	117
6	428	293	176	-	-	-	-	-	72	104	-
7	361	205	92	-	-	-	-	-	-	-	54
8	543	-	-	239	-	33	-	-	12	47	-
9	628	179	115	-	-	-	-	-	59	42	-
10	586	320	189	-	-	-	-	-	83	62	-
11	542	182	-	208	-	-	-	-	14	56	-
12	735	266	113	-	-	-	-	-	-	23	77
13	554	112	-	87	-	-	-	236	13	42	-
14	604	-	242	-	-	45	86	-	09	-	-
15	636	224	94	-	-	-	-	-	56	59	-
16	598	-	243	-	-	-	-	167	-	26	22
17	752	-	212	339	-	-	-	-	81	76	13
18	588	56	-	94	-	-	121	-	18	52	-
19	572	-	294	-	-	59	-	-	51	73	-
20	599	273	-	136	-	-	-	-	64	-	42
21	689	-	412	-	-	97	-	-	134	106	-
22	645	304	186	-	-	-	-	-	47	21	-
23	501	-	236	-	112	-	-	-	93	24	128
24	556	114	-	79	-	-	137	-	21	53	-
25	713	294	277	-	-	-	-	-	56	112	-

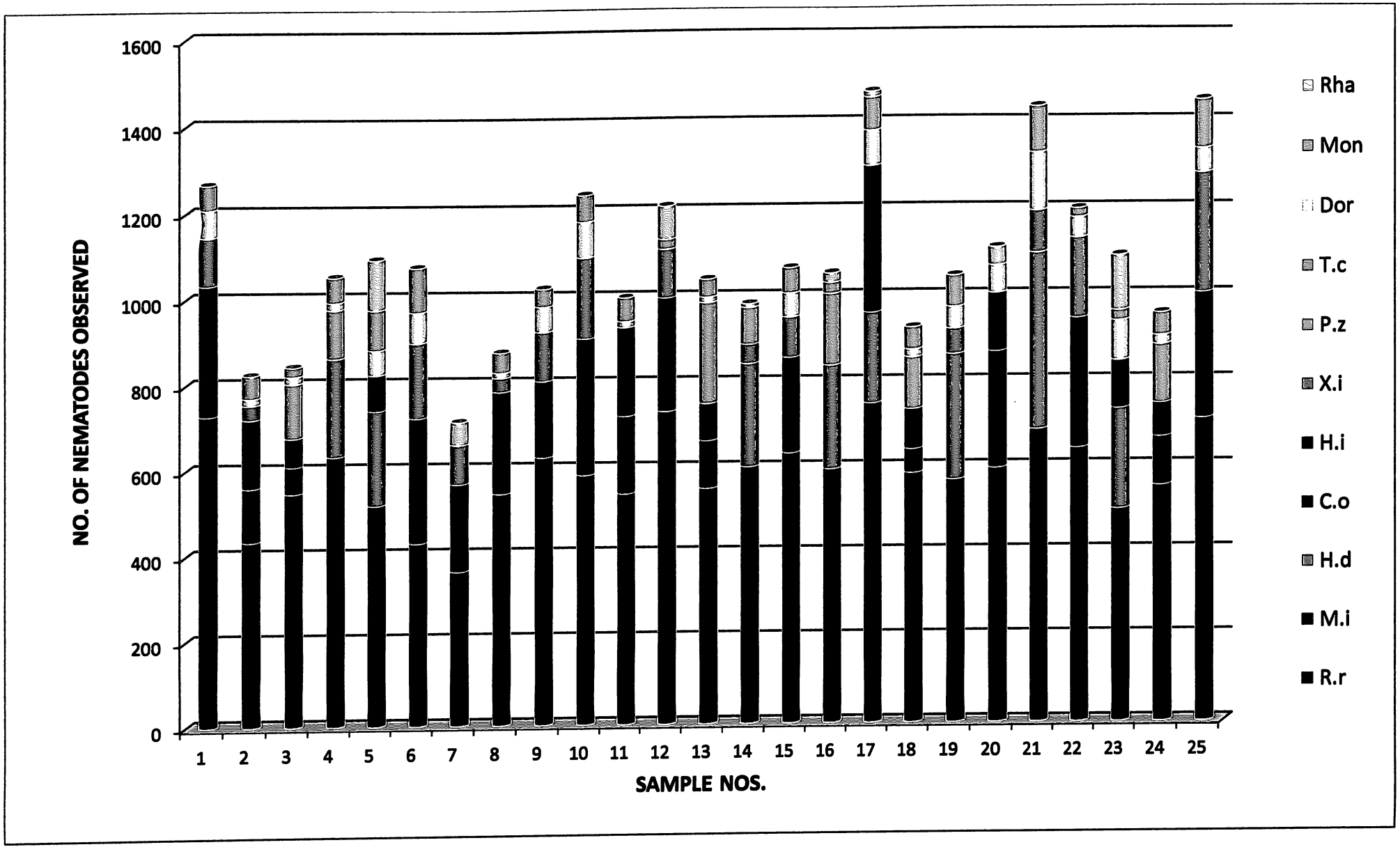

Figure 2 - Nematode population observed during the month of November, 2013

Table 3. Nematode diversity of lemon plants during February, 2014

Sample No.	Rr	Mi	Hd	Co	Hi	Xi	Pz	Tc	Dor	Mon	Rha
1	701	288	93	-	-	-	-	-	56	68	-
2	421	-	-	108	149	33	-	-	11	44	-
3	534	56	-	62	-	-	95	-	13	26	-
4	513	-	187	-	-	-	-	88	19	42	-
5	496	-	203	-	76	-	-	-	62	87	99
6	487	279	163	-	-	-	-	-	66	94	-
7	293	195	87	-	-	-	-	-	-	-	38
8	522	-	-	168	-	31	-	-	08	33	-
9	609	172	110	-	-	-	-	-	54	41	-
10	564	303	176	-	-	-	-	-	72	61	-
11	518	164	-	184	-	-	-	-	11	48	-
12	645	197	104	-	-	-	-	-	-	17	81
13	543	98	-	83	-	-	-	212	09	36	-
14	499	-	212	-	-	41	79	-	10	-	-
15	618	183	86	-	-	-	-	-	54	52	-
16	573	-	238	-	-	-	-	157	-	14	19
17	706	-	189	314	-	-	-	-	72	58	11
18	552	46	-	86	-	-	106	-	17	38	-
19	461	-	286	-	-	47	-	-	49	67	-
20	582	254	-	128	-	-	-	-	56	-	36
21	654	-	371	-	-	78	-	-	112	93	-
22	581	245	163	-	-	-	-	-	39	18	-
23	497	-	214	-	83	-	-	-	78	21	117
24	542	106	-	67	-	-	124	-	19	48	-
25	684	261	259	-	-	-	-	-	54	98	-

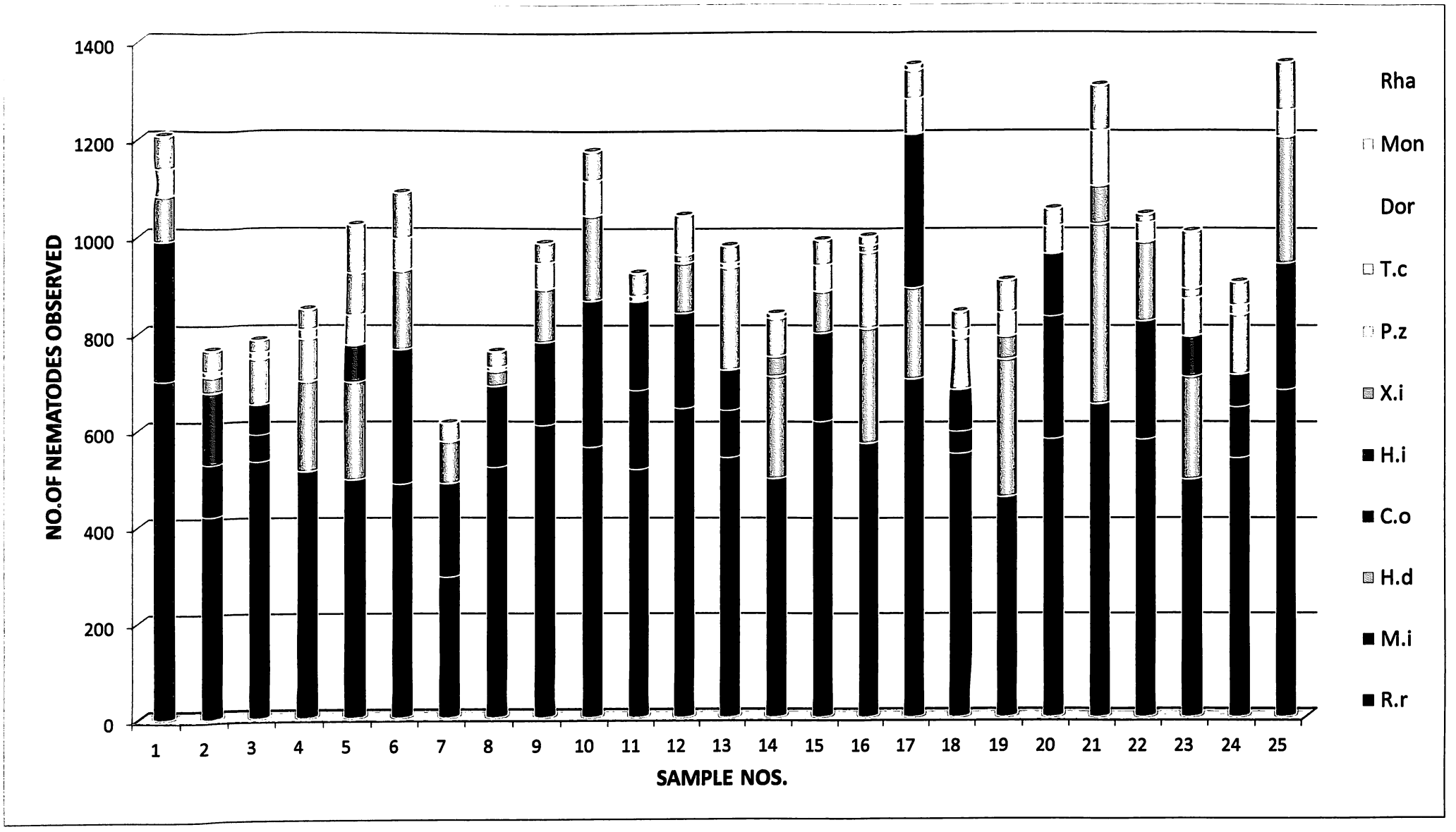

Figure 3 - Nematode diversity of lemon plants during February, 2014

Table 4: Nematode diversity of lemon plants during May, 2014 .

Sample No.	Rr	Mi	Hd	Co	Hi	Xi	Pz	Tc	Dor	Mon	Rha
1	632	204	82	-	-	-	-	-	43	47	-
2	386	-	-	87	124	21	-	-	06	24	-
3	472	44	-	51	-	-	79	-	09	14	-
4	469	-	163	-	-	-	-	76	17	38	-
5	478	-	182	-	66	-	-	-	56	63	72
6	456	251	138	-	-	-	-	-	48	76	-
7	189	157	64	-	-	-	-	-	-	-	28
8	476	-	-	137	-	26	-	-	05	24	-
9	541	123	98	-	-	-	-	-	42	37	-
10	524	286	148	-	-	-	-	-	51	45	-
11	454	143	-	154	-	-	-	-	07	29	-
12	502	177	97	-	-	-	-	-	-	12	63
13	493	82	-	71	-	-	-	187	05	28	-
14	382	-	184	-	-	29	56	-	04	-	-
15	481	166	69	-	-	-	-	-	37	32	-
16	504	-	201	-	-	-	-	136	-	07	14
17	597	-	164	278	-	-	-	-	56	42	04
18	498	33	-	65	-	-	89	-	13	31	-
19	372	-	199	-	-	32	-	-	28	36	-
20	501	189	-	117	-	-	-	-	44	-	31
21	532	-	284	-	-	56	-	-	99	73	-
22	526	191	123	-	-	-	-	-	26	16	-
23	436	-	187	-	64	-	-	-	47	18	102
24	492	88	-	46	-	-	104	-	16	29	-
25	499	208	194	-	-	-	-	-	34	86	-

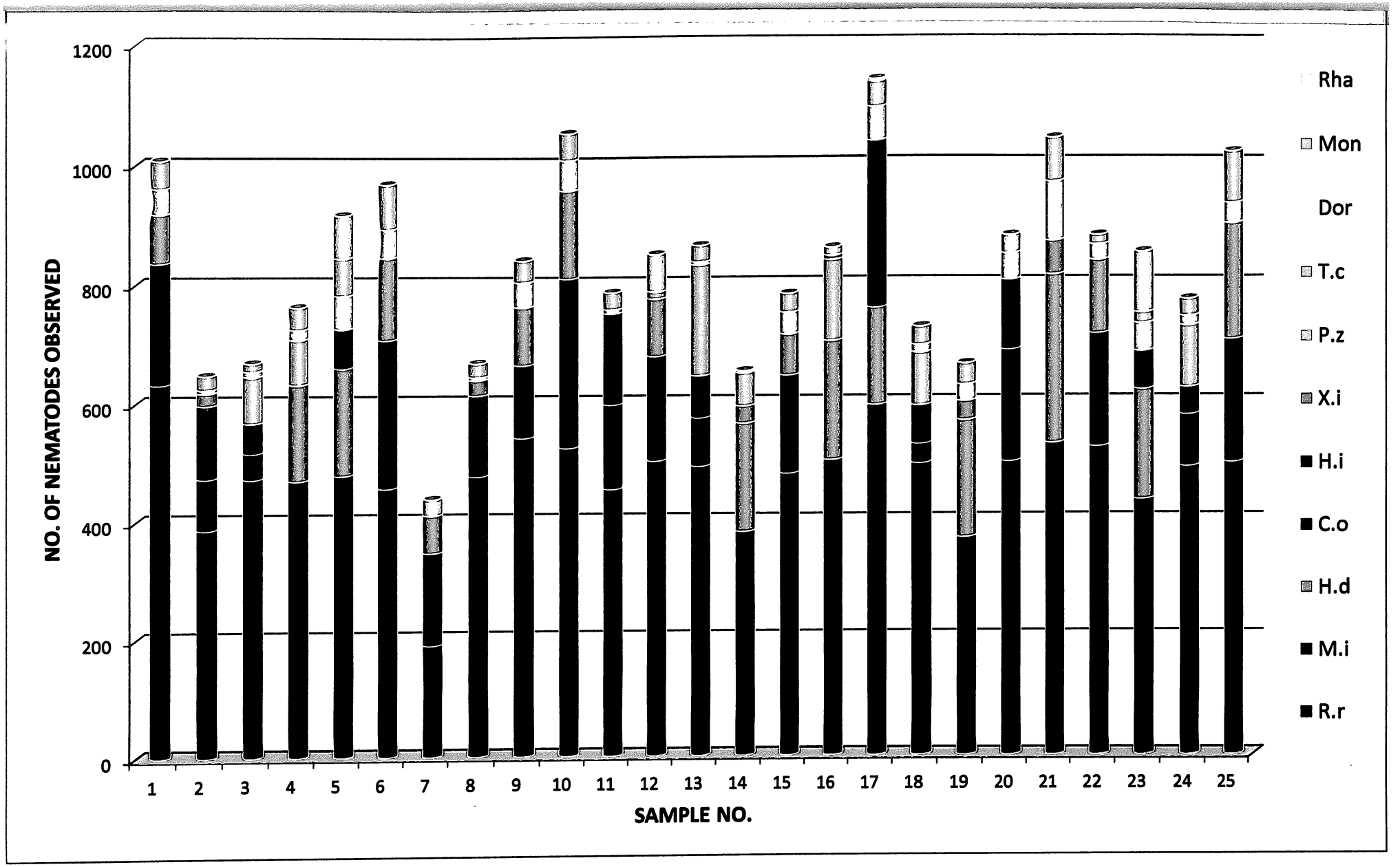

Figure 4 - Nematode diversity of lemon plants during May, 2014 .

Table 5; Total nematode diversity from August, 2013 to May,2014

Smple No.	Rr	Mi	Hd	Co	Hi	Xi	Pz	Tc	Dor	Mon	Rha
01	2748	1010	0355	-	-	-	-	-	255	229	-
02	1556	-	-	0410	588	100	-	-	039	146	-
03	2013	0190	-	0238	-	-	385	-	046	074	-
04	1840	-	0683	-	-	-	-	344	077	178	-
05	1808	-	0731	-	273	-	-	-	247	268	341
06	1879	1097	0659	-	-	-	-	-	249	361	-
07	1127	0689	0307	-	-	-	-	-	-	-	148
08	1973	-	-	0658	-	114	-	-	066	116	-
09	2289	0985	0420	-	-	-	-	-	207	143	-
10	2166	1117	0634	-	-	-	-	-	260	240	-
11	1864	0577	-	0718	-	-	-	-	044	178	-
12	2487	0752	0409	-	-	-	-	-	-	061	305
13	2023	0388	-	0303	-	-	-	749	034	118	-
14	1952	-	0776	-	-	139	279	-	034	-	-
15	2322	0686	0331	-	-	-	-	-	205	189	-
16	2177	-	0755	-	-	-	-	602	-	052	067
17	2723	-	0669	1204	-	-	-	-	262	204	32
18	2097	0162	-	0297	-	-	397	-	055	133	-
19	1838	-	0995	-	-	169	-	-	220	200	-
20	2169	0834	-	0480	-	-	-	-	216	-	122
21	2439	-	1369	-	-	287	-	-	447	355	-
22	2021	1304	0574	-	-	-	-	-	130	060	-
23	1805	-	0779	-	333	-	-	-	289	067	482
24	2029	0355	-	0250	-	-	460	-	064	136	-
25	2500	1049	0912	-	-	-	-	-	197	363	-

Rr - *Rotylenchulus reniformis* ; Mi – *Meloidogyne incognita ;*
Hd – *Helicotylenchus dihystera* ; Co – *Criconemella ornata* ;
Hi – *Hoplolaimus indicus* ; Xi - *xiphinema insigne ;*
Pz – *Pratylenchus zeae* ; Tc – *Tylencorhynchus coffeae* ;
Dor – *Dorylaimid ;* Mon – *Mononchid* ;
Rha – *Rhabditid* .

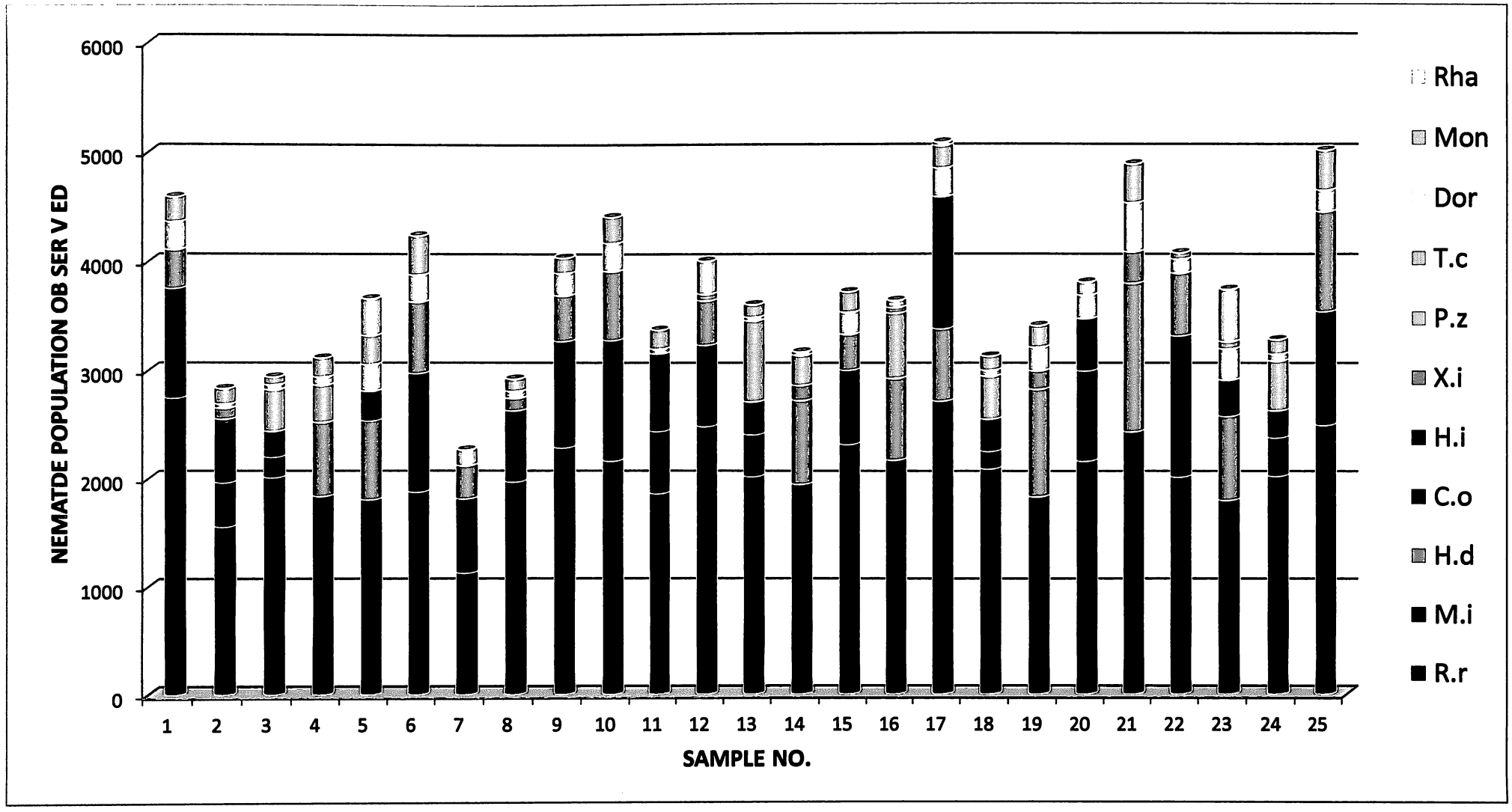

Figure 5- Total nematode diversity from August, 2013 to May, 2014

Community composition :

The results of samples collected from 25 lemon plants of four different period were subjected for analysis of community composition. Informations on parameters of community composition such as frequency, density and prominence value were analysed and are provided in table 6, 7, 8, 9 and 10 . During August,2013 maximum absolute frequency, relative frequency, Absolute density, Relative density and prominence value were observed in case of *Rotylenchulus reniformis*, followed by *Helicotylenchus dihystera* and *Meloidogyne incognita*. Similar, results were also obtained during November,2013 ; February,2014 and May,2014 and so also during total period of study.

Table 6: Community composition of nematodes of lemon plants during August,2013

	No. of samples containing a sps.	**Absolute frequency**	**Relative frequency**	**Absolute density**	**Relative density**	**Frequency**	**Density**	**Prominence value**
R.reniformis	25	100	18.94	457.20	57.43	1.00	457.20	457.20
M.incognita	15	60	11.36	085.64	10.76	0.60	085.64	066.34
H.dihystera	17	68	12.88	093.88	11.79	0.68	093.88	077.42
H.indicus	03	12	02.27	011.00	01.38	0.12	011.00	003.81
C.ornata	09	36	06.82	039.08	04.91	0.36	039.08	023.45
X.insigne	05	20	03.79	005.88	00.74	0.20	005.88	002.63
P.zeae	04	16	03.03	012.68	01.59	0.16	012.68	005.07
T.coffeae	03	12	02.27	012.88	01.62	0.12	012.88	004.46
Dorylaimid	22	88	16.67	036.96	04.64	0.88	036.96	034.67
Mononchid	22	88	16.67	027.80	03.49	0.88	027.80	026.08
Rhabditid	07	28	05.30	013.16	01.65	0.28	013.16	006.96
Total	**132**	**528**	**100.00**	**796.16**	**100.00**	**5.28**	**796.16**	**708.09**

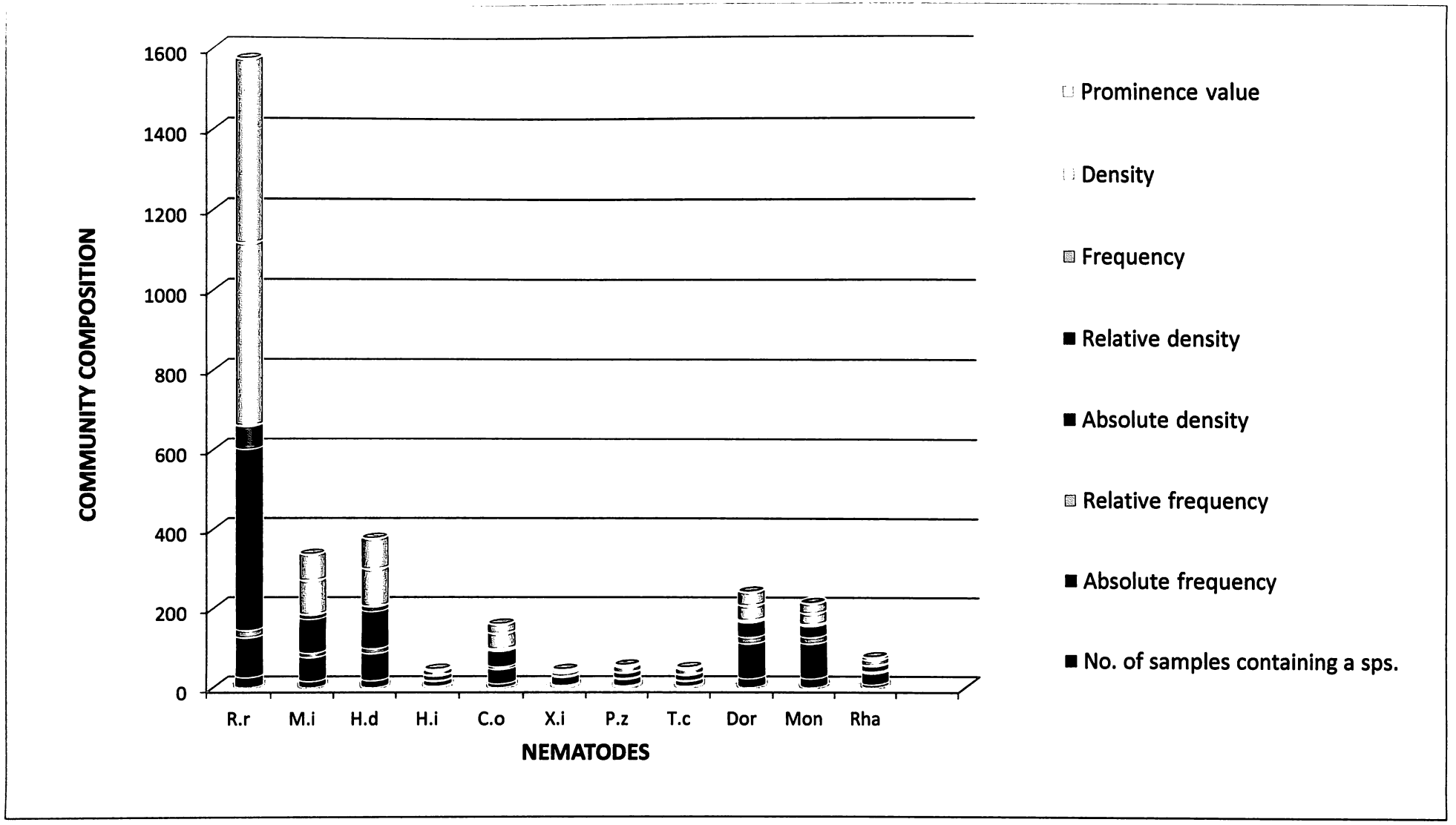

Figure 6 - Community composition of nematodes of lemon plants during August, 2013

Table 7: Community composition of nematodes of lemon plants during November, 2013

	No. of samples containing a sps.	Absolute frequency	Relative frequency	Absolute density	Relative density	Frequency	Density	Prominence value
R.reniformis	25	100	18.94	563.44	53.60	1.00	563.44	563.44
M.incognita	15	60	11.36	127.60	12.14	0.60	127.60	098.83
H.dihystera	17	68	12.88	129.80	12.34	0.68	129.80	107.04
H.indicus	03	12	02.27	014.28	01.36	0.12	014.28	004.95
C.ornata	09	36	06.82	055.00	05.23	0.36	055.00	003.30
X.insigne	05	20	03.79	010.72	01.02	0.20	010.72	004.79
P.zeae	04	16	03.03	018.88	01.80	0.16	018.88	007.55
T.coffeae	03	12	02.27	020.68	01.97	0.12	020.68	007.16
Dorylaimid	22	88	16.67	042.20	04.01	0.88	042.20	039.59
Mononchid	22	88	16.67	050.60	04.81	0.88	050.60	047.47
Rhabditid	07	28	05.30	018.12	01.72	0.28	018.12	009.58
Total	**132**	**528**	**100.00**	**1051.28**	**100.00**	**5.28**	**1051.32**	**893.70**

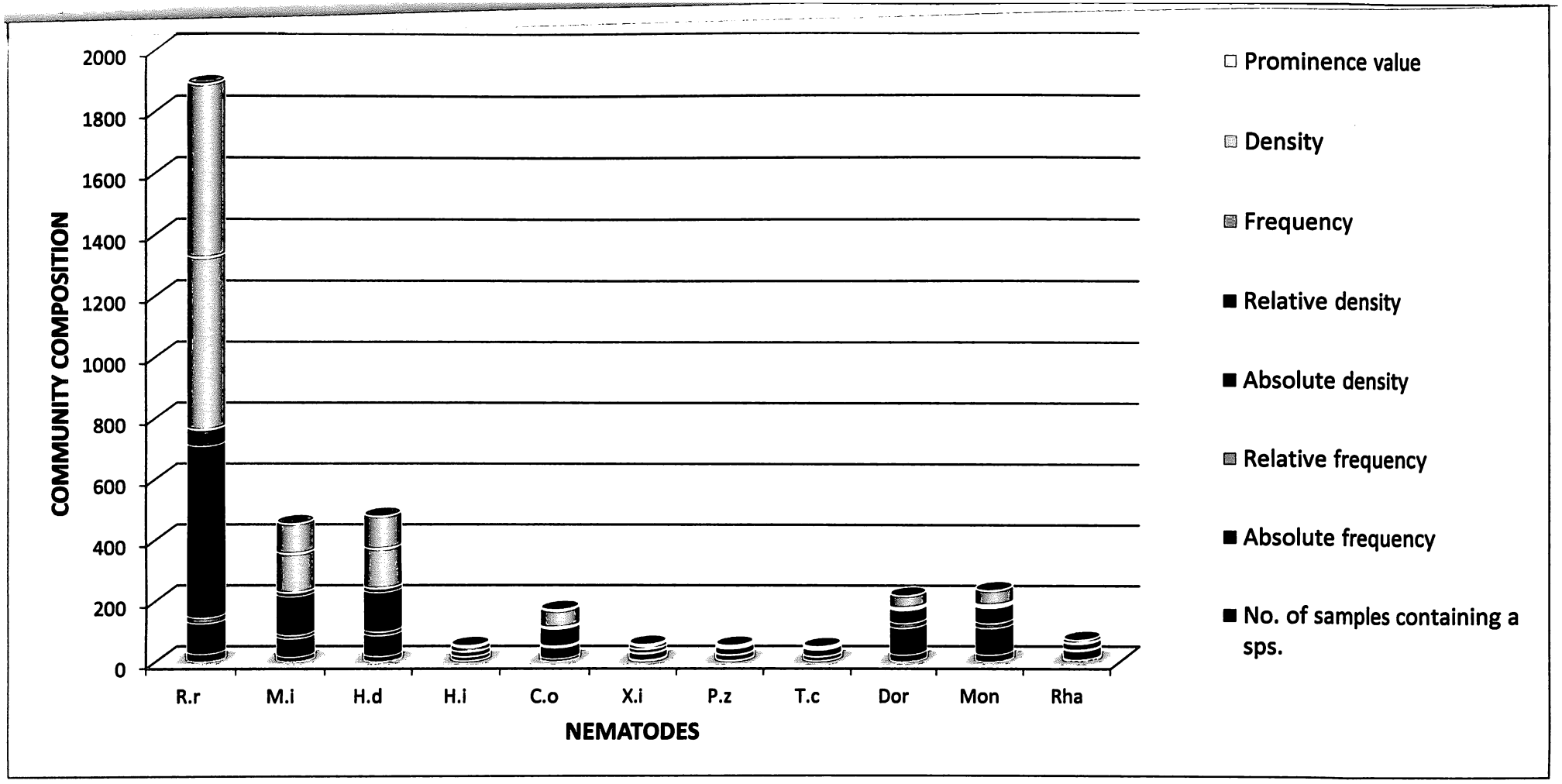

Figure 7 - Community composition of nematodes of lemon plants during November, 2013

Table 8 : Community composition of nematodes of lemon plants during February,2014

	No. of samples contaiing a sps.	Absolute frequency	Relative frequncy	Absolute density	Relative density	Frequency	Density	Prominence value
R.reniformis	25	100	18.94	551.80	55.56	1.00	551.80	551.80
M.incognita	15	60	11.36	113.88	11.47	0.60	113.88	088.21
H.dihystera	17	68	12.88	125.64	12.65	0.68	125.64	103.61
H.indicus	03	12	02.27	012.32	01.24	0.12	012.32	004.27
C.ornata	09	36	06.82	048.00	04.83	0.36	048.00	028.80
X.insigne	05	20	03.79	009.20	00.93	0.20	009.20	004.11
P.zeae	04	16	03.03	016.16	01.63	0.16	016.16	006.46
T.coffeae	03	12	02.27	018.28	01.84	0.12	018.28	006.33
Dorylaimid	22	88	16.67	037.64	03.79	0.88	037.64	035.31
Mononchid	22	88	16.67	044.16	04.45	0.88	044.16	041.43
Rhabditid	07	28	05.30	016.04	01.62	0.28	016.04	008.49
Total	**132**	**528**	**100.00**	**993.12**	**100.01**	**5.28**	**993.12**	**1672.41**

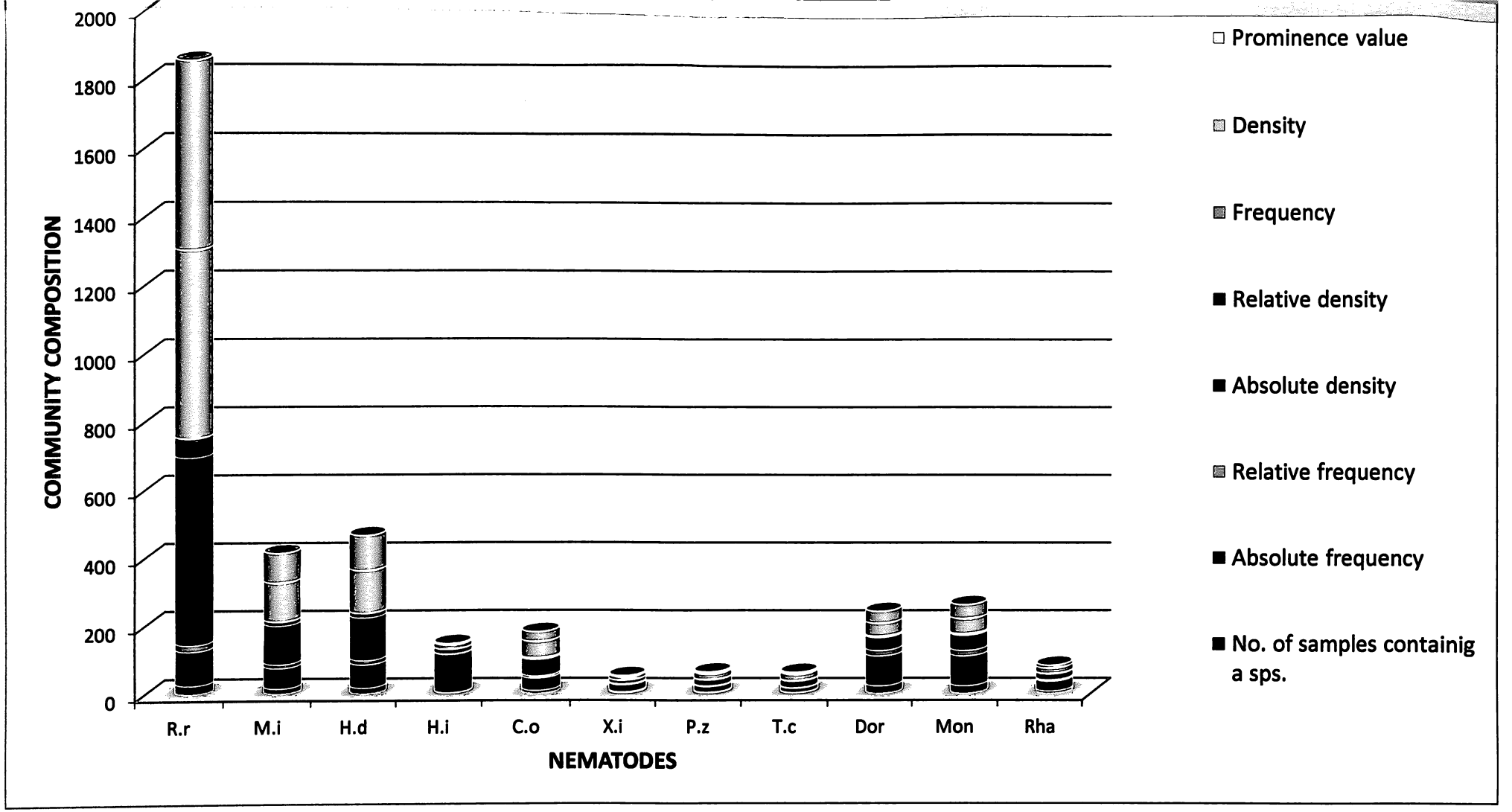

Figure 8 – Community composition of nematodes of lemon plants during February , 2014

Table 9 : Community composition of nematodes of lemon plants during May,2014

	No. of samples contaiing a sps.	Absolute frequency	Relative frequncy	Absolute density	Relative density	Frequency	Density	Prominence value
R.reniformis	25	100	18.94	475.68	57.24	1.00	475.68	21.81
M.incognita	15	60	11.36	093.68	11.27	0.60	093.68	05.81
H.dihystera	17	68	12.88	103.08	12.40	0.68	103.08	06.90
H.indicus	03	12	02.27	010.16	01.22	0.12	010.16	00.38
C.ornata	09	36	06.82	040.24	04.84	0.36	040.24	02.28
X.insigne	05	20	03.79	006.56	00.79	0.18	006.56	00.46
P.zeae	04	16	03.03	013.12	01.58	0.16	013.12	00.58
T.coffeae	03	12	02.27	015.96	01.92	0.12	015.96	00.48
Dorylaimid	22	88	16.67	027.72	03.34	0.88	027.72	04.63
Mononchid	22	88	16.67	032.28	03.88	0.88	032.28	04.99
Rhabditid	07	28	05.30	012.56	01.55	0.28	012.56	00.99
Total	**132**	**528**	**100.00**	**831.04**	**100.03**	**5.28**	**831.04**	**49.31**

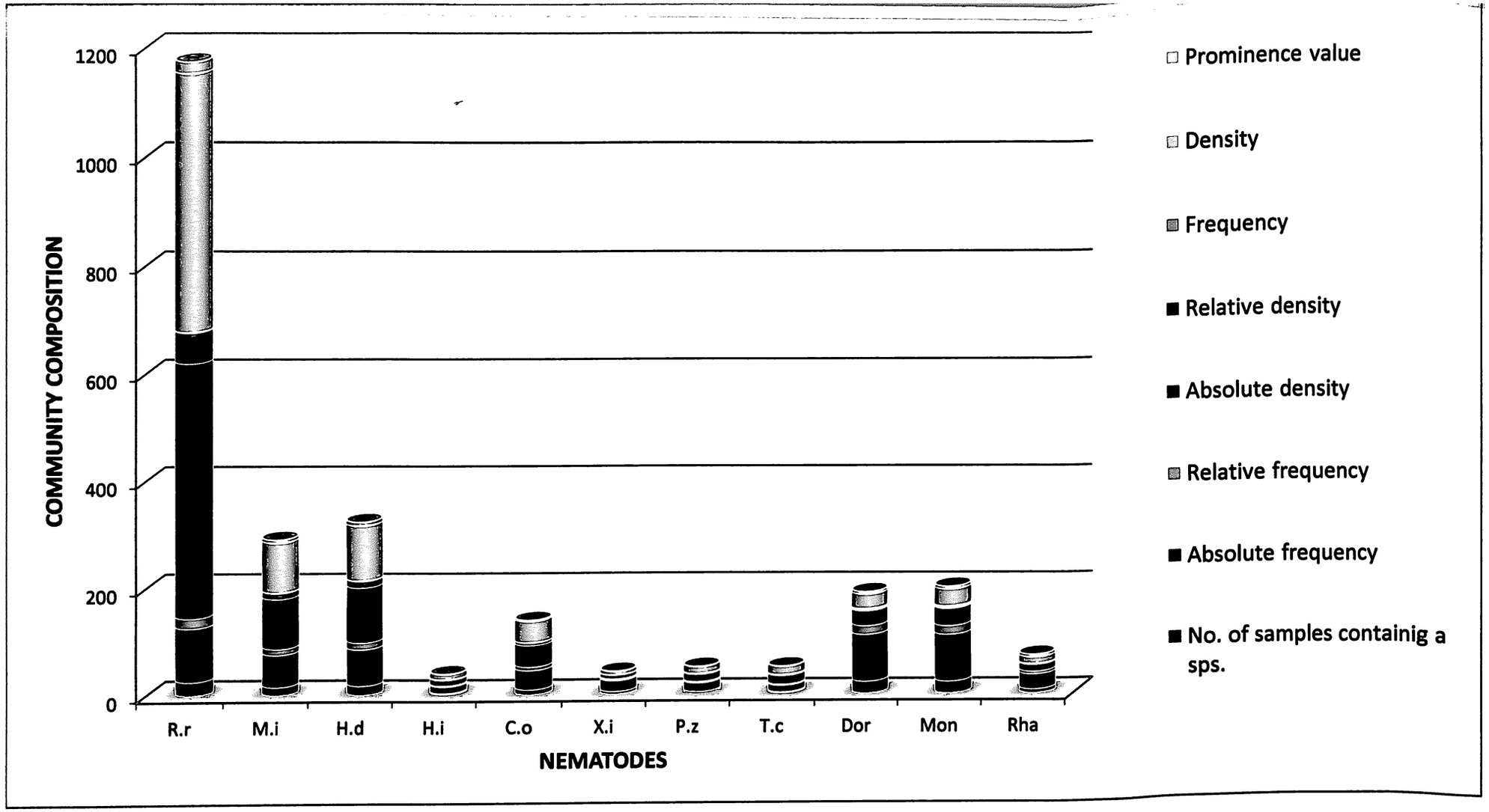

Figure 9 – Community composition of nematodes of lemon plants during May, 2014

Table 10: Community composition of total nematodes associated with lemon plants.

	No. of samples contaiing a sps.	Absolute frequency	Relative frequncy	Absolute density	Relative density	Frequency	Density	Prominence value
R.reniformis	100	100	19.01	518.03	55.95	1.00	518.03	518.03
M.incognita	60	60	11.41	105.30	11.37	0.60	105.30	081.57
H.dihystera	68	68	12.93	115.10	12.43	0.68	115.10	094.91
H.indicus	12	12	02.28	011.94	01.29	0.12	011.94	004.14
C.ornata	36	36	06.84	045.58	04.92	0.36	045.58	027.35
X.insigne	18	18	03.42	008.09	00.87	0.18	008.09	003.43
P.zeae	16	16	03.04	015.21	01.64	0.16	015.21	006.08
T.coffeae	12	12	02.28	016.95	01.83	0.12	016.95	005.87
Dorylaimid	88	88	16.73	036.08	03.90	0.88	036.08	033.85
Mononchid	88	88	16.73	038.71	04.18	0.88	038.71	036.31
Rhabditid	28	28	05.32	014.97	01.62	0.28	014.97	007.92
Total	**526**	**526**	**99.99**	**925.96**	**100.00**	**5.26**	**925.96**	**819.46**

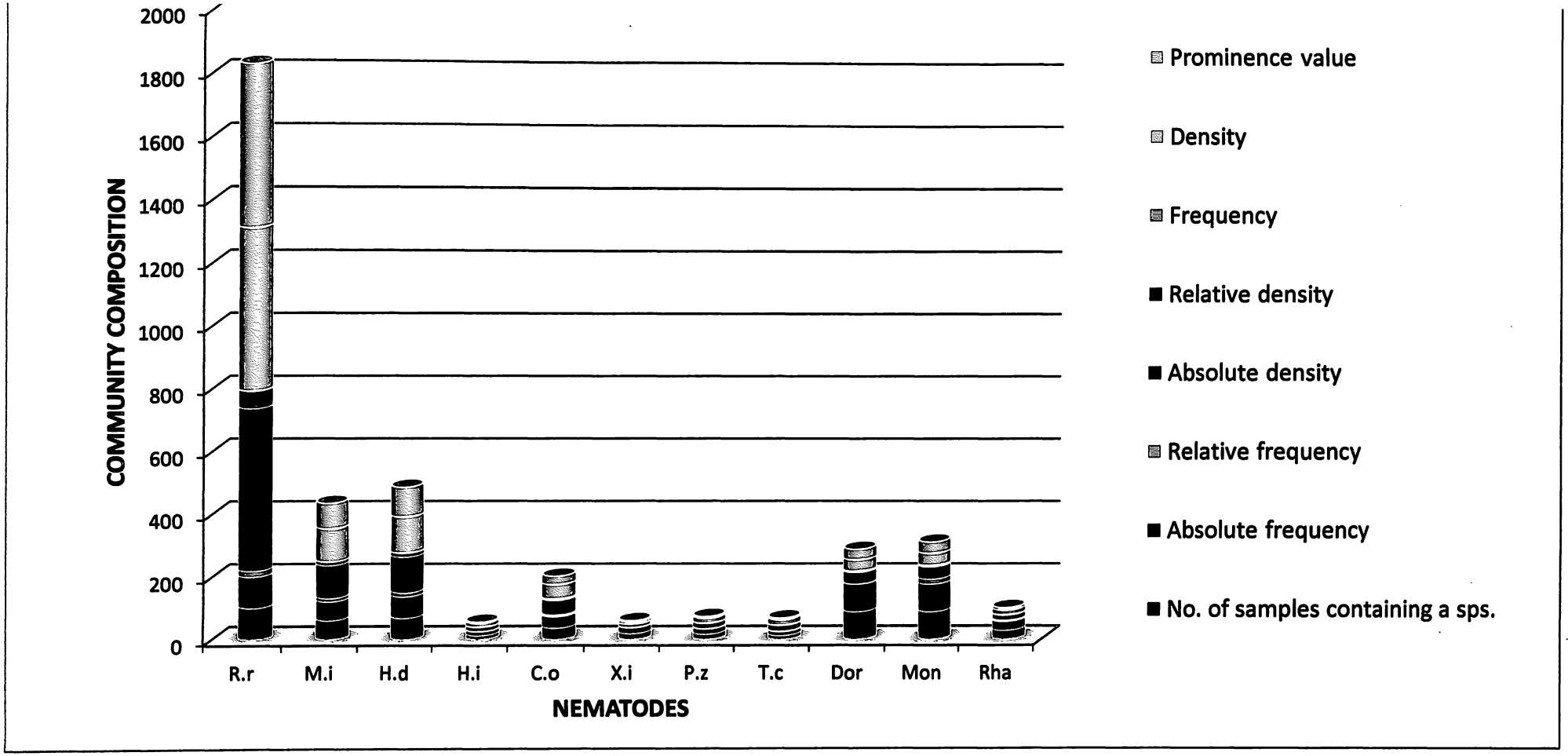

Figure 10 – Community composition of total nematodes associated with lemon plants

Chapter V

DISCUSSION

DISCUSSION

Over the years lemon have become an economically recognized fruit plant in India. Various reports revealed the presence of several plant parasitic nematodes in the rhizospheres of lemon plants. Ever - increasing realization of the economic importance of lemon plants and pathogenic role of nematodes, attempts are now being made to determine the nature of association of both plant parasitic and non - plant parasitic nematodes with lemon plants. So, for a meaningful appreciation of nematode problems associated with lemon plants , which is necessary to study the nematode association and their diversity over the period of native localities to acquire more knowledge about the distribution and diversity pattern as well as community structure of different groups of phytonematodes. So, their population densities can be correlated with the possible logical implications in future .

Results of the present study involving analysis of soil samples of twenty five numbers of lemon plants from different localities in Bhubaneswar during August, 2013; November, 2013; February, 2014 and May, 2014 present a consolidated picture of the nature of nematode association and distribution and their community composition of different groups of nematodes. Eight numbers of plant parasitic nematode species were encountered along with three groups of non – plant parasitic nematodes, during the total period of study extending over a calendar year .

It is evident from the results of this study, *Rotylenchulus reniformis* was the most prominent plant parasitic nematode, found to be associated with all the lemon plants of Bhubaneswar localities along with high rate of occurrence followed by *Helicotylenchus dihystera* and *Meloidogyne incognita.* Various authors have indicated *Tylenchulus semipenetrans* is the most promising and devastating plant parasitic nematode of lemon plants contrary to this study (Davis, 1984; Ganguly, 1988; Davide, 1988; Anwar *et. al*, 1991; Ferguson *et. al*, 1996) . In addition to plant parasitic nematodes, there is also record on the sizable population of groups of non - plant parasitic nematodes. As far as previous surveys are concerned the present study is the first ever record of association and diversity by saprophytic nematodes. Among these nematodes the rate of occurrence of Dorylaimida and Mononchida are almost same but the population of association of Dorylaimida is much higher than that of the Mononchida.

During the period of study maximum number of population of plant parasitic nematodes were recorded during November, 2013 followed by during February,2014 and least population density was observed during May, 2014. This was in confirmatory with the report of Kumar (1991), who obtained similar presence of *Pratylenchus coffeae* on Coorg mandarin orange but contrast to Mukherjee and Dasgupta, (1993) where they recorded the seasonal variation of plant parasitic nematode on *Citrus limettoides* , however the nematode population peaks were observed in August-September and in March- April. Bansa Singh (1997) reported the contrasting data from a 15 year old *Citrus reticulata* rhizosphere that *Tylenchulus semipenetrans* populations in soil gradually decreased from May to October but increased from November, reaching a peak in January and the population then decreased until May .

Generally, nematode association and their densities are correlated with disease development in lemon plants. But, to explain the differences in densities of nematodes having a difficult and challenging task for the ecologists. It is evident from this study plant parasitic nematodes like *Rotylenchulus reniformis*, *Helicotylenchus dihystera* and *Meloidogyne incognita* as compared to other five plant parasitic nematode species, are widely distributed in the rhizospheres of lemon plants situated at different localities of Bhubaneswar. Similarly the trends of absolute density, relative density and prominence value follow the same path. Among non- plant parasitic nematodes Dorylaimids and Mononchids were distributed widely and are similar to each other and comparatively more than Rhabditids . As far as previous survey records are concerned this is the first ever report on community structures of both plant and non – plant parasitic nematodes of lemon plant .

However, this is the preliminary study on nematode diversity and community composition in the rhizosphere of lemon plants around Bhubaneswar localities of Odisha state . The results of the study indicated an interesting association of both plant parasitic and non – plant parasitic nematodes and an encouraging community structure for any management practices in future . Further studies on a wider range of lemon plants in the state will provide a wealth of informations in evaluating the nature of nematode diversity and community composition in the rhizosphere of lemon plants in Odisha state and finally the management strategies against plant parasitic nematodes .

Chapter VI

SUMMARY

SUMMARY

Examination of rhizosphere soil samples obtained from economically important lemon plants growing in different localities of Bhubaneswar revealed existence of nematode diversity and differences in community composition.

It was observed that upto maximum eight plant parasitic nematode genera were associated with 25 lemon plants examined. The three important plant parasitic nematode genera in decreasing order of their frequency in occurrence were *Rotylenchulus reniformis*, *Helicotylenchus dihystera* and *Meloidogyne incognita*. The similar trends were recorded during August, 2013; November, 2013; February, 2014 and May, 2014. However, maximum number of population of plant parasitic nematodes were recorded during November, 2013 followed by February, 2014 and September, 2013 and the least was during May, 2014. Apart from plant parasitic nematodes three groups of non-plant parasitic nematodes were also recovered from the soil samples. These were Dorylaimids, Mononchids and Rhabditids.

In respect to community composition maximum of absolute frequency, relative frequency, absolute density, relative density and prominence value were observed for *Rotylenchulus reniformis* among both plant parasitic and non- plant parasitic nematodes.

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BIBLIOGRAPHY

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