

**SOIL NEMATODE DIVERSITY IN
THREE WILDLIFE SANCTUARIES OF GOA, INDIA**

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DECLARATION

I, Mr. Kiran Suresh Gaude, hereby declare that this thesis represents work which has been carried out by me and that it has not been submitted, either in part or full, to any other University or Institution for the award of any research degree.

Place: Goa University

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Kiran Suresh Gaude

CERTIFICATE

I hereby certify that the above Declaration of the candidate, Kiran Suresh Gaude is true and the work was carried out under my supervision.

Prof. I. K. Pai
(Research Guide)
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PREFACE

Nematode is most abundant metazoans and like insects, they occur in all possible climatic condition and habitats that can sustain life. Their distribution is linked to soil particle size, moisture, availability of mineral nutrients and above ground plant distribution. Nematodes possess number of traits, which includes simple anatomy, transparent body due to permeable cuticle, high species richness, abundance, pervasiveness and tolerance, close association with the soil particles and interstitial water, short generation time, have resistant stage (cysts) also they demonstrate anhydrobiosis, Osmobiosis or Cryobiosis to survive inactively during unfavourable environmental conditions, immense sensitivity to various changes in the soil ecosystem and their ability to reflect difference between disturbed, undisturbed and human-impacted environment makes them imperative and inexpensive organism for biological, ecological and environmental studies as a tool to monitor changes.

In the present study, an attempt was made to document the free living, soil inhabiting nematodes of the three wildlife sanctuary viz. the Bondla Wildlife Sanctuary, the Bhagwan Mahaveer Wildlife Sanctuary and the Mhadei Wildlife Sanctuary. The sampling was done opportunistically and soil samples were collected to analyses physico-chemical parameters and to extract the nematodes for identification. Soil analysis was carried out in soil testing laboratory, Krishi Vigyan Kendra, Margao, Goa. Soil analysis indicates

slightly acidic pH, and high organic carbon, Nitrogen, Potassium and Phosphorous. Microelements like Zinc, Iron, Manganese, Copper and Boron was also found to be high.

A total of 38 free living soil nematode species, belonging to 5 orders and 17 families are reported. Order Dorylaimida was the most dominant order, consisting of 10 families and 28 species of free living soil inhabiting nematodes. A total of 27, 36 and 21 free living soil nematode species were recorded in the Bondla Wildlife Sanctuary, Bhagwaan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary respectively.

Seasonally, number of species was more in winter and post-monsoon compared to summer and in monsoon. When compared with forest soil and plantation soil (*Acacia auriculiformis* plantation), it was observed that, more number of nematode families in forested soil compared to that of plantation. This clearly indicates the suitability of forested soil for nematodes as compared to soil of Acacia plantation.

Change in landscapes could be major threat to soil nematodes. The aim of this present study was to understand free living soil nematode diversity from protected areas of Goa and to understand possible threats for nemafuna. The present study provides basic data and information on the soil inhabiting, free living nematodes of three wildlife sanctuaries, which will form a strong base for further studies and intense research in soil nematology in the state of Goa.

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INTRODUCTION

INTRODUCTION

Soil is a vital natural resource that forms thin mantle over earth's surface which may be described as multiphase, multi-component, multifunctional living systems (Lavelle, 1996; Bardgett, 2005; Kibblewhite *et al.*, 2008). It consist of minerals materials, plant roots and their exudates, water and gases, organic matter and also inhabits many organisms viz. bacteria, fungi, protozoans, nematodes, mites, collembolans, annelids and micro-arthropods (Bongers and Ferris, 1999; Bardgett, 2005; Briones, 2014) together providing series of ecosystem goods and services to various biological processes (Lavelle, 2002; Kibblewhite *et al.*, 2008; Wall *et al.*, 2010).

The underground biota is an important part of soil, responsible for about 30% of mineralization of carbon and nitrogen, apart from helping in availability of nutrients in soil (Gorres *et al.*, 1998). It induces soil physical and chemical properties (Brussaard, 1997; Bongers and Ferris, 1999). It also influence nature of vegetation grows on it (Bardgett, 2005). This soil biota along with vegetation is one of five interactive factors for

soil forming process (Bardgett, 2005). Underground biota is critical for biogeochemical and ecological functioning of terrestrial ecosystem (Wall *et al.*, 2010).

Soil Nematode is one of the important groups of underground biota that belongs to phylum Nematoda (Chew, 1974) in terrestrial, marine and freshwater ecosystem (Hanel, 1999; Tahseen, 2012). Even though placed at lower level of taxonomic hierarchy it is most diverse phylum after Arthropoda (Maggenti, 1981; Morand *et al.*, 2006) and most ecologically successful group (Ahmad, 2001), occupying key position in the detritus food web (Moore and de Rooter, 1991; Nehar, 2001; Rizvi, 2008).

They are very small, ranging from 0.3mm to 8 meters (*Placentonema gigantisma* discovered in placenta of Sperm Whales), which is said to have 32 ovaries. They are structurally simple worm-like animal (Yeast, 1979; Yeast and Bongers, 1999), diverse (Ettema, 1998), ubiquitous inhabitants (Bernard, 1992; Bloemers *et al.*, 1997; Bongers and Ferris, 1999; Rizvi, 2008; Tahseen, 2012) in all soils. Yeast *et al.*,

(1993) trophically classified nematodes as herbivores, bacterivores, fungivores, omnivores and predators.

Nematodes are the most ubiquitous organisms on the earth and include free-living forms as well as parasites of plants, insects, humans and other animals (Basyoni and Rizk, 2014). Adult nematodes are made up of roughly 1000 somatic cells out of which, hundreds of cells are typically associated with the reproductive systems (Basyoni and Rizk, 2014). They have rounded body having one way guts (alimentary canal), with a mouth at one end and an anus at the other (characterized as a *tube within a tube*). Body is covered by complex cuticle having evolutionarily plastic feature which protects, helps in body movement and maintaining their shape (Basyoni and Rizk, 2014). They only have longitudinal muscles which assist them to back and forth movement (Basyoni and Rizk, 2014). Nematodes have digestive, reproductive, nervous and excretory systems, but they lack circulatory or respiratory systems (Basyoni and Rizk, 2014). To orient and to respond with a wide range of

environmental stimuli nematodes use chemosensory and mechanosensory neurons those are embedded in the cuticle (Basyoni and Rizk, 2014).

Nematodes are basically aquatic animal depends on thin layer of water or liquid medium for their survival either in parasitic or free-living form (Yeast and Bongers, 1999; Tahseen, 2012). It is soil particle's ability to hold soil moisture and flexible body of nematodes allow them to bending in interstitial system of soil particles that makes nematode species to adapt for terrestrial mode of life (Tahseen, 2012; Lizzane, 2015).

Nematode is most abundant metazoans (Maggenti, 1981; Abebe *et. al.*, 2011) and like insects, they occur in all possible climatic condition and habitats that can support life (Abebe *et. al.*, 2011; Tahseen, 2012) and their distribution is linked to soil particle size, moisture, availability of mineral nutrients and above ground plant distribution (Matlack, 2001).

Nematodes possess number of traits which includes simple anatomy, transparent body due to permeable cuticle, high species richness, abundance, pervasiveness and tolerance, close association with

the soil particles and interstitial water, short generation time, have resistant stage (cysts) also they demonstrate unhydrobiosis, Osmobiosis or Cryobiosis to survive inactively during unfavourable environmental conditions, immense sensitivity to various changes in the soil ecosystem and their ability to reflect difference between disturb, undisturbed and human-impacted environment makes them imperative and inexpensive organism for biological, ecological and environmental studies as a tool to monitor changes (Porazinska *et al.*, 1999; Nehar, 2001; Abebe *et al.*, 2011).

Being small and microscopic, with simple anatomy and transparent body made nematodes ideal model organism for biological research. Nematodes, specially *Caenorhabditis elegans* regarded as best model organism to understand human genetics and human diseases such as Alzheimer's, Parkinson's and Huntington's, diseases, cancer and aging; metabolic disorders such as obesity and diabetes and genetic diseases such as autosomal dominant polycystic kidney disease, muscular dystrophy and arrhythmia (Lizanne, 2015).

Zullini, (1976) proposed nematodes of freshwater sediments as a biological pollution parameter. Li *et al.*, (2011) reported many species of free living nematodes, which are good indicators of heavy metal pollution in soil (Bongers *et al.*, 2001; Georgieva *et al.*, 2002) and in water (Nehar, 2001). Nematodes are also important in mineralization as it was estimated that 40% nutrient mineralization in certain ecosystems is due to nematodes as they feed on microbial populations (De Rooter *et al.*, 1993). They also plays important role to breakdown dead and decaying matter, recycling of plant nutrient, and replenishing of soil nutrient in the terrestrial ecosystem and are good indicators of soil status and soil functioning (Bongers and Ferris, 1999; Porazinska *et al.*, 1999; Ekschmill *et al.*, 2001; Nehar, 2001; Bohra and Sulthana, 2008; Rizvi, 2008; Abebe *et. al.*, 2011; Sthanu *et al.*, 2013, Kergunteuil *et. al.*, 2016). Ekschmill *et al.*, (2001) demonstrated the high potential of bioindication with nematodes, as it relies on sensible biotic functional pathways, and largely avoids production of chemical wastes, which makes it preferable to analyses.

They also occupy key position in soil food web (Bernard, 1992; Bongers and Ferris, 1999). When nematodes graze on saprophytic bacteria and fungi, they give off CO² and NH₄ contributing to C and N mineralization (Ingham *et al.*, 1985) and are key component to enhance soil fertility and maintain soil ecosystem health thereby improving crop productivity. Ekschmill *et al.*, (2001) demonstrated taxonomic richness of nematode communities which reflects both abiotic condition and biotic activity of soil. Nematode species composition reflects substrate texture, climate, biogeography, organic inputs, and both natural and anthropogenic disturbances (Cobb 1915; Tietjen 1989; Yeates 1984; Nehar 2001) indicating balance functioning of ecosystem. It has been successfully used to assess soil responses in agricultural practices, forestry practices, and mining restoration (Porazinska *et al.*, 1999; Forge and Simard, 2001; Hohberg, 2003).

Unlike temperate region, the understanding of mineral soil diversity of nematodes in tropics is not quantified (Power *et al.*, 2009). A total of around 1,000,000 species of nematode estimated globally (Hugot

et al., 2001; Abebe *et. al.*, 2011). When considering the estimated number of living species of nematode only 5.3% has been described (Morand *et al.*, 2006) and nearly 30,028 species are known. About 2902 species of nematode are identified from India (Pande and Arora, 2014) which is 9.66% of total described species.

Nematological research in India predominantly focussed on plant and animal parasitic groups. The parasitic association of nematodes with all the major crops of India has been reported in earlier literature (Khan, 2012). Lizanne (2015) reported nematode species from paddy fields of Goa. Little work has been done on the free living groups in forest ecosystem, as they don't have direct connection with agriculture or livestock.

Extensive faunal studies in general, have been done in the Goa, but the underground biota (Nematodes) has been neglected in most of the cases, apart from a few sparse and scattered studies. As underground diversity is largely unseen; the incomprehensible diversity of soil organisms resulting taxonomic difficulties faced in identifying the soil's

inhabitants (Brussaard, 1997) and due to which it is difficult to quantify (Wall *et. al.*, 2010).

Three wildlife sanctuaries under study are part of the Western Ghats contributing to its rich biodiversity. It may also incorporate wide diversity of soil nematodes as soil of these sanctuaries laden with high organic matter (Lizzane, 2015) and bestowed with a relatively rich floristic diversity (Alvares, 2002). Understanding faunal diversity of any conservation or protected area is imperative as information gathered has fundamental and applied application (Sharma, 2014). Understanding nematode diversity will help to identify plant parasitic nematodes that can affect wild plant species or seedling production in forest nurseries (Stollarova, 1999; Cram and Fraedrich, 2012). Such study will also highlight the possible effect of forest disturbance on soil nematode diversity (Bloemers *et.al.*, 1997).

The Bhagwan Mahaveer Wildlife Sanctuary, the Mhadei Wildlife Sanctuary and the Bondla Wildlife Sanctuary have been selected to study soil nematode diversity with following objectives as follows:

- 1) To analyse the soil samples from the study sites to assess suitability of the same for the presence of soil nematodes.
- 2) To conduct an opportunistic survey for free living soil inhabiting nemafauna in Bhagwan Mahaveer Wildlife Sanctuary, Mhadei Wildlife Sanctuary and Bondla Wildlife Sanctuary.
- 3) To prepare a check list of nemafauna in the study site.
- 4) To study seasonal variations if any regarding the soil nematode in the study site.
- 5) To propose suitable measures to preserve the nemafauna in the study area.

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

Nematodes are the most abundant, multi-cellular organisms and like arthropods, no other animal has impact on humans directly or through agriculture (Maggenti, 1981). Nematodes are known to humans from ancient times and found its reference in *Rig, Yajur* and *Atharv Vedas* (6000-4000 BC) (Lizanne, 2015).

Belowground soil communities are important natural resource (Chew, 1974) with immense, but largely unexplored, biodiversity (Andre *et al.*, 1994, 2001; Wheeler *et al.*, 2004; Nehar *et al.* 2005) and received immense value as ecological indicator (Nehar *et al.*, 2005) after understanding their functional links to ecosystem processes (Debruyne, 1997), soil community usage in determining a hierarchy of geographic scale (Neher *et al.*, 1998) and measuring their utility across ecosystem boundaries. These soil communities interact together providing series of ecosystem goods and services to various biological processes (Lavelle, 2002; Kibblewhite *et al.*, 2008; Wall *et al.*, 2010).

Soil inhabiting nematodes are one the successful and important group of underground biota (Chew, 1974). Soil inhabiting nematodes have the potential to provide insights into soil processes, soil condition, biotic and functional status of soil (Bongers and Ferris, 1999; Porazinska *et al.*, 1999; Ritz and Tradgill, 1999; Ekschmill *et al.*, 2001; Neher, 2001; Bohra and Sulthana, 2008).

Members of phylum Nematoda are one of the most earliest and diverse types of animals, on the earth's surface (Bernard, 1992; Bloemers *et al.*, 1997; Ettema, 1998; Bongers and Ferris, 1999; Wang *et al.*, 1999). Based on the available evidences it is very clear that nematodes have evolved to occupy almost every conceivable niche on the earth that contains, at least a thin film of water and some organic matter (Tahseen, 2012; Lizanne, 2015).

Although nematology attracted attention and recognition only in 20th century, our knowledge of a few species of nematodes of medical importance dates back to Papyrus Ebers (Circa 1500 BC). The intestinal round worm (*Ascaris lumbricoides*), filarid (*Wucheraria bancrofti*) and guinea worm or fiery serpent of Moses (*Dracunculus medinensis*) were already known to the ancient man. However, marine, freshwater, soil and plant nematodes remained little known groups mainly because of their extremely small size and the difficulties encountered in their isolation, mounting and observation.

Borellus (1656) for the first time observed free-living nematodes *Turbatrix aceti* the 'vinegar eels' which was present in vinegar. Like animal parasitic nematode, plant parasitic nematodes receive less attention from ancient scientists. Needham (1743) for the first has observed plant parasitic nematode from a diseased wheat grains (*Anguina tritici*) which were later named *Vibrio tritici* by Steinbuch (1799). Muller (1783) described several species of free-living freshwater nematodes. In the middle of 19th century

Nematode taxonomy further developed and landmark progress was observed based on the studies of nematodes of Iceland (Leuckart, 1849), the Mediterranean (Eberth, 1863), the English coast (Bastian, 1865), the coast of Brittany (Villot, 1875) and on nematodes collected in various expeditions (Von Linstov, 1900). Freshwater nematodes received further interest around 1890 with the papers of Daday (1897) on the Hungarian fauna. Dujardin (1845) and many other researchers viz., Bastian (1865), Schneider (1866), de Man (1884, 1912, 1920 and 1927), Daday (1905) and Maupas (1900) were the pioneers of the field.

Dujardin (1845) reported for the first time relationship between free-living and plant parasitic nematodes and his early work on the free-living nematodes included careful descriptions of *Enoplus*, *Oncholaimus*, *Rhabditis* and *Dorylaimus*. Bastian (1865) grouped the free-living nematodes into soil, freshwater and marine forms and described 100 new species of 30 genera in which 23 were new to science. de Man (1884) listed eight families of free-living nematodes.

Cobb (1920) “Father of Nematology” proposed a significant change in classification and he placed nematodes under separate Phylum Nemata. In Chitwood’s (1933, 1937) classification, ‘Nematoda’ was treated as a phylum with two classes, ‘Phasmidia’ and ‘Aphasmidia’. Andrásy (1984) has contributed extensively to the nematode taxonomy and provided keys to most

species of terrestrial and freshwater nematodes. Siddiqui, (1986) and Nickle (1991) gave taxonomic keys for Tylenchids identification up to genus level. Jairajpuri and Ahmad (1992) gave identification keys to free-living, predacious and plant-feeding dorylaimids.

Barber (1901) first who reported plant parasitic nematode from India *Heterodera dicicola* (the then name of *Meloidogyne*) infesting tea in South India. Milne (1919) reported seed gall nematode (*Anquira tritici*) of wheat. Ayyar (1926, 1933) recorded root knot nematode on vegetables and other crops in south India. Dastur (1936) reported white tip disease of rice. ‘Molya’ disease of wheat and Barley which was caused by nematode species, *Heterodera avenae*, was reported by Vasudeva in 1958. Organized research on plant nematodes started only after the end of 1950. In India, 1960s could be regarded as the most active phase for the growth of nematode taxonomy because of the outstanding contributions of Siddiqui (1959), Prasad *et al.*, (1959), Jones (1961), Jairajpuri and Ahmad (1992) and several other young taxonomists. Their work greatly helped in establishing and developmental work in Nematology in India.

The parasitic association of nematodes with all the major crops of India has been reported in earlier literature (Khan *et. al.*, 1971; Anandi and Dhanachand, 1992; Khan *et. al.*, 1993; Sundararaju, 2006, Lizanne, 2015). Nematodes are known to cause 12.3% of annual yield loss in world’s major

crop and in developing countries is about 14% (Sasser and Freckman, 1987). Agriculture is an important sector and plays a vital role in the Indian economy as it contributes about 17% to the total GDP and provides employment to over 60% of the population (Jain *et al.*, 2007). Jain *et al.*, (2007) estimated total losses caused due to plant parasitic nematodes on different crops. Hence, nematological research in India is predominantly focused on plant and animal parasitic groups. Little work has been done on the free-living group in forested (Vaid *et al.*, 2014) or in social forestry areas (Australian Acacia plantation), probably because they have little direct concern with agriculture and livestock.

A total of about 1,000,000 species of nematode are estimated globally (Hugot *et al.*, 2001), of which around 30,028 species are known. About 2902 species of nematode are identified from India (Pande and Arora, 2014), which accounts for 9.66% of total described species. A large number of earlier workers have reported soil-inhabiting nematodes as well as parasitic nematodes from various parts of the world (Lownsbery and Lownsbery, 1985; Hanel, 1996; Yeates, 1996; Ettema *et al.*, 1998; Gorres *et al.*, 1998; Hanel, 1999; Stollarova, 1999; Yeates and Bongers, 1999; Popovici *et al.*, 2000; Power *et al.*, 2009; Cram and Fraedrich, 2009; Hanel and Cerevkova, 2010; Zhang *et al.*, 2012; Kergunteuil *et al.*, 2016).

Forests are a precious natural resource and play a vital role in the survival of every life on the planet earth (Khan, 2012). Several workers such as Johnson *et*

al., 1972; Yeates, 1972, 1996; Boag, 1974; Sohlenius, 1977; Sohlenius and Wasilewska, 1984; Lownsbery and Lownsbery, 1985; Reuss, 1995; Stollarova, 1999; Power *et al.*, 2009; Zhang *et al.*, 2012 extensively studied nematode diversity in forest ecosystem. In India little work has been done on nematode diversity in forested ecosystem (Rizvi, 2008). Pradhan and Dash (1987) reported 17 species of nematodes in tropical deciduous forest of Sambalpur with nematode density ranging from $15.1 \times 10^4/m^2$ (May) to $66.1 \times 10^4/m^2$ (November). They also found out that, of the total nematode 88.4% were in top 10cm during the peak period of density. Baqri, (1999) reported a total of 191 species belonging to 102 genera, 45 families and 10 orders from West Bengal. Bohra and Baqri (2005) reported 23 species of plant and soil nematode belonging to 21 genera of 12 families under four Orders Viz. Tylenchida, Aphelenchida, Dorylaimida and Mononchida and their result indicates great diversity of plant soil nematode in Ranthambhore National Park. Khan *et al.*, (2005) reported two new monohysterid species (nematoda) from Keoladeo national park, Rajasthan, India. Baniyamuddin *et al.* (2007) reported 85 genera of nematodes from the natural forest of Arunachal Pradesh, where they found out that, it is dominated by taxonomic group Dorylaimida (54%), followed by Rhabditida (7%), Alaimida (5%), Araeolaimida (4%), Enoplida (2%), Aphelenchida and Monohysterida (1% each). Rizvi (2008), studied community analyses of soil inhabiting nematodes from tropical Siwalik Sal forest soil in Dehradun (Uttarakhand), India. In his

study, randomly selected composite samples yielded 59 nematode genera belonging to 11 orders dominated by order Dorylaimida (31%) followed by order Rhabditida (22%). Mohilal *et al.*, (2009) studied plant and soil nematodes from Lokchao Yangoupokpi Wildlife Sanctuary, Manipur, India. Their study showed rich nematode diversity and reported about 25 genera of nematodes from Lokchao Yangoupokpi Wildlife Sanctuary. Nusrat *et al.*, (2013) reported, four known and one new species of Mononchida (Nematoda) from Silent Valley National Park, India. Sharma (2014) reported 18 species of dorylaims from Govind Wildlife Sanctuary. Vaid *et al.*, (2014) reported 43 genera of soil inhabiting nematodes in Dera Ki Gali forest of Poonch district in Jammu and Kashmir. In their study, Vaid *et al.*, (2014) reported Rhabditida, were representing the highest percentage (34%), followed by Dorylaimida and Mononchida. In terms of abundance, Rhabditida was the most dominant group (51%), followed by Dorylaimida, Mononchida and the least was Monhysterida and Enoplida (2%). In terms of trophic groupings, the bacterivore genera representing the highest percentage (48%), followed by predators (20%), omnivores (18%) and plant parasites (11%) (Vaid *et al.*, 2014). Mendam and Kavitha (2015) studied biodiversity of nematodes of Adilabad district forest were they reported many species of nematodes such as *Xiphinema*, *Hemicycliophora*, *Hoplolaimus*, *Paralongidorus* and *Longidorus* associated with *Tectona grandis*. Sharma and Dubey (2015), for the first time, reported a total of 26 species of terrestrial nematodes from Rajaji National Park (RNP),

Uttarakhand, India, of which 15 were belonging to order Dorylaimida and 11 were belonging to order Mononchida. Of these, *Granonchulus subdecurrens*, was recorded from first time from India. In South Goa district 52 species of nematode are reported which is about 0.01% of total species in India (Lizanne and Pai, 2014). Pai and Gaur (2010), for the first time reported occurrence of economically important spiral nematode (*Helicotylenchus multicinctus* Cobb.) from Goa. Lizanne (2015) reported 69 species of free living nematodes of which 21 were from forested areas of Goa. Gaude and Pai (2018) reported 18 genera of nematodes from Bhagwan Mahaveer Wildlife Sanctuary.

Soil inhabiting free-living nematode communities and their structural changes are best known biological tools for assessing soil disturbances, including heavy-metal pollution (Bongers *et al.*, 2001; Georgieva *et al.*, 2002; Bohra and Sulthana, 2008), agricultural and extensive grazing activities (Yeates and Bongers, 1999; Kandji *et al.*, 2001; Mills and Adl, 2006) in the terrestrial ecosystems (Gupta and Yeates, 1997; Neher, 1999). Due to their immense sensitivity to various changes in the soil ecosystem and their ability to reflect differences among disturbed, undisturbed and human-impacted environments, the free-living nematodes are considered to be quite useful and inexpensive organisms for ecological research (Porazinska *et al.*, 1999; Abebe *et al.*, 2006).

Nematode infestation in forested area has been neglected area of research (Khan, 2012). The major reasons could be non appearance of visible symptoms and difficulties in accessing the sampling site for damage assessment. Forested soil due to diversified flora and has high organic content, adequate moisture and moderate temperature are the conducive conditions for nematode survival in forested habitat. Few studies have been conducted on forested tree species such as Acacia, Sal, Teak and Sandalwood (Khan, 2012). Nematodes have wide host range and attack crop plant and also numerous angiosperms and gymnosperm trees. Hence it is imperative to understand nematode diversity in forested area.

STUDY AREA

STUDY AREA

Goa, a small state of India with an area of 3,702 sq. Km, is a part of west coast region. Broadly, there are three physical division of Goa. The mountainous region of the Sahyadris in the east, the middle level plateaus in the centre and the low lying river basins with coastal plains (Alvares, 2002). Sahyadris lying in Goa is a part of the Western Ghat region and is catchment area of the rain and nature has covered the area with forests largely characterised by moist deciduous species. Goa receives around 3000mm of rainfall from southwest monsoon for four months from June to September, followed by dry period of six to eight months experiencing warm and humid tropical climate. Goa has six wildlife sanctuaries Viz., the Bhagwan Mahaveer Wildlife Sanctuary (BMWS), the Cotigao Wildlife Sanctuary (CWS), the Bondla Wildlife Sanctuary (BWS), the Mhadei Wildlife Sanctuary (MWS), the Netravali Wildlife Sanctuary (NWS) and the Salim Ali Bird Sanctuary (SABS) protecting all of its forested area.

For the present studies, three wildlife sanctuaries of Goa viz., the Bondla wildlife sanctuary, the Bhagwan Mahaveer Wildlife Sanctuary and the Mhadei Wildlife Sanctuary was selected.

Bondla Wildlife Sanctuary:

It is located at 15°26'23.95"N and 74°06'22.37"E and is about 47 km. from Panaji, the state capital of Goa. It is a bowl shaped valley surrounded by hills (Alvares, 2002) at the junction of three talukas viz. Ponda, Sangeum and Sattari, with an area of 8 km². This sanctuary is bestowed with rich floral and faunal diversity. Forest type includes moist-deciduous forest with small patches of semi-evergreen forest. Dominant plant species are *Terminalia crenulata*, *Xylia xylocarpa*, *Terminalia tomentosa* and *Strobilanthus* sps. Commonly sighted larger mammalian species are Sambar, Chital and Wild Boar.

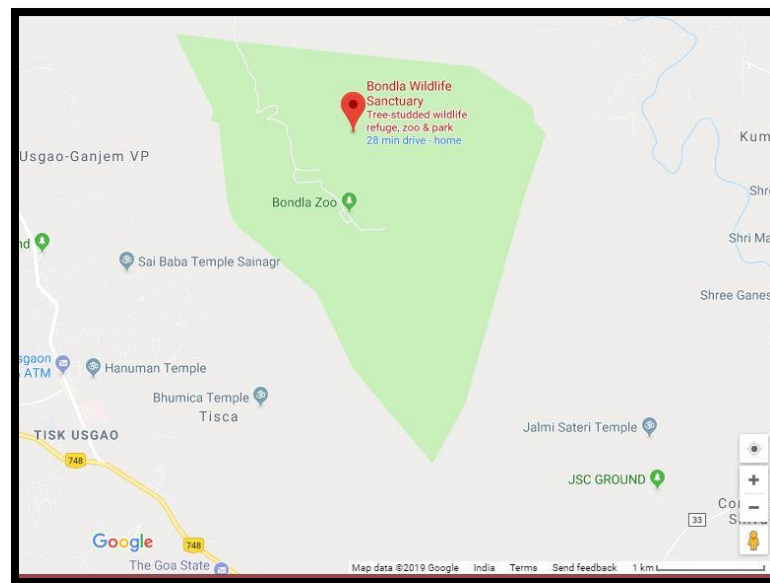


Image 1: Google Image of Bondla Wildlife Sanctuary
<https://goo.gl/maps/umVr58xyWLSfoTZ87>

Bhagwan Mahaveer Wildlife Sanctuary:

It is located at 15°20'28.79" and 74°19'02.26" in Sanguem taluka that covers 240 sq. km area of which about 107 km² of core area of the sanctuary declared as Mollem National Park. Forest types includes West Coast tropical evergreen forest, west coast semi-evergreen forest and moist deciduous forest. This sanctuary is rich in flora and fauna and has dense canopy.

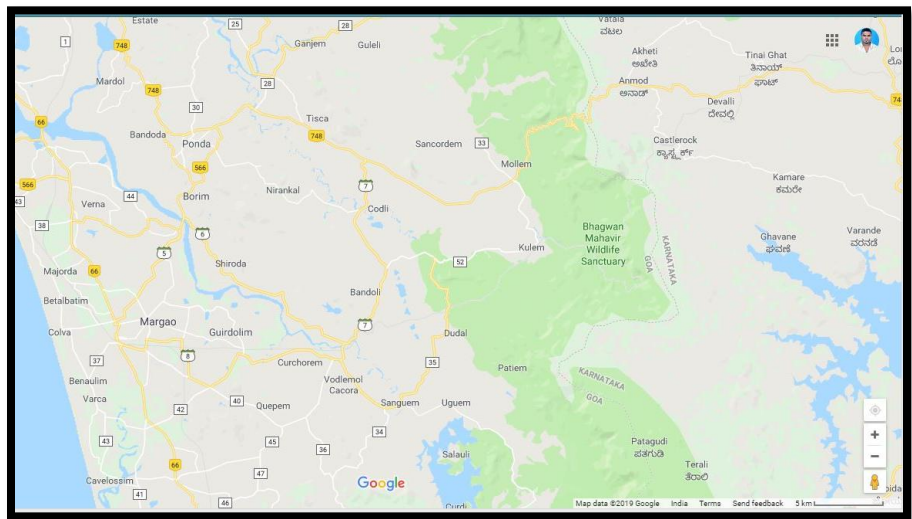


Image 2: Google Image of Bhagwan Mahaveer Wildlife Sanctuary
<https://goo.gl/maps/zVshjvYvcmDMSRSG8>

Mhadei Wildlife Sanctuary:

This located in the North Goa District, Sattari Taluka at $15^{\circ}34'06.86''N$ and $74^{\circ}13'46.82''E$ with an area of about 208.48 km². This sanctuary serve as a connecting link between the reserve forest of Sawantwadi and also the hilly ranges of this sanctuary serve as a corridor for animal movement from Karnataka into Maharashtra and vice versa (Alvares, 2002)

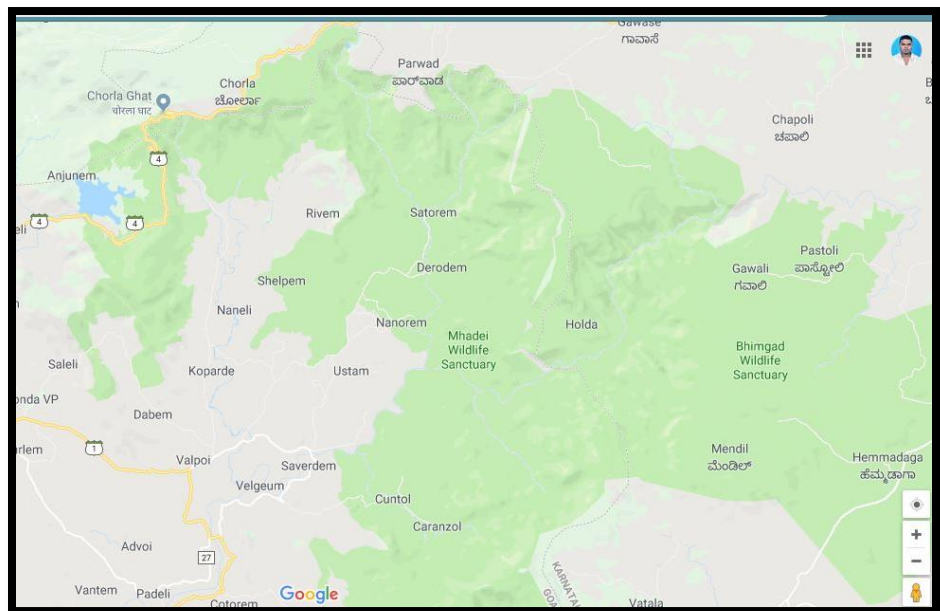


Image 3: Google Image of Bhagwan Mahaveer Wildlife Sanctuary
<https://goo.gl/maps/65dFC3aDweUiBGgp9>

STUDY AREA

- **Bondla Wildlife Sanctuary**
- **The Bhagwan Mahaveer Wildlife Sanctuary**
- **Mhadei Wildlife Sanctuary**



Google Image of Bondla Wildlife Sanctuary



Google Image of Bhagwan Mahaveer Wildlife Sanctuary

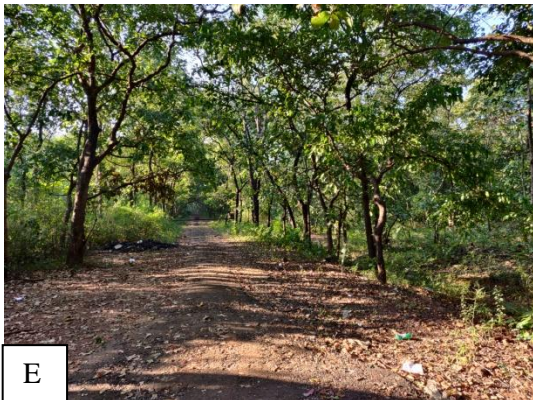
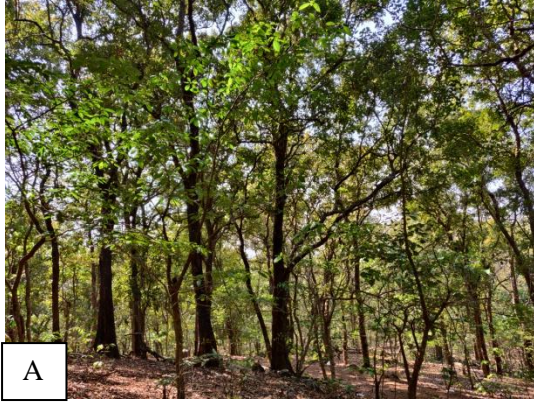


Google Image of Mhadei Wildlife Sanctuary



BWLS- Bondla Wildlife Sanctuary
BMWLS- Bhagwan Mahaveer Wildlife Sanctuary
MWLS- Mhadei Wildlife Sanctuary

Photo Plate 1: Photo images from the study sites







MATERIALS
AND
METHODS

MATERIALS AND METHODS

TOOLS FOR SAMPLING

Sampling tools includes a hand trowel, knives for cutting roots, scissors, polythene sample bags, tags, marker pens for labeling the sample bag and a pencil and notebook for recording information.

TAKING SOIL SAMPLES AND NUMBER OF SAMPLES

The soil, which is very wet or too dry, was avoided and slightly moist soil was collected for soil analysis and for nematode extraction. Enough number of samples was taken to ensure they are representative of the situation in the study area. For more accurate assessment, number of sub-samples were combined for each field sample. For soil nematodes extraction from an area of 0.5 to 1 hectare, collected a minimum of 20 sub-samples. These sub-samples were combined to make one composite sample to represent the field area sampled. Bulking of samples in this way helps to preserve nematodes by maintaining the temperature and moisture of samples (de Maeseneer and Herde, 1963).

SAMPLING PATTERN

Nematodes are rarely distributed evenly in a field, and samples should therefore be collected from several areas, within the field, hence random sampling method was followed.

CARE OF SAMPLES

Soil samples were collected in strong plastic bags, and were labelled systematically directly on the plastic bag with a permanent marker pen a sample number or reference. After collection, care was taken to keep soil samples in cool and dry condition, if soil samples were not processed immediately by storing them in a refrigerator (approx. 10°C) up to two weeks.

FOR SOIL ANALYSIS

Opportunistic field trips were made in Bondla Wildlife Sanctuary, Bhagwan Mahaveer Wildlife Sanctuary and Mhadai Wildlife Sanctuary. Soil samples were collected to analyze soil for its suitability for the existence of soil Nematodes (1 Kg). Collected soil samples were air dried for 15 to 20 days. Dried soil was then sieved using coarse sieve to remove unwanted dry plant parts, stones and pebbles etc. Further soil analysis was

carried out in Soil Testing Laboratory, Krishi Vigyan Kendra, Margao, Goa.

SOIL PROCESSING AND NEMATODE EXTRACTION

The collected soil samples were subjected to sieving and decantation (gravity) method as prescribed by Cobb's (1915). This method takes the advantage of the difference in size and specific gravity between nematodes and soil components. Soil sample about 1000g was placed in a bucket and added 5 liter of water which was mix thoroughly, until all clods and peats are broken up and to separate the nematodes from the soil particles and to suspend them in the water. Bucket was kept idle for 30 seconds to one minute, so as to heavy soil particles sink to the bottom of the bucket and the nematodes remain suspended in the water. Later water was poured through No.20 mesh sieve into the second bucket leaving the heavy soil particles in the first bucket (No. 1). Nematodes will pass easily through the coarse sieve. Most of the nematodes will now be in water of the second bucket. Wash the residue on the coarse sieve with more water and it flow into the second bucket. The purpose is to make sure that no

nematodes remain in the residue on the coarse sieve. Residue on the coarse sieve as well as from the first bucket was discarded. Water from the second bucket gently pour through the fine sieve of 200 to 250 mesh BSS (British Standard Specification) and allow the water to run down the drain. If this is carefully done, most of the nematodes will be caught on the fine sieve. Fine sieve was washed with a gentle stream of water to remove fine soil particles. Residue on the sieve was collected in a beaker and the nematodes were then isolated using modified Baermann's funnel technique (Baermann, 1917). The Baermann funnel is a regular glass or plastic funnel, about 7.5 to 15cm in diameter, with a piece of rubber tubing attached to the stem and closed with a clump or a pinchcock. A molded wire gauge is placed in the funnel. The funnel is filled with fresh tap water. Care was taken to ensure that there are no air bubbles in the funnel or in the rubber tubing as the nematodes can be caught in the air bubble and die. The wire gauge was lined with tissue paper in the form of a cross (+). The edge of the tissue paper should not protrude out of the funnel otherwise water will flow out. The residue that was collected in the beaker was poured over the tissue paper. The entire

set-up was kept undisturbed for a day or two at room temperature. Freshwater was added to the funnel to compensate for loss by evaporation. The nematodes being active migrate to the bottom through the tissue paper and get accumulated in the stem of the funnel and in the rubber tubing at the bottom. At the end of the waiting period of 48 hours, through the rubber tubing a small amount of water was drawn into a cavity block. This water suspension along with nematodes was used for further studies i.e., isolated nematodes were collected for counting, fixing and processed for making permanent slides.

KILLING AND FIXATION OF NEMATODES

Collected nematodes were killed and fixed simultaneously by pouring hot fixative in a cavity block where the fixative was prepared with 90ml distilled water, 8ml formalin and 2ml glycerol and heated for 60°C (Seinhorst, 1959) and was kept for 24 hours.

MOUNTING, SEALING AND PREPARATION OF PERMANENT SLIDES

The nematodes were transferred to a dehydrating agent of glycerine-alcohol which was prepared with 79 parts distilled water, 20 parts 96% ethanol and 1 part glycerol. This block was then kept in a desiccator containing anhydrous calcium chloride for about 4 to 5 weeks. After 4 to 5 weeks, the nematodes were dehydrated and were mounted on a tiny drop of anhydrous glycerin which was placed in the centre of a clean glass slide (1mm thickness). The dehydrated nematodes were transferred into this drop using a horse hair mounted on a handle. Care was taken to make sure that the nematodes were gently pressed to the bottom of the glycerine drop as there is possibility that they can move into the molten wax, if they are on the surface of the glycerine drop. To seal this glycerine drop with nematode at its center a small pieces (4) of wax were kept on four sides of the drop and a cover slip was gently placed on the wax pieces which was then kept on a hot plate (60°C) till the wax melted. Melted wax sealed the drop of glycerin with

the nematodes at its centre and these sealed permanent slides were then used for further study.

TECHNIQUE TO HANDLE A LIVE NEMATODE

Using a compound binocular research microscope; select the nematode of your choice from the cavity block containing the nematode suspension (step 3, as mentioned earlier). Loosen the chosen nematode from the bottom of the cavity block with a short twitch of the horse hair mounted on a handle and gently lead it to the surface of the solution, until it is more or less horizontal, whilst simultaneously changing the focus of the microscope, with the fine tuning knob, to make the nematode visible, as it is led to the surface of the solution. Then tactfully position the tip of the horse hair for the final twitch. The nematode curls round the horse hair, if the tip of the hair is positioned just below them and at right angle to their body axis. Gently lift the handle with the nematode curled round the horse hair and place where needed. The viscosity and the surface tension of the liquid make the nematode to stick to the horse hair instead of pulling it down to the

bottom of the cavity. Mastering this technique is important for any one working with the nematodes, as it is easy to identify them, when they are alive, than when they are killed, since there is a possibility that, some parts of the nematode may be damaged, whilst killing and fixing them.

IDENTIFICATION OF NEMATODES

After the preparation of permanent slide the nematodes were identified and classified according to the following literature (Goodey, 1951; 1963; Bajaj and Jairajpuri, 1979; Andrásy, 1982 (a & b); Jairajpuri and Khan, 1982; Andrásy, 1984, 1999; Siddiqi, 1986; Jairajpuri and Ahmad, 1992; Ahmad, 1996; Mai *et al.*, 1996; Rawat and Ahmad, 2000; Siddiqi, 2000; Esquivel, 2003; Choudhary *et al.*, 2010; and Rizvi, 2010)

Information was also collected from the websites like NEMAPLEX (http://nemaplex.ucdavis.edu/Uppermnus/nematamnu.htm#Taxonomic_Keys), Nema Species Masterlist A-Z.

STATISTICAL ANALYSIS

The monthly data obtained for moisture content, pH and nitrogen / org. content were subjected to Principal Component Analyses to determine, which of the variables contributed significantly for nematode diversity in soil. The test was carried out using XLSTAT software. The correlation between the various parameters within the each study site was analyzed using correlation coefficient.

TO DETERMINE THE DIVERSITY OF SOIL-INHABITING NEMATODES

The following parameters were calculated to determine the diversity of free living soil-inhabiting nematodes:

Frequency (N) is frequency of nematode genus (the number of samples in which the genus was present) (Vaid *et al.*, 2014).

Absolute Frequency (AF %) is frequency of genus / total number of samples counted X 100 (Vaid *et al.*, 2014).

Density (MD) is the number of nematode specimens of the genus counted in all samples / total number of the samples collected (Vaid *et al.*, 2014).

Relative density (RD %) is the mean density of the genus / sum of mean density of all nematodes genera X 100. (Tomar *et al.*, 2006; Vaid *et al.*, 2014).

Photo plate 2: Photo images of basic requirements in present study



A-Seives (75, 250, 850 microns)



B- Cavity Block



C- Horse hair Brush



D- Baermann Funnel

RESULTS

RESULTS

Soil samples from the study sites were analysed for the suitability for the existence of soil nematodes and the results obtained was as follows:

Moisture Content:

Moisture content of the soil from study area was found to be between 10-46% being highest during Monsoon i.e., from June to September (40-46%) and lowest during summer i.e., from March to May (10-12%). During the Post Monsoon and winter the moisture content was between 26-31% and 16-21% respectively.

Moisture content of the soil samples from Bondla wildlife sanctuary was found to be between 11-47% (Fig. 1). Highest being recorded in Monsoon (41-42%) and Post Monsoon (25-27%) and lowest being recorded in summer (13-15%) (Fig. 2).

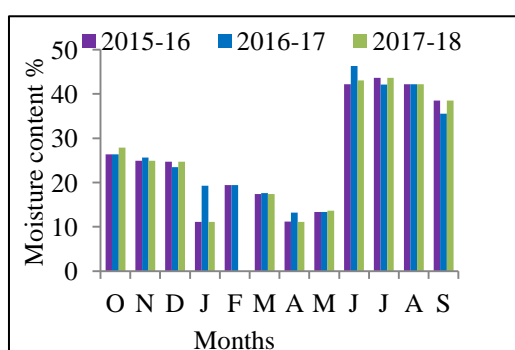


Fig. 1: Graph showing Moisture content in Bondla Wildlife Sanctuary

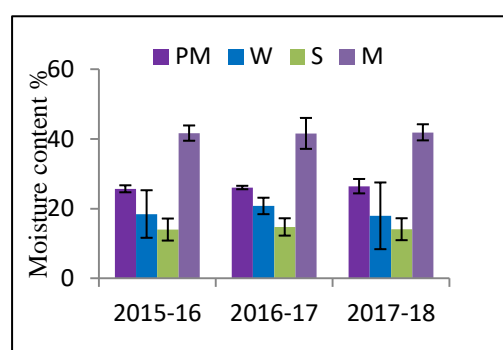


Fig. 2: Graph showing Moisture content in Bondla Wildlife Sanctuary through Seasons

Moisture content of the soil samples from Bhagwan Mahaveer Wildlife Sanctuary follow the same trend as in Bondla wildlife sanctuary

and was found to be between 10- 46% (Fig. 3.). Highest being recorded in monsoon (44-46%) and post monsoon (29-31%) and lowest being recorded in summer (10-12%) (Fig.4). During winter, moisture content was between 19-21%.

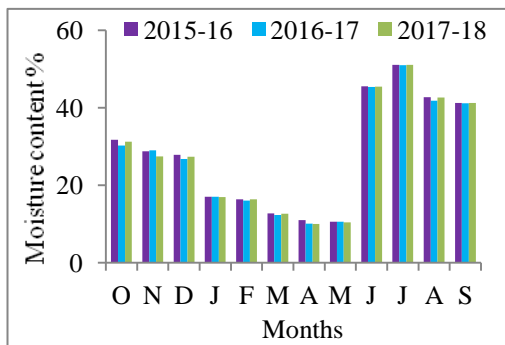


Fig. 3: Graph showing Moisture content in Bhagwan Mahaveer Wildlife Sanctuary

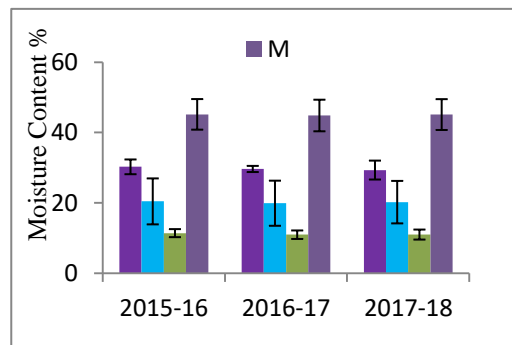


Fig. 4: Graph showing Moisture content in Bhagwan Mahaveer Wildlife Sanctuary through seasons

In Mhadei wildlife sanctuary moisture content of soil samples was between 10 to 46% (Fig. 5.). Highest being recorded in Monsoon (42-43%) followed by in Post Monsoon (30-31%), in winter (20-21%) and lowest being recorded in summer (10-12%) (Fig. 6).

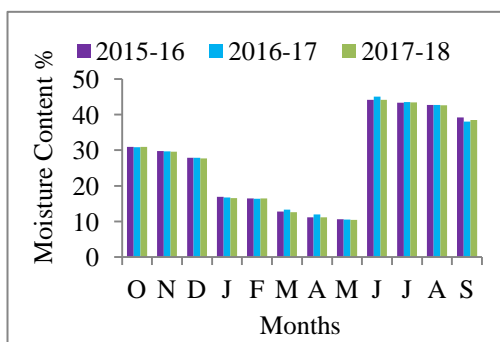


Fig. 5: Graph showing Moisture content in Mhadei Wildlife Sanctuary

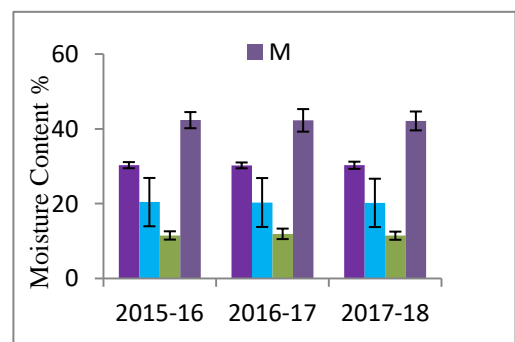


Fig. 6: Graph showing Moisture content in Mhadei Wildlife Sanctuary through seasons

Soil pH:

In the present study, pH of the soil in study area was found to ranging from 5.5~6.6 (mildly acidic).

In Bondla Wildlife Sanctuary pH was in the range of 5.2 to 6.8 (Fig. 7). In Bhagwan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary it was in the range of 5.2 to 6.0 respectively (Fig. 8 and 9). All three study sites pH is negatively correlated with moisture content ($r=-0.589$, $p<0.05$). An insignificant seasonal change with regards to pH throughout the study period was observed (Fig. 10, 11 and 12).

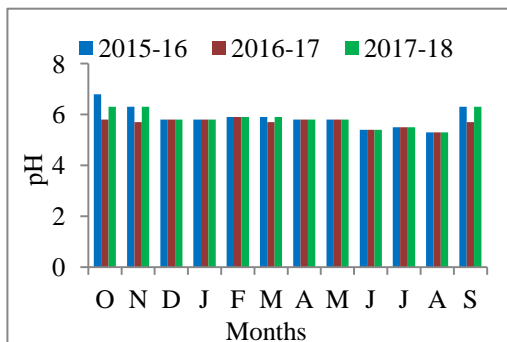


Fig. 7: Graph showing pH in Bondla Wildlife Sanctuary

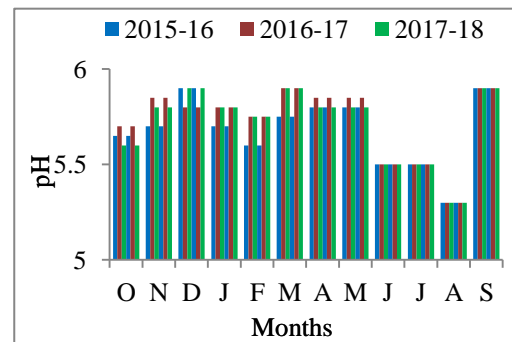


Fig. 8: Graph showing pH in Bhagwan Mahaveer Wildlife Sanctuary

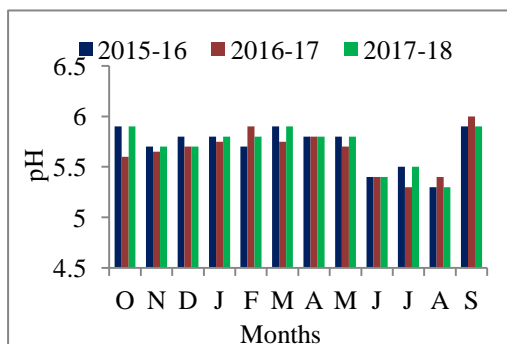


Fig. 9: Graph showing pH in Madhai Wildlife Sanctuary

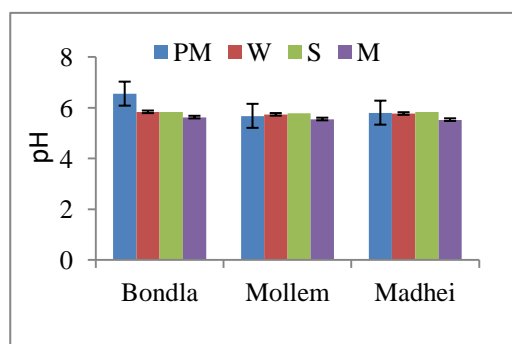


Fig. 10: Graph showing pH in three wildlife sanctuaries through seasons (2015-2016)

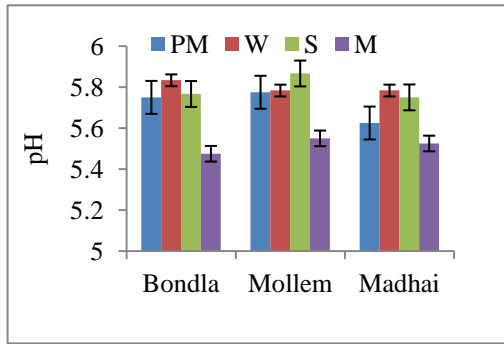


Fig. 11: Graph showing pH in three wildlife sanctuary through seasons (2016-2017)

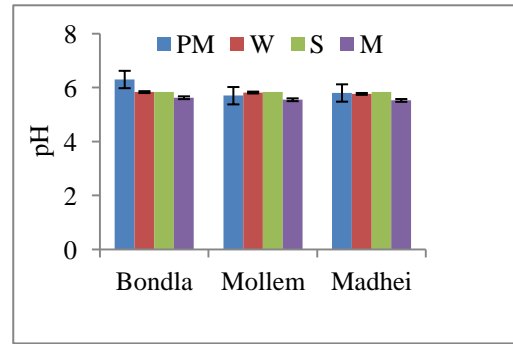


Fig. 12: Graph showing pH in three wildlife sanctuary through seasons (2017-2018)

Nitrogen / Organic carbon:

In the present study nitrogen / organic carbon was between 2.5 to 3.8% (Fig. 13, 14, and 15). Seasonal changes in nitrogen / organic carbon content in soil samples from each of three wildlife sanctuary is given in figure 16, 17 and 18.

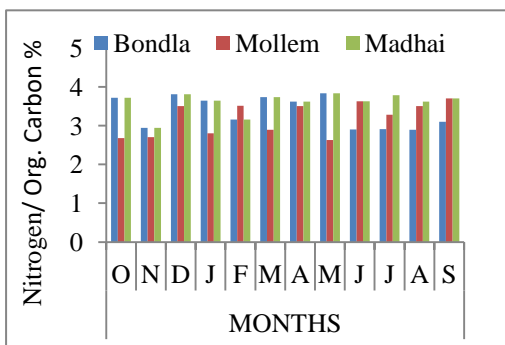


Fig. 13: Graph showing Nitrogen/Org. Carbon in three wildlife sanctuary (2015-2016)

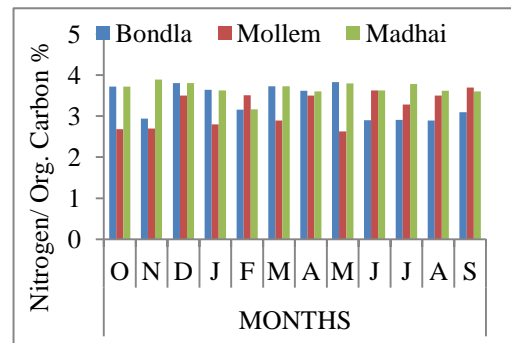


Fig. 14: Graph showing Nitrogen/Org. Carbon in three wildlife sanctuary (2016-2017)

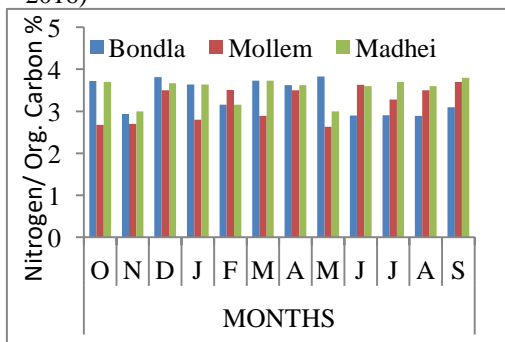


Fig. 15: Graph showing Nitrogen/Org. Carbon in three wildlife sanctuary (2017-2018)

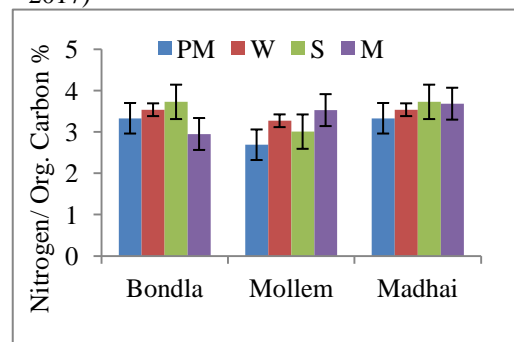


Fig. 16: Graph showing Nitrogen/Org. Carbon in three wildlife sanctuary through seasons (2015-2016)

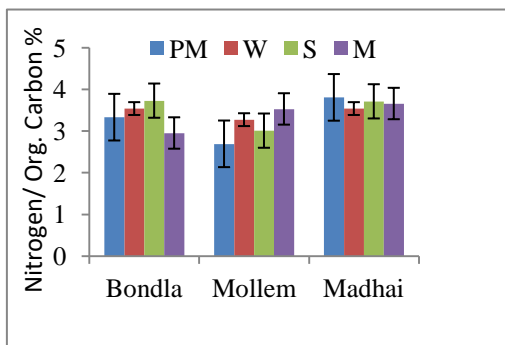


Fig. 17: Graph showing Nitrogen/Org. Carbon in three wildlife sanctuary through seasons (2016-2017)

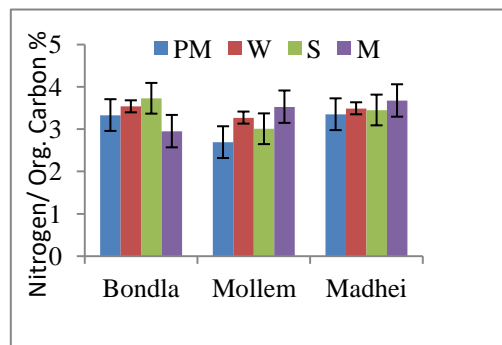


Fig. 18: Graph showing Nitrogen/Org. Carbon in three wildlife sanctuary through seasons (2017-2018)

Potassium, Phosphorous and other Microelements:

In the present study Soil samples were also analysed for Potassium, Phosphorous and other Micronutrients (Iron, Manganese, Copper and Boron) and result of soil for Potassium, Phosphorous and other Microelements is high as recommended by Soil testing laboratory and result of the same is depicted in Table no. 1.

Table 1: Soil analyses of Phosphorus, Potassium and microelements

Study Area	Macroelements		Microelements				
	Phosphorous (Kg/Ha)	Potassium (Kg/Ha)	Zinc	Iron	Manganese	Copper	Boron

Bond la WLS	88.5±4 1.85	343.4± 144.43	4.1002 ±1.744	29.908 ±1.282	29.984 ±0.427	5.584± 6.456	0
Bhag wan Maha veer WLS	26.8±0 .345	100.8	4.579± 0.6434	31.43± 2.560	30.15± 0.4101	1.871± 0.629	0.35±0 .4949
Mhad ei WLS	27±0.5 63	99.8	4.539± 0.6430	31.42± 2.346	30.11± 0.101	1.869± 0.729	0.34±0 .4949

Soil samples were also analysed from the study sites to understand free living Soil Nematode diversity.

Free living Soil Nematode diversity from three wildlife sanctuaries:

A total of 38 free living soil nematode species belonging to 5 orders and 17 families were reported (Table 2, Fig. 18). Nematode diversity in three wildlife sanctuary is given in Table no. 3. Order Dorylaimida was the most dominant order consisting of 10 families and 28 species of free living nematodes and it represents about 73% total nematode species reported (Fig. 19).

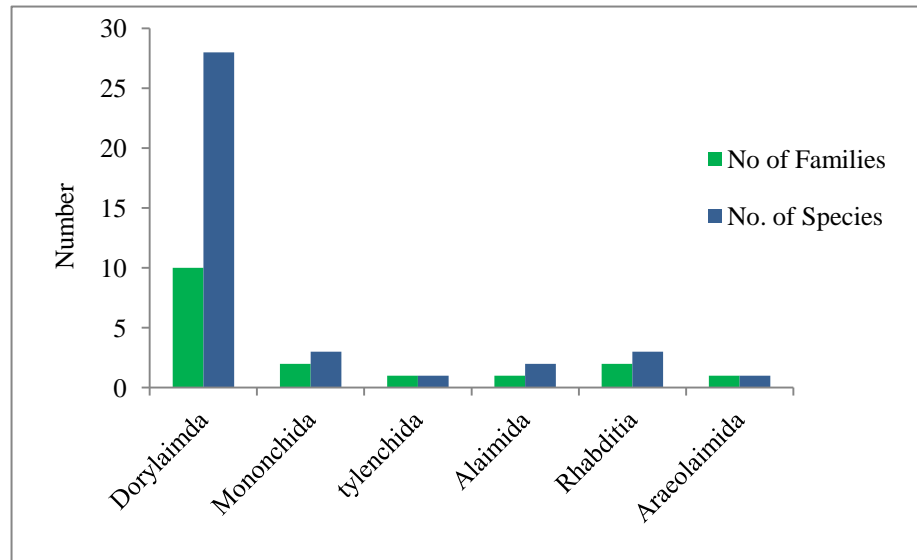


Fig. 19: Graph showing number of families and species in Each Order recorded

Order Mononchida and Rhabditida consist of two families and three species respectively and it represents about 15% of total nematode species reported. Order Alaimida consist of one family and two species while Order Tylenchida and Order Araeolaimida consist of one family and one species each.

Table 2: Checklist of Free living Nematode species from three wildlife sanctuary

Order	Family	Genus	Species
DORYLAIMIDA	Belondri dae	<i>Oxydirus</i>	<i>Oxydirus oxycephaloides</i> (De Man, 1921) Thorne, 1939
		<i>Axonchium</i>	<i>Axonchium amplicolle</i> Cobb, 1920
	Dorylaim idae	<i>Dorylaimus</i>	<i>Dorylaimus stagnalis</i> Dujardin, 1845
			<i>Dorylaimus afganicus</i> Andrassy, 1960
		<i>Mesodorylaimus</i>	<i>Mesodorylaimus chamoliensis</i> , Ahmad 1995
		<i>Sicaguttar</i>	<i>Sicaguttar thornei</i>

		<i>Sicaguttar sp.</i>	
Qudsianematidae	<i>Qudsinema</i>	<i>Qudsinema sp</i>	
		<i>Eudorylaimus sp</i>	
	<i>Microdorylaimus</i>	<i>Microdorylaimus sp.</i>	
Crateronematidae	<i>Chrysonema</i>	<i>Chrysonema sp.</i>	
Nordiidae	<i>Echodelus</i>	<i>Echodelus sp</i>	
	<i>Longidorella</i>	<i>Longidorella sp</i>	
	<i>Thornedia</i>	<i>Thornedia sp</i>	
Thornematidae	<i>Fuscheila</i>	<i>Fuscheila sp.</i>	
Aporcelaimidae	<i>Aporcelaimium</i>	<i>Aporcelaimium baqrii</i>	
	.	<i>Aporcelaimium clamus</i>	
		<i>Aporcelaimium sp</i> Loof and Coomans, 1970.	
	<i>Akortonus</i>	<i>Akortonus vigor</i> Thorne, 1974	
Actinolaimidae	<i>Neoactinolaimus</i>	<i>Neoactinolaimus agilis</i> Thorne, 1967	
		<i>Neoactinolaimus attenuates</i> Ahmad and Jairajpuri, 1994	
	<i>Hexactinolaimus</i>	<i>Hexactinolaimus aneityi</i> Yeates, 1973	
Longidoridae	<i>Paralongidorus</i>	<i>Paralongidorus sali</i> Sissiqi, Hooper and Khan, 1963	
	<i>Longidorus</i>	<i>Longidorus elongatus</i> Thorne and Swanger, 1936	
Xiphinematidae	<i>Xiphinema</i>	<i>Xiphinema americanum</i> Cobb, 1913	
		<i>Xiphinema elongatum</i> Stekhoven and Teunissen, 1938	
		<i>Xiphinema orbum</i> Siddiqi, 1964	
		<i>Xiphinema laevistriatum</i> Lamberti et Bleve Zacheo, 1979	
MONONCHIDA	Monochidae	<i>Clarkus</i>	<i>Clarkus elongatus</i>
		<i>Mononchus</i>	<i>Mononchus truncatus</i>

	Iotonchidae	<i>Iotonchus</i>	<i>Iotonchus indicus</i> Jairajpuri, 1969
TYLENCHIDA	Tylenchidae	<i>Tyloporous</i>	<i>Tyloporus sp.</i>
Alaimida	Alaimidae	<i>Alaimus</i>	<i>Alaimus parvus</i>
			<i>Alaimus indicus</i> , Choudhary & Jairajpuri, 1983
RHABDITIA	Rhabditidae	<i>Mesorhabditis</i>	<i>Prismatolaimus andrassyi</i> Khera & Chaturvedi, 1977
	Panagrolaimidae	<i>Panagrellus</i>	<i>Panagrellus dorsobidentata</i> (Rühm, 1956)
		<i>Panagrolaimus</i>	<i>Panagrolaimus fuchsi</i> Rühm, 1956
Araeolaimida	Plectidae	Plectus	<i>Plectus cirratus</i> Bastian, 1865

-Bondla wildlife sanctuary, BMWLS-Bhagwan Mahaveer wildlife sanctuary, MWLS-Mhadei wildlife sanctuary

Table 3: Nematode Diversity in three wildlife Sanctuary

Sr. No	Species	BW LS	BMW LS	MW LS
1	<i>Oxydirus oxycephaloides</i> (De Man, 1921) Thorne, 1939	-	+	-
2	<i>Axonchium amplicolle</i> Cobb, 1920	+	+	+
3	<i>Dorylaimus stagnalis</i> Dujardin, 1845	+	+	+
4	<i>Dorylaimus afganicus</i> Andrassy, 1960	+	+	+
5	<i>Mesodorylaimus chamoliensis</i> , Ahmad 1995	-	+	-
6	<i>Sicaguttar thornei</i>	+	-	+
7	<i>Sicaguttar sp.</i>	+	-	+
8	<i>Qudsinema sp</i>	+	+	+
9	<i>Eudorylaimus sp</i>	-	+	-
10	<i>Microdorylaimus sp.</i>	+	+	-
11	<i>Chrysonema sp.</i>	-	+	-
12	<i>Echodelus sp</i>	-	+	-
13	<i>Longidorella sp</i>	+	+	-
14	<i>Thornedia sp</i>	+	+	+
15	<i>Fuscheila sp.</i>	-	+	-
16	<i>Aporcelaimium baqrii</i>	+	+	+
17	<i>Aporcelaimium clamus</i>	+	+	+
18	<i>Aporcelaimium sp</i> Loof and Coomans,	+	+	+

	1970.			
19	<i>Akortonus vigor</i> Thorne, 1974	-	+	-
20	<i>Neoactinolaimus agilis</i> Thorne, 1967	+	+	-
21	<i>Neoactinolaimus attenuates</i> Ahmad and Jairajpuri, 1994	+	+	+
22	<i>Hexactinolaimus aneityi</i> Yeates, 1973	+	+	-
23	<i>Paralongidorus sali</i> Sissiqi, Hooper and Khan, 1963	+	+	
24	<i>Longidorus elongatus</i> Thorne and Swanger, 1936	+	+	+
25	<i>Xiphinema americanum</i> Cobb, 1913	+	+	+
26	<i>Xiphinema elongatum</i> Stekhoven and Teunissen, 1938	+	+	+
27	<i>Xiphinema orbum</i> Siddiqi, 1964	+	+	+
28	<i>Xiphinema laevistriatum</i> Lamberti et Bleve Zacheo, 1979	+	+	+
29	<i>Clarkus elongates</i>	+	+	-
30	<i>Mononchus truncates</i>	+	+	+
31	<i>Iotonchus indicus</i> Jairajpuri, 1969	-	+	-
32	<i>Tylodorus sp.</i>	+	+	+
33	<i>Alaimus parvus</i>	+	+	+
34	<i>Alaimus indicus</i> , Choudhary & Jairajpuri, 1983	+	+	+
35	<i>Prismatolaimus andrassyi</i> Khera & Chaturvedi, 1977	+	+	-
36	<i>Panagrellus dorsobidentata</i> (Rühm, 1956)	-	+	-
37	<i>Panagrolaimus fuchsi</i> Ruhm, 1956	-	+	-
38	<i>Plectus cirratus</i> Bastian, 1865	-	+	+

+ = present/reported, - + absent

In terms of feeding guilds (Table 4, Fig. 20) of soil Nematodes predators were most dominant (24%) which includes genus like *Hexactinolaimus*, *Aporcelaimium*, *Mononchus*, *Neoactinolaimus*, followed by omnivore and bactivorous 17% each. Plant parasitic (14%) and least was fungivore (7%).

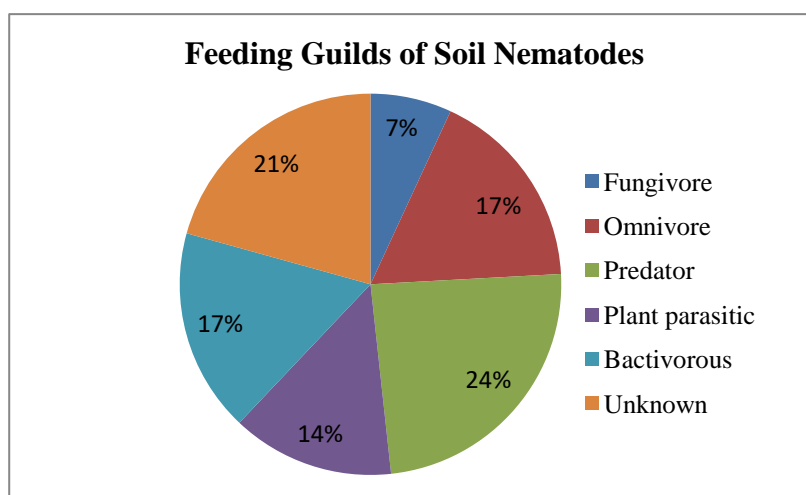


Fig. 20: Feeding guilds of Soil Nematodes

Table 4: Feeding guilds of soil Nematodes reported from three wildlife sanctuary

ORDER	FAMILY	GENUS	Feeding Guilds
DORYLAIMIDA	Belondiridae	<i>Oxydirus</i>	Fungivore
		<i>Axonchium</i>	Fungivore
	Dorylaimidae	<i>Dorylaimus</i>	Omnivore
		<i>Mesodorylaimus</i>	Omnivore
		<i>Sicaguttar</i>	Omnivore
	Qudsianematidae	<i>Qudsinema</i>	Unknown
		<i>Microdorylaimus</i>	Unknown
	Crateronematidae	<i>Chrysonema</i>	Omnivore
	Nordiidae	<i>Echodelus</i>	Unknown
		<i>Longidorella</i>	Unknown
		<i>Thornedia</i>	Unknown
	Thornematidae	<i>Fuscheila</i>	Unknown
	Aporcelaimidae	<i>Aporcelaimium</i>	Predator
		<i>Akortonus</i>	Predator
	Actinolaimidae	<i>Neoactinolaimus</i>	Predator
		<i>Hexactinolaimus</i>	Predator
	Longidoridae	<i>Paralongidorus</i>	Plant parasitic
		<i>Longidorus</i>	Plant parasitic
Xiphinematidae	<i>Xiphinema</i>	Plant parasitic	
MONONCHIDA	Monochidae	<i>Clarkus</i>	Predator
		<i>Mononchus</i>	Predator

	Iotonchidae	<i>Iotonchus</i>	Predator
TYLENCHIDA	Tylenchidae	<i>Tylodoros</i>	Plant parasitic
ALAIMIDA	Alaimidae	<i>Alaimus</i>	Bactivorous
RHABDITIA	Rhabditidae	<i>Mesorhabditis</i>	Bactivorous
	Panagrolaimidae	<i>Panagrellus</i>	Bactivorous
		<i>Panagrolaimus</i>	Bactivorous
Araeolaimida	Plectidae	<i>Plectus</i>	Bactivorous

(Source: Lizanne, 2015)

Nematode Diversity in Bondla Wildlife Sanctuary

A total of 27 species were reported from Bondla Wildlife Sanctuary belonging to 4 orders, 12 families and 18 genera (Table 5). Order Dorylaimida was the most dominant order consisting of 21 species belonging to eight families and which represents 77.77% of total Nematode species reported from this sanctuary. Among the 18 genera reported genus *Dorylaimus* was the most frequent and most dominant genus with absolute frequency of 97%, mean density of 14 and relative density of 25.72% followed by *Quadsinema* with absolute frequency of 77.77%, Mean density of 5.08 and relative density of 9.17%. Genus *Longidorella* was the third most frequent genus with absolute frequency of 61.11%, mean density of 2.05 and with relative density of 3.84%. Genus *Aporcelaimium*, *Xiphinema* and *Neoactinolaimus* were also frequent genus with absolute frequency of 56% and 53% respectively. Genus *Mesorhabditis* was the least frequent and least dominant genus with absolute frequency of 5.5%, mean density of 0.05 and relative density of 0.07%. *Dorylaimus stagnalis* (97%), *Qudsinema sp* (78%),

Longidorella sp (61%), *Aporcelaimium baqrii* (56%), *Xiphinema elongatum* (53%), *Neoactinolaimus agilis* (53%), *Monochus truncates* (50%), *Tylodorus* sp. (50%), *Clarkus elongates* (47%), *Xiphinema americanum* (44%), *Sicaguttar thornei* (42%), *Hexactinolaimus aneityi* (42%), *Xiphinema laevistriatum* (42%), were some the most frequent free living soil nematode species reported from Bondla Wildlife Sanctuary.

Table 5: Soil Nematode Diversity in Bondla Wildlife Sanctuary

Order s	Family	Genus	Species	BWL S
DORYLAIMIDA		<i>Axonchium</i>	<i>Axonchium amplicolle</i> Cobb, 1920	+
	Dorylaimidae	<i>Dorylaimus</i>	<i>Dorylaimus stagnalis</i> Dujardin, 1845	+
			<i>Dorylaimus afganicus</i> Andrassy, 1960	+
		<i>Sicaguttar</i>	<i>Sicaguttar thornei</i>	+
			<i>Sicaguttar</i> sp.	+
	Qudsianematidae	<i>Qudsinema</i>	<i>Qudsinema</i> sp	+
		<i>Microdorylaimus</i>	<i>Microdorylaimus</i> sp.	+
		<i>Longidorella</i>	<i>Longidorella</i> sp	+
		<i>Thornedia</i>	<i>Thornedia</i> sp	+
	Aporcelaimidae	<i>Aporcelaimium</i>	<i>Aporcelaimium baqrii</i>	+
		.	<i>Aporcelaimium clamus</i>	+
			<i>Aporcelaimium</i> sp Loof and	+

			Coomans, 1970.	
	Actinolaimidae	<i>Neoactinolaimus</i>	<i>Neoactinolaimus agilis</i> Thorne, 1967	+
			<i>Neoactinolaimus attenuates</i> Ahmad and Jairajpuri, 1994	+
		<i>Hexactinolaimus</i>	<i>Hexactinolaimus aneityi</i> Yeates, 1973	+
	Longidoridae	<i>Paralongidorus</i>	<i>Paralongidorus sali</i> Sissiqi, Hooper and Khan, 1963	+
		<i>Longidorus</i>	<i>Longidorus elongatus</i> Thorne and Swanger, 1936	+
	Xiphinematidae	<i>Xiphinema</i>	<i>Xiphinema americanum</i> Cobb, 1913	+
			<i>Xiphinema elongatum</i> Stekhoven and Teunissen, 1938	+
			<i>Xiphinema orbum</i> Siddiqi, 1964	+
			<i>Xiphinema laevistriatum</i> Lamberti et Bleve Zacheo, 1979	+
MONONCHIDA	Monochidae	<i>Clarkus</i>	<i>Clarkus elongatus</i>	+
		<i>Mononchus</i>	<i>Mononchus truncatus</i>	+
TYLENCHIDA	Tylenchidae	<i>Tylogorus</i>	<i>Tylogorus sp.</i>	+
Alaimida	Alaimidae	<i>Alaimus</i>	<i>Alaimus parvus</i>	+
			<i>Alaimus indicus</i> , Choudhary & Jairajpuri, 1983	+
RHABDITIA	Rhabditidae	<i>Mesorhabditis</i>	<i>Prismatolaimus andrassyi</i> Khera & Chaturvedi, 1977	+

BWLS- Bondla Wildlife Sanctuary, + = present/ reported

In terms of feeding guilds (Fig. 21) of soil Nematodes reported from Bondla wildlife sanctuary predators were most dominant (29%) followed by plant parasitic (23%), omnivore and bacterivorous 12 % each and the least was fungivore (6%).

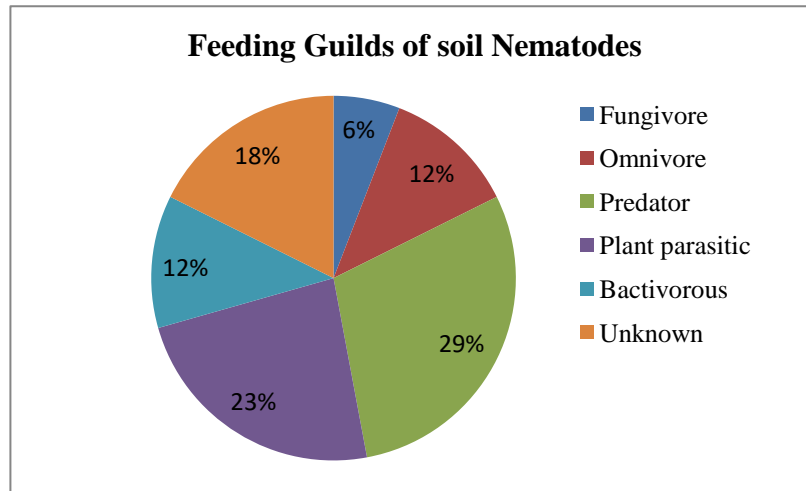


Fig. 21: Feeding guilds of Soil Nematodes reported from Bondla Wildlife Sanctuary

Nematode Diversity in Bhagwan Mahaveer Wildlife Sanctuary

A total of 36 species were reported from Bhagwan Mahaveer Wildlife Sanctuary belonging to 6 orders, 17 families and 27 genera (Table 6). Order Dorylaimida was the most dominant order consisting of 26 species belonging to ten families and which represents 72.22% of total Nematode species reported from Bhagwan Mahaveer Wildlife Sanctuary. In terms of dominance of free living nematode species similar trend was seen like in Bondla Wildlife Sanctuary. Among the 27 genera reported genus *Dorylaimus* was the most frequent and most dominant genus with absolute frequency of 100%, mean density of 15.19 and relative density of 36.08% followed by *Longidorella* with absolute frequency of 83.33%, Mean density of 2.13 and relative density of 5.13%. Genus *Qudsinema* was the third most frequent genus with absolute frequency of 80.55%, mean density of 4.61% and with relative density of 10.95%. Genus *Xiphinema*, *Aporcelaimium*, *Hexactinolaimus*, *Monochus* and *Tylogorous*

were also frequent genus with absolute frequency of 75%, 72%, 67%, 61% and 58% respectively. Genus *Akortonus* and *Oxydirus* was the least frequent and least dominant genus with absolute frequency of 5.5% and 2.8%, mean density of 0.06 and 0.03 and relative density of 0.13% and 0.06% each. *Dorylaimus stagnalis* (100%), *Longidorella sp* (83.3%), *Qudsinema sp* (80.5%), *Xiphinema elongatum* Stekhoven and Teunissen, 1938 (75%), *Aporcelaimium baqrii* (72%), *Xiphinema americanum* Cobb, 1913 (66.6%), *Hexactinolaimus aneityi* (53.3%), *Monochus truncates* (58.3%), *Tylogorus sp.* (55.50%), *Alaimus indicus*, (52.7%), *Neoactinolaimus attenuates* (50%), *Xiphinema orbum* (50%), *Clarkus elongates* (50%), *Dorylaimus afganicus* (47.2%), *Neoactinolaimus agilis* Thorne, 1967 (47.2%), and *Alaimus parvus* (47.2%) were some the most frequent free living soil nematode species reported. In Bhagwan Mahaveer Wildlife Sanctuary, predatory nematodes were most dominant (24%) followed by omnivore and bacterivorous 17% each, plant parasitic (14%) and least was fungivore (7%) (same as Fig. 20).

Table 6: Soil Nematode diversity in Bhagwan Mahaveer Wildlife Sanctuary

Order	Family	Genus	Species	BMWLS
DO RYL AIM	Belondiridae	<i>Oxydirus</i>	<i>Oxydirus oxycephaloides</i> (De Man, 1921) Thorne, 1939	+

IDA		<i>Axonchium</i>	<i>Axonchium amplicolle</i> Cobb, 1920	+
	Dorylaimidae	<i>Dorylaimus</i>	<i>Dorylaimus stagnalis</i> Dujardin, 1845	+
			<i>Dorylaimus afganicus</i> Andrassy, 1960	+
		<i>Mesodorylaimus</i>	<i>Mesodorylaimus chamoliensis</i> , Ahmad 1995	+
	Qudsianematidae	<i>Qudsinema</i>	<i>Qudsinema sp</i>	+
			<i>Eudorylaimus sp</i>	+
		<i>Microdorylaimus</i>	<i>Microdorylaimus sp.</i>	+
	Crateronematidae*	<i>Chrysonema</i>	<i>Chrysonema sp.</i>	+
	Nordiidae	<i>Echodelus</i>	<i>Echodelus sp</i>	+
		<i>Longidorella</i>	<i>Longidorella sp</i>	+
		<i>Thornedia</i>	<i>Thornedia sp</i>	+
	Thornematidae*	<i>Fuscheila</i>	<i>Fuscheila sp.</i>	+
	Aporcelaimidae	<i>Aporcelaimium</i>	<i>Aporcelaimium baqrii</i>	+
		.	<i>Aporcelaimium clamus</i>	+
			<i>Aporcelaimium sp</i> Loof and Coomans, 1970.	+
		<i>Akortonus</i>	<i>Akortonus vigor</i> Thorne, 1974	+
	Actinolaimidae	<i>Neoactinolaimus</i>	<i>Neoactinolaimus agilis</i> Thorne, 1967	+
			<i>Neoactinolaimus attenuates</i> Ahmad and Jairajpuri, 1994	+
		<i>Hexactinolaimus</i>	<i>Hexactinolaimus aneityi</i> Yeates, 1973	+
	Longidoridae	<i>Paralongidorus</i>	<i>Paralongidorus sali</i> Sissiqi, Hooper and Khan, 1963	+

		<i>Longidorus</i>	<i>Longidorus elongatus</i> Thorne and Swanger, 1936	+
	Xiphinematidae	<i>Xiphinema</i>	<i>Xiphinema americanum</i> Cobb, 1913	+
			<i>Xiphinema elongatum</i> Stekhoven and Teunissen, 1938	+
			<i>Xiphinema orbum</i> Siddiqi, 1964	+
			<i>Xiphinema laevistriatum</i> Lamberti et Bleve Zacheo, 1979	+
MONONCHIDA	Monochidae	<i>Clarkus</i>	<i>Clarkus elongatus</i>	+
		<i>Mononchus</i>	<i>Mononchus truncatus</i>	+
	Iotonchidae	<i>Iotonchus</i>	<i>Iotonchus indicus</i> Jairajpuri, 1969	+
TYLENCHIDA	Tylenchidae	<i>Tylodorus</i>	<i>Tylodorus sp.</i>	+
ALAIMIDA	Alaimidae	<i>Alaimus</i>	<i>Alaimus parvus</i>	+
			<i>Alaimus indicus</i> , Choudhary & Jairajpuri, 1983	+
RHABDITIDA	Rhabditiidae	<i>Mesorhabditis</i>	<i>Prismatolaimus andrassyi</i> Khera & Chaturvedi, 1977	+
	Panagrolaimidae	<i>Panagrellus</i>	<i>Panagrellus dorsobidentata</i> (Rühm, 1956)	+
		<i>Panagrolaimus</i>	<i>Panagrolaimus fuchsi</i> Ruhm, 1956	+
ARAEOLAIMIDA	Plectidae	<i>Plectus</i>	<i>Plectus cirratus</i> Bastian, 1865	+

BMWLS- Bhagwan Mahaveer Wildlife Sanctuary, + = Present/reported

Nematode Diversity in Mhadei Wildlife Sanctuary

A total of 21 species were recorded from Mhadei Wildlife Sanctuary belonging to five orders, 12 families and 13 genera (Table 7). Order Dorylaimida was the most dominant order consisting of 16 species belonging to eight families and which represents 76.19% of total Nematode species reported from Mhadei Wildlife Sanctuary. Compared to Bondla and Bhagwan Mahaveer Wildlife Sanctuaries, least number of species was reported from the Mhadei sanctuary. In terms of dominance of genus *Dorylaimus* similar trend was seen like in Bondla Wildlife Sanctuary and in Bhagwan Mahaveer Wildlife sanctuary with absolute frequency of 100%, mean density of 12.3 and relative density of 31.7% followed by *Qudsinema* with absolute frequency of 83.3%, Mean density of 4.5 and relative density of 12.03%. Genus *Sicaguttar* was the least frequent and least dominant genus with absolute frequency of 8.3%, mean density of 0.08 and relative density of 0.21%. *Dorylaimus stagnalis* (100%), *Qudsinema sp* (83%), *Xiphinema elongatum* (80.5%), *Aporcelaimium baqrii* (66.6%), *Xiphinema americanum* (66.6%), *Tylodorus sp.* (62.5%), *Alaimus indicus*, (54.16%), *Neoactinolaimus attenuates* (50%) and *Monochus truncatus* (50%) were some the most frequent free living soil nematode species reported from Mhadei Wildlife Sanctuary.

Table 7: Soil Nematode diversity in Mhadei Wildlife Sanctuary

Orders	Families	Genus	Species	MWLS
DOR YLAI MID A	Belondiridae	<i>Axonchium</i>	<i>Axonchium amplicolle</i> Cobb, 1920	+
	Dorylaimidae	<i>Dorylaimus</i>	<i>Dorylaimus stagnalis</i> Dujardin, 1845	+
			<i>Dorylaimus afganicus</i> Andrassy, 1960	+
		<i>Sicaguttar</i>	<i>Sicaguttar thornei</i>	+
			<i>Sicaguttar sp.</i>	+
	Qudsianematidae	<i>Qudsinema</i>	<i>Qudsinema sp</i>	+
		<i>.Thornedia</i>	<i>Thornedia sp</i>	+
	Aporcelaimidae	<i>Aporcelaimium</i>	<i>Aporcelaimium baqrii</i>	+
		.	<i>Aporcelaimium clamus</i>	+
			<i>Aporcelaimium sp</i> Loof and Coomans, 1970.	+
	Actinolaimidae		<i>Neoactinolaimus attenuates</i> Ahmad and Jairajpuri, 1994	+
	Longidoridae	<i>Longidorus</i>	<i>Longidorus elongatus</i> Thorne and Swanger, 1936	+
	Xiphinematidae	<i>Xiphinema</i>	<i>Xiphinema americanum</i> Cobb, 1913	+
			<i>Xiphinema elongatum</i> Stekhoven and Teunissen, 1938	+
			<i>Xiphinema orbum</i> Siddiqi, 1964	+
		<i>Xiphinema laevistriatum</i> Lamberti et Bleve Zacheo, 1979	+	

MON ONC HID A	Mononch idae	<i>Mononc hus</i>	<i>Mononchus truncatus</i>	+
TYL ENC HID A	Tylenchi dae	<i>Tylodor ous</i>	<i>Tylodorus sp.</i>	+
ALAI MID A	Alaimida e	<i>Alaimus</i>	<i>Alaimus parvus</i>	+
			<i>Alaimus indicus, Choudhary & Jairajpuri, 1983</i>	+
ARA EOL AIMI DA	Plectidae	<i>Plectus</i>	<i>Plectus cirratus</i> Bastian, 1865	+

MWLS- Mhadei Wildlife Sanctuary, + = Present/ reported

In Mhadei Wildlife Sanctuary plant parasitic nematodes were most dominant which represents about 25% followed by predators and bacterivorous 17% each, omnivore 16% and least was fungivore 8% (Fig. 22).

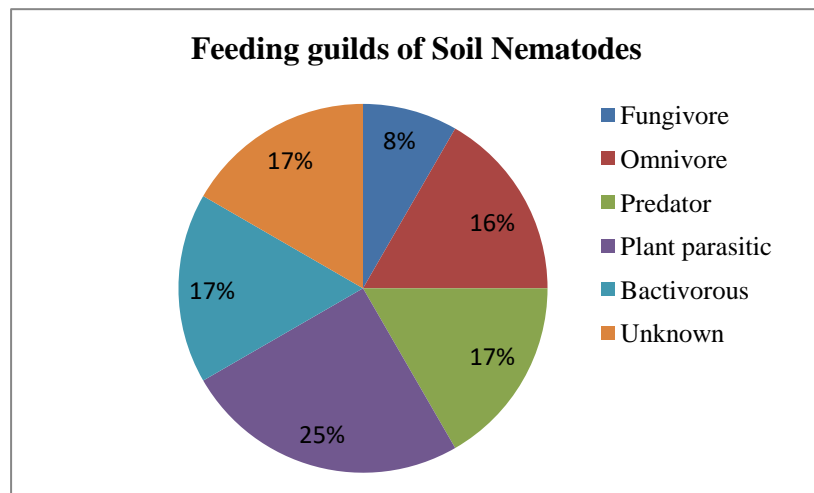


Fig. 22: Feeding guilds of soil Nematodes reported from Mhadei wildlife

Seasonal diversity of nematode species in three wildlife sanctuary

Seasonally, number of species was more in winter and post-monsoon compared to summer and monsoon (Fig. 23).

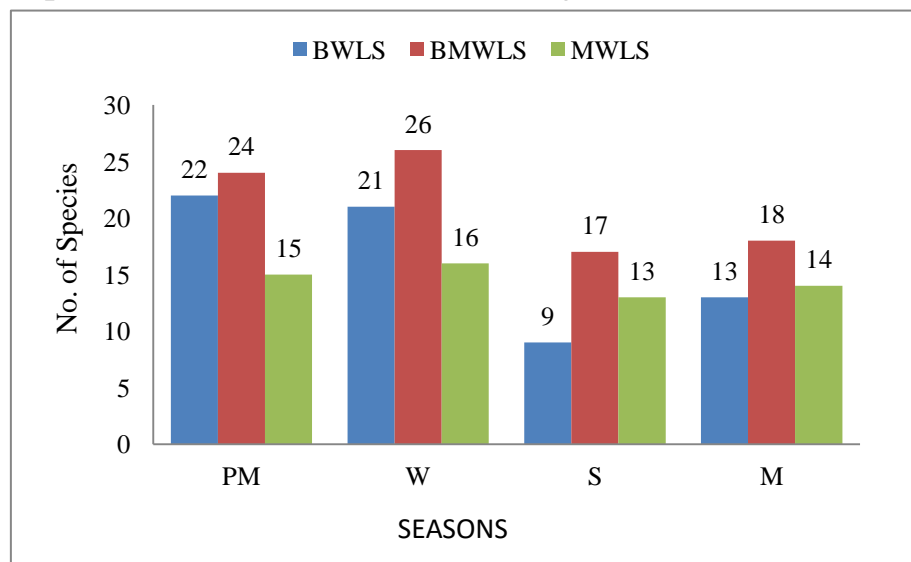


Fig. 23: Graph showing seasonality in number of species

Total of 22 species from Bondla Wildlife Sanctuary, 24 from Bhagwan Mahaveer Wildlife Sanctuary and 15 from Mhadei Wildlife Sanctuary were reported in Post Monsoon. In Winter 21 species from Bondla Wildlife Sanctuary, 26 from Bhagwan Mahaveer Wildlife Sanctuary and 16 from Mhadei Wildlife Sanctuary were reported. In Summer 09 species from Bondla Wildlife Sanctuary, 17 from Bhagwan Mahaveer Wildlife Sanctuary and 13 from Mhadei Wildlife Sanctuary and in Monsoon 13 from Bondla Wildlife Sanctuary, 18 from Bhagwan Mahaveer Wildlife Sanctuary and from Mhadei Wildlife sanctuary 14 species of free living soil nematode were reported. Species such as

Dorylaimus stagnalis with frequency of occurrence between 97-100% were reported throughout the year.

Collected data was subjected to statistical analysis to get better understanding with regards to diversity; evenness and richness were depicted in Table 8, 9 and 10.

Table 8: Species Diversity

SEASON	STUDY AREA		
	BWLS	BMWLS	MWLS
Post- monsoon	2.1662 ± 0.4677	2.3246 ± 0.168	2.09702 ± 0.1517
Winter	2.4651 ± 0.3352	2.47417 ± 0.2323	2.27763 ± 0.2384
Summer	1.2531 ± 0.6166	1.83946 ± 0.3291	1.66253 ± 0.3936
Monsoon	0.8685 ± 0.2585	1.85437 ± 0.2415	1.69731 ± 0.2062

Table 9: Species Richness

SEASON	STUDY AREA		
	BWLS	BMWLS	MWLS
Post-Monsoon	18.267 ± 2.131	20.269 ± 0.694	10.736 ± 0.018
Winter	17.428 ± 3.069	20.102 ± 2.530	11.412 ± 1.544

Summer	2.826 ± 1.705	9.329 ± 3.113	6.978 ± 2.103
Monsoon	1.656±0.485	8.401±0.963	6.332±0.956

Table 10: Species Evenness

SEASON	STUDY AREA		
	BWLS	BMWLS	MWLS
Post-Monsoon	0.728 ± 0.166	0.719 ± 0.039	1.061 ± 0.051
Winter	0.848 ± 0.069	0.856 ± 0.048	0.957 ± 0.062
Summer	0.882 ± 0.143	0.823 ± 0.143	0.896 ± 0.133
Monsoon	0.625 ± 0.297	0.770 ± 0.068	1.044 ± 0.024

Principle Component Analyses

PCA analysis of dataset of Bondla Wildlife Sanctuary (Fig. 24) resulted in PC's (eigenvalue <1) contributing a cumulative variance of 88%. PC1 contribute 70% of total variance due to strong positive loading of pH and Nitrogen /org. carbon PC2 contributed 22 % of total variance due to positive loading of pH and Nitrogen /org. Carbon. PC3 explained 7 % of total variance due to weak positive loading from pH.

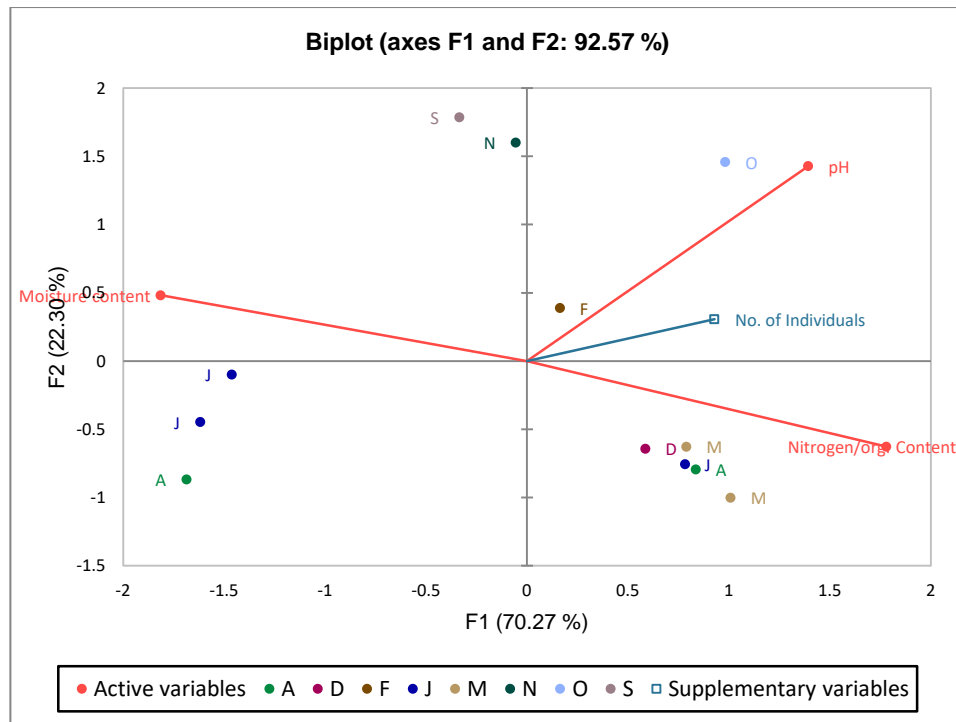


Fig.24 PCA for Bondla Wildlife Sanctuary

PCA analysis of dataset of Bhagwan Mahaveer Wildlife Sanctuary (Fig. 25) resulted in four PC's (eigenvalue >1) contributing a cumulative variance of 84%. PC1 contribute 52% of total variance with positive loading of pH and number of individuals. PC2 contributed 21 % of total variance due to positive loading of Number of Individuals, Moisture content and week negative loading from Nitrogen / org. carbon. PC3 explained 20 % of total variance due to week negative loading from moisture content. PC4 contributed 8% of total variance due to positive loading of Moisture content and pH.

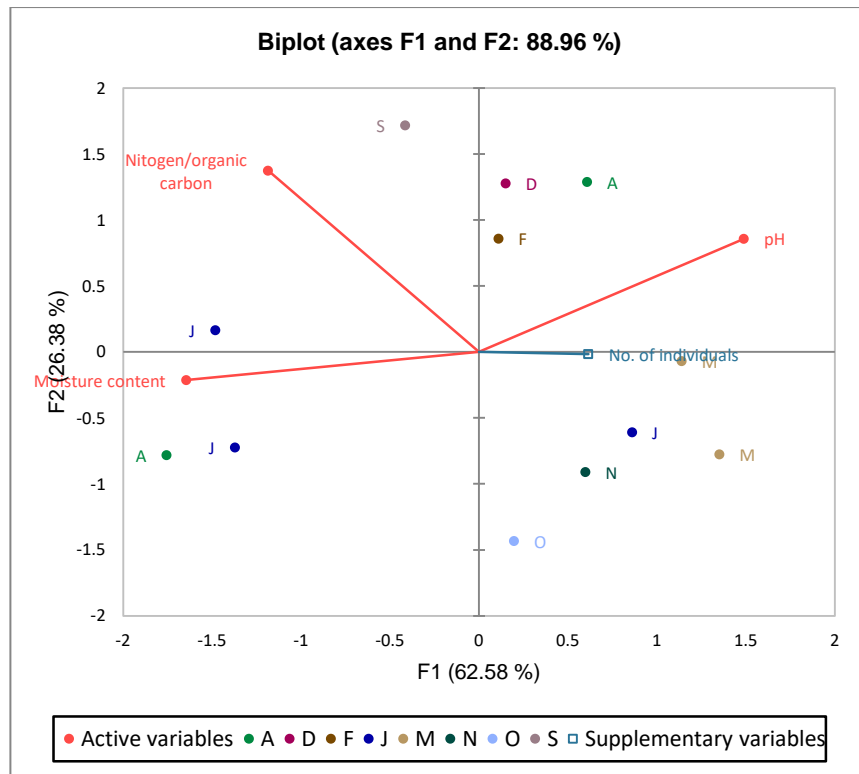


Fig.25 PCA for Bhagwan Mahaveer Wildlife Sanctuary

PCA analysis of dataset of Mhadei Wildlife Sanctuary (Fig. 26) resulted in four PC's (eigenvalue >1) contributing a cumulative variance of 83%. PC1 contribute 58% of total variance with strong positive loading of pH and negative moisture content and nitrogen/org. carbon. PC2 contributed 31 % of total variance due to weak negative loading of Moisture content and strong positive Nitrogen / org. carbon. PC3 explained 10 % of total variance with positive loading of Moisture content and pH.

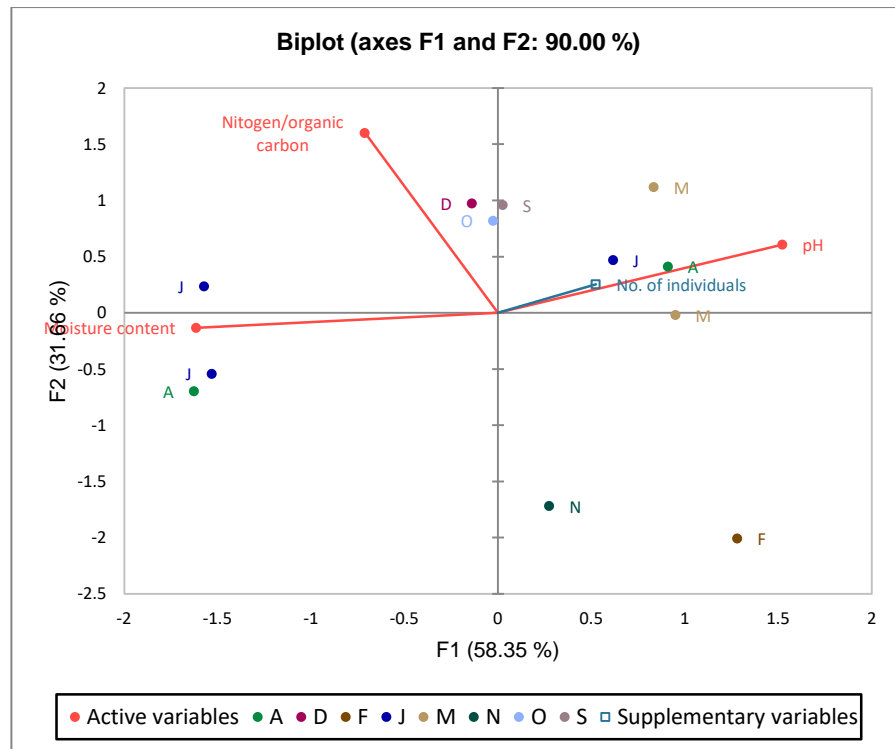


Fig.26 PCA for Mhadei Wildlife Sanctuary

Measures to preserve the nemafuna in the study area

An opportunistic survey has been conducted in the Forested Area (FA) and Social Forest Area (SFA), where Australian Acacia Plants were grown. Separate soil samples were collected for soil analyses and for nematode isolation. Free living soil nematodes were isolated using modified Cobb's (1918) 'sieving and decantation' (gravity) Method.

Soil analyses were conducted at soil testing laboratory, for which the soil samples were collected from Forested and Social Forestry Area, which is depicted in Table 11. Soil analyses result showed that, the soil is acidic in both Forested and Social Forestry area with pH of 5.2 and 5.4 respectively. High Nitrogen / Organic Carbon, low phosphorous and moderate Potassium was recorded in both FA and SFA.

Table 11: Soil analyses from Forested area and Social Forest Area

Sr No	Macro Nutrient Status	Forested area	Social Forest Area
1	Ph	5.2 ± 0.1	5.4 ± 0.17
2	Electronic Conductivity m.mhos / cm	0.095 ± 0.031	0.038 ± 0.033
3	Nitrogen / Org. Carbon%	1.97 ± 0.0	1.45 ± 0.71
4	Phosphorous Kg / Ha	2.77 ± 0.55	2.97 ± 0.86
5	Potassium Kg / Ha	185.8 ± 37.31	129.8 ± 53.42

A total of 138 and 97 individual nematodes were isolated from the soils of Forested and Social Forestry Area respectively. From the isolated nematodes a total of 11 families belonging to four orders are reported from Forested and Social Forestry Area (Table 12).

Table 12: List of families of soil nemafuna from Forested Area and Social Forest Area

Order	Family	Study sites	
		Forested	Social forestry
Dorylaimida	Dorylaimidae	+	+
	Longidoridae	+	+
	Thornematidae	+	+
	Quadsianematidae	+	-
	Nordiidae	+	-
	Aporcelaimidae	+	-
	Actinolaimidae	+	-
	Xiphinematidae	+	-
Tylenchida	Tylenchidae	+	+
Rhabditida	Diplogastridae	+	+
Aphelenchida	Aphelenchoididae	+	+

+ = Present, - = Absent

Order Dorylaimida was the most dominant order consisting of eight families (Fig 27) and represents about (85%) of total soil nemafauna reported of which, 63% was reported from Forested area and 37% were from Social Forestry Area. Second most dominant order was Order Rhabditida (10%), followed by Tylenchida (3%) and least was order Aphelenchida (2%).

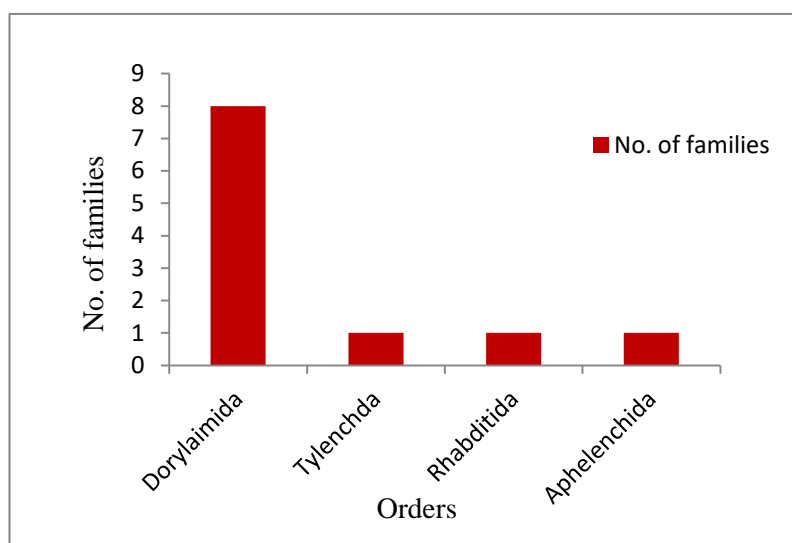


Fig. 27: Showing number of nematode families in each order

Among the families, family Dorylaimidae was the most dominant (MD=17) and with relative density of (RD= 43%) followed by Thornematidae, Longidoridae and Diplogastridae (Fig. 28). The least dominant families were Aprocelaimidae, Actinolaimidae and Xiphinematidae each with mean density of (0.17) and relative density of 0.41%.

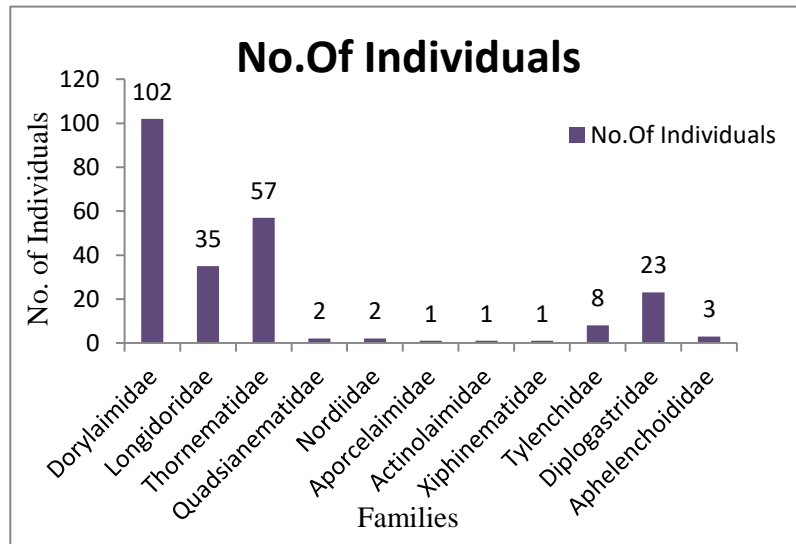


Fig. 28: Showing number of individuals in each family

Forested Area

In forested area Nema fauna was represented by 11 families belonging to four orders (Table 12). Among four orders, Order Dorylaimida was the most dominant and frequent order in all three sites of forested area (Table 13).

Table 13: Showing Site wise list of nematodes in Forested Area

Orders	Families	Forested		
		Site 1	Site 2	Site 3
Dorylaimida	Dorylaimidae	+	+	+
	Longidoridae	+	-	-
	Thornematidae	+	+	+
	Quadsianematidae	+	+	-
	Nordiidae	+	+	-
	Aporcelaimidae	+	-	-
	Actinolaimidae	+	-	-
	Xiphinematidae	+	-	-
Tylenchida	Tylenchidae	+	-	-
Rhabditida	Diplogastridae	+	-	+
Aphelenchida	Aphelenchoididae	+	-	-

+ = Present, - = Absent

Out of the 11 families, Family Dorylaimidae was most dominant (Fig. 29) with mean density of (MD=24.35) and relative density of (53%) followed by Thornematidae with (MD=10.7) and relative density of (23.19%), Longiridae (MD=4.7, RD=10.15) and the least dominant families were Aporcelaimidae, Actinolaimidae, Xiphinematidae and Aphelenchoididae each with mean density of (0.34) and relative density of (0.72%).

Site 1 is most prevalent and almost all 11 families of nematode were reported from this site. Site 2 represented by one order Dorylaimida and four families Dorylaimidae, Thornematidae, Quadsianematidae and Nordiidae. Site 3 represented by two orders and three families.

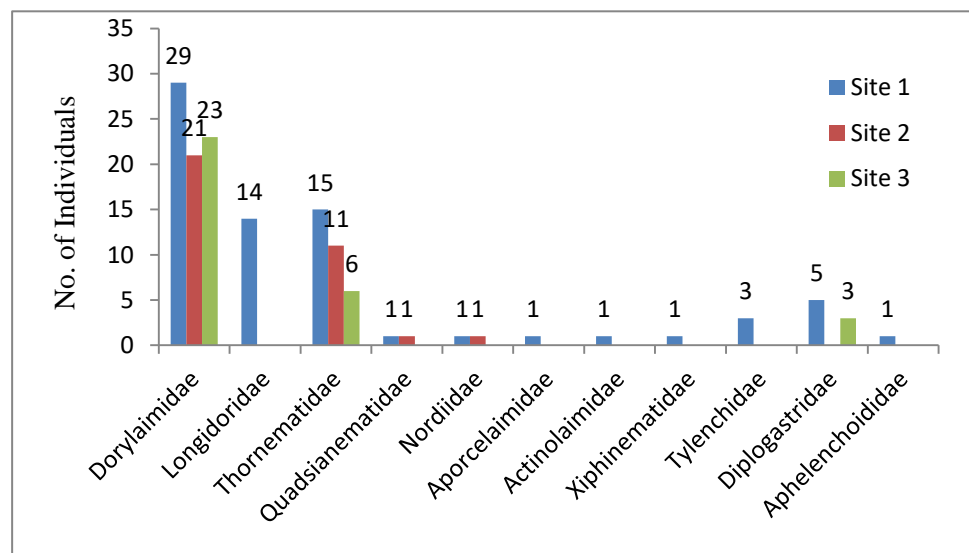


Fig. 29: Showing number of individuals in each family of nematode

Social Forestry Area

Total of six families and four orders were recorded from Social Forestry Area (Table 12). Among the six family order Dorylaimidae was

the most dominant (Fig 30) with mean density of (MD=9.66) and relative density of 29%. Most frequent families were Dorylaimidae (MD=9.66; RD=29.89%) followed by Thornematidae (MD=8.33; RD=25.77%). Most frequent families were Longidoridae, Thornematidae followed by Tylenchidae and Diplogastridae.

Site 1 which was represented by four families belonging to two orders. Site 2 and Site 3 was represented by four families i.e., Longidoridae, Thornematidae, Tylenchidae and Diplogastridae. Former two families belong to order Dorylaimidae and the later ones belong to Order Tylenchida and Rhabditida respectively (Table 14).

Table 14: Showing Site wise list of nematodes in Social Forestry Area

Orders	Families	Social Forestry Area		
		Site 1	Site 2	Site 3
Dorylaimida	Dorylaimidae	+	-	-
	Longidoridae	+	+	+
	Thornematidae	+	+	+
	Quadsianematidae	-	-	-
	Nordiidae	-	-	-
	Aporcelaimidae	-	-	-
	Actinolaimidae	-	-	-
	Xiphinematidae	-	-	-
Tylenchida	Tylenchidae	-	+	+
Rhabditida	Diplogastridae	-	+	+
Aphelenchida	Aphelenchoididae	+	-	-

+ = Present, - = Absent

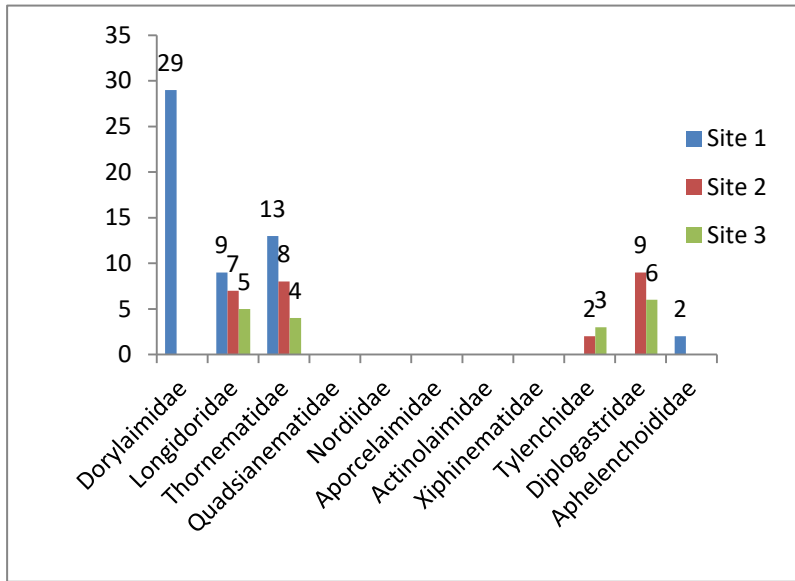
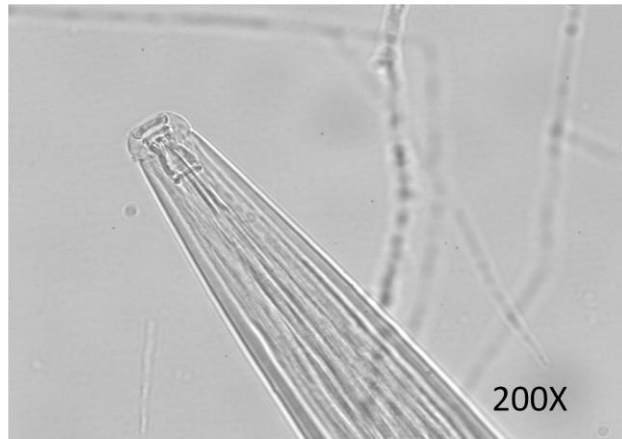


Fig. 30: Showing number of individuals in each family of nematode isolated from social forestry area

Photo plate 3: Photo images of Soil Nematodes reported from study area



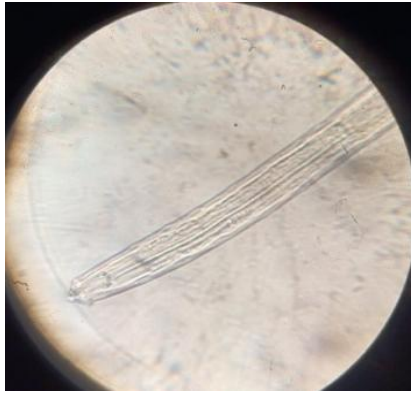
A- *Hexactinolaimus aneityi* Family: Actinolaimoidae



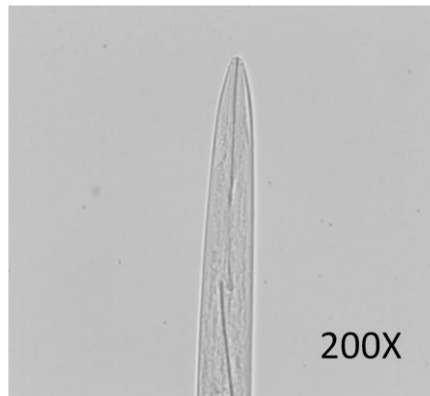
B- *Plectus cirratus* Family: Plectidae



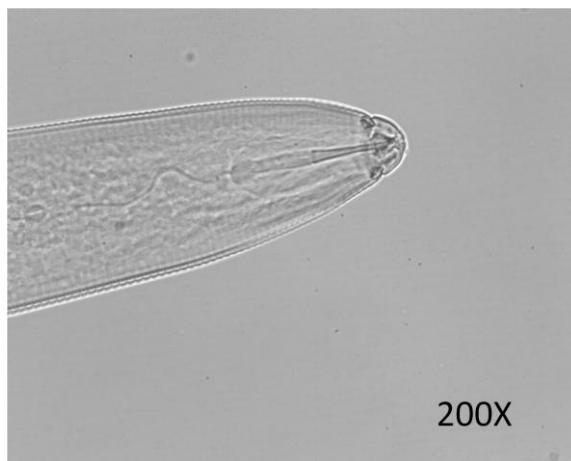
C- *Longidorus elongates* Family: Longidoridae



D- *Paralongidorus sali* Family: Longidoridae



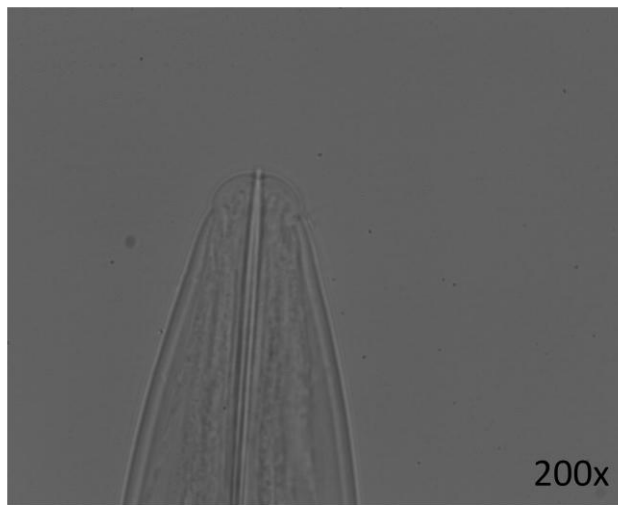
E- *Xiphinema laevistriatum* Family: Xiphinematidae



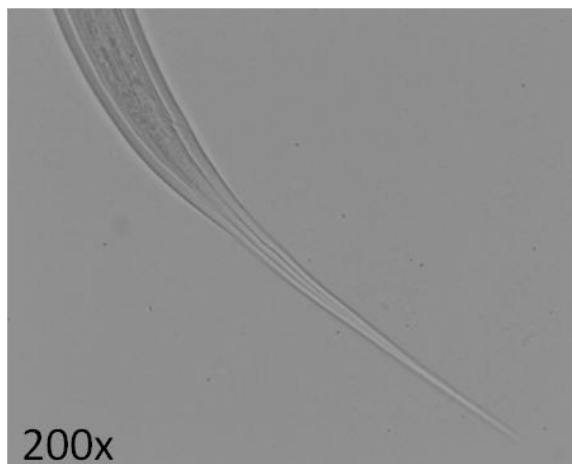
F- *Tylogonus sp.* Family: Tylenchidae



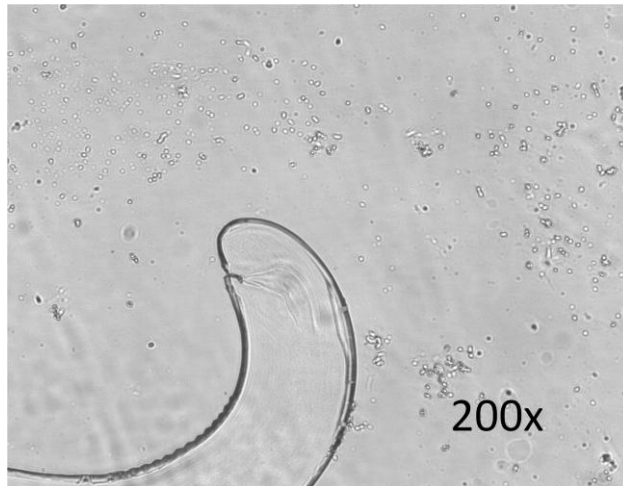
G- *Mononchus truncatus* Family: Mononchidae



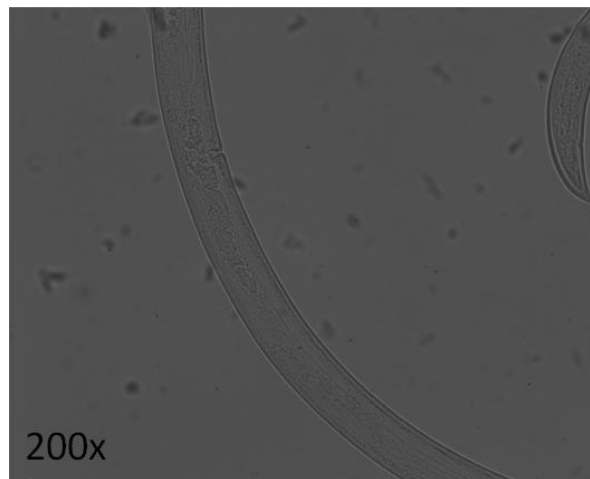
H- *Aporcelaimium baqrii* Family- Aporcelaimid



I- *Dorylaimus stagnalis* Family- Dorylaimidae



J- *Dorylaimus afganicus* Family: Dorylaimidae



K- *Xiphinema elongatum* Family: Xiphinematidae

DISCUSSION

DISCUSSION

Nematodes are seen in all possible climatic condition and habitats, which can support life (Abebe *et. al.*, 2011; Tahseen, 2012). Their distribution is linked to simple soil properties (Zhang, *et. al.*, 2012). Soil properties such as soil moisture, pH and C:N ration can explain to a great extent the distribution of soil organisms (Wall *et. al.*, 2010; Li *et al.*, 2010; Zhang *et. al.*, 2012). Soil properties like other fauna, affect soil nematodes in their diversity and distribution directly or indirectly. Underground biota is critical for biogeochemical and ecological functioning of terrestrial ecosystem (Wall *et al.*, 2010). Soil Nematode is one of the important groups of underground biota, most diverse (Maggenti, 1981; Morand *et al.*, 2006) and ecologically successful group (Ahmad, 2001).

Nematodes are basically aquatic animals, adapted to a variety of terrestrial habitats, provided there is a thin film of water around them (Tahseen, 2012). Their existences either as parasitic or free-living depend on availability of water or liquid medium. Moisture holding capacity of the soil particles is a reason that allows nematode species to adapt and survive in terrestrial mode of life (Lizanne, 2015). Moisture content of the soil from study area was found to be between 10 to 46% being highest during Monsoon i.e., from June to September (40 to 46%) and lowest during summer i.e., from March to May (10-12%). During the Post Monsoon and winter the moisture

content was between 26 to 31% and 16 to 21% respectively. In the present study moisture content between 16 to 31% was found to be optimum for nematode existence.

Nematodes are known to survive in wide range of pH (4-8) (Burns, 1971) and it affects nematode community indirectly via other components of the soil food web. In the present study, pH of the soil in study area was found to be between 5.5 and 6.6 (mildly acidic) optimum for free living soil nematode existence.

The environmental changes, arising from nitrogen (N) deposition and precipitation, influences soil ecological processes in forest ecosystems (Sun *et al.*, 2013). Sun *et al.*, (2013), studied responsive mechanisms of soil biota, to N deposition and precipitation, soil nematode communities. Their results showed that, water combined with N addition treatment decreased the total nematode abundance in the organic horizon (O); while the opposite trend was found in the mineral horizon (A). Significant reductions in the abundances of fungivores, plant-parasites and omnivores-predators were also found in the water combined with N addition treatment. The significant effect of water interacted with N on the total nematode abundance and trophic groups indicated that, the impacts of N on soil nematode communities were mediated by water availability. The synergistic effect of precipitation and N deposition

on soil nematode communities was stronger than each effect alone. Structural equation modelling suggested that, water and N additions had direct effects on soil nematode communities. The feedback of soil nematodes to water and nitrogen addition was highly sensitive and their results indicate that, minimal variations in soil properties such as, those caused by climate changes can lead to severe changes in soil nematode communities. In the present studies nitrogen / organic carbon was between 2.5 to 3.8% which was higher, as recommended by Soil Testing Laboratory. Effect of same was poorly understood on soil biota (Sun *et al.*, 2013).

Humid climate, mildly acidic pH, deciduous forest tree leaves which provide higher quality of water soluble nutrients which are useful to soil nematodes (Keith *et al.*, 2009; Zhang *et al.*, 2012), moist soil which is abundant with decaying and decomposing organic matter, can make forest soils of Goa a favourable and rich habitat to sustain enormous diversity and abundance of nematodes species (Lizanne, 2015).

FREE LIVING SOIL NEMATODE DIVERSITY

In the present study, a total of 38 free living soil nematode species, belonging to 5 orders and 17 families are reported. Order Dorylaimida was the most dominant order consisting of 10 families and 28 species of free living nematodes which represents about 73% total nematode species reported.

Dominance of order Dorylaimida was due to fewer disturbances in this region as most of the forested area of Goa, is under protection. Dorylaims were found in every conceived type of habitat and usually dominate both in numbers and in species over all other soil-inhabiting nematodes (Jairajpuri and Ahmad, 1992). Moreover, they can be easily recognized at lower magnification (Lizzane and Pai, 2014; Lizane, 2015). They are unique among the Nematodes as they exhibit most of the feeding guilds represented by nematodes and this allows them to adapt, diversify and allow them to have wide occurrence in soil ecosystem (Jairajpuri, 2002). Dorylaimids are more sensitive to disturbance (Forge and Simard, 2001), therefore played key role as biological indicators of environmental disturbances (Thomas, 1978; Sohlenius and Wasilewska, 1984). A total of 27, 36 and 21 free living soil nematode species was recorded in the Bondla Wildlife Sanctuary, Bhagwaan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary respectively.

Predatory forms are the major group of soil nematodes species reported from study site is followed by bacterivores and omnivores and least was fungivore species, which is in agreement with Lizanne (2015).

Seasonally, number of species was more in winter and post-monsoon compared to summer and monsoon. This could be because of moist soil with moisture content between 16-31%. As summer (March-May) in Goa, are hotter

and nematode species may be die due to dehydration while during monsoon (June-September) receives heavy rainfall from South-West Monsoon as a result of which they might be washed off from top soil layer.

A Shannon Weaver index reflects that, in these three wildlife sanctuaries, i.e., Bondla Wildlife Sanctuary, Bhagwan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary have a relatively high nematode diversity. The result is in agreement with Odum's prediction for forest (Odum, 1969) as the mature stage of an ecosystem with high H' Value (Baniyamuddin *et al.* 2007; Rizvi, 2008; Vaid *et al.*, 2014).

From the PCA analysis in Bondla Wildlife Sanctuary, it could be seen that, factor like Nitrogen / Org. Carbon shows concentration from December to May (Winter to Summer)

From the PAC analysis in Bhagwan Mahaveer and Mhadei Wildlife Sanctuaries it could be seen that, factors like moisture content shows concentration from June to September (Monsoon) and pH showed concentration January to March (winter to summer).

SUGGESTED MEASURES TO PRESERVE THE NEMAFUNA IN THE STUDY AREA

When soil of forested area was compared with soil of Social Forestry Area soil (*Acacia auriculiformis* plantation), it was observed that, more number of nematode families in forested soil (11) compared to that of Social Forestry Area (6). These clearly indicate the suitability of forested soil for

survival of Nema fauna. This is due to higher organic matter, moist and optimum pH in forest soils, compared to soils of Social Forestry Area.

Change in landscapes could also be a major threat to free living Soil Nematodes. One more reason could be the fact that, Bondla Wildlife Sanctuary, Mhadei Wildlife Sanctuary and Bhagwan Mahaveer Wildlife Sanctuary are part of the Western Ghats, as well are the part of protected areas as per Government of Goa, forest rules. Hence Soil Biota especially Nematode fauna is under less anthropogenic pressure.

The present observation is in corroboration with the earlier studies of Khan (2012), who reported high organic content, adequate moisture and moderate temperature of forest soils with diversified flora provide favourable condition for nematodes survival in forest habitats.

CONCLUSION

CONCLUSION

From this present study, it is concluded that, moisture content of the soil at the study area was found to be between 10-46%, being highest during Monsoon (June to September) (40-46%) and lowest during summer (March to May) (10-12%). During the Post Monsoon and winter the moisture content was between 26-31% and 16-21% respectively. In the present study, moisture content between 16-31% was found to be optimum for nematode existence.

pH of the soil in study area was found to be between 5.5-6.6 (mildly acidic), optimum for free living soil nematode existence. In the present study nitrogen / organic carbon was found between 2.5-3.8%, which was higher as recommended by Soil Testing Laboratory. Effect of same was poorly understood on soil biota (Sun *et al.*, 2013). Humid climate, mildly acidic pH, deciduous forest tree leaves provide higher quality of water soluble nutrients, which are useful to soil nematodes. Moist soil, which is abundant with decaying and decomposing organic matter, makes forest soils of Goa, a favourable and rich habitat to sustain enormous diversity and abundance of nematodes species.

In the present study a total of 38 free living soil nematode species, belonging to 5 orders and 17 families were reported. Order Dorylaimida, was the most dominant order consisting of 10 families and 28 species of free living nematodes and it represents about 73% total nematode species reported. A total

of 27, 36 and 21 free living soil nematode species was recorded in the Bondla Wildlife Sanctuary, Bhagwan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary respectively. Seasonally, number of species was more in winter and post-monsoon compared to summer and monsoon. When compared with forest soil and plantation soil (*Acacia auriculiformis* plantation), it was observed that, more number of nematode families in forested soil compared to that of plantation. This clearly indicates the suitability of forested soil which is rich in organic matter, moist with optimum pH which is best suited for nematodes as compared to soil of *Acacia* plantation.

Bondla wildlife sanctuary, Mhadei wildlife sanctuary and Bhagwan Mahaveer Wildlife Sanctuary are part of the Western Ghats and are also part of protected forest land as declared by Government of Goa. Hence, soil biota viz., free living Nematode fauna is under less anthropogenic pressure. Change in landscapes could also be one of the major threats to free living Soil Nematodes.

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INDIA

A thesis submitted to
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By

KIRAN SURESH GAUDE
Research student
Department of Zoology

Goa University

Under the supervision of

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2021 CERTIFICATE This is to certify that KIRAN SURESH GAUDE has worked on the thesis entitled "Studies of soil nematode diversity from three wildlife sanctuaries of Goa, India" under my supervision and guidance. This thesis being submitted to Goa University, Goa for the award of degree of Doctor of Philosophy in Zoology, is as original record of the work carried out by the candidate himself and has not been previously submitted for award of any other degree or diploma of this or any other university in India or abroad.

Date: Prof. I. K. Pai Place: Goa University (Research Guide)

DECLARATION I hereby declare that the thesis entitled "Studies of soil nematode diversity from three wildlife sanctuaries of Goa, India" is my original contribution and the same has not been submitted on any previous occasion for any other degree or diploma of this or any other university / Institute. The literature conceiving the problem investigated has been cited and due acknowledgement has been made wherever facilities and suggestion have been availed of.

Date: Kiran Suresh Gaude Place: Goa University (Research Scholar)

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Date: Kiran Suresh Gaude Place: Goa University (Research Scholar)

INTRODUCTION

INTRODUCTION Soil is a vital natural resource that forms thin mantle over earth's surface which may be described as multiphase, multi-component, multifunctional living systems (Lavelle, 1996; Bardgett, 2005; Kibblewhite et al, 2008). It consist of minerals materials, plant roots and their exudates, water and gases, organic matter and also inhabits many organisms viz. bacteria, fungi, protozoans, nematodes, mites, collembolans, annelids and micro-arthropods (Bongers and Ferris, 1999; Bardgett, 2005; Briones, 2014) together providing series of ecosystem goods and services to various biological processes (Lavelle, 2002; Kibblewhite et al., 2008; Wall et al., 2010). The underground biota is an important part of soil, responsible for about 30% of mineralization of carbon and nitrogen, apart from helping in availability of nutrients in soil (Gorres et al., 1998). It induces soil physical and chemical properties (Brussaard, 1997; Bongers and Ferris, 1999). It also influence nature of vegetation grows on it (Bardgett, 2005). This soil biota along with vegetation is one of five interactive factors for soil forming process (Bardgett, 2005). Underground biota is critical for biogeochemical and ecological functioning of terrestrial ecosystem (Wall et al., 2010). Soil Nematode is one of the important groups of underground biota that belongs to phylum Nematoda (Chew, 1974) in terrestrial, marine and freshwater ecosystem (Hanel, 1999; Tahseen, 2012). Even though placed at lower level of taxonomic hierarchy it is most diverse phylum after Arthropoda (Maggenti, 1981; Morand et al., 2006) and most ecologically successful group (Ahmad, 2001), occupying key position in the detritus food web (Moore and de Ruitter, 1991; Nehar, 2001; Rizvi, 2008). They are very small, worm-like animal (Yeast, 1979; Yeast and Bonger, 1999), diverse (Ettema, 1998), ubiquitous inhabitants (Bernard, 1992; Bloemers et al., 1997; Bongers and Ferris, 1999; Rizvi, 2008; Tahseen, 2012) in all soils. Yeast et al., (1993) trophically classified nematodes as herbivores, bacterivores, fungivores, omnivores and predators. Nematodes are basically aquatic animal depends on thin layer of water or liquid medium for their survival either in parasitic or free-living form (Yeast and Bongers, 1999; Tahseen, 2012). It is soil particle's ability to hold soil moisture and flexible body of nematodes allow them to bending in interstitial system of soil particles that makes nematode species to adapt for terrestrial mode of life (Tahseen, 2012; Lizzane and Pai, 2015). Nematode is most abundant metazoans (Maggenti, 1981; Abebe et. al., 2011) and like insects, they occur in all possible climatic condition and habitats that can support life (Abebe et. al., 2011; Tahseen, 2012) and their distribution is linked to soil particle size, moisture, availability of mineral nutrients and aboveground plant distribution (Matlack, 2001). Nematodes possess number of traits which includes simple anatomy, transparent body due to permeable cuticle, high species richness, abundance, pervasiveness and tolerance, close association with the soil particles and interstitial water, short generation time, have resistant stage (cysts) also they demonstrate unhydrobiosis, Osmobiosis or Cryobiosis to survive inactively during unfavourable environmental conditions, immense sensitivity to various changes in the soil ecosystem and their ability to reflect difference between disturb, undisturbed and human-impacted environment makes them imperative and inexpensive organism for biological, ecological and environmental studies as a tool to monitor changes

(Porazinska et al., 1999; Nehar, 2001; Abebe et al., 2011). Li et al., (2011) reported many species of free living nematodes, which are good indicators of heavy metal pollution in soil and in water (Nehar, 2001). They are also important in mineralization, recycling of plant nutrient and replenishing of soil nutrient in the terrestrial ecosystem and are good indicators of soil status and soil functioning (Bongers and Ferris, 1999; Porazinska et al., 1999; Ekschmill et al., 2001; Neher, 2001; Bohra and Sulthana, 2008; Rizvi, 2008; Abebe et. al., 2011; Sthanu et al., 2013). They also occupy key position in soil food web (Bernard, 1992; Bongers and Ferris, 1999). When nematodes graze on saprophytic bacteria and fungi, they give off CO₂ and NH₄ contributing to C and N mineralization (Ingham et al., 1985) and are key component to enhance soil fertility and maintain soil ecosystem health. It has been successfully used to assess soil responses in agricultural practices, forestry practices, and mining restoration (Porazinska et al., 1999; Forge and Simard, 2001; Hohberg, 2003). Unlike temperate region, the understanding of mineral soil diversity of nematodes in tropics is not quantified (Power et al., 2009). A total of around 1,000,000 species of nematode estimated globally (Hugot et al., 2001; Abebe et. al., 2011). When considering the estimated number of living species of nematode only 5.3% has been described (Morand et al., 2006) and nearly 30,028 species are known. About 2902 species of nematode are identified from India (Pande and Arora, 2014) which is 9.66% of total described species. Nematological research in India predominantly focussed on plant and animal parasitic groups. The parasitic association of nematodes with all the major crops of India has been reported in earlier literature (Khan, 2012). Little work has been done on the free living groups in forest ecosystem, as they don't have direct connection with agriculture or livestock. Extensive faunal studies in general, have been done in the Goa, but the underground biota (Nematodes) has been neglected in most of the cases, apart from a few sparse and scattered studies. As underground diversity is largely unseen; the incomprehensible diversity of soil organisms resulting taxonomic difficulties faced in identifying the soil's inhabitants (Brussaard, 1997) and due to which it is difficult to quantify (Wall et. al., 2010). Three wildlife sanctuaries under study are part of the Western Ghats contributing to its rich biodiversity. It may also incorporate wide diversity of soil nematodes as soil of these sanctuaries laden with high organic matter (Lizzane and Pai, 2015) and bestowed with a relatively rich floristic diversity (Alvares, 2002). Understanding faunal diversity of any conservation or protected area is imperative as information gathered has fundamental and applied application (Sharma, 2014). Understanding nematode diversity will help to identify plant parasitic nematodes that can affect wild plant species or seedling production in forest nurseries (Stollarova, 1999; Cram and Fraedrich, 2012). Such study will also highlight the possible effect of forest disturbance on soil nematode diversity (Bloemers et.al., 1997). The Bhagwan Mahaveer Wildlife Sanctuary, the Mhadei Wildlife Sanctuary and the Bondla Wildlife Sanctuary has been selected to study soil nematode diversity with following objectives as follows: 1) To analyse the soil samples from the study sites to assess suitability of the same for the presence of soil nematodes. 2) To conduct an opportunistic survey for free living soil inhabiting nemafuna in Bhagwan Mahaveer Wildlife Sanctuary, Mhadei Wildlife Sanctuary and Bondla Wildlife Sanctuary. 3) To prepare a check list of nemafuna in the study site. 4) To study seasonal variations if any regarding the soil nematode in the study site. 5) To propose suitable measures to preserve the nemafuna in the study area.

STUDY AREA Goa, a small state with an area of 3,702 sq. Km, is a part of west coast region. Broadly, there are three physical division of Goa. The mountainous region of the Sahyadris in the east, the middle level plateaus in the centre and the low lying river basins with coastal plains (Alvares, 2002). Sahyadris lying in Goa is a part of the Western Ghat region and is catchment area of the rain and nature has covered the area with forests largely characterised by moist deciduous species. Goa receives around 3000mm of rainfall from southwest monsoon for four months from June to September, followed by dry period of six to eight months experiencing warm and humid tropical climate. Goa has six wildlife sanctuaries Viz.,

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Bhagwan Mahaveer Wildlife Sanctuary, Cotigao Wildlife Sanctuary, Bondla Wildlife Sanctuary, Mhadei Wildlife Sanctuary, Netravali Wildlife Sanctuary and Salim Ali Bird Sanctuary

protecting all of its forested area.

In the present studies three wildlife sanctuaries of Goa Viz., Bondla wildlife sanctuary, Bhagwan Mahaveer Wildlife Sanctuary and Mhadai Wildlife Sanctuary was selected. Bondla Wildlife Sanctuary: It is located at 15°26'23.95"N & 74°06'22.37"E and is about 47 km. from Panaji, the state capital of Goa. It is a bowl shaped valley surrounded by hills (Alvares, 2002) at the junction of three talukas viz. Ponda, Sangeum and Sattari, with an area of 8 km². This sanctuary is bestowed with rich floral and faunal diversity. Forest type includes moist-deciduous forest with small patches of semi-evergreen forest. Dominant plant species are Terminalia crenulata, Xylia xylocarpa, Terminalia tomentosa and Strobilanthus sps. Commonly sighted larger mammalian species are Sambar, Chital and Wild Boar.

Image 1: Google Image of Bondla Wildlife Sanctuary Bhagwan Mahaveer Wildlife Sanctuary: It is located at 15°20'28.79" & 74°19'02.26" in Sanguem taluka that covers 240 sq. km area of which about 107 km² of core area of the sanctuary declared as Mollem National Park. Forest types includes West Coast tropical evergreen forest, west coast semi-evergreen forest and moist deciduous forest. This sanctuary is rich in flora and fauna and has dense canopy.

Image 2: Google Image of Bhagwan Mahaveer Wildlife Sanctuary Mhadai Wildlife Sanctuary: This located in the North Goa District, Sattari Taluka at 15°34'06.86"N & 74°13'46.82"E with an area of about 208.48 km². This sanctuary serve as a connecting link between the reserve forest of Sawantwadi and also the hilly ranges of this sanctuary serve as a corridor for animal movement from Karnataka into Maharashtra and vice versa (Alvares, 2002)

Image 3: Google Image of Bhagwan Mahaveer Wildlife Sanctuary

REVIEW OF LITERATURE

Nematodes are the most abundant, multi-cellular organisms and like arthropods, no other animal has impact on humans directly or through agriculture (Maggenti, 1981). Nematodes are known to humans from ancient times and found its reference in Rig, Yajur and Atharv Vedas (6000-4000 BC) (Lizanne, 2015).

Belowground soil communities are important natural resource (Chew, 1974) with immense, but largely unexplored, biodiversity (Andre et al., 1994, 2001; Wheeler et al., 2004; Nehar et al. 2005). Understanding the

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functional links to ecosystem processes (Debruyn, 1997), determining a hierarchy of geographic scale (Neher et al., 1998) and measuring their utility across ecosystem boundaries

has received immense value for soil communities as ecological indicators (Nehar et al., 2005). These soil communities interact together providing series of ecosystem goods and services to various biological processes (Lavelle, 2002; Kibblewhite et al., 2008; Wall et al., 2010).

Soil inhabiting nematodes are one the successful and important group of underground biota (Chew, 1974). Soil inhabiting nematodes have the potential to provide insights into soil processes, soil condition, biotic and functional status of soil (Bongers and Ferris, 1999; Porazinska et al., 1999; Ritz and Tradgill, 1999; Ekschmill et al., 2001; Neher, 2001; Bohra and Sulthana, 2008).

Members of phylum Nematoda are

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one of the most ancient and diverse types of animals, on

the earth's surface (Bernard, 1992; Bloemers et al., 1997; Ettema, 1998; Bongers and Ferris, 1999; Wang et al., 1999). Based on the available evidences it is very clear that nematodes have evolved to occupy almost every conceivable niche on the earth that contains, at least a thin film of water and some organic matter (Tahseen, 2012; Lizanne, 2015; Hillel, 2019). Although nematology attracted attention and recognition only in 20th century, our knowledge of a few species of nematodes of medical importance dates back to Papyrus Ebers (Circa 1500 BC). The intestinal round worm (*Ascaris lumbricoides*), filarid (*Wucheraria bancrofti*) and guinea worm or fiery serpent of Moses (*Dracunculus medinensis*) were already known to the ancient man. However, marine, freshwater, soil and plant nematodes remained little known groups mainly because of their extremely small size and the difficulties encountered in their isolation, mounting and observation. Borellus (1656) for the first time observed free-living nematodes *Turbatrix aceti* the 'vinegar eels' which was present in vinegar. Like animal parasitic nematode, plant parasitic nematodes receive less attention from ancient scientists. Needham (1743) for the first has observed plant parasitic nematode from a diseased wheat grains (*Anguina tritici*) which were later named *Vibrio tritici* by Steinbuch (1799). Muller (1783) described several species of free-living freshwater nematodes. In the middle of 19th century Nematode taxonomy further developed and landmark progress was observed based on the studies of nematodes of Iceland (Leuckart, 1849), the Mediterranean (Eberth, 1863), the English coast (Bastian, 1865), the coast of Brittany (Villot, 1875) and on nematodes collected by various expeditions (Von Linstow, 1876). Freshwater nematodes received further interest around 1890 with the papers of Daday (1897) on the Hungarian fauna.

Dujardin (1845), Bastian (1865), Schneider (1866), de Man (1884), Daday (1905) and Maupas (1900) were the pioneers of the field.

Dujardin (1845) was first to recognize relationship between free-living and plant parasitic nematodes

and his early work on the free-living nematodes included careful descriptions of *Enoplus*, *Oncholaimus*, *Rhabditis* and *Dorylaimus*. Bastian (1865) grouped the free-living nematodes into soil, freshwater and marine forms and described 100 new species of 30 genera in which 23 were new to science. de Man (1876-1927) listed eight families of free-living nematodes.

Cobb (1920) "Father of Nematology" proposed a significant change in classification and he placed nematodes under separate Phylum Nemata. In Chitwood's (1933, 1937) classification, 'Nematoda' was treated as a phylum with two classes, 'Phasmidia' and 'Aphasmidia'. Andr ssy (1984) has contributed extensively to the nematode taxonomy and provided keys to most species of terrestrial and freshwater nematodes. Siddiqi, (1986) and Nickle (1991) gave taxonomic keys for Tylenchids identification up to genus level. Jairajpuri and Ahmad (1992) gave identification keys to free-living, predacious and plant-feeding dorylaimids.

Barber (1901) first who reported plant parasitic nematode from India *Heterodera dicicola* (the then name of *Meloidogyne*) infesting tea in South India. Milne (1919) reported seed gall nematode (*Anquira tritici*) of wheat. Ayyar (1926, 1933) recorded root knot nematode on vegetables and other crops in south India. Dastur (1936) reported white tip disease of rice. 'Molya' disease of wheat and Barley which was caused by nematode species, *Heterodera avenae*, was reported by Vasudeva in 1958. Organized research on plant nematodes started only after the end of 1950. In India, 1960s could be regarded as the most active phase for the growth of nematode taxonomy because of the outstanding contributions of Siddiqi (1959-61), Prasad et al., (1959), Jones (1961), Jairajpuri and Ahmad (1992) and several other young taxonomists. Their work greatly helped in establishing and developmental work in Nematology in India.

The parasitic association of nematodes with all the major crops of India has been reported in earlier literature. Nematodes are known to cause 12.3% of annual yield loss in world's major crop and in developing countries is about 14% (Sasser and Freckman, 1987). Agriculture is an important sector and plays a vital role in the Indian economy as it contributes about 17% to the total GDP and provides employment to over 60% of the population (Jain et al., 2007). Jain et al., (2007) estimated total losses caused due to plant parasitic nematodes on different crops. Hence, nematological research in India predominantly focused on plant and animal parasitic groups. Little work has been done on the free-living group in forested (Vaid et al., 2014) or in social forestry area (Australian *Acacia* plantation), probably because they have little direct concern with agriculture and livestock. A total of about 1,000,000 species of nematode estimated globally (Hugot et al., 2001), of which around 30,028 species are known. About 2902 species of nematode are identified from India (Anonymous, 2014), which accounts for 9.66% of total described species.

Forests are precious natural resources and play a vital role in the survival of every life on the planet earth (Khan, 2012). Pradhan and Dash (1987) reported 17 species of nematodes in tropical deciduous forest of Sambalpur with nematode density ranging from $15.1 \times 10^4/m^2$ (May) to $66.1 \times 10^4/m^2$ (November). They also found out that, of the total nematode 88.4% were in top 10cm during the peak period of density. Baqri, (1999) reported a total of 191 species belonging to 102 genera, 45 families and 10 orders from West Bengal. Bohra and Baqri (2005) reported 23 species of plant and soil nematode belonging to 21 genera of 12 families under four Orders viz. Tylenchida, Aphelenchida, Dorylaimida and Mononchida and their result indicates great diversity of plant soil nematode in Ranthambhore National Park. Khan et al., 2005 reported two new monohysterid species (nematoda) from Keoladeo national park, Rajasthan, India. Baniyamuddin et al. (2007) reported 85 genera of nematodes from the natural forest of Arunachal Pradesh, where they found out that, it is dominated by taxonomic group Dorylaimida (54%), followed by Rhabditida (7%), Alaimida (5%), Araeolaimida (4%), Enoplida (2%), Aphelenchida and Monohysterida (1% each). Rizvi (2008), studied community analyses of soil inhabiting nematodes from tropical Siwalik Sal forest soil in Dehradun (Uttarakhand), India. In his study, randomly selected composite samples yielded 59 nematode genera belonging to 11 orders dominated by order Dorylaimida (31%) followed by order Rhabditida (22%). Mohilal et al., (2009) studied plant and soil nematodes from Lokchao Yangoupokpi Wildlife Sanctuary, Manipur, India. Their study showed rich nematode diversity and reported about 25 genera of nematodes from Lokchao Yangoupokpi Wildlife Sanctuary. Nusrat et al., (2013) reported, four known and one new species of Mononchida (Nematoda) from Silent Valley National Park, India. Sharma (2014) reported 18 species of dorylaimids from Govind Wildlife Sanctuary. Vaid et al., (2014) reported 43 genera

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of soil inhabiting nematodes in Dera Ki Gali forest of Poonch district in Jammu and Kashmir.

In their study, they reported

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Rhabditida, representing the highest percentage (34%), followed by Dorylaimida (20%) and Mononchida (15%). In terms of abundance, Rhabditida was the most dominant group (51%), followed by Dorylaimida (25%), Mononchida (9%), Monhysterida and Enoplida (2%). In terms of trophic groupings, the bacterivore genera representing the highest percentage (48%), followed by predators (20%), omnivores (18%) and plant parasites (11%).

Sharma and Dubey (2015), for the first time, reported a total of 26 species of terrestrial nematodes from Rajaji National Park (RNP), Uttarakhand, India, of which 15 were belonging to order Dorylaimida and 11 were belonging to order Mononchida. Of these, *Granonchulus subdecurrans* Coetzee, 1966, was recorded from first time from India. In South Goa district 52 species of nematode are reported which is about 0.01% of total species in India (Lizanne and Pai, 2014). Pai and Gaur (2010) for the first time reported occurrence of economically important spiral nematode (*Helicotylenchus multicinctus* Cobb.) from Goa. Lizanne (2015) reported 69 species of free living nematodes of which 21 were from forested areas of Goa. Gaude and Pai (2018), reported 18 genera of nematodes from Bhagwan Mahaveer Wildlife Sanctuary.

Soil inhabiting free-living nematode communities and their structural changes are best known biological tools for assessing soil disturbances, including heavy-metal pollution (Bongers et al., 2001; Georgieva et al., 2002; Bohra and Sulthana, 2008), agricultural and extensive grazing activities (Yeates and Bongers, 1999; Kandji et al., 2001; Mills and Adl, 2006) in the terrestrial ecosystems (Gupta and Yeates, 1997; Neher, 1999). Due to their immense sensitivity to various changes in the soil ecosystem and their ability to reflect differences among disturbed, undisturbed and human-impacted environments, the free-living nematodes are considered to be quite useful and inexpensive organisms for ecological research (Porazinska et al., 1999; Abebe et al., 2006).

Nematode infestation in forested area has been neglected area of research (Khan, 2012). The major reasons could be non appearance of visible symptoms and difficulties in accessing the sampling site for damage assessment. Forested soil due to diversified flora and has high organic content, adequate moisture and moderate temperature are the conducive conditions for nematode survival in forested habitat. Few studies have been conducted on forested tree species such as Acacia, Sal, Teak and Sandalwood (Khan, 2012). Nematodes have wide host range and attack crop plant and also numerous angiosperms and gymnosperm trees. Hence it is imperative to understand nematode diversity in forested area.

MATERIALS AND METHODS

MATERIALS AND METHODS

TOOLS FOR SAMPLING

Sampling tools includes a hand trowel, knives for cutting roots, scissors, polythene sample bags, tags, marker pens for labeling the sample bag and a pencil and notebook for recording information.

TAKING SOIL SAMPLES AND NUMBER OF SAMPLES

The soil, which is very wet or too dry, was avoided and slightly moist soil was collected for soil analysis and for nematode extraction. Enough number of samples was taken to ensure they are representative of the situation in the study area. For more accurate assessment, number of sub-samples were combined for each field sample. For soil nematodes extraction from an area of 0.5 to 1 hectare, collected a minimum of 20 sub-samples. These sub-samples were combined to make one composite sample to represent the field area sampled. Bulking of samples in this way helps to preserve nematodes by maintaining the temperature and moisture of samples.

SAMPLING PATTERN

Nematodes are rarely distributed evenly in a field, and samples should therefore be collected from several areas, within the field, hence random sampling method was followed.

CARE OF SAMPLES

Soil samples were collected in strong plastic bags, and were labelled systematically directly on the plastic bag with a permanent marker pen a sample number or reference. After collection, care was taken to keep soil samples in cool and dry condition, if soil samples were not processed immediately by storing them in a refrigerator (approx. 10°C) up to two weeks.

FOR SOIL ANALYSIS

Opportunistic field trips were made in Bondla Wildlife Sanctuary, Bhagwan Mahaveer Wildlife Sanctuary and Mhadai Wildlife Sanctuary. Soil samples were collected to analyze soil for its suitability for the existence of soil Nematodes (1 Kg). Collected soil samples were air dried for 15 to 20 days. Dried soil was then sieved using coarse sieve to remove unwanted dry plant parts, stones and pebbles etc. Further soil analysis was carried out in Soil Testing Laboratory, Krishi Vigyan Kendra, Margao, Goa.

SOIL PROCESSING AND NEMATODE EXTRACTION

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Samples were processed by modified Cobb's (1918) sieving and decantation (

gravity) method. This method takes the advantage of the difference in size and specific gravity between nematodes and soil components. Soil sample about 1000g was placed in a bucket and added 5 liter of water. Soil was mix thoroughly, until all clods and peats are broken up and to separate the nematodes from the soil particles and to suspend them in the water. Bucket was kept idle for 30 seconds to one minute, so as to heavy soil particles sink to the bottom of the bucket and the nematodes remain suspended in the water. Later water was poured through No.20 mesh sieve into the second bucket leaving the heavy soil particles in the first bucket (No. 1). Nematodes will pass easily through the coarse sieve. Most of the nematodes will now be in water of the second bucket. Wash the residue on the coarse sieve with more water and it flow into the second bucket. The purpose is to make sure that no nematodes remain in the residue on the coarse sieve. Residue on the coarse sieve as well as from the first bucket was discarded. Water from the second bucket gently pour through the fine sieve of 200 to 250 mesh BSS (British Standard Specification) and allow the water to run down the drain. If this is carefully done, most of the nematodes will be caught on the fine sieve. Fine sieve was washed with a gentle stream of water to remove fine soil particles. Residue on the sieve was collected in a beaker and the nematodes were then isolated using modified Baermann's funnel technique (Baermann, 1917). The Baermann funnel is a regular glass or plastic funnel, about 7.5 to 15cm in diameter, with a piece of rubber tubing attached to the stem and closed with a clamp or a pinchcock. A molded wire gauge is placed in the funnel. The funnel is filled with fresh tap water. Care was taken to ensure that there are no air bubbles in the funnel or in the rubber tubing as the nematodes can be caught in the air bubble and die. The wire gauge was lined with tissue paper in the form of a cross (+). The edge of the tissue paper should not protrude out of the funnel otherwise water will flow out. The residue that was collected in the beaker was poured over the tissue paper. The entire set-up was kept undisturbed for a day or two at room temperature. Freshwater was added to the funnel to compensate for loss by evaporation. The nematodes being active migrate to the bottom through the tissue paper and get accumulated in the stem of the funnel and in the rubber tubing at the bottom.

At the end of the waiting period

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after 48 hours, a small amount of water suspension was drawn into a cavity block through the rubber tubing. The nematodes thus isolated were collected for counting, fixing and processed for making permanent slides.

KILLING AND FIXATION

OF NEMATODES

Collected nematodes were killed and fixed simultaneously by pouring hot fixative in a cavity block where the fixative was prepared with 90ml distilled water, 8ml formalin and 2ml glycerol and heated for 60°C (Seinhorst, 1959) and was kept for

24 hours.

MOUNTING, SEALING AND PREPARATION OF PERMANENT SLIDES

the nematodes were transferred to a dehydrating agent of glycerine-alcohol which was prepared with 79 parts distilled water, 20 parts 96% ethanol and 1 part glycerol. This

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<https://core.ac.uk/download/pdf/144528636.pdf>

block was then kept in a desiccator containing anhydrous calcium chloride. After 4 to 5 weeks, the nematodes were dehydrated and ready for mounting. A tiny drop of anhydrous glycerin was placed

in the centre of a clean glass slide (1mm thickness) and one by one the dehydrated nematodes were transferred into this drop using a horse hair mounted on a handle. Care was taken to make sure that the nematodes were gently pressed to the bottom of the glycerine drop as there is possibility that they can move into the molten wax, if they are on the surface of the glycerine drop. Small pieces (4) of wax were kept on four sides of

75%

MATCHING BLOCK 9/15

W

<https://core.ac.uk/download/pdf/144528636.pdf>

the drop and a cover slip (0.1mm thickness) was gently placed on the pieces. This slide was then heated on a hot plate (60°C) till the wax melted.

As the wax melted, it sealed the drop of glycerin with the nematodes in the centre. The permanent slides were then used for further study.

TECHNIQUE TO HANDLE A LIVE NEMATODE

Using a compound binocular research microscope; select the nematode of your choice from the cavity block containing the nematode suspension (step 3, as mentioned earlier). Loosen the chosen nematode from the bottom of the cavity block with a short twitch of the horse hair mounted on a handle and gently lead it to the surface of the solution, until it is more or less horizontal, whilst simultaneously changing the focus of the microscope, with the fine tuning knob, to make the nematode visible, as it is led to the surface of the solution. Then tactfully position the tip of the horse hair for the final twitch. The nematode curls round the horse hair, if the tip of the hair is positioned just below them and at right angle to their body axis. Gently lift the handle with the nematode curled round the horse hair and place where needed. The viscosity and the surface tension of the liquid make the nematode to stick to the horse hair instead of pulling it down to the bottom of the cavity. Mastering this technique is important for any one working with the nematodes, as it is easy to identify them, when they are alive, than when they are killed, since there is a possibility that, some parts of the nematode may be damaged, whilst killing and fixing them. IDENTIFICATION OF NEMATODES After the preparation of permanent slide the nematodes were identified and classified according to the following literature (Goodey, 1951; 1963; Bajaj and Jairajpuri, 1979; Andr assy, 1982 (a & b); Jairajpuri and Khan, 1982; Andr assy, 1984, Siddiqi, 1986; Jairajpuri and Ahmad, 1992; Ahmad, 1996; Mai et al., 1996; Rawat and Ahmad, 2000; Siddiqi, 2000; Esquivel, 2003; Choudhary et al., 2010; and Rizvi, 2010b) Information was also collected from the websites like NEMAPLEX (http://nemaplex.ucdavis.edu/Uppermnus/nematamnu.htm#Taxonomic_Keys), Nema Species Masterlist A-Z.

RESULTS

RESULTS Soil samples were analysed from the study sites to assess soil suitability for the presence of soil nematodes. Soil Samples analysis for Moisture Content:

Moisture content of the soil from study area was found to be between 10-46% being highest during Monsoon i.e., from June to September (40-46%) and lowest during summer i.e., from March to May (10-12%). During the Post Monsoon and winter the moisture content was between 26-31% and 16-21% respectively.

2015-16 O N D J F M A M J J A S 26.37 24.95 24.67 11.138999999999999 19.45 17.38 11.15 13.350000000000001 42.21 43.64 42.21 38.51 2016-17 O N D J F M A M J J A S 26.37 25.67 23.459999999999997 19.3 19.45 17.579999999999995 13.2 13.350000000000001 46.3 42.15 42.21 35.57 2017-18 O N D J F M A M J J A S 27.87 24.95 24.67 11.138999999999999 0 17.38 11.129999999999999 13.67 43.1 43.64 42.21 38.51

PM 1.0040916292848916 0.49497474683024345 2.0647518010646877 1.0040916292848916 0.49497474683024345
2.0647518010646877 2015-16 2016-17 2017-18 25.66 26.020000000000003 26.41 W 6.8240882419069937
2.3596680557512992 9.5678618562351776 6.8240882419069937 2.3596680557512992 9.5678618562351776 2015-16
2016-17 2017-18 18.419666666666668 20.736666666666668 17.904499999999995 S 3.159477804954467
2.4866242176895232 3.1431990073808596 3.159477804954467 2.4866242176895232 3.1431990073808596 2015-16
2016-17 2017-18 13.96 14.71 14.06 M 2.1944380449977809 4.4391543864419614 2.313071550990168
2.1944380449977809 4.4391543864419614 2.313071550990168 2015-16 2016-17 2017-18 41.642500000000005
41.557499999999997 41.865000000000002

Moisture content of the soil samples from Bondla wildlife sanctuary was found to be between 11-47% (Fig. 1.). Highest being recorded in Monsoon (41-42%) and Post Monsoon (25-27%) and lowest being recorded in summer (13-15%) (Fig. 2).

Fig. 1: Graph showing Moisture content in Bondla Wildlife Sanctuary Fig. 2: Graph showing Moisture content in Bondla Wildlife Sanctuary through Seasons Moisture content of the soil samples from Bhagwan Mahaveer wildlife sanctuary follow the same trend as in Bondla wildlife sanctuary and was found to be between 10- 46% (Fig. 3.). Highest being recorded in monsoon (44-46%) and post monsoon (29-31%) and lowest being recorded in summer (10-12%) (Fig.4). During winter, moisture content was between 19-21%.

2015-16 O N D J F M A M J J A S 31.75 28.79 27.89 17.059999999999999 16.41 12.739999999999998 10.96 10.61
45.548000000000002 51.13 42.730000000000004 41.3 2016-17 O N D J F M A M J J A S 30.279999999999998 29.05
26.8 17 16 12.350000000000001 10.06 10.55 45.379999999999995 51 41.849999999999994 41.2 2017-18 O N D J F M
A M J J A S 31.25 27.45 27.35 16.95 16.41 12.65 10 10.43 45.5 51.13 42.63 41.230000000000004

2015-16 PM W S M 30.27 20.453333333333322 11.436666666666669 45.177 2016-17 PM W S M 29.664999999999999
19.933333333333323 10.986666666666668 44.857499999999995 2017-18 PM W S M 29.35 20.236666666666665
11.026666666666667 45.122500000000009 PM 2.0930360723122137 0.86974134085963373 2.6870057685088438
2.0930360723122137 0.86974134085963373 2.6870057685088438 2015-16 2016-17 2017-18 30.27
29.664999999999999 29.35 W 6.5280395219391973 6.4070924242852421 6.033517492585351 6.5280395219391973
6.4070924242852421 6.033517492585351 2015-16 2016-17 2017-18 20.453333333333322 19.933333333333323
20.236666666666665 S 1.1422054689649006 1.2058330453812178 1.4221931420638148 1.1422054689649006
1.2058330453812178 1.4221931420638148 2015-16 2016-17 2017-18 11.436666666666669 10.986666666666668
11.026666666666667 M 4.3433776411146505 4.4879718879095929 4.3816539875562066 4.3433776411146505
4.4879718879095929 4.3816539875562066 2015-16 2016-17 2017-18 45.177 44.857499999999995
45.122500000000009

Fig. 3: Graph showing Moisture content in Bhagwan Mahaveer Wildlife Sanctuary Fig. 4: Graph showing Moisture content in Bhagwan Mahaveer Wildlife Sanctuary through seasons

In Mhadei wildlife sanctuary moisture content of soil samples was between 10 to 46% (Fig. 5.). Highest being recorded in Monsoon (42-43%) followed by in Post Monsoon (30-31%), in winter (20-21%) and lowest being recorded in summer (10-12%) (Fig. 6).

2015-16 PM W S M 30.310000000000006 20.426666666666666 11.506666666666668 42.354999999999997 2016-17
PM W S M 30.25 20.32 11.933333333333332 42.292500000000018 2017-18 PM W S M 30.279999999999998
20.216666666666665 11.436666666666669 42.147500000000001 PM 0.82024386617628831 0.77781745930521395
0.96166522241370733 0.82024386617628831 0.77781745930521395 0.96166522241370733 2015-16 2016-17 2017-18
30.310000000000006 30.25 30.279999999999998 W 6.4663771412850215 6.5420256801697105 6.4636702679927405
6.4663771412850215 6.5420256801697105 6.4636702679927405 2015-16 2016-17 2017-18 20.426666666666666 20.32
20.216666666666665 S 1.1094292827095156 1.4011899704655819 1.0943643512712431 1.1094292827095156
1.4011899704655819 1.0943643512712431 2015-16 2016-17 2017-18 11.506666666666668 11.933333333333332
11.436666666666669 M 2.1596064456284996 3.019750265612434 2.5269398489081198 2.1596064456284996
3.019750265612434 2.5269398489081198 2015-16 2016-17 2017-18 42.354999999999997 42.292500000000018
42.147500000000001

2015-16 O N D J F M A M J J A S 30.89 29.73 27.89 16.89 16.5 12.739999999999998 11.19 10.59 44.120000000000005
43.339999999999996 42.730000000000004 39.230000000000011 2016-17 O N D J F M A M J J A S 30.8 29.7 27.87
16.760000000000002 16.329999999999995 13.3 12 10.5 45 43.5 42.67 38 2017-18 O N D J F M A M J J A S
30.959999999999997 29.6 27.68 16.54 16.43 12.629999999999999 11.2 10.48 44.120000000000005 43.43 42.56
38.480000000000004

Fig. 6: Graph showing Moisture content in Mhadei Wildlife Sanctuary through seasons Fig. 5: Graph showing Moisture content in Mhadei Wildlife Sanctuary

Soil samples analysis for pH: In the present study, pH of the soil in study area was found to be between 5.5-6.6 (mildly acidic). In Bondla wildlife sanctuary pH was in the range of 5.2 to 6.8 (Fig. 7). In Bhagwan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary it was in the range of 5.2 to 6 respectively (Fig. 8 & 9). An insignificant seasonal change with regards to pH throughout the study period was observed (Fig. 10, 11 and 12)

. 2015-16 O N D J F M A M J J A S 5.6499999999999995 5.7 5.9 5.7 5.6 5.75 5.8 5.8 5.5 5.5 5.3 5.9 2016-17 O N D J F M A M J J A S 5.7 5.85 5.8 5.8 5.75 5.9 5.85 5.85 5.5 5.5 5.3 5.9 2017-18 O N D J F M A M J J A S 5.6 5.8 5.9 5.8 5.75 5.9 5.8 5.8 5.5 5.5 5.3 5.9 2015-16 O N D J F M A M J J A S 5.6499999999999995 5.7 5.9 5.7 5.6 5.75 5.8 5.8 5.5 5.5 5.3 5.9 2016-17 O N D J F M A M J J A S 5.7 5.85 5.8 5.8 5.75 5.9 5.85 5.85 5.5 5.5 5.3 5.9 2017-18 O N D J F M A M J J A S 5.6 5.8 5.9 5.8 5.75 5.9 5.8 5.8 5.5 5.5 5.3 5.9

2015-16 O N D J F M A M J J A S 6.8 6.3 5.8 5.8 5.9 5.9 5.8 5.8 5.4 5.5 5.3 6.3 2016-17 O N D J F M A M J J A S 5.8 5.7 5.8 5.8 5.9 5.7 5.8 5.8 5.4 5.5 5.3 5.7 2017-18 O N D J F M A M J J A S 6.3 6.3 5.8 5.8 5.9 5.9 5.8 5.8 5.4 5.5 5.3 6.3

Fig. 8: Graph showing pH in Bhagwan Mahaveer Wildlife Sanctuary Fig. 7: Graph showing pH in Bondla Wildlife Sanctuary

PM 0.47324236215001031 0.47324236215001031 Bondla Mollem Madhei 6.55 5.6750000000000007 5.8000000000000007 W 5.091750772173137E-2 5.091750772173137E-2 Bondla Mollem Madhei 5.8333333333333597 5.7333333333333725 5.7666666666666684 S Bondla Mollem Madhei 5.8333333333333597 5.7833333333333661 5.8333333333333597 M 5.204164998665306E-2 5.204164998665306E-2 Bondla Mollem Madhei 5.6249999999999689 5.5500000000000007 5.5249999999999755

2015-16 O N D J F M A M J J A S 5.9 5.7 5.8 5.8 5.7 5.9 5.8 5.8 5.4 5.5 5.3 5.9 2016-17 O N D J F M A M J J A S 5.6 5.6499999999999995 5.7 5.75 5.9 5.75 5.8 5.7 5.4 5.3 5.4 6 2017-18 O N D J F M A M J J A S 5.9 5.7 5.7 5.8 5.8 5.9 5.8 5.8 5.4 5.5 5.3 5.9

Fig. 10: Graph showing pH in three wildlife sanctuary through seasons (2015-2016) Fig. 9: Graph showing pH in Madhai Wildlife Sanctuary

PM 8.0363756341703979E-2 8.0363756341703979E-2 Bondla Mollem Madhai 5.75 5.7750000000000004 5.6249999999999689 W 2.886751345948068E-2 2.886751345948068E-2 Bondla Mollem Madhai 5.8333333333333597 5.7833333333333661 5.7833333333333661 S 6.3098981620003533E-2 6.3098981620003533E-2 Bondla Mollem Madhai 5.7666666666666684 5.8666666666666671 5.75 M 3.8188130791299207E-2 3.8188130791299207E-2 Bondla Mollem Madhai 5.4749999999999996 5.5500000000000007 5.5249999999999755

PM 0.32145502536642001 0.32145502536642001 Bondla Mollem Madhei 6.3 5.6999999999999975 5.8000000000000007 W 3.469443332443542E-2 3.469443332443542E-2 Bondla Mollem Madhei 5.8333333333333597 5.8166666666666664 5.7666666666666684 S Bondla Mollem Madhei 5.8333333333333597 5.8333333333333597 5.8333333333333597 M 5.204164998665306E-2 5.204164998665306E-2 Bondla Mollem Madhei 5.6249999999999689 5.5500000000000007 5.5249999999999755

Fig. 11: Graph showing pH in three wildlife sanctuary through seasons (2016-2017) Fig. 12: Graph showing pH in three wildlife sanctuary through seasons (2017-2018)

Soil Samples analysis for Nitrogen / Organic carbon: In the present study nitrogen / organic carbon was between 2.5 to 3.8% (Fig. 13,14, and 15). Seasonal changes in nitrogen / organic carbon content in soil samples from three wildlife sanctuary is given in figure 16, 17 and 18.

Bondla O N D J F M A M J J A S MONTHS 3.72 2.94 3.8099999999999987 3.64 3.16 3.73 3.62 3.8299999999999987 2.9 2.9099999999999997 2.8899999999999997 3.1 Mollem O N D J F M A M J J A S MONTHS 2.68 2.7 3.5 2.8 3.51 2.8899999999999997 3.5 2.63 3.63 3.2800000000000002 3.5 3.7 Madhai O N D J F M A M J J A S MONTHS 3.72 3.8899999999999997 3.8099999999999987 3.63 3.17 3.73 3.6 3.8 3.63 3.7800000000000002 3.62 3.6

Bondla O N D J F M A M J J A S MONTHS 3.72 2.94 3.8099999999999987 3.64 3.16 3.73 3.62 3.8299999999999987 2.9 2.9099999999999997 2.8899999999999997 3.1 Mollem O N D J F M A M J J A S MONTHS 2.68 2.7 3.5 2.8 3.51

2.8899999999999997 3.5 2.63 3.63 3.2800000000000002 3.5 3.7 Madhai O N D J F M A M J J A S MONTHS 3.72 2.94
3.80999999999999987 3.64 3.16 3.73 3.62 3.82999999999999987 3.63 3.7800000000000002 3.62 3.7

Fig. 14: Graph showing pH in three wildlife sanctuary (2016-2017) Fig. 13: Graph showing pH in three wildlife sanctuary (2015-2016)

Bondla O N D J F M A M J J A S MONTHS 3.72 2.94 3.80999999999999987 3.64 3.16 3.73 3.62 3.82999999999999987 2.9
2.90999999999999997 2.8899999999999997 3.1 Mollem O N D J F M A M J J A S MONTHS 2.68 2.7 3.5 2.8 3.51
2.8899999999999997 3.5 2.63 3.63 3.2800000000000002 3.5 3.7 Madhei O N D J F M A M J J A S MONTHS 3.7 3 3.67
3.64 3.16 3.73 3.62 3 3.6 3.7 3.6 3.8

PM 0.36950417228135862 0.36950417228135862 Bondla Mollem Madhai 3.32999999999999987 2.6900000000000004
3.32999999999999987 W 0.15396007178389776 0.15396007178389776 Bondla Mollem Madhai 3.5366666666666577
3.2699999999999996 3.5366666666666577 S 0.415692193816529 0.415692193816529 Bondla Mollem Madhai
3.7266666666666666 3.0066666666666664 3.7266666666666666 M 0.38602407092477525 0.38602407092477525
Bondla Mollem Madhai 2.9499999999999997 3.5274999999999999 3.6825000000000001

Fig. 16: Graph showing pH in three wildlife sanctuary through seasons (2015-2016) Fig. 15: Graph showing pH in three wildlife sanctuary (2017-2018)

PM 0.55953105365118505 0.55953105365118505 Bondla Mollem Madhai 3.32999999999999987 2.6900000000000004
3.8049999999999997 W 0.15396007178389767 0.15396007178389767 Bondla Mollem Madhai 3.5366666666666577
3.2699999999999996 3.5366666666666577 S 0.41096543965715082 0.41096543965715082 Bondla Mollem Madhai
3.7266666666666666 3.0066666666666664 3.7099999999999995 M 0.37659936714410114 0.37659936714410114
Bondla Mollem Madhai 2.9499999999999997 3.5274999999999999 3.6575000000000002

PM 0.37541088600802791 0.37541088600802791 Bondla Mollem Madhei 3.32999999999999987 2.6900000000000004
3.34999999999999988 W 0.14241306640011342 0.14241306640011342 Bondla Mollem Madhei 3.5366666666666577
3.2699999999999996 3.4899999999999998 S 0.36320079131909727 0.36320079131909727 Bondla Mollem Madhei
3.7266666666666666 3.0066666666666664 3.4499999999999997 M 0.38316391183583093 0.38316391183583093
Bondla Mollem Madhei 2.9499999999999997 3.5274999999999999 3.6749999999999998

Fig. 18: Graph showing pH in three wildlife sanctuary through seasons (2017-2018) Fig. 17: Graph showing pH in three wildlife sanctuary through seasons (2016-2017)

Soil samples were also analysed from the study sites to understand free living Soil Nematode diversity.

Free living Soil Nematode diversity from three wildlife sanctuaries:

A total of 38 free living soil nematode species belonging to 5 orders and 17 families were reported (Table 1, Fig. 18). Order Dorylaimida was the most dominant order consisting of 10 families and 28 species of free living. Also they represents about 73% total nematode species reported (Fig. 19).

Order Mononchida and Rhabditida consist of two families and three species respectively and it represents about 15% of total nematode species reported

No of Families Dorylaimida Mononchida tylenchida Alaimida Rhabditia Araeolaimida 10 2 1 1 2 1 No. of Species Dorylaimida Mononchida tylenchida Alaimida Rhabditia Araeolaimida 28 3 1 2 3 1

Fig. 19: Graph showing number of families and species in Each Order recorded .

Order Alaimida consist of one family and two species, while Order Tylenchida and Order Araeolaimida consist of one family and one species each.

Nematode Diversity in Bondla Wildlife Sanctuary

A total of 27 species were reported from Bondla Wildlife Sanctuary belonging to 4 orders, 12 families and 18 genera (Table 1). Order Dorylaimida was the most dominant order consisting of 21 species belonging to eight families and which represents 77.77% of total Nematode species reported from this sanctuary. Among the 18 genera reported genus Dorylaimus was the most frequent and most dominant genus with absolute frequency of 97%,

36%

MATCHING BLOCK 12/15

W

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mean density of 14 and relative density of 25% followed by *Quadsinema* with absolute frequency of 78%, Mean density of 5 and relative density of 9%. Genus *Longidorella* was the third most frequent genus with absolute frequency of 61%, mean density of 2 and with relative density of 4%.

Genus *Aporcelaimium*, *Xiphinema* and *Neoactinolaimus* were also frequent genus with absolute frequency of 56% and 53% respectively. Genus *Mesorhabditis* was the least frequent and least dominant genus with absolute frequency of 5.5%, mean density of 0.05 and relative density of 0.07%. *Dorylaimus stagnalis* Dujardin, 1845 (97%), *Qudsinema* sp (78%), *Longidorella* sp (61%), *Aporcelaimium baqrii* (56%), *Xiphinema elongatum* Stekhoven and Teunissen, 1938 (53%), *Neoactinolaimus agilis* Thorne, 1967 (53%), *Monochus truncates* (50%), *Tylodorus* sp. (50%), *Clarkus elongates* (47%), *Xiphinema americanum* Cobb, 1913 (44%), *Sicaguttar thornei* (42%), *Hexactinolaimus aneityi* Yeates, 1973 (42%), *Xiphinema laevistriatum* Lamberti et Bleve Zacheo, 1979 (42%), were some the most frequent free living soil nematode species reported from Bondla Wildlife Sanctuary.

Nematode Diversity in Bhagwan Mahaveer Wildlife Sanctuary

A total of 36 species were reported from Bhagwan Mahaveer Wildlife Sanctuary belonging to 6 orders, 17 families and 27 genera (Table 1). Order *Dorylaimida* was the most dominant order consisting of 26 species belonging to ten families and which represents 72.22% of total Nematode species reported from Bhagwan Mahaveer Wildlife Sanctuary. In terms of dominance of free living nematode species similar trend was seen like in Bondla Wildlife Sanctuary. Among the 27 genera reported genus *Dorylaimus* was the most frequent and most dominant genus with absolute frequency of 100%,

36%

MATCHING BLOCK 15/15

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mean density of 15 and relative density of 36% followed by *Longidorella* with absolute frequency of 83%, Mean density of 2 and relative density of 5%. Genus *Qudsinema* was the third most frequent genus with absolute frequency of 81%, mean density of 4.6% and with relative density of 10%.

Genus *Xiphinema*, *Aporcelaimium*, *Hexactinolaimus*, *Monochus* and *Tylodorous* were also frequent genus with absolute frequency of 75%, 72%, 67%, 61% and 58% respectively. Genus *Akortonus* and *Oxydirus* was the least frequent and least dominant genus with absolute frequency of 5.5% and 2.8%, mean density of 0.06 and 0.03 and relative density of 0.13% and 0.06% each. *Dorylaimus stagnalis* Dujardin, 1845 (100%), *Longidorella* sp (83.3%), *Qudsinema* sp (80.5%), *Xiphinema elongatum* Stekhoven and Teunissen, 1938 (75%), *Aporcelaimium baqrii* (72%), *Xiphinema americanum* Cobb, 1913 (66.6%), *Hexactinolaimus aneityi* Yeates, 1973 (53.3%), *Monochus truncates* (58.3%), *Tylodorus* sp. (55.50%), *Alaimus indicus*, Choudhary and Jairajpuri, 1983 (52.7%), *Neoactinolaimus attenuates* Ahmad and Jairajpuri, 1994 (50%), *Xiphinema orbum* Siddiqi, 1964 (50%), *Clarkus elongates* (50%), *Dorylaimus afganicus* Andrassy, 1960 (47.2%), *Neoactinolaimus agilis* Thorne, 1967 (47.2%), and *Alaimus parvus* (47.2%) were some the most frequent free living soil nematode species reported from Bhagwan Mahaveer Wildlife Sanctuary.

Nematode Diversity in Mhadei Wildlife Sanctuary

A total of 21 species were recorded from Mhadei Wildlife Sanctuary belonging to 5 orders, 12 families and 13 genera (Table 1). Order *Dorylaimida* was the most dominant order consisting of 16 species belonging to eight families and which represents 76.19% of total Nematode species reported from Mhadei Wildlife Sanctuary. Compared to Bondla and Bhagwan Mahaveer Wildlife Sanctuaries, least number of species was reported from the Mhadei sanctuary. In terms of dominance of genus *Dorylaimus* similar trend was seen like in Bondla Wildlife Sanctuary and in Bhagwan Mahaveer Wildlife sanctuary with absolute frequency of 100%,

35%

MATCHING BLOCK 13/15

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mean density of 12.3 and relative density of 31.7% followed by *Qudsinema* with absolute frequency of 83%, Mean density of 4.5 and relative density of 12.03%. Genus *Sicaguttar* was the least frequent and least dominant genus with absolute frequency of 8.3%, mean density of 0.08 and relative density of 0.21%.

Dorylaimus stagnalis Dujardin, 1845 (100%), Qudsinema sp (83%), Xiphinema elongatum Stekhoven and Teunissen, 1938 (80.5%), Aporcelaimium baqrii (66.6%), Xiphinema americanum Cobb, 1913 (66.6%), Tyloporus sp. (62.5%), Alaimus indicus, Choudhary & Jairajpuri, 1983 (54.16%), Neoactinolaimus attenuates Ahmad and Jairajpuri, 1994 (50%) and Monochus truncatus (50%) were some the most frequent free living soil nematode species reported from Mhadei Wildlife Sanctuary.

Seasonality in diversity of nematode species in three wildlife sanctuary Seasonally, number of species was more in winter and post-monsoon compared to summer and monsoon (Fig.20).

BWLS PM W S M 22 21 9 13 BMWLS PM W S M 24 26 17 18 MWLS PM W S M 15 16 13 14

Fig. 20: Graph showing seasonality in number of species

Total of 22 species from Bondla Wildlife Sanctuary, 24 from Bhagwan Mahaveer Wildlife Sanctuary and 15 from Mhadei Wildlife Sanctuary were reported in Post Monsoon. In Winter 21 species from Bondla Wildlife Sanctuary, 26 from Bhagwan Mahaveer Wildlife Sanctuary and 16 from Mhadei Wildlife Sanctuary were reported. In Summer 09 species from Bondla Wildlife Sanctuary, 17 from Bhagwan Mahaveer Wildlife Sanctuary and 13 from Mhadei Wildlife Sanctuary and in Monsoon 13 from Bondla Wildlife Sanctuary, 18 from Bhagwan Mahaveer Wildlife Sanctuary and from Mhadei Wildlife sanctuary 14 species of free living soil nematode were reported. Species such as Dorylaimus stagnalis Dujardin, 1845 with frequency of occurrence between 97-100% were reported throughout the year. Collected data was subjected to statistical analysis to get better understanding with regards to diversity; evenness and richness were depicted in Table 2, 3 and 4.

Measures to preserve the nemafauna in the study area

As the diversity of Nemafauna was more in forest area than the Acacia plantation area, it is suggested that, more and more forested area may be preserved or Acacia plantation may be reduced significantly (Fig. 21).

Fig. 21: Graph showing number of families in forested and Acacia plantation No. of nematode families Forested Area Acacia Plantation 17 6

Study Area No. of families

Table 1: Checklist of Free living Nematode species from three wildlife sanctuary Orders Families Genus Species BWLS BMWLS MWLS DORYLAIMIDA Belonidiridae Oxydirus Oxydirus oxycephaloides (De Man, 1921) Thorne,1939 +

Axonchium Axonchium amplicolle Cobb, 1920 + + +

Dorylaimidae Dorylaimus Dorylaimus stagnalis Dujardin, 1845 + + +

Dorylaimus afganicus Andrassy, 1960 + + +

Mesodorylaimus Mesodorylaimus chamoliensis, Ahmad 1995 +

Sicaguttar Sicaguttar thornei + +

Sicaguttar sp. + +

Qudsiematidae Qudsinema Qudsinema sp + + +

Eudorylaimus sp +

Microdorylaimus Microdorylaimus sp. + +

Crateronematidae* Chrysonema Chrysonema sp. +

Nordiidae .Echodelus Echodelus sp +

.Longidorella Longidorella sp + +

.Thornedia Thornedia sp + + +

Thornematidae* Fuscheila Fuscheila sp. +

Aporcelaimidae

Aporcelaimium

Aporcelaimium baqrii

+

+

+

. Aporcelaimium clamus + + +

Aporcelaimium sp Loof and Coomans, 1970. + + +

Akortonus Akortonus vigor Thorne, 1974 +

Actinolaimidae Neoactinolaimus Neoactinolaimus agilis Thorne, 1967 + +

Neoactinolaimus attenuates Ahmad and Jairajpuri, 1994 + + +

Hexactinolaimus Hexactinolaimus aneityi Yeates, 1973 + +

Longidoridae Paralongidorus Paralongidorus sali Sissiqi, Hooper and Khan, 1963 + +

Longidorus Longidorus elongatus Thorne and Swanger, 1936 + + +

Xiphinematidae Xiphinema Xiphinema americanum Cobb, 1913 + + +

Xiphinema elongatum Stekhoven and Teunissen, 1938 + + +

Xiphinema orbum Siddiqi, 1964 + + +

Xiphinema laevistriatum Lamberti et Bleve Zacheo, 1979 + + + MONONCHIDA Monochidae Clarkus Clarkus elongatus + +

Monochus Monochus truncatus + + +

Iotonchidae Iotonchus Iotonchus indicus Jairajpuri, 1969 + TYLENCHIDA Tylenchidae Tylodorous Tylodorus sp. + + + Alaimida Alaimidae Alaimus Alaimus parvus + + +

Alaimus indicus, Choudhary & Jairajpuri, 1983 + + + RHABDITIA Rhabditidae Mesorhabditis Pristomatolaimus andrassyi Khera & Chaturvedi, 1977 + +

Panagrolaimidae Panagrellus Panagrellus dorsobidentata (Rühm, 1956) +

Panagrolaimus Panagrolaimus fuchsi Ruhm, 1956 + Araeolaimida Plectidae Plectus Plectus cirratus Bastian, 1865 + +

-Bondla wildlife sanctuary, BMWLS-Bhagwan Mahaveer wildlife sanctuary, MWLS-Mhadei wildlife sanctuary, + - present/reported

Table 2: Species Diversity SEASONS STUDY AREA

BWLS BMWLS MWLS Post-monsoon 2.1662 ± 0.4677 2.3246 ± 0.168 2.09702 ± 0.1517 Winter 2.4651 ± 0.3352 2.47417 ± 0.2323 2.27763 ± 0.2384 Summer 1.2531 ± 0.6166 1.83946 ± 0.3291 1.66253 ± 0.3936 Monsoon 0.8685 ± 0.2585 1.85437 ± 0.2415 1.69731 ± 0.2062

Table 3: Species Richness SEASONS STUDY AREA

BWLS BMWLS MWLS Post-Monsoon 18.267 ± 2.131 20.269 ± 0.694 10.736 ± 0.018 Winter 17.428 ± 3.069 20.102 ± 2.530 11.412 ± 1.544 Summer 2.826 ± 1.705 9.329 ± 3.113 6.978 ± 2.103 Monsoon 1.656 ± 0.485 8.401 ± 0.963 6.332 ± 0.956

Table 4: Species Evenness SEASONS STUDY AREA

BWLS BMWLS MWLS Post-Monsoon 0.728 ± 0.166 0.719 ± 0.039 1.061 ± 0.051 Winter 0.848 ± 0.069 0.856 ± 0.048 0.957 ± 0.062 Summer 0.882 ± 0.143 0.823 ± 0.143 0.896 ± 0.133 Monsoon 0.625 ± 0.297 0.770 ± 0.068 1.044 ± 0.024

DISCUSSION

DISCUSSION Nematodes are seen in all possible climatic condition and habitats, which can support life (Abebe et. al., 2011; Tahseen, 2012). Their distribution is linked to simple soil properties (Zhang, et. al., 2012). Soil properties such as soil moisture, pH and C:N ration can explain to a great extent the distribution of soil organisms (Wall et. al., 2010; Li et al.,

2010; Zhang et. al., 2012). Soil Nematodes are not an exception and a soil property affects their diversity and distribution directly or indirectly. Underground biota is critical for biogeochemical and ecological functioning of terrestrial ecosystem (Wall et al., 2010). Soil Nematode is one of the important groups of underground biota, most diverse (Maggenti, 1981; Morand et al., 2006) and ecologically successful group (Ahmad, 2001).

Nematodes are basically aquatic animals, adapted to a variety of terrestrial habitats, provided there is a thin film of water around them (Tahseen, 2012). Their existences either as parasitic or free-living depend on availability of water or liquid medium. Moisture holding capacity of the soil particles is a reason, that allows nematode species to adapt and survive in terrestrial mode of life (Lizanne, 2015). Moisture content of the soil from study area was found to be between 10 to 46% being highest during Monsoon i.e., from June to September (40 to 46%) and lowest during summer i.e., from March to May (10-12%). During the Post Monsoon and winter the moisture content was between 26 to 31% and 16 to 21% respectively. In the present study moisture content between 16 to 31% was found to be optimum for nematode existence.

Nematodes are known to survive in wide range of pH (4-8) (Burns, 1971) and it affects nematode community indirectly via other components of the soil food web. In the present study, pH of the soil in study area was found to be between 5.5 and 6.6 (mildly acidic) optimum for free living Soil Nematode existence.

The environmental changes, arising from nitrogen (N) deposition and precipitation, influence soil ecological processes in forest ecosystems (Sun et al., 2013). Sun et al., (2013) studied responsive mechanisms of soil biota, to N deposition and precipitation, soil nematode communities. Their results showed that, water combined with N addition treatment decreased the total nematode abundance in the organic horizon (O); while the opposite trend was found in the mineral horizon (A). Significant reductions in the abundances of fungivores, plant-parasites and omnivores-predators were also found in the water combined with N addition treatment. The significant effect of water interacted with N on the total nematode abundance and trophic groups indicated that, the impacts of N on soil nematode communities were mediated by water availability. The synergistic effect of precipitation and N deposition on soil nematode communities was stronger than each effect alone. Structural equation modelling suggested that water and N additions had direct effects on soil nematode communities. The feedback of soil nematodes to water and nitrogen addition was highly sensitive and their results indicate that, minimal variations in soil properties such as, those caused by climate changes can lead to severe changes in soil nematode communities. In the present studies nitrogen / organic carbon was between 2.5 to 3.8% which was higher, as recommended by Soil Testing Laboratory. Effect of same was poorly understood on soil biota (Sun et al., 2013).

Humid climate, mildly acidic pH, deciduous forest tree leaves which provide higher quality of water soluble nutrients which are useful to soil nematodes (Keith et. al., 2009; Zhang et. al., 2012), moist soil which is abundant with decaying and decomposing organic matter, can make forest soils of Goa a favourable and rich habitat to sustain enormous diversity and abundance of nematodes species (Lizanne, 2015).

FREE LIVING SOIL NEMATODE DIVERSITY:

In the present study, a total of 38 free living soil nematode species, belonging to 5 orders and 17 families are reported. Order Dorylaimida was the most dominant order consisting of 10 families and 28 species of free living nematodes which represents about 73% total nematode species reported. Dominance of order Dorylaimida was due to fewer disturbances in this region as most of the forested area of Goa, is under protection. Dorylaims were found in every conceived type of habitat and usually dominate both in numbers and in species over all other soil-inhabiting nematodes (Jairajpuri and Ahmad, 1992). Moreover, they can be easily recognized at lower magnification (Lizzane and Pai, 2014). They are unique among the Nematodes as they exhibit most of the feeding guilds represented by nematodes and this allows them to adapt, diversify and allow them to have wide occurrence in soil ecosystem (Jairajpuri, 2002). Dorylaimids are more sensitive to disturbance (Forge

88%

MATCHING BLOCK 14/15

W

[https://www.researchgate.net/publication/ ...](https://www.researchgate.net/publication/...)

and Simard, 2001), therefore used as indicators of environmental disturbances (Thomas, 1978; Sohlenius and Wasilewska, 1984).

A

total of 27, 36 and 21 free living soil nematode species was recorded in the Bondla Wildlife Sanctuary, Bhagwaan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary respectively.

Seasonally, number of species was more in winter and post-monsoon compared to summer and monsoon. This could be because of moist soil with moisture content between 16-31%. As summer in Goa, are hotter and during monsoon receives heavy rainfall. During summer nematode species may be die due to dehydration, while during monsoon they may be washed off from top soil layer. SUGGESTED MEASURES TO PRESERVE THE NEMAFUNA IN THE STUDY AREA: When compared with forest soil and plantation soil (*Acacia auriculiformis* plantation), it was observed that, more number of nematode families in forested soil compared to that of plantation area. This clearly indicates, the suitability of forested soil for survival of Nemafuna. This is due to higher organic matter, moist and optimum pH in forest soils, compared to soils of *Acacia* plantation. Change in landscapes could also be a major threat to free living Soil Nematodes. One more reason could be the fact that, Bondla wildlife sanctuary, Mhadei wildlife sanctuary and Bhagwan Mahaveer Wildlife Sanctuary are part of the Western Ghats, as well are the part of protected areas as per Government of Goa, forest rules. Hence Soil Biota especially Nematode fauna is under less anthropogenic pressure.

CONCLUSION

From this present study, it is concluded that, moisture content of the soil at the study area was found to be between 10-46%, being highest during Monsoon (June to September) (40-46%) and lowest during summer (March to May) (10-12%). During the Post Monsoon and winter the moisture content was between 26-31% and 16-21% respectively. In the present study, moisture content between 16-31% was found to be optimum for nematode existence.

pH of the soil in study area was found to be between 5.5-6.6 (mildly acidic), optimum for free living soil nematode existence. In the present study nitrogen / organic carbon was found between 2.5-3.8%, which was higher as recommended by Soil Testing Laboratory. Effect of same was poorly understood on soil biota (Sun et al., 2013). Humid climate, mildly acidic pH, deciduous forest tree leaves provide higher quality of water soluble nutrients, which are useful to soil nematodes. Moist soil, which is abundant with decaying and decomposing organic matter, makes forest soils of Goa, a favourable and rich habitat to sustain enormous diversity and abundance of nematodes species.

In the present study a total of 38 free living soil nematode species, belonging to 5 orders and 17 families were reported. Order Dorylaimida, was the most dominant order consisting of 10 families and 28 species of free living nematodes and it represents about 73% total nematode species reported. A total of 27, 36 and 21 free living soil nematode species was recorded in the Bondla Wildlife Sanctuary, Bhagwan Mahaveer Wildlife Sanctuary and Mhadei Wildlife Sanctuary respectively. Seasonally, number of species was more in winter and post-monsoon compared to summer and monsoon. When compared with forest soil and plantation soil (*Acacia auriculiformis* plantation), it was observed that, more number of nematode families in forested soil compared to that of plantation. This clearly indicates the suitability of forested soil which is rich in organic matter, moist with optimum pH which is best suited for nematodes as compared to soil of *Acacia* plantation.

Bondla wildlife sanctuary, Mhadei wildlife sanctuary and Bhagwan Mahaveer Wildlife Sanctuary are part of the Western Ghats and are also part of protected forest land as declared by Government of Goa. Hence, soil biota viz., free living Nematode fauna is under less anthropogenic pressure. Change in landscapes could also be one of the major threats to free living Soil Nematodes.

2015-16 O N D J F M A M J J A S 31.75 28.79 27.89 17.059999999999999 16.41 12.739999999999998 10.96 10.61 45.548000000000002 51.13 42.730000000000004 41.3 2016-17 O N D J F M A M J J A S 30.279999999999998 29.05 26.8 17 16 12.350000000000001 10.06 10.55 45.379999999999995 51 41.849999999999994 41.2 2017-18 O N D J F M A M J J A S 31.25 27.45 27.35 16.95 16.41 12.65 10 10.43 45.5 51.13 42.63 41.230000000000004

2015-16 O N D J F M A M J J A S 6.8 6.3 5.8 5.8 5.9 5.9 5.8 5.8 5.4 5.5 5.3 6.3 2016-17 O N D J F M A M J J A S 5.8 5.7 5.8 5.8 5.9 5.7 5.8 5.8 5.4 5.5 5.3 5.7 2017-18 O N D J F M A M J J A S 6.3 6.3 5.8 5.8 5.9 5.9 5.8 5.8 5.4 5.5 5.3 6.3

PM 0.36950417228135862 0.36950417228135862 Bondla Mollem Madhai 3.3299999999999987 2.6900000000000004 3.3299999999999987 W 0.15396007178389776 0.15396007178389776 Bondla Mollem Madhai 3.5366666666666577 3.2699999999999996 3.5366666666666577 S 0.415692193816529 0.415692193816529 Bondla Mollem Madhai 3.7266666666666666 3.0066666666666664 3.7266666666666666 M 0.38602407092477525 0.38602407092477525 Bondla Mollem Madhai 2.9499999999999997 3.5274999999999999 3.6825000000000001

PM 8.0363756341703979E-2 8.0363756341703979E-2 Bondla Mollem Madhai 5.75 5.7750000000000004 5.6249999999999689 W 2.886751345948068E-2 2.886751345948068E-2 Bondla Mollem Madhai 5.8333333333333597 5.7833333333333661 5.7833333333333661 S 6.3098981620003533E-2 6.3098981620003533E-2 Bondla Mollem Madhai

5.7666666666666684 5.8666666666666671 5.75 M 3.8188130791299207E-2 3.8188130791299207E-2 Bondla Mollem
Madhai 5.4749999999999996 5.5500000000000007 5.52499999999999755

PM 1.0040916292848916 0.49497474683024345 2.0647518010646877 1.0040916292848916 0.49497474683024345
2.0647518010646877 2015-16 2016-17 2017-18 25.66 26.020000000000003 26.41 W 6.8240882419069937
2.3596680557512992 9.5678618562351776 6.8240882419069937 2.3596680557512992 9.5678618562351776 2015-16
2016-17 2017-18 18.419666666666668 20.736666666666668 17.904499999999995 S 3.159477804954467
2.4866242176895232 3.1431990073808596 3.159477804954467 2.4866242176895232 3.1431990073808596 2015-16
2016-17 2017-18 13.96 14.71 14.06 M 2.1944380449977809 4.4391543864419614 2.313071550990168
2.1944380449977809 4.4391543864419614 2.313071550990168 2015-16 2016-17 2017-18 41.642500000000005
41.557499999999997 41.865000000000002

2015-16 O N D J F M A M J J A S 5.6499999999999995 5.7 5.9 5.7 5.6 5.75 5.8 5.8 5.5 5.5 5.3 5.9 2016-17 O N D J F M A M
J J A S 5.7 5.85 5.8 5.8 5.75 5.9 5.85 5.85 5.5 5.5 5.3 5.9 2017-18 O N D J F M A M J J A S 5.6 5.8 5.9 5.8 5.75 5.9 5.8 5.8 5.5
5.5 5.3 5.9 2015-16 O N D J F M A M J J A S 5.6499999999999995 5.7 5.9 5.7 5.6 5.75 5.8 5.8 5.5 5.5 5.3 5.9 2016-17 O N
D J F M A M J J A S 5.7 5.85 5.8 5.8 5.75 5.9 5.85 5.85 5.5 5.5 5.3 5.9 2017-18 O N D J F M A M J J A S 5.6 5.8 5.9 5.8 5.75
5.9 5.8 5.8 5.5 5.5 5.3 5.9

Bondla O N D J F M A M J J A S MONTHS 3.72 2.94 3.8099999999999987 3.64 3.16 3.73 3.62 3.8299999999999987 2.9
2.9099999999999997 2.8899999999999997 3.1 Mollem O N D J F M A M J J A S MONTHS 2.68 2.7 3.5 2.8 3.51
2.8899999999999997 3.5 2.63 3.63 3.2800000000000002 3.5 3.7 Madhei O N D J F M A M J J A S MONTHS 3.7 3 3.67
3.64 3.16 3.73 3.62 3 3.6 3.7 3.6 3.8

2015-16 O N D J F M A M J J A S 30.89 29.73 27.89 16.89 16.5 12.739999999999998 11.19 10.59 44.120000000000005
43.339999999999996 42.730000000000004 39.230000000000011 2016-17 O N D J F M A M J J A S 30.8 29.7 27.87
16.760000000000002 16.329999999999995 13.3 12 10.5 45 43.5 42.67 38 2017-18 O N D J F M A M J J A S
30.959999999999997 29.6 27.68 16.54 16.43 12.629999999999999 11.2 10.48 44.120000000000005 43.43 42.56
38.480000000000004

2015-16 O N D J F M A M J J A S 5.9 5.7 5.8 5.8 5.7 5.9 5.8 5.8 5.4 5.5 5.3 5.9 2016-17 O N D J F M A M J J A S 5.6
5.6499999999999995 5.7 5.75 5.9 5.75 5.8 5.7 5.4 5.3 5.4 6 2017-18 O N D J F M A M J J A S 5.9 5.7 5.7 5.8 5.8 5.9 5.8 5.8
5.4 5.5 5.3 5.9

No of Families Dorylaimda Mononchida tylenchida Alaimida Rhabditia Araeolaimida 10 2 1 1 2 1 No. of Species Dorylaimda
Mononchida tylenchida Alaimida Rhabditia Araeolaimida 28 3 1 2 3 1

2015-16 O N D J F M A M J J A S 26.37 24.95 24.67 11.138999999999999 19.45 17.38 11.15 13.350000000000001 42.21
43.64 42.21 38.51 2016-17 O N D J F M A M J J A S 26.37 25.67 23.459999999999997 19.3 19.45 17.579999999999995
13.2 13.350000000000001 46.3 42.15 42.21 35.57 2017-18 O N D J F M A M J J A S 27.87 24.95 24.67
11.138999999999999 0 17.38 11.129999999999999 13.67 43.1 43.64 42.21 38.51

Bondla O N D J F M A M J J A S MONTHS 3.72 2.94 3.8099999999999987 3.64 3.16 3.73 3.62 3.8299999999999987 2.9
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2.8899999999999997 3.5 2.63 3.63 3.2800000000000002 3.5 3.7 Madhai O N D J F M A M J J A S MONTHS 3.72 2.94
3.8099999999999987 3.64 3.16 3.73 3.62 3.8299999999999987 3.63 3.7800000000000002 3.62 3.7

2015-16 PM W S M 30.310000000000006 20.426666666666666 11.506666666666668 42.354999999999997 2016-17
PM W S M 30.25 20.32 11.933333333333332 42.292500000000018 2017-18 PM W S M 30.279999999999998
20.216666666666665 11.436666666666669 42.147500000000001 PM 0.82024386617628831 0.77781745930521395
0.96166522241370733 0.82024386617628831 0.77781745930521395 0.96166522241370733 2015-16 2016-17 2017-18
30.310000000000006 30.25 30.279999999999998 W 6.4663771412850215 6.5420256801697105 6.4636702679927405
6.4663771412850215 6.5420256801697105 6.4636702679927405 2015-16 2016-17 2017-18 20.426666666666666 20.32
20.216666666666665 S 1.1094292827095156 1.4011899704655819 1.0943643512712431 1.1094292827095156
1.4011899704655819 1.0943643512712431 2015-16 2016-17 2017-18 11.506666666666668 11.933333333333332
11.436666666666669 M 2.1596064456284996 3.019750265612434 2.5269398489081198 2.1596064456284996
3.019750265612434 2.5269398489081198 2015-16 2016-17 2017-18 42.354999999999997 42.292500000000018
42.147500000000001

Bondla O N D J F M A M J J A S MONTHS 3.72 2.94 3.809999999999987 3.64 3.16 3.73 3.62 3.829999999999987 2.9
2.909999999999997 2.889999999999997 3.1 Mollem O N D J F M A M J J A S MONTHS 2.68 2.7 3.5 2.8 3.51
2.889999999999997 3.5 2.63 3.63 3.2800000000000002 3.5 3.7 Madhai O N D J F M A M J J A S MONTHS 3.72
3.889999999999997 3.809999999999987 3.63 3.17 3.73 3.6 3.8 3.63 3.7800000000000002 3.62 3.6

PM 0.37541088600802791 0.37541088600802791 Bondla Mollem Madhei 3.329999999999987 2.6900000000000004
3.349999999999988 W 0.14241306640011342 0.14241306640011342 Bondla Mollem Madhei 3.536666666666577
3.269999999999996 3.489999999999998 S 0.36320079131909727 0.36320079131909727 Bondla Mollem Madhei
3.726666666666666 3.006666666666664 3.449999999999997 M 0.38316391183583093 0.38316391183583093
Bondla Mollem Madhei 2.949999999999997 3.527499999999999 3.674999999999998

PM 0.47324236215001031 0.47324236215001031 Bondla Mollem Madhei 6.55 5.6750000000000007
5.8000000000000007 W 5.091750772173137E-2 5.091750772173137E-2 Bondla Mollem Madhei 5.833333333333597
5.733333333333725 5.766666666666684 S Bondla Mollem Madhei 5.833333333333597 5.783333333333661
5.833333333333597 M 5.204164998665306E-2 5.204164998665306E-2 Bondla Mollem Madhei 5.624999999999689
5.5500000000000007 5.524999999999755

No. of nematode families Forested Area Acacia Plantation 17 6

Study Area No. of families

2015-16 PM W S M 30.27 20.45333333333322 11.436666666666669 45.177 2016-17 PM W S M 29.664999999999999
19.93333333333323 10.986666666666668 44.857499999999995 2017-18 PM W S M 29.35 20.236666666666665
11.026666666666667 45.122500000000009 PM 2.0930360723122137 0.86974134085963373 2.6870057685088438
2.0930360723122137 0.86974134085963373 2.6870057685088438 2015-16 2016-17 2017-18 30.27
29.664999999999999 29.35 W 6.5280395219391973 6.4070924242852421 6.033517492585351 6.5280395219391973
6.4070924242852421 6.033517492585351 2015-16 2016-17 2017-18 20.45333333333322 19.93333333333323
20.236666666666665 S 1.1422054689649006 1.2058330453812178 1.4221931420638148 1.1422054689649006
1.2058330453812178 1.4221931420638148 2015-16 2016-17 2017-18 11.436666666666669 10.986666666666668
11.026666666666667 M 4.3433776411146505 4.4879718879095929 4.3816539875562066 4.3433776411146505
4.4879718879095929 4.3816539875562066 2015-16 2016-17 2017-18 45.177 44.857499999999995
45.122500000000009

PM 0.32145502536642001 0.32145502536642001 Bondla Mollem Madhei 6.3 5.699999999999975
5.8000000000000007 W 3.469443332443542E-2 3.469443332443542E-2 Bondla Mollem Madhei 5.833333333333597
5.816666666666664 5.766666666666684 S Bondla Mollem Madhei 5.833333333333597 5.833333333333597
5.833333333333597 M 5.204164998665306E-2 5.204164998665306E-2 Bondla Mollem Madhei 5.624999999999689
5.5500000000000007 5.524999999999755

PM 0.55953105365118505 0.55953105365118505 Bondla Mollem Madhai 3.329999999999987 2.6900000000000004
3.804999999999997 W 0.15396007178389767 0.15396007178389767 Bondla Mollem Madhai 3.536666666666577
3.269999999999996 3.536666666666577 S 0.41096543965715082 0.41096543965715082 Bondla Mollem Madhai
3.726666666666666 3.006666666666664 3.709999999999995 M 0.37659936714410114 0.37659936714410114
Bondla Mollem Madhai 2.949999999999997 3.527499999999999 3.6575000000000002

BWLS PM W S M 22 21 9 13 BMWLS PM W S M 24 26 17 18 MWLS PM W S M 15 16 13 14

8/15	SUBMITTED TEXT	25 WORDS	100% MATCHING TEXT	25 WORDS
<p>functional links to ecosystem processes (Debruyn, 1997), determining a hierarchy of geographic scale (Neher et al., 1998) and measuring their utility across ecosystem boundaries</p>		<p>functional links to ecosystem processes (Debruyn. 1997), determining a hierarchy of geographic scale i (Neher et al., 1998) and measuring their utility across ecosystem boundaries.</p>		
<p>W https://core.ac.uk/download/pdf/144528636.pdf</p>				

4/15	SUBMITTED TEXT	12 WORDS	100% MATCHING TEXT	12 WORDS
<p>one of the most ancient and diverse types of animals, on</p>		<p>one of the most ancient and diverse types of animals on</p>		
<p>W https://www.apsnet.org/edcenter/disandpath/nematode/intro/Pages/IntroNematodes.aspx</p>				

3/15	SUBMITTED TEXT	44 WORDS	70% MATCHING TEXT	44 WORDS
<p>Dujardin (1845), Bastian (1865), Schneider (1866), de Man (1884), Daday (1905) and Maupas (1900) were the pioneers of the field. Dujardin (1845) was first to recognize relationship between free-living and plant parasitic nematodes</p>		<p>Dujardin (1845), Bastian (1865), Schneider (1866), de Man (1884), Maupas (1900) and Daday (1905) fly were the pioneers of the field of nematode taxonomy. Dujardin (1845) was first to recognize the close relationship of free-living and plant parasitic nematodes.</p>		
<p>W https://core.ac.uk/download/pdf/144528636.pdf</p>				

10/15	SUBMITTED TEXT	17 WORDS	96% MATCHING TEXT	17 WORDS
<p>of soil inhabiting nematodes in Dera Ki Gali forest of Poonch district in Jammu and Kashmir.</p>		<p>of soil-inhabiting nematodes in Dera Ki Gali (DKG) forest of Poonch district in Jammu and Kashmir,</p>		
<p>W https://www.researchgate.net/publication/272460334_Diversity_of_soil_inhabiting_nematodes_in_Dera ...</p>				

ANNEXURE

- Research paper published:

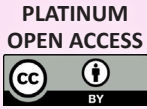
Gaude, K. and Pai, I. K. (2020) A preliminary survey of soil nemafuna of Bhagwan Mahaveer Wildlife Sanctuary, Goa, India. *Journal of Threatened Taxa* 12(8): 15932–15935.

Bowalkar, D., Gracy Michael, N. A., Gaude, K. & I.K. Pai (2017). A checklist of butterflies (Insecta: Lepidoptera) from Taleigao Plateau, Goa, India. *Journal of Threatened Taxa* 9(8): 10626–10630.

- Research papers presented:

Kiran Gaude and I. K. Pai (2016) Linking Vegetation type, Soil Properties and Nematodes abundance from Moist-deciduous and Semi-evergreen forest type of Goa, India. *International Symposium on “Understanding the Molecules of Life in the Era of New Biology” & 28th All India Congress of Zoology (AICZ)*, Departments of Life Sciences, Davangere University, Davangere, Karnataka State, India, 30 Oct-02 Nov: 64pp.

Kiran Gaude and I. K. Pai (2018) A Preliminary Survey of Soil Nemafuna of the Bhagwan Mahaveer Wildlife Sanctuary, Goa, India. *International Conference on Materials and Environment Science*, Shivaji University, Kolhapur, 07-08 December: 154pp.



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SHORT COMMUNICATION

A PRELIMINARY SURVEY OF SOIL NEMAFUNA OF BHAGWAN MAHAVEER WILDLIFE SANCTUARY, GOA, INDIA

Kiran Gaude & I.K. Pai

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A preliminary survey of soil nemafauna of Bhagwan Mahaveer Wildlife Sanctuary, Goa, India

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Abstract: Nematological research in India is primarily focussed on major crops and animal parasitic groups, while ignoring free living groups in forest ecosystems. In the present study, soil nemafauna of Bhagwan Mahaveer Wildlife Sanctuary, Goa, India was assessed. A total of 18 genera, 14 families, and five orders were recorded. Among four orders, Dorylaimida was the most dominant one, which consists of 12 genera and nine families. Among the 18 genera *Sicaguttur*, *Qudsinema*, *Microdorylaimus*, *Longidorella*, *Paralongidorus*, *Xiphidorinae*, *Fuscheila* and *Chrysonema* are reported for the first time from the state. More such intensive survey will add more numbers of nematode species.

Keywords: Invertebrate, Nematoda, protected area, underground biota.

Nematodes are one of the important groups of invertebrate in both terrestrial and freshwater ecosystems (Hanel 1999). They are small, worm-like animals (Yeast 1979; Yeast & Bonger 1999), diverse (Ettema 1998), and ubiquitous inhabitants (Bernard 1992; Bloemers et al. 1997; Bonger & Ferris 1999) in nature. A total of 1,000,000 species of nematodes is estimated globally (Hugot et al. 2001); nearly 30,028 species are known. Around 2,900 species of nematodes are identified from India (MoEF 2014) which is 9.66% of the total described species. Nematological research in India predominantly focuses on plant and animal

parasitic groups. The parasitic association of nematodes with all the major crops of India has been reported in earlier literature. Little work has been done on the free living groups in forest ecosystems as they do not have a direct connection with agriculture or livestock (Pradhan & Dash 1987; Baniyamuddin et al. 2007; Vaid et al. 2014).

Goa, a small state with an area of 3,702km², in the Western Ghats and on the coast of the Arabian Sea, contributes a rich biodiversity (Alvares 2002). Extensive faunal studies, in general, have been done in Goa but the underground biota (Nematoda) has been neglected in most cases. In South Goa District, 52 species of nematodes are reported which is about 0.01% of total species in India (Lizanne & Pai 2014). These sanctuaries are part of the Western Ghats and may incorporate a wide diversity of soil nematodes.

STUDY AREA

Bhagwan Mahaveer Wildlife Sanctuary (Image 1) is a 240km² protected area located at 15.319° & 74.288°. It contains several temples and the Dudhsagar Fall. This sanctuary is famous for its snakes particularly the King Cobra. Vegetation is classified as west coast tropical evergreen forests, west coast semi-evergreen forests, and moist deciduous forests (Alvares 2002). The

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Image 1. Bhagwan Mahaveer Wildlife Sanctuary, the study area, in South Goa

predominant species are *Terminalia*, *Lagerstroemia*, *Xylia*, *Strobilanthus*, and *Dalbergia*. The forest canopy is almost closed, pH of soil samples from Bhagwan Mahaveer Sanctuary is slightly acidic (pH6.12) and has high deposits of Phosphorous (88.5 Kg/Ha) and macronutrient viz., Iron (29.908 ppm), Zinc (4.1002ppm), Copper (5.584ppm) and Manganese (29.984ppm) (Soil Testing Laboratory, Ela, Old Goa)

MATERIALS AND METHODS

Soil collection and processing for nematode extraction and identification was as per Lizanne & Pai (2014) and Vaid et al. (2014). Ten soil samples were collected randomly in a self-sealing plastic bag. Each soil sample comprises 20 sub-samples. These sub-samples were combined to make one composite sample. The soil samples were processed using modified Cobb's sieving and decantation and modified Baermann's funnel techniques for the extraction of nematodes (Ravichandra 2015). A small amount of water suspension from a funnel was drawn into a cavity block through a rubber tubing. The nematodes thus isolated were collected for counting, fixing, and processed for making permanent slides. For counting nematodes, water was added to the extracted nematode suspension to make

its volume 25ml. The suspension was stirred thoroughly and then 5ml volume was sucked by a pipette to pour in a Syracuse dish. Counting was done thrice for each sample and finally the mean was calculated. Individuals belonging to a genus were counted separately. Counted nematodes were then killed and fixed in 4% formalin and dehydrated in glycerine-alcohol (Seinhorst 1959). Dehydrated nematodes were mounted in anhydrous glycerine. Permanent slides of the specimens were prepared using paraffin wax ring method and were studied under Olympus BX51 microscope. The identification of nematodes was done consulting relevant literature (Jairajpuri & Ahmad 1992; Lamberti et al. 2002; NEMAPLEX, Nema Species Masterlist).

RESULTS AND DISCUSSION

A total of 18 genera, 14 families and five orders of nematodes were reported from Bhagwan Mahaveer Wildlife Sanctuary (Table 1) (provide photographs/images if available for publication). Among four orders Dorylaimida is the most dominant order (Figure 1) consisting of 13 genera and 10 families followed by Mononchida consisting of two genera and one family. Dominance of order Dorylaimida is due to fewer disturbances in this region. Dorylaims are found in every

Table 1. Soil nematode genera from Bhagwan Mahaveer Wildlife Sanctuary.

Orders	Families	Genera	Feeding type
Dorylaimida	Swangeriinae	<i>Oxydirus</i> Thorne, 1939	Plant parasite
	Dorylaimidae	<i>Dorylaimus</i> Dujardin, 1845	Omnivore
		<i>Sicaguttar</i> Siddiqi, 1971	-
	Quadsianematidae	<i>Qudsinema</i> Jairajpuri, 1965	-
		<i>Microdorylaimus</i> Andrassy, 1986	Omnivore
	Nordiidae	<i>Longidorella</i> Thorne, 1939	Omnivore
	Aporcelaimidae	<i>Aporcelaimium</i> Loof & Coomans, 1970	Predator
	Actinolaimidae	<i>Hexactinolaimus</i> Yeates, 1973	Predator
	Longidoridae	<i>Longidorus</i> Micoletzky, 1922	Plant parasite
		<i>Paralongidorus</i> Siddiqi, Hooper & Khan, 1963	Plant parasite
	Xiphinematidae	<i>Xiphinema</i> , Cobb, 1913	Plant parasite
	Thornematidae	<i>Fuscheila</i> Siddiqi, 1982	-
	Crateronematidae	<i>Chrysonema</i> Thorne, 1929	Not known
Tylenchida	Tylenchidae	<i>Tylenchus</i> Bastian, 1865	Plant parasite
Alaimida	Alaimidae	<i>Alaimus</i> de Man, 1880	Bacterivore
Rhabditida	Rhabditidae	<i>Mesorhabdtis</i>	Bacterivore
Mononchida	Monochidae	<i>Clarkus</i> Jairajpuri, 1970	Predator
		<i>Monochus</i> Bastian, 1865	Predator

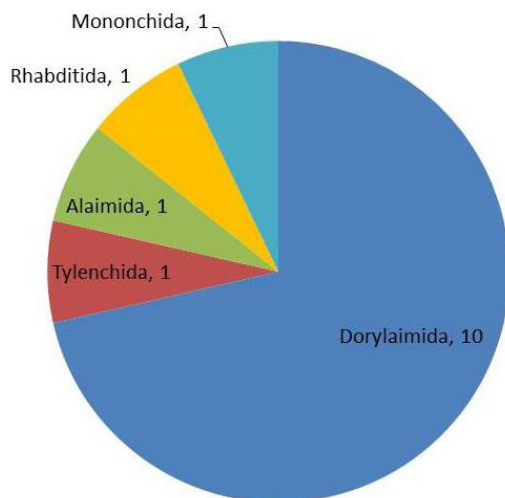


Figure 1. Dominance of orders of soil nematodes

conceived type of habitat and usually dominate both in numbers and in species over all other soil-inhabiting nematodes (Jairajpuri & Ahmad 1992). Dorylaimids and mononchids are more sensitive to disturbance (Forge & Simard 2001), therefore, they are used as indicators of environmental disturbances (Thomas 1978; Sohlenius & Wasilewska 1984). All these 18 genera are reported for the first time from this protected area. Genus *Dorylaimus* Dujardin was the most dominant among

all (Figure 2) followed by *Xiphinema* Cobb, *Tylenchus* Bastian, *Longidorus* Micoletzky, and *Longidorella* Thorne. Genera like *Sicaguttar* Siddiqi, *Qudsinema* Jairajpuri, *Microdorylaimus* Andrassy, *Longidorella* Thorne, *Paralongidorus* Siddiqi, *Fuscheila* Siddiqi, and *Chrysonema* Thorne are reported for the first time from the state. Lizanne & Pai (2014) reported 69 species belonging to 48 genera. The addition of these eight genera will take the tally to 56 genera for the state of Goa. On assigning 18 genera to the trophic grouping using secondary data collected (Neher & Weight 2013; Vaid et al. 2014), trophic groups reported were plant parasites, predators, and omnivores (Table 1). Plant parasites were the most dominant (five genera) followed by predators (four genera), omnivore (three genera), and bacterivores (two genera). In terms of number, omnivores dominated the area (Figure 3) followed by predators. According to Vaid et al. (2014), the abundance of predators is uncommon in forest ecosystems and is clearly due to the absence of anthropogenic activities.

CONCLUSION

This is a preliminary study on this forest, more such intensive survey in the sanctuary will yield more species of nematodes.

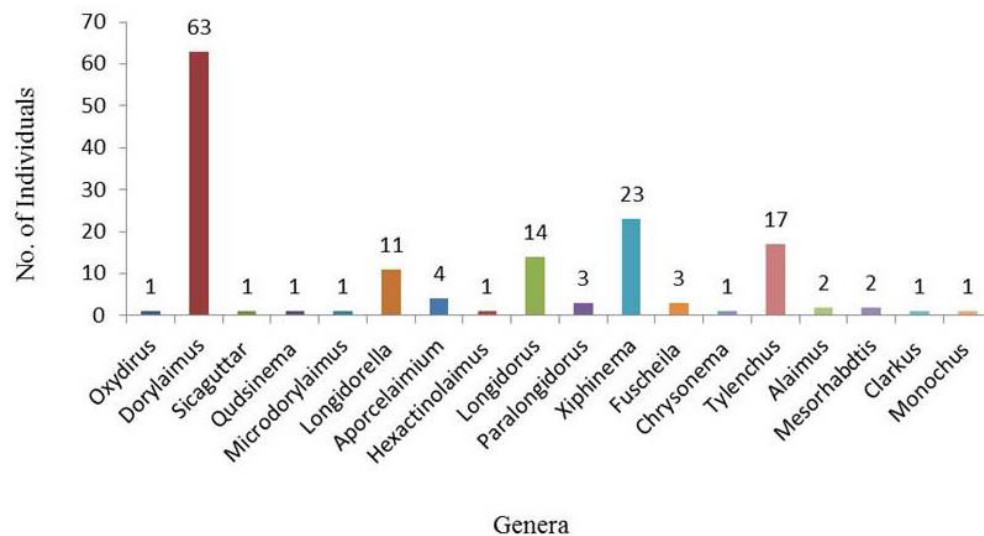


Figure 2. Genera-wise dominance of soil nematodes

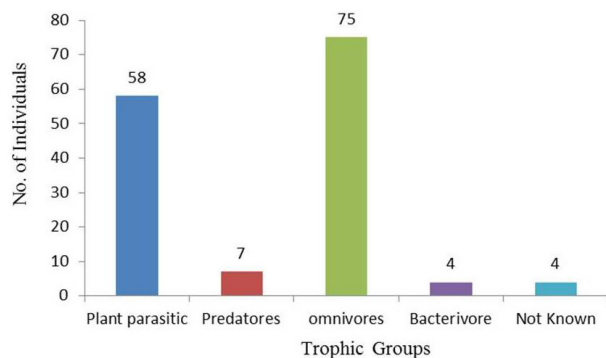


Figure 3. Number of individuals of soil nematodes as per trophic groups

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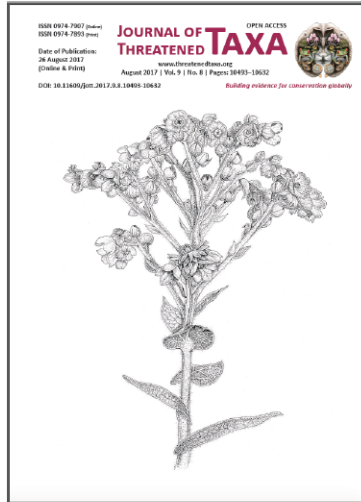
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NOTE

A CHECKLIST OF BUTTERFLIES (INSECTA: LEPIDOPTERA) FROM TALEIGAO PLATEAU, GOA, INDIA

Dipak Bowalkar, Nadar Anal Gracy Michael, Kiran Gaude & I.K. Pai

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Plateaus are characteristic features of Goa (Alvares 2002). They are intermediate areas between the Western Ghats and the coastal plains and are known to harbor endemic plants of the Western Ghats (Joshi & Janarthanam 2004). The most prominent plateaus in Goa are Pernem, Mopa, Morgim, Assonora, Ponda, Kundaim, Betul, Sanvordem and Quepem. Plateaus are often considered as barren lands and hence they were the natural choice for setting up developmental projects (Alvares 2002; Desai & Shanbhag 2012). Taleigao plateau (Fig. 1) is not an exception to this and several state institutions, hostels and residential areas have been set up in this area. It covers an area of about 296ha with moist deciduous forest mixed with evergreen species, scrub jungle and lateritic vegetation and is surrounded by sloping valleys and alluvial plains of two rivers—Mandovi in the north and Zuari in the south (Desai & Shanbhag 2012). This plateau encompasses Goa University campus spanning an area of 173ha, residential buildings and Dr. Shyama Prasad Mukherjee Indoor Stadium. With regards to the biodiversity of Taleigao plateau, the flora (Joshi & Janarthanam 2004) and avifauna (Shanbhag & Gramopadhye 1993; Shyama & Gowthaman 1995; Desai & Shanbhag 2012) is well documented.

Gaonkar (1996) documented 251 species from the state. Subsequently, Pai & Mehndiratta (2001) have documented 52 species. Later Borkar & Komarpant (2004) reported 97 butterfly species from Bondla Wildlife Sanctuary. Recently, Gaude & Janarthanam (2015) reported 33 butterfly species from four sacred groves of Goa, viz., Nirankarachi Rai, Alvatiniichi Rai, Mharinginichi Rai and Azobachi Rai. Rangnekar (2007)

in his photographic guide dealt with common butterfly species of Goa, though he did not mention the total number of species. Recently Rangnekar & Dharwadkar (2009) reported three new butterfly species, Black-Vein Sergeant *Athyma ranga* Moore, White-banded Awl *Hasora taminatus* (Hubner) and Coon *Psolos fuligo* (Mabille), making a total of 254 species to the butterfly fauna of Goa. However, there is hardly any report of butterfly diversity from this regions. It was in this context that the present work was undertaken.

Field investigations at Taleigao plateau (Fig. 1) at 15.4588333 N & 073.8340556 E carried out from June 2014 to July 2015. During the study period Sunday mornings between 07:00–10:30 hr were utilized for the study purpose. The butterflies were documented by direct observation, random walks and opportunistic sightings (Murugesan et al. 2013). Visually encountered butterflies were identified on the field using photographic guides of Rangnekar (2007) and Kehimkar (2008).

A total of 98 species belonging to 72 genera were recorded (Table 1), which constitutes about 39% of the known butterfly fauna for the state. This includes 34 species of Nymphalidae, followed by Lycaenidae (25

A CHECKLIST OF BUTTERFLIES (INSECTA: LEPIDOPTERA) FROM TALEIGAO PLATEAU, GOA, INDIA

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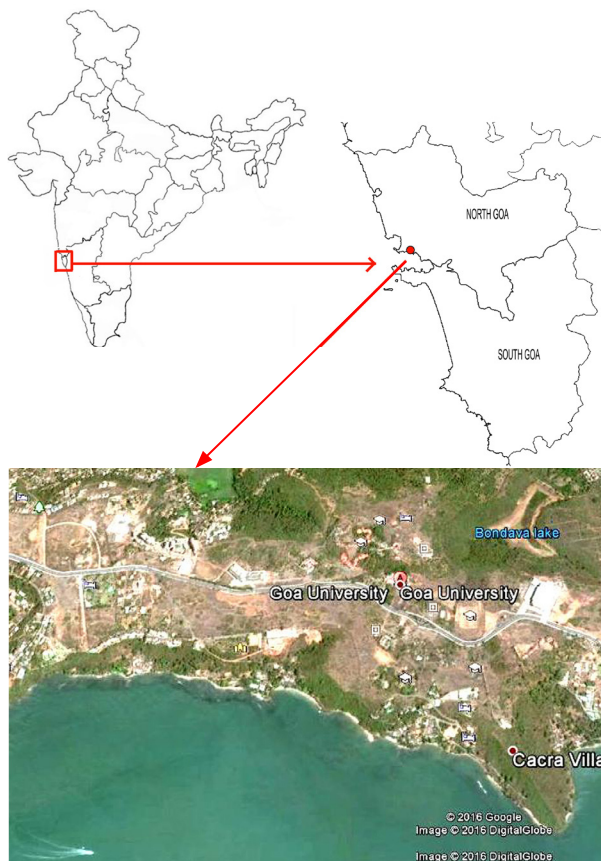


Figure 1. Study Area - Taleigao Plateau

species), Hesperidae (16 species), Pieridae (13 species), and Papilionidae with 11 species. Of the 98 butterfly species, two species, the Malabar-banded Peacock *Papilio buddha* (Image 1e) and the Southern Birdwing *Troides minos* (Image 1h) are endemic to the Western Ghats and 10 species, viz., Southern Birdwing, Crimson Rose *Altrophaneura hector* (Image 1d), Common Pierrot *Castalius rosimon* (Image g), Danied Eggfly *Hypolimnna misippus*, Pea Blue *Lampides boeticus*, Gram Blue *Euchrysops cnejus* (Image 1f), Common Cerulean *Jamides celeno*, Common Wanderer *Pareronia valeria* (Image 1c), Common Gull *Cepora nerissa* (Image 1b), Common Crow *Euploea core* (Image 1a) are protected under the Indian Wildlife (Protection) Act (1972). Of these, *Troides minos*, *Altrophaneura hector*, *Castalius rosimon*, *Hypolimnna misippus* have been placed as Schedule I; *Lampides boeticus*, (*Euchrysops cnejus*), *Jamides celeno*, *Pareronia valeria*, and *Cepora nerisa* in Schedule II and *Euploea core* under Schedule III species.

Family Nymphalidae was the most dominant among the families reported. Availability of larval host plants and adult nectar plants could be one of the reasons for its dominance (Murugesan et al. 2013). Different authors

in their respective studies observed a similar pattern of dominance (Kunte 1997; Kunte et al. 1999; Eswaran & Pramod 2005; Dolia et al. 2008; Krishnakumar et al. 2008; Gaude & Janarthanam 2015). Plateaus in Goa are known for their rich floral diversity (Joshi & Janarthanam 2004). In the present study, family Lycaenidae was the second largest family, with 25 butterfly species; Nimbalkar et al. (2011) got similar results. It is known that members of Lycaenidae largely feed on grasses (Nimbalkar et al. 2011) and the vegetation of Taleigao Plateau is also dominated by herbs, shrubs and rough grass species interspersed with trees. At the study site grass species persist from June to late December, hence it could be a good host for the members of the Lycaenidae family. This is followed by the family Hesperidae with 16 species. This clearly indicates the importance of the plateaus for the members of the family Hesperidae. This plateau is infested with invasive plant species such as *Chromolaena odorata*, i.e., known for its high nectar production (Laxmi & Raju 2011) and *Lantana camara* that flowers throughout the year and is a good source of nectar for butterflies (Day et al. 2003), which could be some of the reasons for the wide assemblage of butterfly species.

Findings of the present study underline the importance of Taleigao plateau as a preferred habitat for butterflies. The presence of endemic and schedule butterfly species, viz., *Papilio Buddha*, *Troides minos*, *Altrophaneura hector*, *Castalius rosimon*, *Hypolimnna misippus*, *Lampides boeticus*, *Euchrysops cnejus*, *Jamides celeno*, *Pareronia valeria*, *Cepora nerissa*, *Euploea core* also indicates the importance of this plateau for butterflies. The management of landscape, as well as of their food plants, may help to maintain and increase the butterfly diversity on the plateau. In the present scenario, plateau after plateau has been encroached upon for various mega projects, which doesn't bode well for conservation of biodiversity of these unique habitats. It is imperative to carry out systematic studies on the flora and fauna on a number of plateaus in the region, identify them as protected sites, such that, these plateaus with grassland patches can be conserved.

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Image 1 a–h. (a) Common Crow *Euploea core*; (b) Common Gull *Cepora nerisa*; (c) Common Wanderer *Pareronia valeria*; (d) Crimson Rose *Altrophaneura Hector*, (e) Malabar-banded Peacock *Papilio buddha*; (f) Gram Blue *Euchrysops cnejus*; (g) Common Pierrot *Castalius rosimon*; (h) Southern Birdwing *Troides minos*

Table 1. Checklist of butterflies of Taleigaon Plateau

	Common name	Scientific name
	Family: Papilionidae	
	Subfamily: Papilioninae	
1	Common Blue bottle	<i>Graphium sarpedon</i> (Linnaeus)
2	Tailed Jay	<i>Graphium agamemnon</i> (Linnaeus)
3	Common Mime	<i>Chilasa clytia</i> (Linnaeus)
4	Malabar-banded Peacock #	<i>Papilio buddha</i> Westwood
5	Common Mormon	<i>Papilio polytes</i> Cramer
6	Red Helen	<i>Papilio helenus</i> Linnaeus
7	Blue Mormon	<i>Papilio polymnestor</i> Cramer
8	Lime Butterfly	<i>Papilio demoleus</i> Linnaeus
9	Common Rose	<i>Atrophaneura aristolochiae</i> (Fabricius)
10	Crimson Rose	<i>Atrophaneura hector</i> (Linnaeus)*
11	Southern Birdwing#	<i>Troides minos</i> (Cramer)*
	Family: Pieridae	
	Subfamily: Coliadinae	
12	Small Grass Yellow	<i>Eurema brigitta</i> (Cramer)
13	Common Grass Yellow	<i>Eurema hecabe</i> (Linnaeus)
14	Spotless Grass Yellow	<i>Eurema ta</i> (Boisduval)
15	Common Emigrant	<i>Catopsilia pomona</i> (Fabricius)
16	Mottled Emigrant	<i>Catopsilia pyranthe</i> (Linnaeus)
	Subfamily: Pierinae	
17	Small Salmon Arab	<i>Colotis amata</i> (Fabricius)
18	Great Orange Tip	<i>Hebomoia glaucippe</i> (Linnaeus)
19	Dark wanderer	<i>Pareronia ceylanica</i> (C. & R. Felder)
20	Common Wonderer	<i>Pareronia valeria</i> (Cramer)**
21	Common Gull	<i>Cepora nerisa</i> (Fabricius)**
22	Common Jezebel	<i>Delias eucharis</i> (Drury)
23	Psyche	<i>Leptosia nina</i> (Fabricius)
	Family: Lycaenidae	
	Subfamily :Miletinae	
24	Apefly	<i>Spalgis epius</i> (Westwood)
	Subfamily : Curetinae	
25	Indian sunbeam	<i>Curetis thetis</i> (Drury)
	Subfamily: Theclinae	
26	Large Oakblue	<i>Arhopala amantes</i> (Hewitson)
27	Yamfly	<i>Loxura atymnus</i> (Stoll)
28	Monkey Puzzle	<i>Rathinda amor</i> (Fabricius)
29	Common Silverline	<i>Spindasis vulcanus</i> (Fabricius)
	Subfamily: Polyommatae	
30	Angled pierrot	<i>Caleta caleta</i> Hewitson
31	Common pierrot	<i>Castalius rosimon</i> (Fabricius)*
32	Zebra Blue	<i>Leptotes plinius</i> Fabricius
33	Rounded Pierrot	<i>Tarucus nara</i> Kollar
34	Common Cerulean	<i>Jamides celeno</i> (Cramer)**

	Common name	Scientific name
35	Forget me not	<i>Catochrysops strabo</i> (Fabricius)
36	Pea Blue	<i>Lampides boeticus</i> (Linnaeus)**
37	Dark Grass Blue	<i>Zizeeria karsandra</i> (Moore)
38	Pale Grass Blue	<i>Pseudozizeeria maha</i> (Kollar)
39	Lesser Grass Blue	<i>Zizina otis</i> (Fabricius)
40	Tiny grass blue	<i>Zizula hylax</i> (Fabricius)
41	Indian Cupid	<i>Everes lacturnus</i> (Godart)
42	Red pierrot	<i>Talicauda nyseus</i> (Guerin-Meneville)
43	Quaker	<i>Neopitheops zalmora</i> (Butler)
44	Common Hudge Blue	<i>Acytoplepis puspa</i> (Horsfield)
45	Gram Blue	<i>Euchrysops cnejus</i> (Fabricius)**
46	Plains Cupid	<i>Chilades pandava</i> (Horsfield)
47	Suffused double banded Judy	<i>Abisara bifasciata suffuse</i> (Moore)
48	Dakhan Common Acacia Blue	<i>Surendra quercetorum bipagiata</i> Butler
	Family : Nymphalidae	
	Subfamily : Danainae	
49	Blue Tiger	<i>Tirumala limniace</i> (Cramer)
50	Dark Blue Tiger	<i>Tirumala septentrionis</i> (Butler)
51	Stripped Tiger	<i>Danaus gnutia</i> (Cramer)
52	Plain Tiger	<i>Danaus chrysippus</i> (Linnaeus)
53	Glassy Tiger	<i>Parantica aglea</i> (Stoll)
54	Common Crow	<i>Euploea core</i> (Cramer)***
	Subfamily: Charaxinae	
55	Common Nawab	<i>Polyura athamas</i> (Drury)
56	Black Raja	<i>Charaxes solon</i> (Fabricius)
	Subfamily: Satyrinae	
57	Common Evening Brown	<i>Melanitis leda</i> (Linnaeus)
58	Common Treebrown	<i>Lethe rohria</i> (Fabricius)
59	Common Palmfly	<i>Elymnias hypermnestra</i> (Linnaeus)
60	Common Bushbrown	<i>Mycalesis perseus</i> (Fabricius)
61	Dark Banded Bushbrown	<i>Mycalesis mineus</i> (Linnaeus)
62	Common Four ringed	<i>Ypthima huebneri</i> Kirby
	Subfamily: Heliconinae	
63	Towny Coster	<i>Acraea violae</i> (Fabricius)
64	Rustic	<i>Cupha erymanthis</i> (Drury)
65	Common Leopard	<i>Phalanta phalantha</i> (Drury)
	SabFaamily: Limenitinae	
66	Commander	<i>Moduza procris</i> (Cramer)
67	Common Lascar	<i>Pantoporia hordonia</i> (Stoll)
68	Common Sailer	<i>Neptis hylas</i> (Linnaeus)
69	Common Baron	<i>Euthalia aconthea</i> (Cramer)
70	Grey Count	<i>Tanaecia lepidea</i> (Butler)
	Subfamily: Biblidinae	
71	Angled Castor	<i>Ariadne ariadne</i> (Linnaeus)

	Common name	Scientific name
72	Common Castor	<i>Ariadne merione</i> (Cramer)
	Subfamily: Nymphalinae	
73	Painted Lady	<i>Vanessa cardui</i> (Linnaeus)
74	Blue Pansy	<i>Junonia orithiya</i> (Linnaeus)
75	Yellow Pansy	<i>Junonia hierta</i> (Fabricius)
76	Chocolate Pansy	<i>Junonia iphita</i> (Cramer)
77	Grey Pansy	<i>Junonia atlites</i> (Linnaeus)
78	Lemon Pansy	<i>Junonia lemonias</i> (Linnaeus)
79	Peacock Pansy	<i>Junonia almana</i> (Linnaeus)
80	Great Eggfly	<i>Hypolimnas bolina</i> (Linnaeus)
81	Daniad Eggfly	<i>Hypolimnas misippus</i> (Linnaeus)*
82	Autumn Leaf	<i>Doleschallia bisaltide</i> (Cramer)
	Family: Hesperidae	
	Subfamily :Coeliadinae	
83	Common Banded Awl	<i>Hasora chromus</i> (Cramer)
84	Brown Awl	<i>Badamia exclamationis</i> (Fabricius)
	Subfamily : Hesperinae	
85	Common banded redevye	<i>Gangara lebadea</i> (Hewitson)
86	Chestnut Bob	<i>Lambrix salsala</i> (Moore)
87	Giant redevye	<i>Gangara thyrus</i> (Fabricius)
88	Grass Demon	<i>Udaspes folus</i> (Cramer)
89	Pygmy Scrub hopper	<i>Aeromachus pygmaeus</i> (Fabricius)
90	Bush hopper	<i>Ampittia dioscorides</i> (Fabricius)
91	Tamil grass dart	<i>Taractrotera ceramas</i> (Hewitson)
92	Rice Swift	<i>Borbo cinnara</i> (Wallace)
	Subfamily : Pyrginae	
93	Golden Angle	<i>Caprona ransonnetti</i> (C. & R. Felder)
94	Fulvous piedflat	<i>Pseudocoladenia dan</i> (Fabricius)
95	Tricolour Flat	<i>Coladenia indrani</i> (Moore)
96	Common small Flat	<i>Tagiades jopetus</i> (Stoll)
97	Water snow Flat	<i>Tagiades litigiosa</i> Moschler
98	Indian Skipper	<i>Spialia galba</i> Fabricius

* - Schedule I; ** - Schedule II; *** - Schedule III;

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Article

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OP22 Lifestyle, Health and Environmental Quality

Linking Vegetation type, Soil Properties and Nematodes abundance from Moist-deciduous and Semi-evergreen forest type of Goa, India

Kiran Gaude and I. K. Pai

Department of Zoology, Goa University, 403206

Abstract

Understanding faunal diversity of any conservation or protected area is imperative as information gathered has fundamental and applied application. Goa is a small state with and it amidst the Western Ghats and bordered by Arabian Sea, contributing rich bio-diversity. It has five wildlife sanctuaries and one Bird sanctuary which provide legal protection to most of the areas. Unlike temperate region, the understanding of mineral soil diversity of nematodes in tropics is not quantified. Apart from few work no major contribution is come in the field of nematology except from South Goa district. Extensive faunal studies have been done in the Goa, but the underground biota (Nematode) has been neglected in most of the cases, apart a very few studies. These sanctuaries are part of the Western Ghats and it may incorporate wise diversity of soil nematodes. The Bhagwan Mahaveer Wildlife Sanctuary and the Bondla Wildlife Sanctuary have been selected for the study purpose. The objective of the study is to understand the relationship between vegetation type (Semi evergreen & Moist-deciduous forest type), soil properties and nematodes abundance. 1 Kg of soil samples were collected from Semi evergreen and Moist deciduous forest in a self-sealing plastic bag with a label containing necessary field information. The processing involved soaking the samples in freshwater for a few minutes and then collecting the nematode from these samples by Cobb's decanting and sieving method followed by the modified Baermann's funnel method. To analyse physio-chemical parameters 1 kg of soil samples were collected and then sun dried. After drying these samples were sieved to remove unwanted materials like stones, plant parts *etc.* Further soil analysis was carried out in Soil Testing Laboratory, Ela, Old Goa. Two Orders of nematodes Doryolimida and Monochida has been reported from the

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A Preliminary Survey Of Soil Nema fauna Of The Bhagwan Mahaveer Wildlife Sanctuary, Goa, India

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Abstract:
Soil-inhabiting nematodes are the most abundant Metazoans and are among important groups of invertebrates. A total of 30,028 species are known worldwide while about 2902 species of nematode are reported from India. Nematological research in India is primarily focussed on major crops and animal parasitic groups, while ignoring free living group in forest ecosystem. Understanding faunal diversity of any conservation or protected area is imperative, as information gathered has fundamental and applied application. Although an extensive faunal study has been carried out in Goa, underground biota has been neglected in most of the cases. In the present study, the Bhagwan Mahaveer Wildlife Sanctuary, a 240 sq km protected area east of Panaji Goa was selected as study area. The core area of the sanctuary covering 107 sq km was notified as Mollem National park in 1978 and the forest canopy is almost close. Soil collection and processing for Nematode extraction and identification was as per Lizanne and Pai (2014). The Physico-chemical parameters of soil was analysed as per standard testing procedure at KVK South Goa, which showed that the soil contains high deposits of phosphorous and macronutrient viz., Iron, Zinc, Copper and Manganese with an overall acidic pH. A total of 16 species belonging to 16 genera, 12 families and four orders were reported. Among four orders, Dorylaimida was most dominant one, which consists of 12 genera and nine families. Among the 16 genera Sicaguttur, Qudsinema, Microdorylaimus, Longidorella, Paralongidorus, Xiphidorinae, Fuscheila and Chrysonema are reported for the first time from the state. More such intensive survey will add more number of nematode species.

Keywords: Protected Area, Soil, Nema fauna

Use Of Some Antibiotics And Fertilizers In The Management Of Leaf Spot Of Ginger Caused By Phyllosticta Zingiberi Resistant To Carbendazim

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Abstract:
The minimum inhibitory concentration of carbendazim among 13 isolates of Phyllosticta zingiberi Ramkr. causing leaf of ginger, was different on the agar plates and on ginger plants. MIC on agar plates ranged from 2 to 9 % while it was 2 to 8 % on ginger plants. Isolate Pz-11 was highly resistant with resistance factor 4. Use of carbendazim in mixture with some agrochemicals such as, antibiotics (streptomycin, cefotaxime, cefixime and erythromycin) and fertilizers (NPK, potash and urea) showed inhibition of the growth of resistant isolate of Phyllosticta zingiberi causing leaf spot of ginger on agar plates whereas there was cent percent control of disease of ginger plants.

Keywords: Phyllosticta zingiberi, fertilizers, antibiotics, MIC, isolates.