Chapter -I

INTRODUCTION

Nematodes are a diverse group of round worms occurring worldwide, in almost every environment, ranging from hot springs to icy Antarctica and soil to marine or fresh waters. They are free living, parasitic or predacious animals, which include algae, bacteria, fungi, animal and plant tissues and other nematodes species as their food sources (Joshi Prabha.M 1971). Nematodes are microscopic, complex, diverse and ubiquitous organisms found worldwide in essentially all environments. They are also known as eelworms in Europe, Nemas in United States and round worms by zoologists.

The name nematode was derived from Greek word ‘nema’ (thread) and ‘oides’ (resembling). Nematodes are triploblastic, bilaterally symmetrical, unsegmented, pseudocoelomates, vermiform and colourless animals. The body cavity is permanently under pressure. Usually cylindrical and circular in cross section. Sexes are separate which pass through four larval stages.

Nematodes are lower invertebrate animals. They are highly diversified and perhaps the most numerous multicellular animals on the earth. Like insects, they are found in almost all types of biotypes and occur in unimaginable numbers and in a wide variety of shapes and sizes.

Nematodes are generally free-living in marine, freshwater or soil environments, but a large number of species are parasitic on different kinds of plants and animals. The parasitic species are of considerable agricultural, clinical and veterinary importance as pests of plants and parasites of Man and livestock respectively.

The plant parasitic nematodes are slender, elongate, spindle shaped or fusion tapering towards both ends and circular in cross section invertebrate that possesses digestive system, nervous system, excretory system, reproductive system and a set of longitudinal muscles but lack respiratory system and circulatory system as well appendages.

They represent a simple body organization with relative smaller number of cells and possess a system of inter and intracellular control like those encountered in higher
metazoan. The unsegmented body is covered with tough and resistant cuticle secreted by epidermal (hypodermal) cells. The combination of outer flexible cuticle, the underlying hypodermis and longitudinal muscles surrounding the fluid filled pseudocoelom, facilitate a unique form of locomotion (sinusoidal-type) in these roundworms. The somatic musculature consists of smooth muscle cells, which extend between the epidermal chords and are directly connected to nerve chords. Oral aperture is terminal and surrounded by lips with sensilla.

The digestive system consists of a feeding apparatus (stoma), pharynx, intestine and rectum, with modifications according to feeding habits and modes. The central nervous system comprises of a group of ganglia associated with nerve ring that usually encircles the pharynx (circum-pharyngeal). From the nerve ring, arise longitudinal nerves extending anteriorly and posteriorly, also connecting to the peripheral network in some groups.

The pseudocoelomic fluid balances the absence of circulatory and respiratory systems. Excretory system constitutes of renette cells or tubular excretory canals while reproductive system represents tubular gonads with gonoducts. In general, nematodes are a diaceous group, but exceptions exist in the form of hermaphrodites and intersexes. Females are generally oviparous or ooviviparous. Cleavage is determinate and post-embryonic growth involves four moults.

The nematodes hatch from the egg as J2 and continue to feed, molt and reproduce, extensively macerating and distorting the plant tissue. Once the plant is destroyed or winter arrives, the stem and bulb nematode juveniles arrest their development at the environmentally resistant J4 stage and overwinter. Fluffy masses of dried (cryptobiotic) Ditylenchus can be seen on the surface of bulbs and are known as "nematode wool." Once environmental conditions are favorable, the cryptobiotic J4 become active and their life cycle resumes (Neher D.A. 2001).

Inside the nematode there is an inner tube, the alimentary canal, which runs inside the nematode from head to tail. Between the alimentary canal and the body wall is fluid that provides pressure against the wall to maintain body shape and allow
movement. At the head of a plant-parasitic nematode is a hollow mouth spear (like a hypodermic needle) called a stylet.

The nematode uses this stylet to puncture plant cells, to withdraw food and also to secrete protein and metabolites that aid the nematode in parasitizing the plant. The stylet is connected to the pharynx that, in turn, is connected to the intestine. The intestine ends at the rectum in the female nematode and the cloaca in the male. Attached to the pharynx are three - five salivary glands which produce secretions that may be emitted from the stylet and that assist the nematode in plant invasion and parasitism. (*M R Khan, RK Jain and RV Singh* 2010).

In females the reproductive organs are used as traits for identification because the number of ovaries and the position of the vulva in the female nematode's body are easily seen under the light microscope, male nematodes have one or two testes and they are easily identified by the presence of spicules. Spicules are copulatory structures that are used during mating to guide, size of the head tail, and number and position of ovaries in the female. More slight characters may include number of lines on the nematode’s cuticle or the presence or absence of pore-like sensory organs. Nematodes feed on all parts of the plant, including roots, stems, leaves, flowers and seeds.

Nematodes feed from plants in a variety of ways, but all use a specialized spear called a stylet. The infectious stage of the stem and bulb nematodes is the fourth stage juvenile. This stage often enters emerging plant tissues below ground, but can crawl up stems in a film of water and enter shoots via buds, petioles, or stomata. Once in the host plant, they destructively feed as migratory endoparasites, molt into adults and reproduce. Nematodes are found at the bottom of lakes, rivers and at enormous depths in the oceans. Some species can with stand temperatures constantly below freezing point while others live in the waters of hot springs. By 1930 some 4,500 species of nematode had been described. This rose to 9,000 by 1950 and the present-day number of known species of nematodes is well over 15,000. The estimated number of existing species ranges from 500,000 to several million, but the truth is that nobody has much of an idea of their total number (*Reddy Parvatha* 1983).
Many species are important parasites of plants and animals. The most common groups, the plant parasitic nematodes are microscopic, vertically invisible to naked eye when in the soil and range from 0.2 to 0.4 mm length to about 30 µm diameters. They feed by using a spear-like structure; the stylet located on the head end to puncture the plant cells and withdraw plant juices. The presence of the stylet differentiates plant parasites from the free-living ones in the soil.

The nematodes are further categorized into those that feed from the outside of plant tissues (ectoparasites) and those that enter plant tissues to feed and live within (endoparasites), thus damaging the plant in number of ways. Feeding causes wounding and creates openings through which other pathogens may enter the plant. They also interrupt the vascular tissue, thus reducing the transport of water and minerals from the root system up to the leaves and stems of the plant. Symptoms of a nematode infested plant include: lesions, stubby, curled, or galled roots, and a reduced root system.

The first record of a plant parasite, the ear cockle nematode of wheat, dates back to 1743 when Needham observed *Anguina tritici* causing galls in wheat. It was described in 1799 by Steinbuch. In 1855 root-knot nematode, *Meloidogme* was discovered by Berkeley in Cucumber plants. The first record on the involvement of nematodes in disease complexes was reported in 1892, when Atkinson observed that cotton wilt (*Fusaroium oxysporum vasinfectum*) was more severe in the presence of root-knot nematode. Injuries (micro punctures) caused by presence of root-knot nematodes on surface play a key role in inciting many bacterial diseases (*Joshi Prabha, M. 1971*).

Although a living host is required by nematodes for growth and reproduction, most plant-parasitic nematodes contain large food reserves enabling them to survive for long periods of time even in the absence of a living host. Nematodes that feed shallowly on the root cortex or epidermis e.g. *Helicotylenchus* Pests and parasites often threaten agricultural production. Reduction in agriculture productivity on account of pests is alarming. The country is losing about 30% of its total agricultural production worth Rs.1.50 lakhs crores every year due to pests and diseases, according to Crop Foundation of India. (*MR Khan, RK Jain and RV Singh 2010*)
Annual crop losses due to these obligate parasites have been estimated to be about $78 billion worldwide and $8 billion for U.S. growers (Graham R. Stirling 2011).

Mechanical wounding (without nematodes) and the presence of Helicotylenchus increased bacterial wilt of carnations. Now this nematode has been found to cause considerable damage to many plants.

It is perfectly suited for puncturing the cells of the plant roots to facilitate passage of plant material through the narrow lumen of the stylet, on feeding the median bulb pumps digestive juices, excreted by the doro-oesophageal gland, through the stylet into the plant cell for predigestion of the cell contents. Plant parasitic nematodes need free water for locomotion and host finding. Below ground, within the soil environment, the relative humidity present at wilting point is more than adequate to allow movement. For survival in the absence of water and a host, plant parasitic nematodes have a survival stage.

This survival stage may be the egg, any of the four juvenile stages, or the adult, depending on the species. The survival stage is capable of slowing down its metabolism and may be capable of anhydrobiosis. Because of this adaptability, damp soils, including rehydrated soils from the driest deserts, may be found teeming with nematodes. Typical survival stages are best known in non-criconematina. Tylenchs infecting above ground plant parts may survive for many years in their stored host. Cyst-forming species, in the absence of a host, may survive in soil for more than 10 years (M. Baniyamuddin et al 2007).

Because nematodes are difficult or impossible to see in the field, and their symptoms are often non-specific, the damage they inflict is often attributed to other, more visible causes. Farmers and researchers alike often underestimate their effects.

A general assessment is that plant parasitic nematodes reduce agricultural production by approximately 11% globally (A.K Shrivastva and Shyam Singh 2004), reducing production by millions of tonnes every year.

As nematodes feed on a wide range of the soil organisms and are dependent on the continuity of soil water films for movement, their activities are largely controlled by
soil biological and physical conditions. Given the ease of recovering nematodes from soils and the ability to identify them to meaningful taxa or functional group, soil nematodes offer great potential for the use as indicators of biodiversity and for assessing the impact of changing land use on soil conditions. They may produce complex disease symptoms to the plants. Some nematodes have been known to be able to transmit viruses from one plant to another (*Longidorus sp.* and *Xiphinema sp.*).

In India the first report on plant parasitic nematodes can be traced back to 1901, when Barber (1901) reported an eel worm found infesting tea plantations. This was followed by Butler (1913) and Dastur (1930) who made further reports on nematodes of plants. The study of plant parasitic nematode receiving attention since few Das Gupta (1987) on pineapple and Samathanan and Chawala (1982) on soil and plant parasitic nematodes of hilly areas in Tamilnadu, Bilgrami *et al.* (1997) on community analysis of predaceous and free living nematodes, Mukherji *et al.* (2000) on rubber nurseries, Rama and Das Gupta (2000) on coconut and arecanut Sundararju (2006) on banana plantation of Andhra pradesh. Devi (2007) on pine apple in Megalaya, Rao *et al.* (2007), Patel *et al.* (2000) on agricultural crops in Junagadh of Gujarat and vegetable crop of Andhra Pradesh; and Naidu *et al.*, (2007) and Kadela (2008) in Kawas of Rajasthan (*Reena sahu2012*). From India in Manipur, such work had been done on mulberry by Lokapam Bina (2012) and S. B. Avhad (2014) from Gangapur mulberry farm (Aurangabad), on citrus by Lokesh Zalpuri *et al.* (2013) from Jammu on hill trees by Puneet Kumar (2012) from North India, But no such precise work on the soil and plant parasitic nematodes of *Ficus plants*. So the present study was carried out to fill the lacuna.

*Ficus carica* is commonly referred as “Fig”. Various parts of the plant like bark and leaves are important. The fig is a very nourishing food and used in industrial products. It is rich in vitamins, mineral elements, water, and fats. Figs are one of the highest plant sources of calcium and fiber. According to USDA data for the Mission variety, dried figs are richest in fiber, copper, manganese, magnesium, potassium, calcium, and vitamin K, relative to human needs. The genus, Ficus, consists of over 800 species and is one of about 40 genera of the mulberry family, Moraceae. There is
significant genetic diversity among different varieties of fig, which contain remarkable pharmacological activities and are of commercial importance. Literature survey indicated that figs have been cultivated over 1100 years and these are among the earliest cultivated plants for human use (I.M. de O. Abrantes et al 2008).

Ficus carica Linn belongs to the family Moraceae. It is commonly known as Anjeer, which is a medium tree widely distributed in sub-tropical and tropical countries. It has been used as fruit and medicine for several centuries (Jim Kamas et al 2000). Phytoconstituents like flavonoids, phenolics, fatty acids, proanthocyanidine, Phytosteroles (campesterol, stigma sterol, and sitosterol) xanthotoxin, psoralens, bergapten, and xanthotol have been extracted from leaves and fruits, and peptides from latex. The fruit extract possess hypoglycemic, diuretic, antioxidant, immunity, hepatoprotective activities, latex has anthelmintic and anticarcinogenic activities. In English the word “fig” means giving care about something. The word ficolin, which appears similar to Ficus and refers to a lectin like compound combining the first parts of the words for fibrinogen and collagen Ficus is a genus of about 850 species of woody trees, shrubs, vines, epiphytes and hemi – epiphytes in Moraceae family. Its english name is Fig and common name is anjeer they are native throughout the tropics with a few species extending into warm temperature zone Fig is a temperate species from middle east (mostly ukraine) which has been wildly cultivated from acient time for its fruite and its constitute extremly important food resource for wild life (Sadhu M.K. 1990).

Fig are also of paramount cultural important through out the tropics, both as object to worship and for their many practical uses. Figs occupy a wide variety of ecological niches. Take for example, the common fig, a small temperate deciduous tree whose fingered fig leaf is well known in art and iconography or the weeping fig ficus carica. is ordinarily deciduous and commonly referred to as “FIG” (F. carica ) belongs to the order of Urticales and family of Moraceae with over 1400 species classified into about 40 genera The common fig is a tree native to southwest Asia and the eastern Mediterranean, and it is one of the first plants that were cultivated by humans (Jim Kamas et al 2000).
The Fig is an important harvest worldwide for its dry and fresh consumption. Fig (ficus carica) commonly called anjeer, is of high medicinal value. Its fruit, root, and leaves are used in traditional medicine to treat various ailments such as gastrointestinal (colic, indigestion, loss of appetite, and diarrhea), respiratory (sore throats, coughs, and bronchial problems), and cardiovascular disorders and as anti-inflammatory and antispasmodic remedy (Shukranul Mawa et al 2013).

In India, Maharashtra ranks first in the production of the fig. The fig is cultivated in 2242 hectares of land in Maharashtra especially in the districts of Pune, Nasik, Aurangabad and Ahmednagar. Profit of Fig production is estimated Rs. 2.5 lakhs per hectare. In other fruits this is about Rs. 1 to 1.5 lakhs its cultivation is one of the fastest growing agricultural crop, becoming an agricultural industry in and around Aurangabad.

In spite of its economic and nutritive importance, progress in research on the fauna of nematode would be worth while to identify some of the Lacunae which required depth studies in future research programme. It is apparent that very little is known about the nematodes with particular references to those found in this region, crop losses by plant parasitic nematodes.

The problem caused by nematodes in fig tree (Ficus carica) are reduction in growth yield, roots have distinctive swelling called galls. Study of nematodes in relation to fig has its strength in agricultural application because of the economic implication it would be worth while to identify some of the Lacunae which required depth studies in future research programme.

According to Khan and Jain (2010), 73 million loss of crop (national level) due to these lacunae. It is apparent that very little is known about the nematodes with particular references of those found in the region, crop losses by plant parasitic nematodes in relation to fig has its strength in agricultural application because of the economic implication. The symptoms are indicative of the nematodes problem it was found nematodes cause damage a considerable loss to fruit yield stunting and yellowing of leaves defoliation poor fruiting and die back.
Plant effected by nematode appear sick with fungal look due to nutrient deficiency. Lesion nematodes infested trees may appear stunned with very few feeder roots the problem caused by nematodes are, no doubt of varied kind and must have existed in our cultivated crops for a long time but very limited studies have been conducted so far and precise information is yet available. Basic studies specially identification of nematodes is of vital importance. To develop and suggest appropriate control measures.

Present work distributed into following parts

- An introduction about the topic.
- Review of the work done in relation to present study.
- Topography of selected sites.
- Material and method.
- Taxonomy of identified species by identification key given by DeLey and Blaxter (2002). Identification up to generic level was done mainly using Goodey (1963); Jairajpuri & Khan (1982); Andrássy (1984); Siddiqi (1986); Jairajpuri & Ahmad (1992); Ahmad (1996). Trophic groups were allocated according to Yeates et al. (1993), Bongers (1990).
- Result and discussion in relation to physicochemical parameter of soil, prevalence of incidence.
- Summary
- References and Bibliography

The taxonomy part represents a study of the nematodes belonging to the orders Tylenchida, Doryloimeda, Monochida. Samples were collected from fig orchards and different residential areas. The nematodes were isolated by Cobb’s (1918) sieving and decantation and modified Baermann’s funnel techniques. The extracted nematodes were examined under stereoscopic microscope. Nematodes were simultaneously killed and fixed in hot FA fixative. Later, they were transferred into a mixture of glycerine alcohol and kept in a desiccator for dehydration. Dehydrated nematodes were mounted in anhydrous glycerine on glass slides and sealed under a
circular glass cover slip using nail polish and stick fast as sealing material. All measurements were made on specimens mounted in dehydrated glycerine with an ocular micrometer. De Man’s (1884) formula was used to denote the dimensions of nematodes.

All morphological observations and drawings were made on camera lucida, microscope and photograph was taken by Panasonic linux camera mounted on stereo microscope. In all eighteen species belonging twelve genera, falling under three orders five sub- orders nine supper families, eight families and nine sub families have been described. Of these species have been described and illustrated. One known species is being reported for the first time from Aurangabad. The terminology used in the text to describe the parts of stoma is of De Ley.

Among the taxonomic groups recorded, the order was the most dominant in terms of individual abundance as well as in generic diversity. Root knot nematodes are difficult to control and can be spread easily from garden to garden in soil (for example, on tools, boots, etc.) and plant parts. Root knot nematodes survive from season to season primarily as an egg in the soil. After the eggs hatch, the second stage juveniles invade roots, usually at root tips, causing some of the root cells to enlarge where the nematodes feed and develop. Root knot nematodes usually cause distinctive swellings, called galls, on the roots of affected plants. The nematodes feed and develop within the galls, which may grow to as large as 1-inch in diameter on some plants.