WORK REPORT ON STUDENT INDUSTRIAL WORK EXPERIENCE SCHEME (SIWES)

UNDERTAKEN AT

THE INTERNATIONAL INSTITUTE OF TROPICAL AGRICULTURE, IDI-OSE,

OFF OYO EXPRESSWAY, IBADAN.

BY

OLASUPO TOMIWA EMMANUEL

MATRIC NO: 222370

THE DEPARTMENT OF BOTANY, FACULTY OF SCIENCE

UNIVERSITY OF IBADAN

SUBMITTED TO INDUSTRIAL TRAINING COORDINATING CENTRE (ITCC),

UNIVERSITY OF IBADAN.

JULY 2024.

Department Of Botany,

Faculty of Science,

University Of Ibadan.

10th JULY, 2024.

The Director,

Industrial Training Coordinating Centre (ITCC),

University Of Ibadan.

Dear Sir,

SUBMISSION OF INDUSTRIAL TRAINING WORK REPORT

I, OLASUPO TOMIWA EMMANUEL, a 300-level student in the Department of Botany with the matriculation number 222370 hereby present a work report which contains the expertise I acquired during my industrial training at the Nematology Unit, International Institute of Tropical Agriculture.

Thank you for the privilege of being part of this program.

Yours faithfully,

OLASUPO TOMIWA.

ACKNOWLEDGMENT

I appreciate God almighty for the gift of life, protection and strength to successfully go through my industrial training. I want to specially appreciate my family members for their support and encouragement all throughout the program. I also appreciate the Industrial Training Coordinating Centre for this initiative which has opened my eyes to the practical aspects of my course of study and also the work environment.

I also appreciate everyone that contributed to the success of my industrial training one way of the other: My lecturer – Dr Popoola for securing a spot for me at the institute, my unit supervisor – Dr Omowunmi Adewuyi for the guidance and teachings given to me and my colleagues to help shape our skills and carry out assigned tasks. However, my training wouldn't have been possible without the help of other skilled and casual staff with whose experience I was able to learn more and maneuver difficult situations.

Lastly, I would like to appreciate my fellow IT students from University of Ibadan and the friends I made from other schools for their cooperation and support.

TABLE OF CONTENT

ABSTRACT

This report is based on my experience working at the International Institute of Tropical Agriculture (IITA), Ibadan, in the Nematology/Striga Unit, under the Students Industrial Work Experience Scheme.

It spotlights nematodes, a broad class of worm-like creatures that can be found both as free-living animals and as parasites in nearly every kind of habitat. Because they are notoriously hard to manage, nematodes seriously jeopardize the yields of sustainable agriculture. In comparison to temperate conditions, these difficulties are more severe in tropical regions, which makes it more difficult to identify and understand individual nematode species and groups.

During my time at the Nematology Unit, I gained hands-on experience in culturing nematodes, observing their effects on plants, extracting nematodes from plant and soil samples, preserving and identifying different nematode species, and implementing various control methods.

INTRODUCTION

SIWES: The Students' Industrial Work Experience Scheme is a skills training program designed to prepare students of universities and other tertiary institutions for the work environment they are going to meet after graduation. The main objective of the program is to bridge theory with practice by making it possible for students to get themselves exposed to the practical aspects of their courses of study, jobs in their various field and the work environment.

IITA: The International Institute of Tropical Agriculture (IITA) is a non-profit institution that generates agricultural innovations to meet Africa's most pressing challenge of hunger, malnutrition and poverty. Since 1967, IITA has worked with various international and national partners, to improve livelihoods, enhance food and nutrition security, increase employment and preserve natural resource integrity.

IITA is guide by an ambitious strategy to lift 11.5 million people out of poverty and revitalize millions of hectares of farm land. As one of the 15 research centers in the Consultative Group on International Agricultural Research (CGIAR) which is a global partnership for a food secure future, IITA is engaged in several research programs and has delivered more than 70% of the CGIAR's impact in sub-saharan Africa and is committed to science-driven improvement of agriculture and other food value chains.

Fig 1.0: IITA main entrance.

IITA as a research-for-development (R4D) organization has implemented programs that are focused on four areas: Biotechnology and genetic improvement, Natural Resource management, Social science and Agribusiness, Plant production and plant health. There are several units based on the major research crops undertaken by the the institute viz: Cassava Breeding Unit, Yam Breeding Unit, Banana/Plantain Breeding Unit, Cowpea Unit, Maize Breeding Unit, Soybean Breeding Unit; others include those based on crop protection and improvement: Pathology Unit, Virology Unit, Entomology Unit, Nematology/Striga Unit, Soil Microbiology Unit etc. Others include Agronomy Unit, Research Farm Unit, Crop Transformation/Utilization Unit, Genetic Resources Center, Communication/Publishing Unit, Bio-sciences Unit, Analytical Services Unit etc.

During the course of my SIWES program, The Nematology / Striga unit which falls under the forth areas in which IITA focuses on allowed me to have a practical experience on nematodes, its culturing, effects and various methods involved in extracting it from infested plants.

Having my Industrial Training at IITA was an awesome experience as I got the opportunity of learning new things and meeting new people from all over the world.

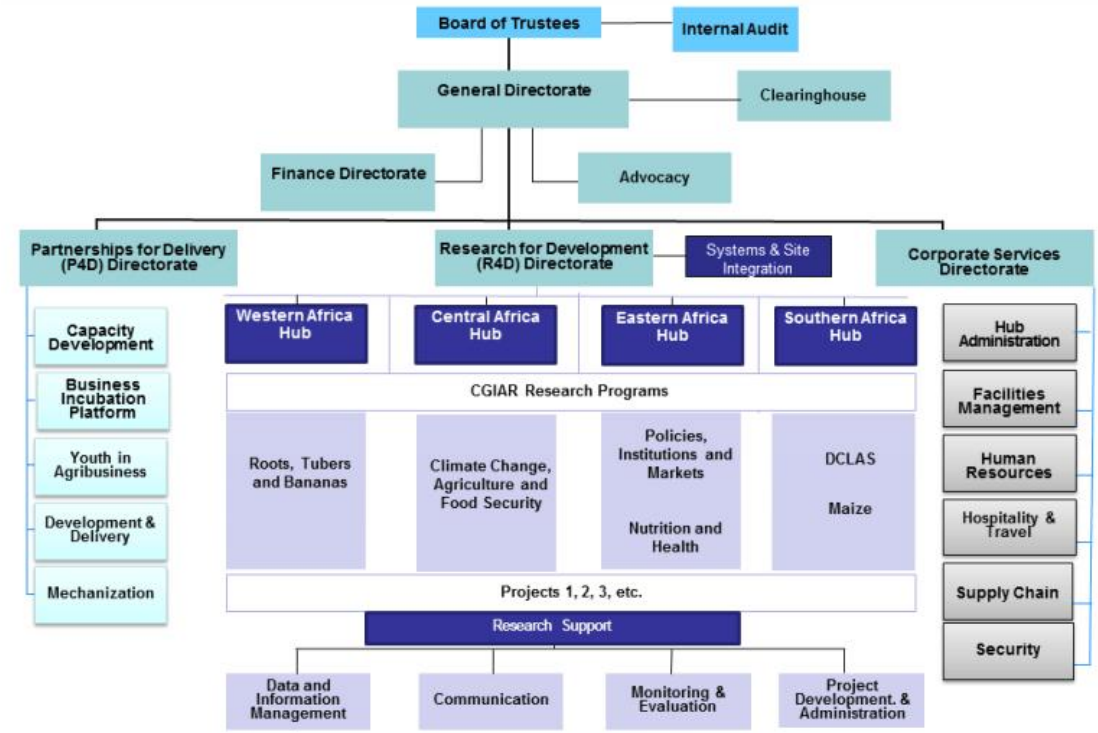


Fig 1.1: IITA Organogram

CHAPTER ONE

BIOLOGY OF PLANT PARASITIC NEMATODES

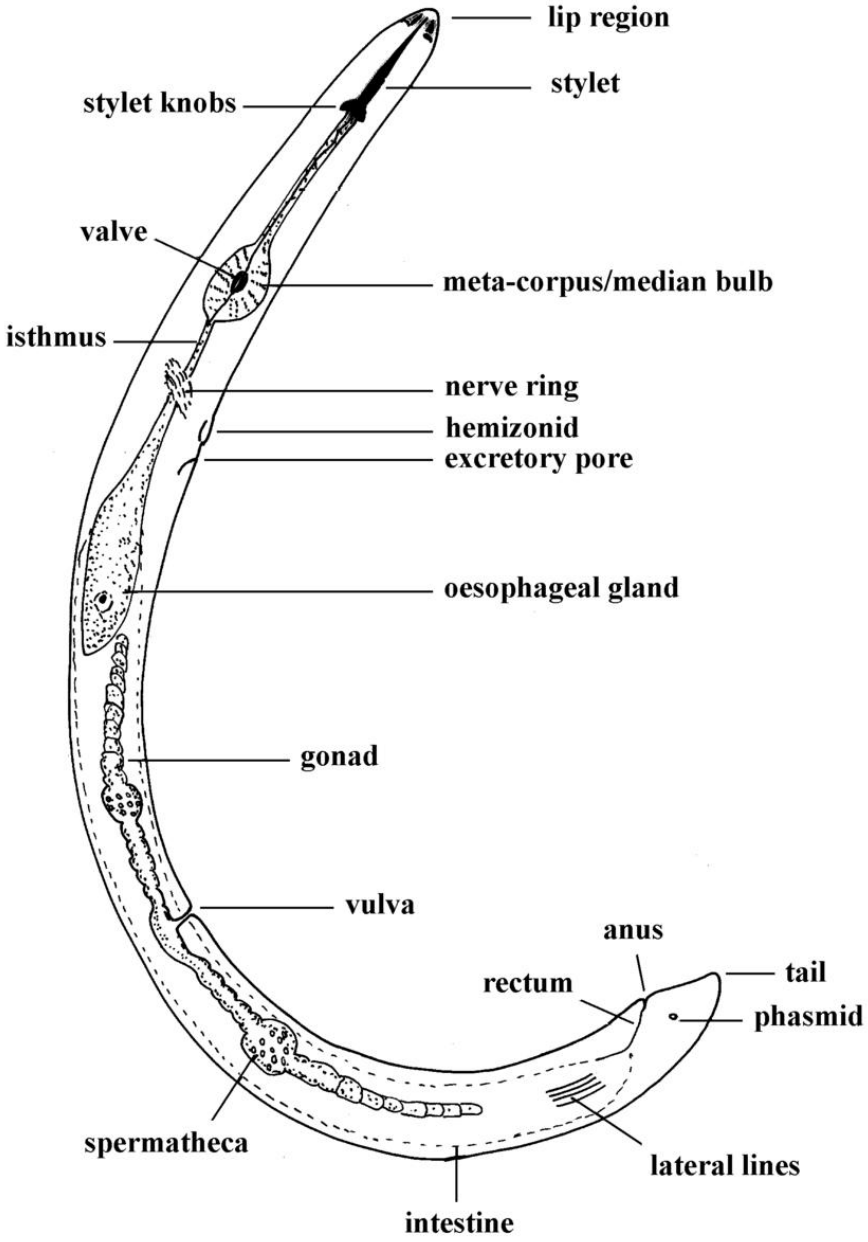
Nematodes are a diverse group of worm-like animals. They are found in virtually every environment, both as parasites and as free-living organisms. They are generally minute, but some species can reach several meters in length.

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 (Agrios, 2005), reducing production by millions of tonnes every year.

The amount of damage nematodes cause depends on a wide range of factors, such as their population density, the virulence of the species or strain, and the resistance (ability of the plant to reduce the population of the nematode) or tolerance (ability of the plant to yield despite nematode attack) of the host plant. Other factors also contribute to a lesser extent, including climate, water availability, soil conditions, soil fertility, and the presence of other pests and diseases. However, although we have some knowledge on the nematode–crop relationship and influencing factors, much remains to be learned. Damage thresholds for nematodes on various crops in various parts of the world, for example, are often unknown, and the threat nematodes pose often requires an educated guess.

Appearance and structure

Plant parasitic nematodes are mostly thread-like worms ranging from 0.25 mm to >1.0 mm long, with some up to 4.0 mm. Although most taper toward the head and tail, they come in a variety of shapes and sizes . Females of some species lose their worm-like shape as they mature, becoming enlarged and pear-, lemon- or kidney-shaped or spherical as adults. Like all animals, nematodes have circulatory, respiratory and digestive systems . Plant parasitic nematodes differ from nematodes that feed on bacteria and fungi in that they have a specialized feeding structure, the spear or stylet . This is used to inject enzymes into plant cells and tissues and then to extract the contents, in a similar way that aphids feed on plants.



Life cycle

The nematode life cycle is typically divided into six stages: the egg, four juvenile stages and the adult (Fig. 4). The duration of any of these stages and of the complete life cycle differs for different species, and also depending on factors such as temperature, moisture and plant host. Under favorable conditions in the tropics many species have relatively short life cycles, with several generations possible per season.

This can lead to rapid population build up from just one (if self-fertilizing) or two nematodes.

Nematodes can survive unfavorable conditions, such as a dry season or a cold winter. Different species survive best at different life stages, for example *Heterodera* species survive best as eggs encapsulated within cysts, *Ditylenchus* species as fourth stage juveniles, and *Anguina* species as second stage juveniles.

Types of Nematodes

Plant parasitic nematodes can be separated into aerial parasites

– those feeding on above-ground parts of plants and root and tuber parasites

– those feeding on below-ground parts.

They can also be grouped by their feeding behavior and motility into three main groups:

• Migratory endoparasites – These are mobile nematodes that feed inside the plant root tissue. All life stages of migratory endoparasitic nematodes are mobile except the egg. The nematodes burrow through the plant from cell to cell, or may leave the plant tissue in search of new feeding sites. Whilst feeding they commonly lay eggs both inside the plant cortical tissue and also in soil surrounding the root tissue. Damaged cells release toxins which kill neighboring cells, resulting in small spots or lesions of necrotic tissue. Root rot fungi and bacteria are often associated with infestations of migratory endoparasitic nematodes, which enter the plant tissues through areas damaged by nematodes.

• Sedentary endoparasites – These are nematodes that, once they have reached a feeding site inside the plant, cease to be mobile and feed from a fixed location.

Sedentary endoparasitic nematodes invade plant tissue usually as newly hatched second-stage juveniles – the 'infective' wormlike stage. They move through the soil to locate host roots, and then through the plant tissue to find a feeding site. At the feeding site the female develops, remaining permanently sited for the duration of her life. As she develops, her body swells to a spherical, lemon, kidney, or ovoid form. The nematode feeds on a relatively small number of cells, which are regulated by the

nematode with growth substances. Some groups (e.g. cyst and root-knot nematodes) cause ‘giant’ feeding cells to form in the host plant.

• Ectoparasites – These are nematodes that feed on the plant from the outside of the plant. Ectoparasitic nematodes feed externally, on the surface of the plant, usually on root hairs or cortical tissue. They are often found in high densities, but do not always pose a problem. However, they may cause serious damage if the plant is suffering from other biotic or abiotic stresses (e.g. fungal attack or low water availability). Examples of ectoparasitic nematodes are ring nematodes (*Criconemoides* spp.), spiral nematodes (*Helicotylenchus* spp.) and the aerial rice white-tip nematode *(Aphelenchoides besseyi*). It is well recognized that some ectoparasites transmit plant viruses, for example some species of dagger nematodes (*Xiphinema* spp.), needle nematodes (*Longidorus* spp.) and stunt nematodes (*Trichodorus* and *Paratrichodorus* spp.).

Symptoms of plants affected with Nematodes

Symptoms of nematode damage are found both above and below ground.

✧ Above-ground symptoms

Above-ground symptoms fall into two categories: those caused by aerial nematodes attacking foliage and those caused by root nematodes attacking plant roots.

Symptoms caused by aerial nematodes .These are often specific symptoms associated with the nematode pest and therefore may be diagnostic. They include:

• Gall formation, or abnormal swelling of seeds (e.g. *Anguina*) or leaves (e.g. *Cynipanguina*)

• Leaf stripe, bleaching and discoloration of leaves (especially in temperate climates) (e.g. *Aphelenchoides*)

• Swollen, crinkled and disorganized tissue growth (e.g. *Ditylenchus*)

• Internal stem necrosis, signified with a red ring (*Bursaphelenchus cocophilus*) • Inflorescence necrosis

• Chlorosis/browning of leaves (needles in pines) and eventual tree death (*Bursaphelenchus xylophilus*).

✧ Symptoms caused by root nematodes

Root nematodes almost always cause varying degrees of abnormal above-ground growth, but these symptoms alone are generally not enough to diagnose a root nematode problem. Most symptoms reflect or can be mistaken for other problems, such as reduced water uptake or disturbed mineral absorption. They include:

• Chlorosis (yellowing) or other abnormal coloration of foliage

• Patchy, stunted growth

• Thin or sparse foliage

• Symptoms of water stress, such as wilting or leaf rolling.

Below-ground symptoms

These are due to root nematodes, and may be specific enough to allow diagnosis of the root nematode problem. Uprooting of plants or excavation of roots is needed to observe symptoms. Symptoms include:

• Galling

• Shortened, stubby or abbreviated roots

• Root lesions

• Root or tuber necrosis, rotting or death

• Root or tuber cracking

• Cysts or 'pearly' root

• Deformed roots

• Altered root architecture.

Root galls Root galls are caused mostly by the root-knot nematodes (*Meloidogyne* spp.), although other nematodes such as *Nacobbus aberrans* may also cause galling . Feeding by some nematodes, such as *Xiphinema spp*., may result in swellings or less defined galls, often at the root tips.

CHAPTER TWO

NEMATOLOGY / STRIGA UNIT

The IITA Nematology / Striga unit, led by Dr. Omowumi Adewuyi, is tasked with conducting research on nematode-infested plants with the goal of developing innovations to control the effects of nematodes on agricultural produce, which is a threat to long-term crop production. The fundamental aim of IITA is to ensure Sub-Saharan Africa's future stability and food security. As a result, the unit conducts research on staple food crops afflicted by nematodes, such as banana and plantain, cassava, cowpea, maize, soybean, and yam. The unit also carry out research on Striga which are parasitic weeds that affects cereal crops in many parts of Africa. However, during the course of my SIWES program, the unit worked mainly on nematodes.

Fig 2.0: Outer view of the unit

The unit conducts crop protection and improvement research, analyzes samples, sterilizes soil sample, evaluates data, and promotes healthy planting practices. They support research fellows (MSc, PhD) and provide professional nematode control instructions to other divisions within the institute, as well as farmers. The unit also provides services such as soil sterilization, nematode disease detection and assessment, as well as capacity building programs for NYSC, IT students, and student excursions.

The unit has three sub division which are as follows:

- Screen Houses
- Laboratory
- Micro-plot

Screen Houses

These are enclosed buildings that shield the plants from unfavorable biotic elements mostly using net along the sides and nylon coverings. Here, experimental research is carried out which include culturing of nematodes by transplanting plants grown on nursery beds into pots with nematode-infested soil, measuring the plant's algorithm parameters, and collecting soil samples for study. It is significant to remember that when research is to be done on a particular plant, that plant is made available in various representative quantities, allowing us to have a broad understanding of the impact of nematodes on the plant.

Fig 2.0 : Picture showing one of the screen houses

Laboratory

This is where where scientific experiments, analysis, and research are carried out. Activities carried out in the laboratory include; extraction of nematodes from samples,

counting of nematodes, microscopic identification of various nematodes. The table below show various equipment used in the Laboratory

S/N	EQUIPMENT	USES
1	Microscope	For observing nematodes
2	Sterilizer	To purify materials used in the lab e.g petri dish
3	Pipette	To obtain extract containing nematodes
4	Weighing scale	To weigh the yield obtained from the screen house
5	Desktop	To record data obtained from research carried out
6	Vortex	To mix samples when necessary
7	Blender	To reduce root samples into smaller sizes
8	Desiccator	To dry samples under atmospheric pressure

Fig 2.0 : Picture showing the Laboratory

Micro-plot

This is a farm field where experiments are carried out. As opposed to the screen houses where plants are planted into pots, here plants are planted directly into the soil for experimental and research purposes.

CHAPTER THREE

NEMATODES ASSESMENT AND MANAGEMENT

Upon harvesting of the experimental plants from the screen houses and after noticing signs that point to a potential or probable nematode infestation, samples from the afflicted plants and the soil surrounding the roots are collected and taken to the lab for examination in order to identify the type of nematodes and their density. The next stage is to extract nematodes from the samples. This should be done as soon after collection has been rinsed and air dried (in case of root samples). There are many extraction techniques, however, the following were used during the course of my stay at the nematology unit:

- Sodium Hypochlorite (NaOCL) Extraction Method
- Pie pan Extraction Method

Sodium Hypochlorite Method

This method is used to extract nematodes and eggs from infected plant roots usingm liquid solution containing sodium hypo-chlorite like Jik or Hypo. This method of extraction is mainly used on *Meloidogyne spp* (Root Knot Nematode) due to the presence of the galls on the plant roots. This sodium hypo-chloride breaks the gelatinous matrix in which the eggs are enclosed during the extraction so as to be visible under microscope.

Equipment

Required equipment for sodium hypo-chlorite method of extraction are:

- Knife or scissors.
- Measuring cylinder.
- Conical flask.
- NaOCl solution.
- Water.

- Labelled cup.
- Stack of Sieves.

Procedures

- Collect the infected root.
- Chop the collected root into smaller sizes.
- Prepare the NaOCl solution (10ml of NaOCl and 90ml water).
- Shake the chopped roots in the solution inside a jar for 5 minutes.
- Pour the resulting solution into a stack of sieves (212, 90, and 25) µm.
- Apply water with pressure from the tap or wash bottle.
- Carefully wash and collect nematodes from the smallest sieve into a labeled cup.
- Then count under the microscope after adjusting the objective lens to a suitable magnification

Fig 2.0 Picture showing the measurement of 10ml of NaOCL

Pie pan Extraction Method

This method is sometimes also called the modified Baermann technique, or the Whitehead tray method.

Equipment required are:

• A domestic sieve

- A dish/tray/plate, slightly larger than the basket
- Extraction paper
- Permanent marker for labelling the sample
- Knife/scissors

Procedure for extraction of nematodes from soil samples using Pie pan extraction method

a) Remove roots from sample and place in a separate dish. Label.
b) Using a coarse sieve, remove stones and debris from soil and break up soil lumps.
c) In a plastic container (basin, bucket) thoroughly mix the soil sample. Remove a measure of soil (e.g. 100 ml).
d) Place extraction paper in the plastic sieve/basket (placed on a plastic plate) ensuring that the base of the sieve is fully covered by the tissue
e) Place the soil measured on the tissue in the sieve. It is important that the soil remains on the tissue paper as spillover results in dirty extractions.
f) Add water to the extraction plates . Take care to gently pour water into the plate (dish) and not onto the tissue paper or soil (between the edge of the sieve and the side of the tray).
g) Leave undisturbed for a set period (48 hours)
h) Nematodes from the soil or plant tissue will move through the tissue paper into the water below, resting on the tray/plate. After the extraction period, drain excess water from the sieve and the soil into the extraction.
i) Remove the sieve and dispose of plant tissue/soil. Pour the water from the plate into a labeled beaker (or cup), using a water bottle to rinse the plate.
j) Leave samples to settle .
k) For counting the nematodes in the extraction under microscope, reduce the volume of water by gently pouring off and then pick the extract onto slides for counting under microscope after adjusting the objective lens to a suitable magnification

Procedure for extraction of nematodes from root samples using Pie pan extraction method

a. Gently tap soil off the roots/tubers or rinse under a tap and then gently dab dry with tissue paper.

b. Chop the roots finely with a knife or scissors and place in a labeled dish .

c. Mix all chopped root material thoroughly. Remove and weigh a sub-sample (e.g. 5 g) of chopped root material using measuring scales

d. Place weighed sub-sample on the tissue paper in the labeled sieve/basket .

e. Follow the rest of the procedure for soil extraction above (steps g–k)

Fig 2 : Picture showing pouring of 100ml of soil into the extraction paper

CHAPTER FOUR

IDENTIFICATION OF NEMATODES

There are various means of nematodes identification such as the morphological, anatomical and molecular analysis. In International Institute of Tropical Agriculture Nematology Unit, morphological features used to identify the nematodes are:

- The shape of the head e.g. offset etc.
- The shape of stylet e.g. long, short, thin and thick.
- The gland overlapping e.g. ventral or dorsal etc.
- The vulva position e.g. 50%, 70% etc.
- The shape of the tail e.g. coniod, round etc

During the course of my training, different species of nematodes identified are as follow:

- *Pratylenchus spp*
- *Radopholus spp*
- *Scutellonema spp*
- *Hoplolaimus spp*
- *Helicotylenhus spp*
- *Meloidogyne spp*

Morphological Features of *Pratylenchus Spp*

- Head shape: Offset and low
- Stylet: Short and thick
- Gland overlap: Ventral

- Vulva position: 70-75%

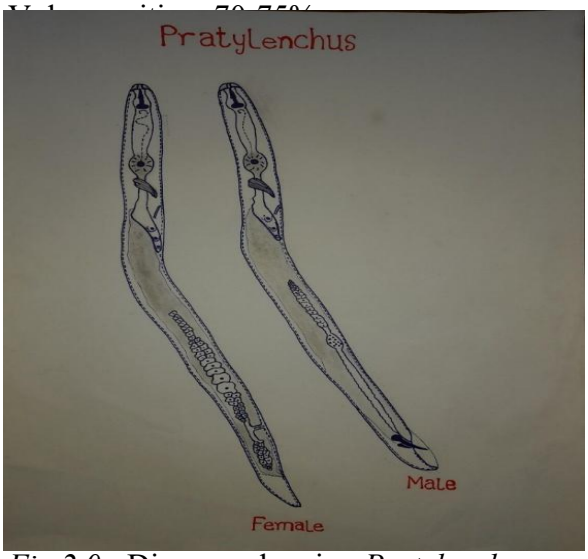


Fig 2.0 : Diagram showing *Pratylenchus spp*

Morphological Features of *Scutellonema Spp*

- Head shape: offset
- Stylet: Long
- Gland overlap: Dorsal
- Vulva position: 50%
- Tail shape: Rounded

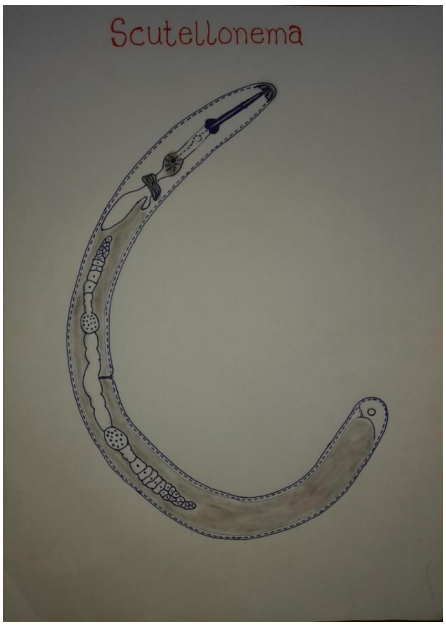


Fig 2.2: Diagram showing *Scutellonema Spp*

Morphological Features of *Hoplolaimus Spp*

- Head shape: Offset
- Stylet: Long and thin
- Gland overlap: Dorsal
- Vulva position: 50%
- Tail shape: Rounded

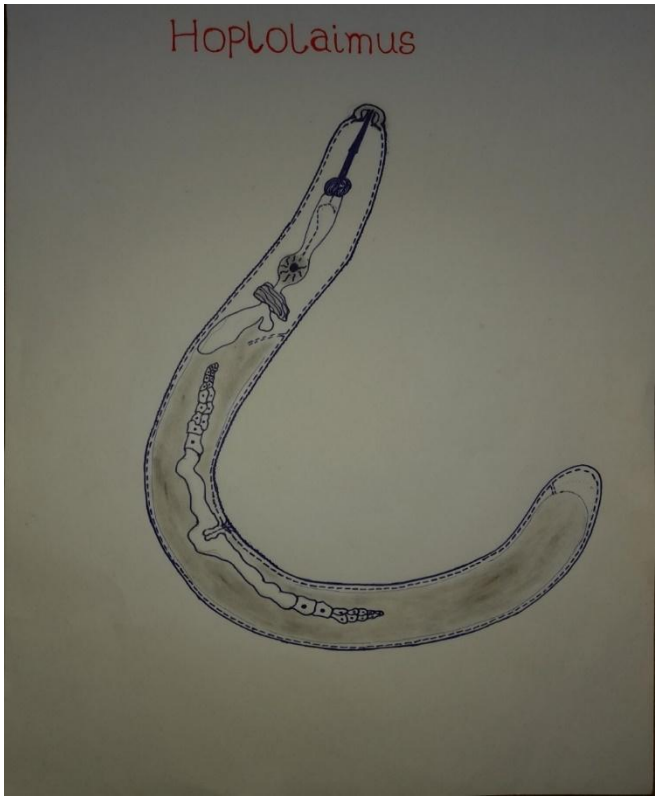


Plate 25. Hand drawing of Hoplolaimus.

Morphological Features of *Helicotylenchus Spp*

- Head shape: Offset
- Stylet : Long and thin
- Gland overlap : Ventral
- Vulva position: 70%
- Tail shape: Conoid

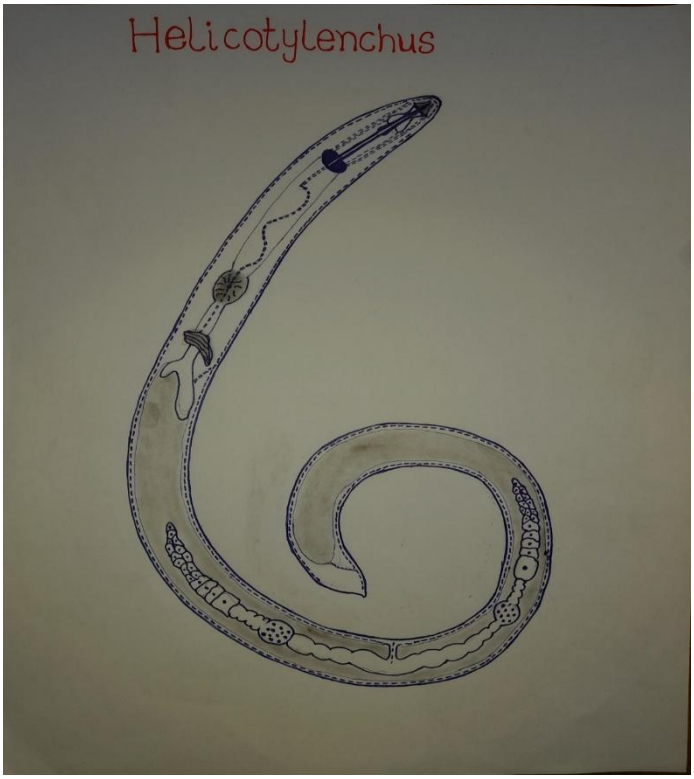


Fig 2.6. Diagram showing *Helicotylenchus Spp*

Morphological Features of *Radopholus Spp*

- Head shape: Offset (male), flat (female)
- Stylet: Short and thick
- Gland Overlap: Dorsal
- Vulva position: 50-60%

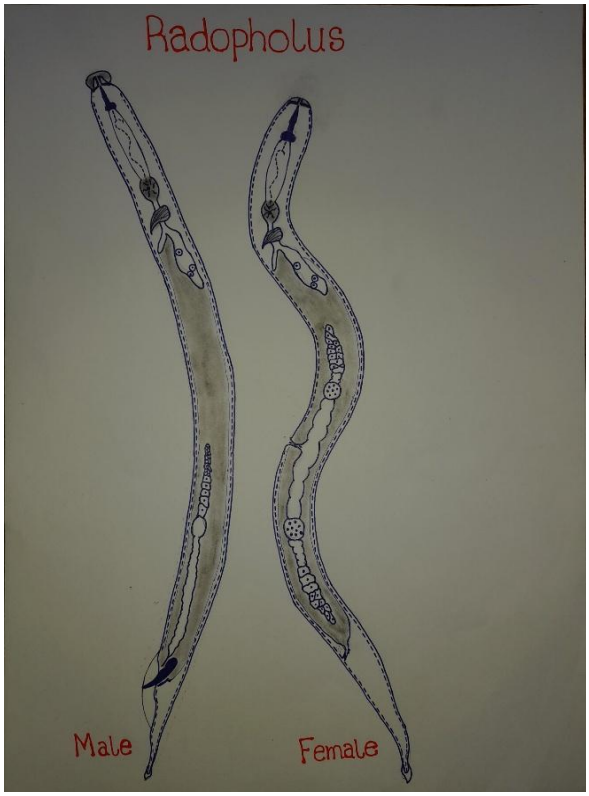


Plate 23. Hand drawing of Radopholus.

Radopholus spp exhibits sexual dimorphism (males are different from females)