Chapter 1: Introduction

1.1. Banana

Bananas (*Musa* spp.) are an important fruit in agriculture and to the world economy. This fruit provides carbohydrates, proteins, vitamin and minerals to more than 400 million people worldwide (Musabyimana *et al*., 1999). A banana plant is considered as a herbaceous perennial plant; herbaceous because this plant do not have any woody parts and after the ripening of the fruits, the aerial parts would wither and die while at the same time being perennial due to the fact that new shoots would sprout from the base mature banana and forming a new tree.

A mature banana plant can be divided into three integral parts, namely a corm, pseudostems with leaves and a bunch with fruits (Speiger *et al*., 1997). A corm is the underground part of a banana plant that consists of the roots and suckers. The corm is actually the true stem of the plant because it is the apical meristem or the growing point of the leaves which are developed from the tip of the corm. The corm is composed of a middle cylinder bounded by a cortex. Primary roots separate from the central cylinder and the cortex. Primary roots are formed in three to four waves and the majority of them are created within four months after planting. Primer roots grow horizontally all over the cortex and stay above 50 cm of soil. They are one centimeter thick and are three to four meters in length. A subsequent system of secondary and tertiary roots and root hairs would emerge from the primary roots.

Banana is the major the staple food crop for people in developing countries. It is produced mostly in the tropical countries across Asia, Africa and Latin America, with India leading the world production of banana and plantains in 2007 with 21.7 million metric tons (http://faostat.fao.org/site/339/default.aspx, retrieved July 2009). But like any other major crop, threats from natural disasters and diseases can cause significant loss to the industry. Diseases caused by fungal, nematode and virus infection as well as low tolerance of pathogens to pesticides prompts the need for comprehensive disease and pest management system (Raut *et al*., 2004).

1.2. What are nematodes?

One of the largest groups of animals that can live in the marine, fresh water and terrestrial habitats are nematodes. They are present at a variety of trophic levels and have important function in numerous ecosystems (Holterman *et al*., 2006). Nematodes have brief generation time, high density and incessant reproduction and also they are maintained and sensitive to numerous toxicants (Beyrem *et al*., 2007). At present, only 30,000 species of nematodes have been recognized. The greatest part of them is living free in soil and water. They feed on different microorganisms such as bacteria, algae, fungi, other nematodes and organic remains. 50% of them live in marine salt water and 25% can be found in freshwater and soil. From 25% leftover of the known species, 15% are parasitic in animal including in humans and insects while 10% are of plant parasitic nematodes. Plant parasitic nematodes are obligate parasites because in order to complete their life cycle, they have to feed on tissue of living plants at some point in their life cycle (Tylka *et al*., 2001).

1.3. Classification of plant nematodes

Phylum Nemata can be divided into two classes, Secernentea and Adenophorea. Class Secernentea contains the subclass Diplogasteria and placed under this subclass is the order Tylenchida. The order Tylenchida can be split into two suborders, Tylenchina and Aphelenchina. Further subdivision of the suborders Tylenchina would give rise to two super families, Tylenchoidea and Criconematoidea. The super family Tylenchoidea is comprised of six families, namely Anguinidae, Dolichodoridae, Belonolaimidae, Pratylenchidae, Hoplolaimidae and Heteroderidae, while the super family Criconematoidea consists of two families, Criconematidae and Tylenchulidae. The other suborder, which is suborder Aphelenchina on the other hand, is subdivided into a super family called Aphelenchoidoidea that includes the family Aphelenchoididae.

The class Adenophorea is divided into a subclass, Enoplia and this subclass is separated into two orders, Triplonchida and Dorylaimida. The order Triplonchida is divided respectively into suborder, Diphtherophorina, superfamily Trichodoroidea and family Trichodoridae. Order Dorylaimida is separated into suborder Dorylaimina, superfamily Dorylaimoidea and family Longidoridae (Kleynhans, 1999).

1.4. Physical structures of a nematode

Majority of nematodes are so tiny and to see them requires the use of a microscope. But many of animal parasitic nematodes are large enough to be observed easily with bare eye and without using a microscope. For example, the length of a nematode species that parasitizes on whales can reach up to 10 meters (Tylka *et al*., 2001). Nematodes are not

segmented like some worms and normally are colorless. They are long, cylindrical and tapered at both ends and normally do not have a distinct body cavity lined by epithelial tissues. The body structure of a nematode comprises of a body wall (that is composed of somatic muscles, cuticle and hypodermis), nervous, digestive, excretory and reproductive systems but at the same time lacks the typical circulatory, respiratory and endocrine systems. The whole of its outside surface cover with cuticle and also cuticle lines the buccal activity, rectum, vagina, esophagus, excretory hole and cloaca. Usually the cuticle is soft smooth but different structures may be present on it, such as bristles, spines, lumps, papillae and ridges. The innermost layer of the body wall is formed by somatic muscles. Somatic muscles appear in the form of spindles and have a role in body motion. The digestive system comprises three sections, which are the foregut, midgut and hindgut. The foregut region contains esophagus, stoma and esophagi-intestinal valve while in the hindgut, the parts that are present include rectum, valve and several other associated structures. Plant parasitic nematodes possess a stylet (which comprises a cone, shafts and knobs as well as a fine lumen that extends through the stylet) used to penetrate plant cell in order to feed. The muscles of nematodes have direct function in the feeding process because these muscles are connected to the knobs and control the tightening strength of the pointed cone in a host. In females, the hindgut region comprises a rectum, which ends in a form of a simple opening or anus. But in males, the digestive and reproductive system in the hindgut would merge and form a cloaca (Baldwin, 1978).

1.5. Biology of plant parasitic root nematodes

Plant parasitic root nematode can be categorized into three types based on their biological attributes. Ectoparasitic nematodes are parasitic worms that live outside of plant tissues. They feed by inserting their stylet into plant cell layers. Migratory endoparasitic are nematodes that attack plant tissues. This type of parasitic nematodes can move in and out of the plant tissue layers as they feed on the cells inside the plant. These parasites would also lay their eggs either within or outside the plant tissue layers. Most of plant parasitic nematodes that attack banana are of migratory endoparasitic. Another type of plant parasitic nematodes is called sedentary endoparasitic nematodes. In this case, these parasitic nematodes can attack the plant tissues and feed on a few special cells within the plant. Mature females would become sedentary and eggs are laid together outside of the plant in a single egg sac (Speiger *et al*., 1997).

Table 1.1: Categorization of plant parasitic nematodes

Types	Biology	Genus	Species
Endoparasitic	Migratory	Radopholus	*Radopholus similis*
		Pratylenchus	*Pratylenchus coffeae* *Pratylenchus goodeyi*
		Helicotylenchus	*Helicotylenchus multicinctus*
	Sedentary	Meloidogyne	*Meloidogyne incognita* *Meloidogyne javanica* *Meloidogyne arenaria* *Meloidogyne hapla*

1.6. Damage of nematode

Plant parasitic nematodes are the main pathogen accountable for the damage of a broad range of crops. They cause high crop production losses that are close to US$ 100 billion yearly in agricultural industry (Wang *et al*., 2007). Nematodes are the important pests of banana. They cause high losses in banana crop with estimated average in one year of about 20% of banana crop losses worldwide (Speiger *et al*., 1997).

Plant parasitic nematode can bring damage to plants via different ways. They can stunt the growth of the roots and confuse the root system and vascular tissue of the roots, resulting in decreased transference of water and minerals from the root system to the leaf and stems of the plant. Among the signs of nematodes feeding on the roots tissue are lesions on the surface of the roots, stubby and curling of roots, formation of galls on the root surface and decreased root system. Nematodes move very slowly but they are able to travel in great distance and spread in the soil and attack plant tissues. Plants, during photosynthesis, produce water, minerals and nutrients that are required to support its own growth but it becomes insufficient when the nematodes start to feed on it. Nematodes also can produce enzymes and other disease-inducing compounds that are harmful to the resulting in stunting of plant growth, decrease of crop yields and in the end possibly lead to plant death. At times when infection is not too severe, a plant can still grow but at a very slow pace and need longer time to bear any fruits besides suffers from loss of weight and reduce the productive life of the farm. The majority of nematodes parasitize on the root tissues but it has been observed that some of the nematodes do feed on the plant parts above the ground. It is possible that plant parasitic nematode invade a plant together with other

plant pathogenic microorganism and also some of them transmit viruses so at times infect host plants with viruses that they carry (Tylka *et al*., 2001).

1.7. Chemical and biological treatment against nematodes

Crop rotation and chemical-based control methods are the basis of nematode management initiatives in banana but where bananas are grown incessantly, crop rotation cannot be applied. Another way to control nematodes population is by the use of nematicides, but the majority of nematicides available are known to be toxic to the environment. Although numerous agriculture crops have natural resistance and tolerance against nematodes, this has not yet been exploited to assist in nematode management in bananas due to limited knowledge of genes that confer tolerance against nematode in *Musa* genepool (Speisger *et al*., 1997).

Nematicides are costly, very toxic and damaging to the environment. For example in the United States during 1982, 50,000 tones of nematicide active components were used to treat the crops which costed more than $US 1 billion. In recent decades, many reports have been published that address issues such as contamination of ground water as well as toxicity to mammals and birds (Bird *et al*., 2003). Other ways that were used to control and reduce nematodes population that employed natural agents such as removal of infected tissue, treatment with direct sunlight, the use of hot water, and crop rotation using non-host plants.

By removing the roots and superficially paring the corm of infected plant as well as exposing the planting material to direct sunlight for duration of two weeks can reduce the density of nematode in the planting material. Additional treatment with hot water (53°C to 55°C) for 20 minutes can eliminate almost all nematodes from the plant material. Nematode inhabitants may be decreased to an effective level by planting non-host plants for a period of time. For example, in Africa, by planting non-host plants such as *Chromolaena odorata* for a year, the population of nematodes in the infested soil was observed to have shrunken (Bridge *et al*., 1997; Sarah *et al*., 1996).

1.8. Different types of plant parasitic nematodes

1.8.1. *Meloidogyne* spp.

The root knot nematodes belong to the Meloidogyne genus. They are prevalent in numerous parts of the world and cause considerable yield losses mostly in tropical and subtropical regions (Tesarova *et al*., 2003).These worms are of the sedentary endoparasites kind and have compound trophic relationship with their host by forming the specific nourishing cells that are recognized as giant cells in root tissues (Piotte *et al*., 1999; Wang *et al*., 2007). Root knot nematodes invade host plants, migrate between the root cells and create large and multinucleate cells called giant cells. Giant cells block root vascular system and disturb function of root and also retardation and reduction of plant growth but if soil has been highly infested soil plant die before gall formation (Scholl *et al*., 2003; Tesarova *et al*., 2003; Wrona *et al*., 1996).

The shape of root knot nematodes is different from most nematodes because their adult females would change shape from slender shape to swollen. One of the symptoms of infection in by root knot nematode in plants is the appearance of galls around the swollen mature female that can easily be seen on infected root (Tylka *et al*., 2001). Root knot nematode has short generation cycle that depends on temperature. Normally the life cycle of a root knot nematode can be completed within 4 weeks when the soil temperature is 80F°. If the temperature of the soil is under 50°F or over 100°F, the life cycle of this parasitic worms will be reduce or completely stopped. These parasites hardly exist in chilly or frozen soil and as a result, their numbers will dwindle during winters when the soil is frozen.

Nearly 90% of this parasite may die between the transition period of harvesting one crop and planting the next. But by having a short life cycle and large number eggs being laid by adult females, these parasites can multiply and increase again in numbers quickly in favorable condition. A mature female lays around 300 to 1000 eggs that are secured in a gelatinous matrix (Wrona *et al*., 1996). After hatching, the juveniles would first migrate into the soil to search for an appropriate host plant. Upon finding one, they will enter the host through the root tip following a stable pathway. They are settling down close to the protophloem where they persuade the separation of specialized feeding sites and continue to grow until reaching the adult stage. After they become adults, females would remain inside to plant root but males migrate out of the root to live free in the soil (Piotte *et al*., 1999).

It is not clear what gene is responsible for the parasitic ability of *Meloidogyne* spp. Analyses based on phylum-wide phylogenetic study revealed that plant parasitic nematode species arise independently for multiple times over the route of nematode evolution. Therefore, this cannot be supported if any gene or set of genes have parasitic ability in certain nematode species will also be or have likewise function in another species. As a result, several mechanism such as gene loss, gene duplication, adaptation of pre-existing genes to encode new function, change in genes regulation metabolic or development pathway, acquisition of genes from other species via horizontal (or lateral) gene transfer could drive the evolution of nematodes to parasitism (Bird *et al*., 2003).

1.8.2. *Rotylenchulus reniformis*

Rotylenchulus reniformis nematodes belong to the Rotylenchulus genus and are semi-endoparasitic. *Rotylenchulus reniformis* is dispersed in tropical and subtropical as well as in regions with warm climate. They are also called reniform nematodes, referring to the mature female body that resembles the shape of a kidney. Average body length of a male juvenile nematode is about 0.34 to 0.42 mm, while the average body length for mature female nematode is 0.38 to 0.52 mm. The females have stylets of 16 to 21μm long and are of moderate strength with small rounded knobs but in males, the stylets and knobs are feebler. Immature female, male and juveniles are usually in the energetic vermiform phase and they can be found in the soil. If there are no hosts for *Rotylenchulus reniformis*, this nematode can stay alive for 6-8 months. In these species, the females become sedentary because they will penetrate to the root cortex and establish a constant feeding site in the

stele region of the root. The head of the female will remain in the root but its tail from root tissue and remain outside and swells during maturation (Inserra *et al*., 1989; Wang, 2007).

In the life cycle of *Rotylenchulus reniformis*, one or two weeks are needed for them to become adults once females enter the host root. Male nematodes that remained outside the root can then fertilize the female adult and the sperms are stored in the spermatheca. After the female gonads matured, the eggs are fertilized with the sperms before being stored in a gelatinous matrix. There are about 60 to 200 eggs in a gelatinous matrix. One to two weeks after spawning, the eggs will hatch but the first stage of juvenile molting still take place within the eggs then following the second stage juvenile that juvenile will leaves the egg and in the life cycle of this parasitic nematodes, infectious activity will start one to two weeks after hatching. Ordinarily in a population, the numbers of females and males are equal but in some populations, *Rotylenchulus reniform* reproduce parthenogenetically.

Life cycle of this parasite depends on several factors such as soil temperature. Life cycle of this nematode takes less than three weeks and also this parasite can live for at least two years in condition such as absence of host plants and dry soil. Only the females of this species infect plant roots. Infection by these nematodes form a feeding site composed of syncytial cells which are a multinucleated cell that are formed due to cells wall disintegration of several adjoining cells (Wang, 2007).

Although gelatinous matrix produced by *Rotylenchulus reniformis* has similar appearance to the ones produced by root knot nematode, it differs in several aspects. In the genus Meloidogyne, gelatinous matrix is produced by rectal gland but in *Rotylenchulus reniformis,* it is formed by its vulval gland. And gelatinous matrix of *Rotylenchulus reniformis* can be located outside of root whereas the gelatinous matrix root knot nematode can be found perfectly encased in the tissue of plant (Agudelo *et al*., 2004).

1.8.3. *Pratylenchus* spp.

Pratylenchus coffeae and *Pratylenchus goodeyi* belong to genus Pratylenchus and are major pests of *Musa* spp. wherever they occur. Both species are migratory endoparasitic of corms and root cortex of banana. Both these nematode species can attack and feed in the root and corm tissue at every stages of their life regardless of their sexes. The female laid eggs in the root and corm tissue (Bridge *et al*., 1997). Another species of this genus is *Pratylenchus filipjev*, which also is migratory root endoparasite that attack and migrate through root cortical. They cause necrotic lesion by direct feeding on root tissues and creating a favorable condition for other pathogenic microorganisms to infect the plant (Subbotin *et al*., 2008).

At the temperature of 25°C-30°C, the life cycle of *Pratylenchus coffeae* is less than 30 days. The damages of infection by *Pratylenchus coffeae* and *Pratylenchus goodeyi* is very similar to that caused by *Radopholus similis*. This loss and decreasing of the root system leads to dwarfing of plants, enlargement of the production cycle, reduced bunch yield and fall over or uprooting. Damages incurred by *Pratylenchus goodeyi* nematode are

high in banana from genetic groups of Pelipita (ABB), Plantains (AAB) and Bluggoe (ABB) but low in others. Plants in different geographic conditions also have different tolerance to this plant parasitic nematode. For instance bananas of the old East African highland, some of them are more 100 years of age, are found to be tolerant to high density of *Pratylenchus goodeyi*, while in neighboring part of Tanzania, observation revealed relatively high incidence of plant toppling despite the low density of *Pratylenchus goodeyi* population at the site (Bridge *et al*., 1997).

Removing *Pratylenchus coffeae* or *Pratylenchus goodeyi* from contaminated soil prior to replanting is hard because these nematodes have a broad range of host. *Pratylenchus coffeae* and *Pratylenchus goodeyi* densities can be decreased by removing the root and apparently paring the corm and additional treatment with sunlight and hot water. All the nematicides that are used to control *Radopholus similis* can be useful to control *Pratylenchus coffeae* and *Pratylenchus goodeyi* although they have negative effect to the environment (Bridge *et al*., 1997).

1.8.4. *Helicotylenchus* spp.

Helicotylenchus are known as spiral nematodes. They are most common plant parasitic in temperate and tropical region. While several species of Helicotylenchus genus have been implicated with several plant diseases, the majority of them are not considered invasive parasites. *Helicotylenchus multicinctus*, *Helicotylenchus dihystera, Helicotylenchus digonicus* and *Helicotylenchus pseudorobustus* are four species of this genus that have been consistently linked with plant growth repression. Usually

Helicotylenchus are migratory ectoparasitic feeders and their main host is banana. Helicotylenchus lives outside the host root and feed on the young epidermis tissues using their stylets. Eggs are laid on the surface of the root tissues or in the soil close with the root tissue and they will hatch under suitable temperature. After two or three days, the juveniles will start feeding on the root tissue and become matured. But a few species of Helicotylenchus are migratory and semiendoparasitic. They have been observed incompletely implant within the root tissue where they feed on cortical cell tissue, as much as 4-6 cells deep. The nematodes that nourished on the cell may cause damaged feeder functions of the roots because the nematodes feed on the cell contents, causing the cell wall to collapse and eventually die. Usually in many crops infested by *Helicotylenchus* spp., there are no noticeable indications aboveground. But in some instances, several symptoms were described and linked to infection of this species, such as dwarfed and sparse looking as well as yellowing foliage. Reliable data on plant symptoms and injuries characteristic to infection of this nematode is hard to be established because spiral nematodes infection is almost always associates with other nematodes (O'Bannon *et al*., 1989).

1.8.5. *Aphelenchus* spp.

In Aphelenchus genus, the stylet is without basal knobs and lips are somewhat offset. In this genus, esophageal glands partly cover intestine dorsally. Females have short tail and bluntly rounded. Frequently males of this genus are rare. Gubernaculums of this genus is V form (Nickle, 1970). *Aphelenchus avenae* is a famous species of this genus. They are found in soil, leaf sheaths and also in plant crowns as well as in the cortex of some

roots particularly if the roots are contaminated with fungi. *Aphelenchus avenae* are primarily fungivorous, i.e. they feed on fungi and using their stylets. There are a few records about their pathogenicity on higher plant. *Aphelenchus avenae* can be found in the roots of different plants particularly ones that grow on sandy soil, but it is more frequently found in roots that have been infected with *Pratylenchus* spp. However there are reports about *Aphelenchus avenae* that feed on higher plant tissue but its major ecological position appears to be fungivorous. They can be cultured easily, and for this reason, they are used as appropriate test organism for nematicides. Usually, this species reproduce parthenogenetically and meiotic parthenogenesis.

In another species, *Aphelenchus radicicolus* which is also widely spread in a parthenogenetic population, but the males are more commonly found in soil with temperature of 30°C. Females can produce 225 eggs in a single spawning. Usually, their eggs are unsegmented when stored and will hatch in 275-282 hours at 10°C and in 28-30 hours at 36°C. They have four larval stages. Females need a continuous supply of food to produce eggs and in an unsuitable situation, the egg production time becomes longer but do not affect the total eggs number (Hooper, 1974).

1.8.6. *Xiphinema* spp.

Xiphinema spp. feed on the root tip of host plants. These activities frequently result in swollen root tip and such that the indications may be confused with root galls caused by root knot nematode. When *Xiphinema* spp. feed of host cell, cells normally become enlarged and multinucleated with dense cytoplasm compared to the neighboring cells,

similar to the root knot nematode and other sedentary nematodes. These nematodes have long stylets used for feeding off cells in the root while their bodies remain outside the root. These nematodes have long life cycle but are closely affected by environmental factors and host plant conditions. Usually the life cycle of a female takes one year to grow from an egg to become adult and laying eggs. Length of females ranges from 1 to over 10mm. Several species of this genus can act as vector for plant viruses (Bridge *et al*., 2007). The most common species in Xiphinema genus is *Xiphinema index*. *Xiphinema index* is parthenogenetic and their eggs are produced in the Spring and adults into four juvenile steps. Generally, male nematodes are extremely rare. *Xiphinema index* are ectoparasitic nematode that parasitize on actively growing root tips and create galls that comprise distended multinucleate cells with dense cytoplasm. *Xiphinema index* are plant parasitic nematode and also act as vectors for viruses. The juvenile and adult *Xiphinema index* nematodes can acquire and transmit the Grapevine fanleaf virus (GFIV) to healthy plant (Belin *et al*., 2001; Demangeat *et al*., 2004).

1.9. Secondary infection

When plant parasitic nematode feeds on the root surface, they create orifices on the roots that may permit other pathogens to penetrate the plant. Parasitic nematode invasion will also reduce tolerance of plants against other stresses. It is even possible sometimes that plant parasitic nematode attacks to jointly attack a plant with other plant pathogenic microorganisms such as fungi to give rise to diseases that can hardly inflicted by either organism on their own. Also some plant parasitic nematode transmits viruses contracted

when feeding on a virus-infected plant and afterward passing the virus on other plant when they feed (Tylka *et al*., 2001).

1.10. Characteristic and identification of plant parasitic nematode

Conventional methods have been used to observe morphological characteristic for nematode identification. Using methods that are based on morphology and morphometrics for identification of plant parasitic nematodes are time consuming and difficult due to the overlapping of numerous characteristics and not to mention their plasticity. Therefore identification of different nematode species needs significant experience and skill. Besides that, detection of root knot nematodes based morphometrics is complicated and tedious (Kumari *et al*., 2004; Tesarova *et al*., 2003). Such methodology needs skilled personnel to be able to recognize organisms to the genus and species levels. And most of the time, it would require the nematodes to be at a specific stage of life, usually as adults (Berry *et al*., 2008).

Identification methods based on DNA offer the best solution to overcome problems related with nematode identification. Lately, nematode identification methods are done based on polymerase chain reaction (PCR). These methods have been productively employed for nematode diagnostics (Kumari *et al*., 2004). Some of the advantages of this PCR-based method are its speed, high sensitivity, and reliable diagnosis. It can also facilitate better understanding and discovery of genetic relationship between genus and species of nematodes (Berry *et al*., 2008; Tesarova *et al*., 2003).

1.11. Internal transcribed space (ITS)

Sequence analysis for nuclear ribosomal DNA (rDNA) has been assessed as a source to elucidate phylogenetic kinship between populations and species of many organisms including nematodes (Kaplan *et al*., 2000). The Internal transcribed spacer (ITS) region has been used from a broad taxonomic range of nematodes and was assessed as a taxonomic marker. The size of the amplified ITS regions assisted in the first determination of the group member. The size of the amplified ITS regions would also be able to indicate groups that may need taxonomic reassessment. The ITS region is situated between the 18S and 28S ribosomal DNA genes. The *small ribosomal subunit* gene (18S) and the *large ribosomal subunit* gene (28S) are joined respectively to ITS1 and ITS2 while the 5.8S ribosomal gene is located in between ITS1 and ITS2 (Powers *et al*., 1997). Highly conserved sequences established in the rDNA genes have been utilized to successfully design universal primers for amplification of this region (Kaplan *et al*., 2000).

1.12. Polymerase chain reaction and gene cloning

The polymerase chain reaction (PCR) is a significantly important molecular technique and it has a wide application in biological, biotechnological and biomedical areas of science. PCR creates numerous copies of a specific region of a DNA template and permits that particular portion of DNA to be manipulated and studied in greater detail (Hamilton *et al*., 2006).

PCR is carried out by mixing DNA with a set of specific reagents followed by incubation in a thermal cycler. In the first step of thermal cycling at 94°C, DNA is denatured because at this temperature, hydrogen bonds that hold together two strands of DNA are broken causing the double stranded DNA to become single stranded DNA. In the next step, temperature is lowered to 50°C-60°C, allowing the primers to anneal to the specific region of DNA template. Next, the temperature is increased to 74°C, which is the optimum working temperature for *Taq* DNA polymerase. *Taq* DNA polymerase binds to the 3' end of each primer and synthesizes new strands of DNA, producing double stranded DNA at the end of this step (one strand is the original molecule, the other is the new strand of DNA). This cycle is repeated multiple times until many copies of specific region are generated.

The basic step of gene cloning is the insertion of a fragment of DNA, containing the gene to be cloned, into a cloning vector to produce a chimerical or recombinant DNA molecule. Therefore, the vector acts as a vehicle to transfer the gene into a host cell. The common cell being used as host is bacterium although other types of living cells can be used as well. When the vector containing the insert DNA was successfully taken into the host cell, it can multiply itself that consequently having many identical copies of itself as well as the gene that the vector is carrying. Copies of the recombinant DNA molecule are passed down to its daughter cells and further vector replication takes place when they divide. A colony or clone of identical host cells is produced after a multiple division of cells with each cell in the clone having one or more copies of the recombinant DNA (Brown, 2001).

1.13. Objective

The main objective of this study is to identify and characterize banana parasitic nematodes by using DNA-based methods. The specific objectives are to:

- Assess the effectiveness of different DNA extraction methods on diverse species plant parasitic nematodes.
- Examine and inspect the ability of ITS regions as taxonomic molecular marker for identification and characterization banana parasitic nematodes.