II. LITERATURE REVIEW

2.1 Root-knot Nematode

Root knot nematode had already been reported by 1885 to cause damage to various plant species, majorly in the tropical and subtropical regions. According to Chitwood (1949) root-knot nematodes consist of four main species based on the perineal morphology pattern of the female adult nematodes and other morphological characteristics, the four species are *Meloidogyne javanica*, *M. arenaria*, *M. incognita*, *M. hapla*. By the year 1988 as much as 61 species of *Meloidogyne* had been noted (Eisenback & Triantaphyllou 1991). The root-knot nematode forms the most important plant parasitic nematode with wide host range, that is around 2000 plant species (Agrios 2005) and most of these crops are cultivated crops (Jensen 1972). In Indonesia root-knot nematodes of *Meloidogyne incognita* has a wide distribution area with 45.4% prevalence and *M. arenaria* has 38.6% prevalence (Hadisoeganda 1989).

Root knot nematodes has been known as a disease of vegetable crops since 1855, when (Berkeley 1855) in England first described the disease on cucumber *Cucumis sativus* L. roots, (Eisenback & Triantaphyllou 1991). The causal organism was described as *Heterodera radicicola*. From 1884 to 1949, root knot nematodes were considered a single species in combination with cyst nematodes and referred to by a number of designations (Johnson & Fassuliotis 1984). Chitwood (1949) described morphological differences among populations, and re-assigned the root knot nematode to the genus *Meloidogyne*. At this time *Meloidogyne incognita*, *M. arenaria*, *M. javanica*, *M. hapla* and *M. exiqua* were recognized primarily on the basis off perineal pattern and other morphological characteristics.

Initially all root knot nematodes were considered to one extremely phylophagous species, *Heterodera marioni* until (Chitwood 1949) re-established the genus *Meloidogyne*, although 51 species of *Meloidogyne* have been described to date (Jepson 1987), four species are of particular economic importance to vegetable production, *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*, *Meloidogyne hapla*. The root-knot nematode forms the most important plant parasitic nematode with wide host range, that is around 2000 plant species (Agrios 2005) and most of these crops are cultivated crops (Jensen 1972). In Indonesia root-knot nematodes of *Meloidogyne incognita* has a wide distribution area with 45.4% prevalence and *M. arenaria* has 38.6% prevalence (Hadisoeganda 1989).
Meloidogyne arenaria, and Meloidogyne hapla. Out of the 1000 root knot population collected from 75 countries 52% were identified as Meloidogyne incognita, 30% as Meloidogyne javanica, 8% as Meloidogyne arenaria, 8% as Meloidogyne hapla and 2% as Meloidogyne exigua or other species (Taylor & Sasser 1978). M. incognita consists of four races; M. arenaria has two races, M. javanica and M. hapla show no clearly defined races M. incognita, M. javanica M. arenaria and M. hapla have the widest host ranges. M. incognita and M. javanica are commonly found in the tropics, while M. hapla is a species commonly found in the temperate regions and occasionally in the cooler upland tropics.

2.2 Mechanism of Infection by Root-knot Nematodes Meloidogyne incognita

The direct mechanical injury inflicted by nematodes while feeding causes only slight damage to plants. Most of the damage is caused by secretion of saliva injected into the plant while the nematodes are feeding. The nematodes puncture cell walls using their stylet, inject saliva into the cells, withdraw cell contents, they remain sedentary at their feeding site for the whole of their life while feeding at the site.

The feeding process causes the affected plant cells to react, resulting to dead or devitalized root tips, lesion forming and tissue break down, swelling and gall formation, these are caused by the dissolution of the infected tissues by nematode enzymes which causes tissue disintegration and death of the cells, others are caused by abnormal cell enlargement (hypertrophy) by suppression of cell division, or by stimulation of cell division proceeding in a controlled manner and resulting in the formation of galls, or large number of lateral roots at or near the point of infection.

2.3 Taxonomy of Root-knot Nematode (Meloidogyne incognita)

Kingdom animalia, Phylum Nematoda, Class: Secernentea, Order Tylenchida, Family Heteroderidae, Genus Meloidogyne, Species: M. incognita (Franklin 1982).
2.4 Morphology of Root-knot Nematode (*Meloidogyne incognita*)

*Meloidogyne incognita* like other a plant parasitic nematode has a colourless body that is cylindrical in shape (Wallace 1963). Adult female, adult male and the larva can be differentiated based on their body form.

2.4.1 Larva. First instar (L1) has a blunt tail and molts within the egg, the second instar larva (L2) is hatched and live freely in the soil and look for a host, according to (Walker 1975) the length of the (L2) is between 375-500 μm with a diameter of 12-15 μm. The third and fourth larval instas develop within the host plant tissues.

2.4.2 Male adult. *Meloidogyne incognita* adult males are stretched cylindrical and are threadlike with the length 1.2-1.5 mm (Agrios 2005). The male head is composed of head cap and head region provides many good diagnostic features. The head cap includes labial disk surrounded by lateral and medial lips, a centrally located prestoma leads to a slit like stoma, four sensory organs terminate on medial lips (cephalic sensilia), and the head region may or may not be set off from the remainder of the body.

2.4.3 Female adults. Name of the genus *Meloidogyne* originated from greek language with the meaning that literally means apple and female because the body form of the female nematode is apple or pear shaped, with the length of 0.40-1.30 mm and diameter of 0.27-0.75mm (Walker 1975; Agrios 2005) with the neck of 0.15-0.24 mm wide (Walker 1975), the name *Meloidogyne* was given for the first time by Goeldi in the year 1887 (Franklin 1982).

2.5 Lifecycle of the Root-knot Nematode

Root-knot nematodes display marked sexual dimorphism i.e. the females are pyriform or saccate, the males vermiform. These general differences in the body form between male and female become established during the post embryonic development of *Meloidogyne incognita*. The embryonic development results in the first stage juvenile which molts once in the egg and hatches as a second stage
juvenile. This motile vermiform, infective stage migrates through the soil and enters roots of the suitable host plant, it moves through the plant tissue to a preferred feeding site and establishes a complex host parasite relationship with the plant. The second stage juvenile becomes sedentary and as it feeds on special nurse cells (giant cells), it undergoes more morphological changes, and become flask-shaped, without further feeding it molts three times into third and fourth stage juveniles and finally becomes an adult. Shortly after last molt the saccate adult female resumes feeding and continues to feed for the remainder of her life, during this post embryonic development, the reproductive system develops and grows into functional gonads, the sexes can be differentiated based on the number of gonads (females have two gonads; males only one gonad). The change in shape from saccate male juvenile to vermiform adult male takes place during the fourth juvenile stage. The adult male does not feed it will leave the root and move freely through the soil. Depending on type and mode of reproduction, of a particular species, amphimixis or parthenogenesis, males may search for females and mate or remain in the soil and finally die. Length of life cycle of root-knot nematodes is greatly influenced by temperature, for *Meloidogyne incognita* is about 29°C, the first adult females appear 13-15 days after root penetration. The lifespan of egg-producing females may extend from 2-3 months and they lay upto 2000 eggs, but that of males maybe shorter.

Figure 2. Lifecycle of the root-knot nematodes *Meloidogyne incognita*  
(Source; The American Phytopathological Society 2003)  
(Photo courtesy of V. Brewster)
2.6 Anatomy of Root-knot Nematodes

The nematode body is more or less transparent; it is covered by a colourless cuticle that molts when the nematode goes through successive juvenile stages. The cuticle is produced by the hypodermis which consists of living cells and extends into the body cavity as four chords separating four bands of longitudinal muscles, the muscles enable the nematode to move.

The body cavity contains fluid through which circulation and respiration takes place, the digestive system is a hollow tube extending from the mouth through the esophagus, rectum and anus. Lips usually six in number, surrounds the mouth. Most plant parasitic nematodes have a hollow stylet or spear that they use to puncture holes in plant cells and through which to withdraw nutrients from the cell.

The reproductive system of the nematodes is well developed, females have one or two ovaries followed by an oviduct terminating in a vulva. The male reproductive structure is similar to that of the females, but there is a testis, seminal vesicle, and a terminus in a common opening with the intestine. A pair of protrusible and, copulatory spicules is also present in males. Reproduction in plant parasitic nematodes is through eggs and may be sexual or parthenogetic to the species that lack males.

2.7 Factors Influencing Development of Root Knot Nematodes

Many factors limits the growth development of root-knot nematodes, however there are two major important factors that is temperature and host suitability (Chrystie 1959).

2.7.1 Temperature. Meloidogyne incognita is sedentary endoparasite and completes its lifecycle in 20-25 days within the root cortex at a temperature of 27°C (Agrios 2005). Between 27°C-30°C the development of female root-knot nematodes begins from infective larva up to egg hatching going on for 17 days, at temperature of 24°C, egg hatching goes for 31 days, the longest development takes place at around 15.4°C and it takes up to 54 days. However in environments
with temperatures below 15.4°C and above 33.5°C the development in root-knot nematodes will fail to take place up to adult stage.

2.7.2 Host suitability. In suitable host plants, eggs produced by the Meloidogyne incognita are many, the more suitable the host plant the more eggs produced (Chrystie 1959). Occurrence of continuous infection is influenced by the host suitability. When the larva has already entered the non-suitable host tissues, in about 4-6 days this infective larva will leave that plant tissue and invade another plant, or stay in the latter plant tissues with its life development experiencing disturbance (Dropkin 1980).

2.7.3 Soil moisture. This will influence the development of the Meloidogyne incognita by determining the time taken for the start of egg hatching. Egg hatching will be impeded in dry conditions with low moisture levels (Chrystie 1959). Sufficient soil moisture content (in the field capacity), forms the best condition for the development of root-knot nematodes, but flooded soils also will have bad consequences, or even cause death. According to Dropkin (1980) best conditions for the development of root-knot nematodes is in the soils with little sand, and not good in clay soils.

Water availability will really determine the life process and at the same time it is an important media for movement of root-knot nematodes in the soil (Norton 1978). Low moisture conditions will influence mobility acceleration of Meloidogyne incognita but has not resulted to death, it only changes physiological mechanisms of root-knot nematodes. Soil moisture content best for the existence of the root-knot nematodes ranges between 40-60% from field capacity (Wallace 1963).

2.7.4 Nutrition availability: Total nutrient availability shows much influence in the population ratio between males and females. According to Norton (1978) host plants tissue that gives abundant nutrition leads to increased development of the larva to female while host plant tissue that gives less plant nutrition, leads to increased development of the larva mostly into males.
Based on the experimental results it is known that giving of mineral nutrients to plants is influential to nematode development. Giving solutions of N, P and K to tomato and potato host plants increases the production of root-knot nematode eggs (Dropkin 1980). In plants with much nitrogen, nematode development also increases, however on the contrary in plants with less nitrogen availability development of *Meloidogyne incognita* is impeded (Dropkin 1980).

Existence of excess potassium like in cucumber shows increase in the development of *Meloidogyne incognita* although this case does not occur with *M. hapla* and *M. javanica*. In pea plant with excess potassium hatching of the first egg takes place on the 16th day after inoculation, very different from plants with less potassium, hatching of eggs takes place 40 days after inoculation (Chrystie 1959).

### 2.8 Root Knot Nematodes as Pests of Tomato Plants

The species of root knot nematodes found to be most detrimental to tomato plants are those involved in the destruction of primary roots, disrupting the anchorage system and divitalization of the root tips and eventually death of the plant in severe cases. The most wide spread and important are *Meloidogyne incognita*, it is found worldwide in tropical and sub-tropical regions and occurs wherever tomatoes are grown (Bridge & Gowen 1993). Areas where the nematode is known to occur on tomatoes include Africa, parts of Asia, Central and South America, Cuba, Australia and several countries in Southern Europe.

The root-knot nematode second stage juveniles are short (400-600µm) the cephalic framework is weakly sclerotized, and has indistinct knobs. The esophageal gland lobe overlaps the intestine ventrally, and tail tapers to a pointed tip with a clear terminus. The males of root-knot nematodes are 1.0 to 2.0 mm long, the stylet is about 18-24µm and has distinct knobs. The esophageal gland lobe overlaps the intestine ventrally. The tail is short and rounded and lacks bursa. The spicules of root-knot male nematodes open a short distance from the tail tip, unlike those of the cyst nematodes which opens near the terminus. The females of *Meloidogyne incognita* are swollen and pear shaped, pearly white, and sedentary. They deposit all of their eggs in gelatinous mass that usually
protrude from the galled root tissues, unlike cyst nematodes the female root-knot nematodes usually remain completely endoparasitic.

The species has a pronounced sexual dimorphism in which males are warm like vermiform and about 1.0 to 2.0 mm long by 30 to 36 micrometer in diameter (Agrios 2005). Each female lays approximately 2000 eggs in a gelatinous substance the first stage juveniles develop inside each egg, the second stage juvenile emerges from the egg into the soil and this is the only infective stage of the nematode, if it reaches a susceptible host the juvenile enters the roots become sedentary and grows thick like a sausage (Agrios 2005). *Meloidogyne incognita* is a sedentary endoparasite and completes its life cycle in 20-25 days within the root cortex at a temperature of 27°C (Agrios 2005). Females lay 20-30 eggs per day for a period of two weeks (Niere 2001). The eggs hatch in 8-10 days and the juvenile stages are completed in 10-13 days, the nematode cannot survive more than six months in soil deficient of the host (Ssango & Speijer 1997).

**2.9 Symptoms of Root-knot Nematodes in Tomato Plant**

Root-knot nematode infection of plants results in appearance of symptoms, typical symptoms of nematode injury can involve both above ground and below ground plant parts.

**2.9.1 Above ground symptoms:** Infected plants will show inhibited growth (stunting), yellowing (chlorosis) of leaf, reduced yield, poor quality and quantity of crop products like the tomato fruits, premature leaf fall, erratic stands, wilting during the day.

**2.9.2 Underground symptoms:** Infected plants will show excessive branching of secondary roots, overall development of root galls, injured root tips and egg masses on the root surface, rough root surfaces with club appearance, infected roots are small and show necrosis.

Interactions involving fungal plant pathogens and plant parasitic nematodes have been reviewed previously (Powell 1971a; Webster 1985; Mai & Abawi 1987; Rowe *et al.* 1987; Evans & Haydock 1993; Francle &
Interaction between *Meloidogyne incognita* and Fusarium wilt fungi have received special attention and were documented in 20 crop species. Interactions of these pathogens were especially obvious when the root knot nematode infection preceded those of the Fusarium wilt pathogens by 3 to 4 weeks. Majority of studies have established that the presence of root-knot nematodes increases the incidence and rate of development and severity of wilt or the mortality of the *Fusarium*-susceptible and tolerant crops. However the role of root-knot nematodes in the breakdown or alteration of the monogenic type of resistance to Fusarium wilt fungi (such as tomato cultivars with the dormant I-genes against *F. oxysporum* f.sp. *lycopersici*) remains controversial and requires further investigation (Mai & Abawi 1987).

Many example of disease complexes are known (Pitcher 1963; Powell 1971a; Powell 1971b; Taylor 1979; Webster 1985). Tomato plants wilt more quickly and can be killed when *Fusarium oxysporium* is simultaneously present along side with nematodes, resistance of tomato cultivars to fungal wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* was reduced in the presence of *Meloidogyne incognita* (Jenkins & Coursen 1957; Sidhu & Webster 1977). Damage to the root system caused by root knot nematode attack has been considered responsible for the increase in the intensity of bacterial wilt caused by *Pseudomonas solanacearum* (Valdez 1978) and bacterial canker caused by *Corynebacterium michiganense* (Moura et al. 1975). Several of the viruses that are transmitted by the nematodes cause significant economic losses on major food crops such as tomato and tobacco ring spot virus. *Meloidogyne incognita* race 1 was shown to increase wilt caused by both *R. solanacearum* and *F. oxysporum* f.sp. *lycopersici* on resistant tomato cultivars when inoculated simultaneously (Chindo et al. 1991). Van Gundy *et al.* (1977) demonstrated that leaching of nematode infected plants applied to tomato inoculated with *Rhizoctonia* sp resulted in the appearance of severe rots. The presence of root-knot nematodes play a major role in increasing the incidence and severity of bacterial wilt diseases caused by *Pseudomonas solanacearum* on various crops including tomato, potato, egg plants and tobacco.
Yield loss in tomatoes due to root-knot nematodes in the world has been estimated to be approximately $100 billion world wide annually (Sasser & Freckman 1987). The root knot nematodes have a worldwide distribution but are more abundant in warm temperate and tropical soils. Losses due to *Meloidogyne incognita* in tomatoes can be as high as 50% (Niere 2001). In addition to the direct crop damage caused by the nematodes, many nematode species have also been shown to predispose plants to various infections by fungal or bacterial pathogens, or to transmit virus diseases.

Several control methods are available for the control of the tomato root-knot nematodes. The most important cultural control method is use of clean planting materials, only seedlings with roots free of galls should be selected for transplanting, the nurseries should also be free from root knot nematodes and seed beds should be selected on sites where previously there were no host plants. Crop rotation reduces the impact of root knot nematodes in tropical region and it’s the main management strategy to regulate the population of nematodes but its success is often limited because of the wide host range of most root knot nematodes species and the frequent occurrence of infestation composed of more than one species (Sikora *et al.* 1988), galled roots remaining in the field after harvest should be eliminated by uprooting and destruction, other control practices include bare fallowing and flooding.

Nematicides are widely used by growers producing fruits for export trade, a number of organophosphates and carbamates are used. However, their use is often prohibitive for many resources poor small scale farmers, registered products are highly toxic, expertise is required for application and most of them have been phased out of the market. The pesticide usually inactivates the nematode within the plant tissue or in the soil, which after microbial degradation the nematode recovers and damage continues (Sikora & Pocasangre 2004).

Possible agents for biological control of root knot nematodes are fungal antagonists that include nematode trapping or predacious fungi, endoparasitic fungi, parasites of nematode eggs, and fungi that produce enzymes and metabolites toxic to the nematode (Coosemans 1993). Research on way of exploiting these agents on field scale has yielded little success. Development of
root knot nematode resistant cultivars would substitute the toxic nematicides currently in use and permit cultivation where farmers could not previously afford nematode control (Sikora et al. 2003). Research on the control of root knot nematodes suggests that no single control strategy will provide complete control (Sikora et al. 2003). A broad integrated pest management (IPM) approach including new components of pest control is necessary to safeguard sustainable tomato production. The biological enhancement of tomato plants with mutualistic fungal endophytes is a new approach that seems as a strong option for sustainable and ecologically sound nematode control. Inoculation of tomato plants with endophytes has resulted in reduced nematode reproduction, numbers and damage in pot experiments by more than 30% over controls (Sikora et al. 2003). Conducted research on ability of the fungal endophytes to persist in the tissues of inoculated plants and the interactions between the fungal endophytes and tomato plants has yielded conclusive results (Paparu et al. 2004; Niere et al. 1999).

2.10 Possibility of Biocontrol by Endophytic Fungi

Several definitions of endophytism have been proposed (Carroll 1988; Clay 1990), for the purpose of this research, the term endophyte refers to fungi or bacteria, which for all or part of their life cycle invade and live inside tissues of living plants without causing any disease symptom or any apparent injury to the host (Petrini 1991; Wilson 1995), while epiphytes are bacteria or fungi that colonize plant surface tissues, in contrast to the epiphytes, endophytes are contained entirely within plant tissues, are asymptomatic and may be described as mutualistic (Clay 1990), fungi associated with root rhizospheres of the plants are called plant growth promoting fungi (PGPF). Some of the important PGPF belong to the genus Trichoderma and Gliocladium and the arbuscular mycorrhizal fungi (AMF), which form symbiotic association with plant roots and are also capable of colonizing the roots of their hosts (Gera Hol & Cook 2005).

Estimated yield loss due to plant parasitic nematodes range from 20-80% in tomato production systems (Swennen & Vulysteke 2001). The control methods for root knot nematodes are not completely effective in subsistence farming
system (Niere 2001). The strategic use of naturally occurring antagonistic organisms to control pest population and increase crop production represents a viable option (Marshall et al. 1999). Endophytic fungi are potentially effective biological control agents for plant parasitic nematodes management (Niere et al. 1999; Sikora et al. 2003). A wide diversity of endophytic fungi has been isolated from healthy tomato tissues with majority of isolates being from the genus non pathogenic *Fusarium* sp (Niere 2001; Pocasangre et al. 2000).

Culture filtrates for a number of isolates of non-pathogenic *Fusarium oxysporum* screened for *in vitro* activity against the root knot nematodes have shown high nematicidal activity causing mortality rates of 82-100%. Production of secondary metabolites by endophytic fungi is thought to be one of the mechanisms leading to plant pest control, these has been shown for grass endophytes but has not been elucidated for endophytes of crop plants (Niere et al. 2002).

The use of endophytes for control of plant parasitic nematodes is relatively a new approach. Since endophytes spend most of their life cycle inside plant tissues they are less exposed to the environment factors, hence they don’t entirely depend on the environment for multiplication and survival (Siddiqui & Shaukat 2003a). Endophytes occupy a similar niche as pests and thus are in close contact with the pest which make them an edge over other biological control agents (Hallmann et al. 1996b; Hallmann et al. 1997b). Inside the plant tissues the host plant provides relatively uniform and protected environment enabling the endophytes to avoid microbial competition and extreme environmental conditions such as fluctuations of temperature and moisture (Ramamoorthy et al. 2001).

The endophytic fungi are easy to culture *in vitro* and can be applied as seed treatments or on transplants, reducing the inoculum levels required (Sikora 1992; Sikora & Schuster 1999). Another advantage is that once developed, farmers will not need to apply the control products themselves as this may be done by public or private organizations engaged in commercial tissue culture production. Also fungal endophytes can easily be inoculated into tomato plants hence leading to production of naturally occurring biocontrol into one
strategy. The use of endophytic fungi from both environmental and economic point of view has a major advantage over other biological control agents that are applied directly to the soil. The latter, due to the high levels of inoculums is needed to treat the soil, are more costly, have to be applied more frequently, and their efficacy is often strongly influenced by environmental factors. Another advantage is that endophytic fungi live in plant tissue, thereby reducing the risk of side effects on non-target organisms (Niere et al. 2002). Once the endophytic fungi has established and colonized the plant tissues they can be used as biocontrol agents potential for controlling the root knot nematode in the tomato plants.

In spite of these advantages of endophytes over other biological control agents, the potential of fungal endophytes in pest and disease management in crops remains largely unexplored. Mutualistic endophytic fungi (MEF) can therefore be defined as fungus that live some time in their lifecycle in a plant tissues without producing symptoms of a disease, but simultaneously demonstrate antagonistic activity towards one or more pest or disease affecting the root system (Sikora et al. 2003). It is assumed that mutualistic endophytes have evolved from plant pathogenic fungi and that most if not all higher plants host endophytic fungi (Isaac 1992). Majority of endophytic species which have been successfully identified are Ascomycetes, Deutromycetes with few Basidiomycetes and Oomycetes (Isaac 1992). Among the best studied endophytes are intercellular symbionts in the family Clavicipitaceae found in many cool season grasses which are known to benefit the host with improved tolerance to heavy metals, increased drought resistance, systemic resistance to pests and pathogens and enhanced growth (Arnold et al. 2003).

Endophytes are known to confer resistance to their host against pathogens through a number of mechanisms that include competitive exclusion, parasitism, metabolites production and induced resistance. Due to this, they are potential pest control tools and scientists are using beneficial endophytes as biological control agents against crop pest such as nematodes, borers and plant pathogenic fungi (IITA 1998). Their presence has been proven in all plants investigated such as rice, maize, tomato and banana (Niere et al. 2002).
Mutualistic endophytic fungi have been shown to biologically control root knot nematodes of tomatoes (IITA 1998). The root knot nematodes attack tomato plants through the roots, therefore biological enhancement of the tomato plant using mutualistic fungal endophytes will increase plant resistance to infection (Sikora & Pocasangre 2004). Endophytes are well adapted to the life inside the plant and share the same ecological niche with endoparasitic nematodes, thus they are effective at the exact site of the pest or disease attack (Sikora & Pocasangre 2004).

2.11 Plant Tissue Colonization Process by Endophytic Fungi

The process of colonization of plant tissues by endophytic fungi are complex and include host recognition, spore germination, penetration and colonization. Endophytes penetrate their host plants through natural openings or wounds or actively using hydrolytic cellulases and pectinases (Hallmann et al. 1997b), forming inconspicuous infection within healthy plant tissues for all or part of their life cycles. Plant wounding induced by biotic factors such as plant-parasitic nematodes also constitute a major factor for the entry of the endophytic microorganisms (Hallmann et al. 1998).

For many years endophytic microorganisms colonizing plant tissues have been thought to be weekly virulent pathogens (Sinclair & Cerkauskas 1996). The distinction between endophytic infection and latent infection is that in latent infections, the host plant does not show any symptoms, with the infection persisting latently until symptoms are prompted to appear by environmental or nutritional stress conditions. The state of host plant and the pathogen may also provide signals for symptom expression. Since the production of disease symptoms is dependent upon the interaction between the host, parasite and the environment over time endophytic colonization is considered not to cause any disease (Sinclair & Cerkauskas 1996).

To detect endophyte colonization of plants, several methods for in situ detection of fungal endophytes in plant tissues have been developed. A simple method involves microscopic examination of differentially stained samples of endophyte infected plants (Saha et al. 1988). This method is however time
consuming and less reliable since histological staining is not endophyte specific (Hahn et al. 2003). Other methods for in situ detection of endophytes include the use of monoclonal antibodies (Hiat et al. 1997; Hiat et al. 1999) tissue printing immunoblotting (Gwinn et al. 1991) tissue print immunoassay (Hahn et al. 2003), electron microscopy (Sardi et al. 1992) and autoradiography (You et al. 1995).

Figure 3. Light micrographs of stained endophytic mycelium inside plant tissue showing intercellular colonization by endophytic fungi. A, B. Mycelium(arrow) running along the host vascular bundle (VB) x1000. PM: palisade mesophyll, SM: spongy mesophyll, T: tracheids Bars = 10μm. (Source; Review Iberoam Micology 2007)

Majority of endophytic fungi isolated from healthy tomato tissues belong to the genus Fusarium, followed by Acremonium, others include soil fungi belonging to the genera Penicillium, Aspergillus and Gongronella also Trichoderma which has biological potential is usually isolated (Niere et al. 2002).

The most dominant species is Fusarium oxysporum, which has been reported as an endophyte of many crop plants including banana, tomato, rice and maize and is an effective colonizer of plant roots (Niere et al. 2002). However, Fusarium sp are also notorious as causal agent of Fusarium wilt of many crops these are distinguished as specialised forms and physiological races, but majority of isolates of F. oxysporum are non-pathogenic (Niere et al. 2002). Two fungal endophytes F. oxysporum and Fusarium solani when added to tissue culture plants were found to be highly effective in immobilizing root knot nematodes (IITA 1998).
2.12 Interaction between Endophytic Fungi and Plant Parasitic Nematodes

Inhibitory effects against some species of migratory and sedentary endoparasites occur in grasses infected by *Neotyphodium* endophytes (West *et al*. 1988; Kimmons *et al*. 1990). *Neotyphodium* species infect aerial tissues, not roots. Therefore the inhibitory effects observed in the infected plants were interpreted as a result of fungal alkaloids being translocated to roots.

Non pathogenic *Fusarium oxysporum* isolated from roots are other groups of endophytic fungi known to be implicated in the antinematode activity. Culture filtrates of *F. oxysporum* have an inhibitory effect on *Meloidogyne incognita* suggesting that fungal toxins could be the mechanism of interaction (Hallmann & Sikora 1996). However the mechanism of *Fusarium* inhibition of nematodes appears to be more complex than toxin operated system.

2.13 Antagonistic Mechanisms of Endophytic Fungi Against RKN


2.13.1 Antibiosis: The production of toxic compounds is an important mechanism of action of beneficial endophytic organisms against plant parasitic nematodes. Grass endophytes mainly those belonging to *Neotyphodium* sp produces a wide range of metabolites both in culture and in plants. The production of alkaloids toxic to both insects and herbivores by grass endophytes has been documented (Breen 1994). These toxins have been isolated successfully from pure cultures of grass endophytes. Infection of tall fescue plants by *N. coenophialum* resulted in both qualitative and quantitative differences in the production of volatile compounds between endophyte-infected and endophyte free plants (Yue *et al*. 2001).
The ability of the endophyte infected plants to produce biologically active compounds depends on the location and concentration of endophyte in plants. Distribution of these compounds in the plant may also vary depending on the compound itself and the season. Toxins produced in endophyte-infected plants may be translocated elsewhere and exuded into the surrounding soil, affecting the nematode population.

Although toxic metabolites produced by most endophytic fungi in culture may show antagonistic activity against nematodes in vitro, the role of these compounds in nematode reduction in plants can only be shown if they are present in detectable concentrations in plant tissues. Secondary metabolites from endophytic isolates obtained from tomato cultivars have been shown to have inactivating or killing effects on the root knot nematode and mortality rates of up to 80-90% have been recorded (Niere et al. 2002). Majority of isolates that produce nematoxic or entomotoxic metabolites are *F. oxysporum*, others include *F. solani, F. concentricum* and *Acremonium* sp (Niere et al. 2002).

Both the type and quantity of secondary metabolites produced in endophyte infected plants might depend on the fungal genotype. For example tall fescue endophytes grown in vitro differed in the production of ergot alkaloid (Bacon 1988). Hill et al. (1990) also found that different isolates of *A. coenophialum* from tall fescue plants differed in the amounts and types of ergopeptine alkaloids produced. The host plant may also affect the production and concentration of the secondary metabolites and therefore its very important to determine a compatible host-endophyte-genotype combinations inorder to maximize the benefits of the association (Hill et al. 1990; Breen 1994; Siddiqui & Shaukat 2003a).

### 2.13.2 Changes in host physiology:
Endophyte infected plants have improved physiological responses to nematode parasitism; endophyte infected tall fescue plants has been associated with enhanced root growth and osmotic adjustments in growing points of the plant, thereby reducing the effects of drought on the host plant (Elmi et al. 2000).
Endophytes have also been shown to influence photosynthesis rate in host plants as seen in tall fescue plants infected by \textit{N. coenophialum} photosynthesised faster and flowered earlier than the non-infected ones (Newman \textit{et al.} 2003), also endophyte infected tall fescue plants exhibited higher survival and flowering frequency (Hill \textit{et al.} 1991). Such attributes of endophyte infection confer an ecological advantage to the endophyte infected plants, enabling their survival and dominance over endophyte free plants.

2.13.3 \textbf{Induced resistance:} Induction of systemic resistance by non-pathogenic microorganisms against pests and diseases is well documented phenomenon (Rammamorthy \textit{et al.} 2001; Compant 2005a). For example non-pathogenic \textit{F. oxysporum} isolates induced resistance in tomato plants to \textit{F. oxysporum} f.sp. \textit{lycopersici} Jarvis et Shoem, when inoculated prior to infection by the pathogen. Induced systemic resistance (ISR) can be defined as the resistance in plants induced by localized infection or treatment with microbial components or their products, or chemicals compounds (Rammamorthy \textit{et al.} 2001). ISR can be differed from systemic acquired resistance (SAR). SAR develops in plants in response to both biotic (pathogen attack) and abiotic factors (Chemicals) and depends on the accumulation of the salicylic acid (Van Loon \textit{et al.} 1998), the onset of SAR is characterized by the expression of the genes for the PR-proteins such as PR-1, PR-2, Chitinase and peroxidase (M’Piga \textit{et al.} 1987; Rammamorthy \textit{et al.} 2001; Jeun \textit{et al.} 2004). ISR on the other hand is dependent on the jasmonic acid and phenylpropanoid pathways (Pieterse \textit{et al.} 1998; Van Loon \textit{et al.} 1998; Rammamorthy \textit{et al.} 2001) ISR leads to the synthesis of plant defence products including peroxidases, polyphenol oxidases and phenylalanine ammonia-lyases (PAL). Polyphenol oxidase catalyses the formation of lignin through polymerization of phenols while PAL are involved in synthesis of phytoalexins and phenolic compounds.

2.13.4 \textbf{Competition:} Competition for plant space and resources may occur between resident endophytes and incoming plant pathogens this could eventually lead to the reduction of various pathogens in plants by the endophytes.