# BIOLOGY AND MANAGEMENT OF *Meloidogyne incognita (*KOFOID &WHITE, 1919) CHITWOOD, 1949 ON *Cucumis sativus* L. USING CROP ROTATION

**BY**

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## ABSTRACT

*Cucumis sativus* (Cucumber) is an edible fruit vegetable grown for its nutritional and economic values, but root-knot nematodes such as *Meloidogyne incognita* (MI) constitute a major constraint to its production. Nematicides, the most effective control option, are expensive and not environment friendly. Cultural practices such as crop rotation could reduce nematode infection but its effect on MI on cucumber has not been adequately documented. Therefore, biology, pathogenicity and management of *Meloidogyne incognita* with crop rotation were investigated.

A survey of plant-parasitic nematodes on cucumber in Lagos, Ogun, Osun, Oyo, Kaduna and Plateau States where cucumber is mostly cultivated was carried out using systematic sampling technique. Pathogenicity of *M. incognita* on Marketer, Ashley, Tokyo and Marketmore varieties of cucumber were assessed in pot and field experiments. Sixty-four one week-old seedlings of each variety was inoculated at four inoculum densities: 0, 10,000, 20,000 and 40,000 eggs of MI in pots (20 kg soil) using a 4x4 factorial arrangement in a completely randomised design in four replicates. On the field, split-plot design was used with main plots (nematode-infested and nematode-free) and sub-plots (cucumber varieties). Healthy and nematode-infected (unhealthy) cucumber roots of 5 weeks-old were processed for histopathology using standard procedures. Life cycle of MI in Marketer was studied to assess generation time (GT). Twenty-one economic crops were evaluated for resistance to MI (5,000 eggs/5kg soil) using Canto-Saenz's host designation scheme (Resistant = Gall index (GI)≤2 and Reproduction Factor (RF)≤1; Susceptible = GI≥2 and RF≥1; Tolerant = GI≤2 and RF≥1). Potentials of three resistant crops were evaluated in rotation with cucumber for MI management in the field. Data were collected on fresh shoot weight (FSW), marketable fruit yield (MFY, t/ha), GT, GI and RF. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Eleven plant-parasitic nematode genera were identified and the most prevalent were *Meloidogyne* spp. (45.5%), while the least were *Rotylenchus* spp. (0.3%). The four cucumber varieties were susceptible and susceptibility was in order of Marketer (GI=4.0, RF=20) >Ashley (GI=3.8, RF=14) >Marketmore (GI=3.5, RF=10) >Tokyo (GI=3.0, RF=15). However, MI was highly pathogenic at 10,000 eggs/20 kg soil on all cucumber varieties with MFY ranged from zero (Marketer) to 32.5±9.9 t/ha (control). The MI

infection significantly reduced FSW (62.1%) and MFY (77.7%) on the field. The *M. incognita* infection collapsed and shrank cells in the cortex, endodermis, vascular and formation of giant cells in unhealthy roots compared to healthy roots. The GT of MI was 30.0±0.0 days. Out of the 21 crops, 10 were resistant, 8 susceptible and 3 tolerant. Cucumber planted in rotation with resistant crops; marigold, sesame and maize each reduced GI by 75.0%, while FSW increased by 60.8% in marigold, 57.2% in sesame and 57.7% in maize. The MFY of cucumber planted after marigold and sesame increased by 96.0% each and 97.0% in maize compared to control (36.0%).

The cucumber varieties were susceptible to *Meloidogyne incognita* infection with more generations per growing season. However, cultivating cucumber in rotation with marigold, sesame or maize reduced *Meloidogyne incognita* infection in cucumber plots.

**Keywords**: Crop rotation, Cucumber fruit yield, Nematode infection, *Meloidogyne incognita*,

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This work is dedicated to my husband, Francis Taiwo Aminu, my children: Dominic, Samuel, Anthony, Antonia, John and Peter and to the lovely memory of my beloved parents, Late Alhaji Jimoh Aremu and late Mrs. Ebunoluwa Adijat Adeyemi.

## CERTIFICATION

I certify that this work was carried out by Mrs B. R. Aminu-Taiwo in the Department of Crop Protection and Environmental Biology, University of Ibadan

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# CHAPTER ONE

# INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an edible fruit vegetable belonging to the family Cucurbitaceae. It is the second most important crop of the family, after watermelon. It is believed to have originated in Northern India (Anon, 2000). It was known to ancient Egyptians, Greeks and Romans and was available in China in the sixth century AD. It has now spread throughout the world. Its areas of cultivation include Northern and Southern India, South East Asia, China, Central and South America and Africa (Anon, 2000). Cucumber production in Nigeria is fast becoming popular since it is a useful ingredient in the preparation of salad. It also has medicinal and therapeutic values, such as in the cure of hypertension and skin diseases. It is also a valuable source of potassium, sodium, magnesium, silicon, phosphorous, chlorine and fluorine (Rai and Yadav, 2005). Taking cucumber with other vegetables, cereals, fruits, nuts and salads enhances the nutritional value of food items (Anon, 2000). It is widely cultivated in the Northern States of Nigeria, such as Kaduna, Kano, Plateau and in peri-urban sites of Lagos State. It is planted both in dry and rainy seasons.

Despite the importance of this crop in the diets of Nigerian consumers, pests and diseases constitute major threats to its production. Cucumber is one of the most susceptible hosts of root–knot nematodes, *Meloidogyne* spp. (Darekar and Bele, 1990). Other nematodes, such as sting nematodes (*Belonolaimus* spp.) occasionally cause some losses in cucumber production (Natarajan *et al*., 2006). Though these nematodes alone rarely cause death, they predispose the plants to infection by other pathogens, such as fungi, viruses and bacteria, which eventually lead to wilting of crops. *Meloidogyne* spp. are notoriously difficult to control because of their wide host range and high rates of reproduction, with generation times of typically between 20 and 30 days in tropical soils, and females capable of producing a thousand eggs (Natarajan *et al*., 2006). By their activities in the roots of susceptible plants, they

cause galls on roots which results in reduction in water and nutrient uptake, manifesting in yellowing of leaves and patchiness in the field. This results in reduction in yield and quality of susceptible crops (Jonathan and Hedwig, 1991). *Meloidogyne* spp., are becoming a real threat to almost all vegetable crops and they have been considered as limiting factors in crop production (Ibrahim 2011).

Many of the nematicides used for the management of root-knot nematodes are effective but are expensive, highly toxic, pose human and environmental risk or have been withdrawn from the market (Greco *et al*., 1992; Ploeg, 2002; Wang *et al*., 2002; Abd-Elgawad, 2008). The over use of these pesticides also compound pest and disease problems due to the fact that many non-target organisms acting as natural predators are wiped out (Dhalmini *et al*., 2005). Apart from the aforementioned problems, it is not applicable where low value crops are involved (Hague and Growen, 1987). With the phase-out of these broad-spectrum fumigant nematicides and increased need for agricultural producers to improve environmental and food safety, alternatives to the application of fumigant chemicals to the soil is essential. Sequential cropping in crop rotation is widely regarded as a good agricultural practice in traditional and modern agriculture (Rahman *et al*., 2007). Crop rotation systems are useful in maintaining soil fertility, reducing or preventing disease and pest build-up in the soil (Kimenju *et al*., 2008).

In nematode management, the principle that guides the use of crop rotation is the reduction of populations of damaging nematode species to levels that allow subsequent crops to complete early growth before being heavily attacked (Bridge, 1996). This can be achieved by alternating poor hosts, non-hosts or resistant crops with susceptible crops (Ateka *et al*., 2001). The adoption of sequential cropping in root-knot nematode control is restricted among the smallholder farms due to scarcity of arable land, coupled with market-driven demand for particular crops and/or varieties (Bridge, 1996). More so, training is required to design and implement effective crop cycles to control pathogens such as root-knot nematodes that have a wide host range (Kerry, 1990; Kimenju *et al*., 1999; Yamada, 2002). Meanwhile, previous studies have focused on plants such as *Tagetes* spp., (Marigold), *Crotolaria* spp. (Sunn hemp), *Asparagus* spp., *Sesamum indicum* (Sesame), and *Azadirachta indica* (neem) that are antagonistic to root-knot nematodes through release of root exudates toxic to the nematodes (Varges-Ayala *et al*., 2000). However, a major

hindrance to their adoption into most cropping systems is low or lack of commercial value of the most intensively studied plants (Johnson *et al*., 1992; Otipa *et al*., 2006). Suitability of crops incorporated into crop rotation system for nematode management is not only determined by their efficiency in nematode suppression, but also by the economic returns they bring to the farmer. Therefore, the challenge is to identify nematode-suppressive crops that satisfy the economic considerations in crop production systems. In an effort to address the above challenges, this study was conducted to identify potential rotation crops with food, forage or commercial value and incorporate them into cropping cycles for root-knot nematode management in cucumber production. Food and environmental safety is paramount to sustainable development and healthy citizenry. Nigerian literature is very scanty in the use of economic crops in rotation for the management of root-knot nematode which is devastating to most of the vegetables crops in the tropics. This research work therefore was set to investigate various aspects of cucumber and *M. inco*gnita relationship, as well as, its management using some selected plants/crops that are of economic value in rotation.

In view of the economic importance of root-knot nematode, *Meloidogyne incognita* as a serious biotic factor in cucumber production, the objectives of study were to:

1. Identify the plant-parasitic nematodes associated with cucumber production in South Western and some parts of Northern States in Nigeria.
2. Investigate the effects of different population densities of *M. incognita* on growth and yield of cucumber
3. Study the histopathology of *M. incognita* on infected cucumber root.
4. Investigate the life cycle of *M. incognita* in the root of cucumber
5. Screen the potential crops/plants for resistance to root- knot nematode (*Meloidogyne incognita*)
6. Determine the effect of growing cucumber in rotation with resistant crops in managing root-knot nematode in naturally infested field.

# CHAPTER TWO
# LITERATURE REVIEW

## 2.1 Origin and history of cucumber

Cucumber is an indigenous vegetable to India (DeCandole, 1967). Purseglove (1969) reported that the cultigen, *Cucumis sativus* L. originated in Northern India where the related *C. hardwicki* Royle occurs as wild, although this might be a 'weedy' form of *C. sativus*, which has escaped cultivation. In China, cucumber was known by the 6$^{th}$ century AD. Cucumber has been cultivated in India for at least three thousand years. From India, it spread rapidly to China and even earlier and more rapidly to the West. It has now spread throughout the world (Rai and Yadav, 2005).

## 2.2 Nutritive value and uses of cucumber

Cucumber is rich in Vitamins B and C, as well as minerals such as calcium, phosphorous, iron and potassium (Table 2.1). It has 2.5% carbohydrate, 0.4% protein, 0.1% fat and 0.4% fibre content (Rai andYadav, 2005). Cucumber is consumed generally as fruit. Cucumber is an important ingredient in making salad and is used in beauty therapy i.e. the extract has cleansing, soothing, and softening properties (Van Luijk, 2004). Young or ripe cucumber fruits are used as cooked vegetables or made into chutney (Van Luijk, 2004). Young shoots are consumed as leafy vegetable, and seeds are consumed or used to extract edible oil, but these uses have not been recorded for Africa (Van Luijk, 2004). It also prevents constipation, jaundice and indigestion.

Two compounds, -2-6-nonadienol and 2-hexenol, are attributed to flavor of cucumbers. Some volatile compounds have also been identified in cucumber, namely inonaol, trans-2-neonal-1-ol, cis-3-nones and C10-15 saturated chain of aldehydes (Rai and Yadav, 2005). Cucumber is also characterized by the presence of bitter principles called cucurbitacins. They occur in nature as free glycosides or in bound form. High concentration of bitter principles is found in the fruits. The pollen also

contains bitter principles and when this pollen fertilizes non-bitter ovules, the resulting fruit becomes bitter. This phenomenon is called metaxenia. During fruit maturation, several changes occur. These include changes in concentration of bitter principles, ascorbic acid, minerals, etc. (Rai and Yadav, 2005)

**Table 2.1: Nutritive composition of cucumber (amounts per 100 g edible portion)**

| Food value | Slicing cumber | Pickling cucumber |
|---|---|---|
| Moisture | 96% | 96% |
| Energy | 13kcal | 12kcal |
| Protein | 0.5g | 0.7g |
| Fat | 0.1g | 0.1g |
| Carbohydrate | 2.9g | 2.4g |
| Fibre | 0.6g | 0.6g |
| Ca | 14mg | 13mg |
| P | 17mg | 24mg |
| Fe | 0.3mg | 0.6mg |
| Na | 2mg | 6mg |
| K | 149mg | 190mg |
| Vitamin A | 45IU | 270IU |
| Thiamine | 0.03mg | 0.04mg |
| Riboflavin | 0.02mg | 0.2mg |
| Nacin | 0.30mg | 0.4mg |
| Ascorbic Acid | 4.7mg | 19.0mg |
| Vitamin B6 | 0.05mg | 0.4mg |

Sources: Gebhardt *et al*., (1982); Haytowitz and Matthews (1984)

## 2.3 Common cucumber cultivar types

There are several varieties of cucumber which differ in shape, size and colour. The colours vary from whitish green to dark green. The fruits turned orange, yellow or brownish yellow when mature (Van Luijk, 2004). There are three major cucumber cultivar types produced today: processing (pickling), fresh market (slicing) and greenhouse (slicing). The fruits of pickling cucumbers are blunt and angular, warty and light green in colour. They have either black or white spines on their skin. e.g. cultivars Calypso, Saladin and Little Leaf**.** Slicing cucumbers are usually longer, smooth and have more uniform green skin colour and they are tougher than picklers. e.g. Marketmore, Poinsett 76, Beith Alpha, Ashley etc. Greenhouse cucumbers are greenhouse-grown cucumbers which are parthenocarpic and do not require pollination e.g. Marketer, Murano, Tokyo (Van Luijk, 2004).

## 2.4 Characteristics of cucumber

Cucumber is a warm season, annual vining plant that produces stiff hairs on the leaves and stems. When touched by human skin, cucumber plant can be itchy and irritating. The plant is herbaceous, so it is easily susceptible to moisture stress. Cucumber has moderately deep root, like many of the other cucurbits, which have long taproots as well as shallow fibrous root systems. The stems of cucumber are vining. Therefore, they can be trained on trellises to save space and improve yield and fruit quality (Valenzuela *et al*., 2007). The leaves also produce bristly hairs, which are simple, alternate and lobed. They are triangular, palmate, and located at the base of the main axils. Perfect flowers are rare in cucumbers. Many of the older cultivars are monoecious which means they produce separate male and female flowers on the same plant (Plate 2.1). Most of the current cultivars are gynoecious i.e have mostly female flowers (only 5% are male). Production of male flowers is promoted by long days and high temperatures. Male flowers are also produced when the plant is stressed or has a high fruit load. Female flowers are produced during the short days with cool temperatures and low light (Van Luijk, 2004).

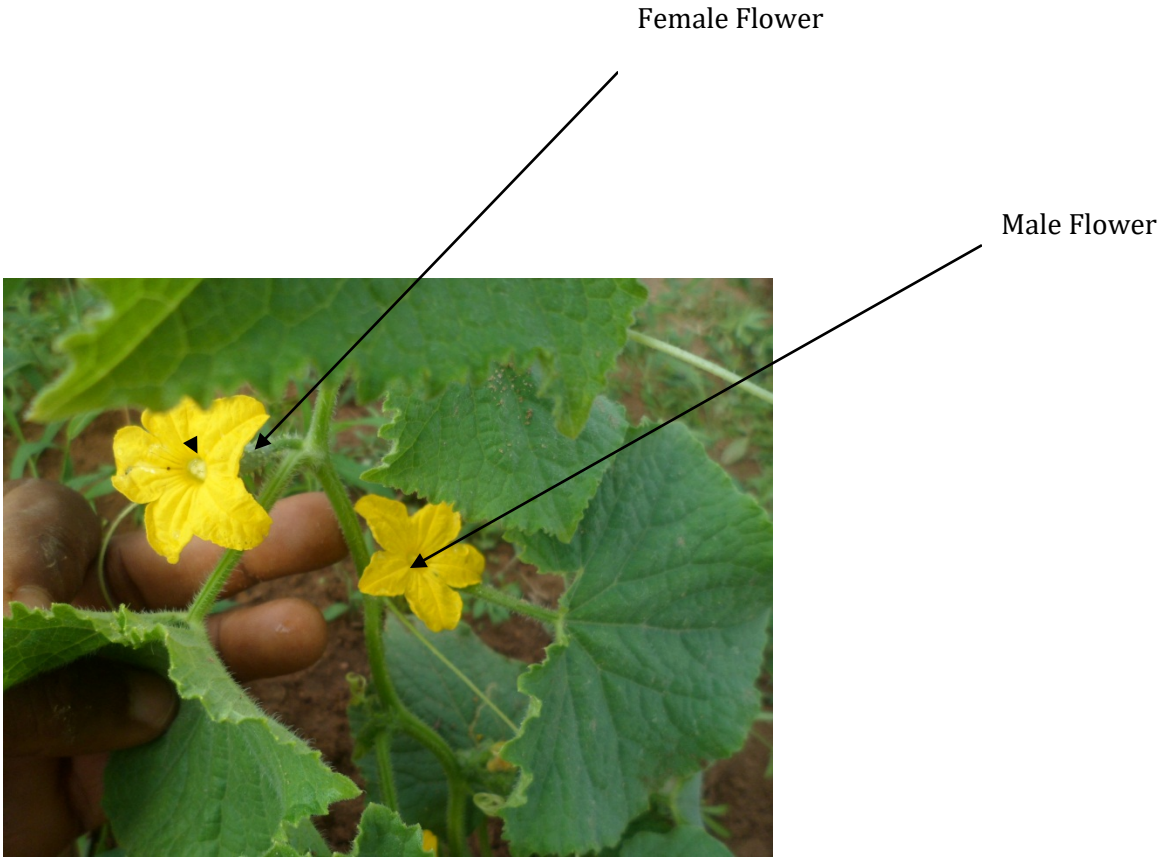



**Plate 2.1: Monoecious cucumber plant showing both the male and female flowers.**

## 2.5 Growth and development of cucumber

Germination of cucumber seeds takes three days at optimum temperatures. Flowering starts 40-45 days after sowing. The female flowers develop later than the more numerous male flowers. The ratio of male/female flowers largely depends on day length, temperature and cultivar. Long days and high temperatures tend to keep the plants in the male phase or change the ratio to a higher male proportion (Van Luijk, 2004). Fruits are harvested 1-2 weeks after flowering, depending on the genotype, usually before they are physiologically mature.

## 2.6 Propagation, planting and management of cucumber

Cucumber is propagated by seed. The 1000-seed weight ranges from 20-35g. One – three (1- 3) kg of seed is needed per hectare depending on the method of sowing. Planting on raised beds improves drainage during rainy season and can support good root development. Weed control is necessary until the plants cover the soil. The plant can be staked. This will improve fruit quality, reduce disease incidence and make it easier to pick during harvesting (Van Luijk, 2004).

## 2.7 Harvesting and yield of cucumber

Cucumber fruits for fresh consumption are harvested before they are fully mature. Depending on the type, this can be 1-2 weeks after flowering. First harvest is 40-60 days after sowing, depending on climate and cultivar. Harvesting is done every other day to every few days. The fruits are allowed to mature on the plant for seed production. World average fruit production in 2013 reached 71 million MT (FAOSTAT, 2015). In Africa, few data are available. Estimated fruit yield for Cameroon and Ghana are 1.1t/ha and 12t/ha, respectively (FAOSTAT, 2015). However, Thailand and Indonesia have an estimated average yield of just above 10t/ha while India produces 6.34t/ha. European Union produces an average yield of 53.20t/ha (FAOSTAT, 2015).

## 2.8 Cucumber production areas and figures

Areas of cultivation of cucumber include Northern and Southern India, South East-Asia, China, Central and South America, Africa, Central and South America, the Caribbean and most tropical areas. In 2013, the world area under cultivation was about 2.12 million hectares with total production of 71.4 million metric tonnes (MT)

(FAOSTAT, 2015). In 2013, Netherlands ranked first for cucumber production in the world. Egypt, South Africa, Ghana and Cameroon were the only major African producers with an average yield of 22.83, 16.64, 12.00 and 1.10 t/ha, respectively (Table 2.2). There was no cucumber and gherkins production data for Nigeria in FAO data base (FAOSTAT, 2015).

## 2.9 Pests and diseases of cucumber

The cucumber plant is susceptible to the flea beetle (*Podagrica* spp) that greatly decreases leaf mass and inhibits photosynthesis. Other insects that attack cucumber are aphids and pickle worm. Cucumber plants are prone to many diseases such as anthracnose, angular leaf spot, bacterial wilt, downy mildew, cucumber mosaic virus and powdery mildew. Root-knot, caused by *Meloidogyne* spp., is a serious disease of cucumber (Walter *et al*., 1996). In North Carolina State, USA, this nematode destroys about 11% of cucumber crop annually (Walter *et al*., 1996). In Nigeria, the root-knot nematodes (*Meloidogyne* species) are important pests of vegetables. Root-knot nematodes attack almost all the cultivated plants and can cause high losses (Adegbite and Adesiyan, 2001; Adegbite, 2003). Other nematodes that infect cucumber are the sting nematode, *Belonolaimus* spp; the root-lesion or meadow nematode, *Pratylenchus* spp.; the stubby root nematode, *Trichodorus* spp., and the pin nematode, *Paratylenchus* spp. (Valenzuela *et al*., 2007).

**Table 2.2: World production of cucumber and gherkins in 2013**

| Rank | Country | Production (T/ha)* |
|---|---|---|
| 1. | Netherlands | 666.67 |
| 2. | Denmark | 349.09 |
| 3. | Canada | 120.21 |
| 4. | Spain | 93.14 |
| 5. | Germany | 80.98 |
| 6. | France | 75.11 |
| 7. | Saudi Arabia | 72.29 |
| 8. | Republic of Korea | 70.15 |
| 9. | Japan | 50.43 |
| 10. | China | 46.60 |
| 11. | Mexico | 41.06 |
| 12. | Australia | 34.95 |
| 13. | Denmark | 34.91 |
| 14. | Turkey | 27.87 |
| 15. | Egypt | 22.83 |
| 16. | Chile | 22.77 |
| 17. | South Africa | 16.64 |
| 18. | United States of America | 15.12 |
| 19. | Colombia | 14.83 |
| 20. | Ghana | 12.00 |
| 21 | Indonesia | 10.19 |
| 22. | Iraq | 9.29 |
| 23. | Cuba | 8.89 |
| 24. | India | 6.34 |
| 25. | Cameroon | 1.10 |
| 26. | Nigeria | No Data |

*T/ha = Tonnes per Hectare
Source: FAOSTAT (2015)

## 2.10 Root-knot nematodes (*Meloidogyne* spp.)

Root-knot nematodes are a group of plant-parasitic nematodes that feed on roots resulting in the formation of irregular, knotty enlargement of the roots (Scurrah *et al*., 2005). The first observation of root-knot infection was made on cucumber in 1850's (Mai, 1985). The size and shape of the knots or galls depend on the number of nematodes present in the roots, the species of the nematodes present and the plant species involved (Mitkowski and Abawi, 2003). Root-knot nematodes are amongst the most economically important nematode pests in Nigeria. They have a very wide host range (Adesiyan *et al*., 1990, Netscher and Sikora, 1990, Mitkowski and Abawi, 2003). This makes them very difficult to control because they can survive and reproduce on other host crops including weeds (Mitkowski and Abawi, 2003). Some of the reasons adduced to their high ranking include their world-wide distribution, extensive host range, the debilitating nature of the diseases caused by them and their role in many destructive diseases complex (Mai, 1985, Mitkowski and Abawi, 2003).

They attack a wide range of hosts including both cultivated and uncultivated crops. Root-knot nematodes parasitize more than 2000 plant species including monocotyledons, dicotyledons, herbaceous and woody plants (Hussey, 1985, Mitkowski and Abawi, 2003). Quite a number of weeds also serve as hosts to root-knot nematodes (Ogbuji, 1978). Other favoured hosts of the root-knot nematodes in Nigeria and other places include cowpea, tomato, sugarcane, tobacco, cotton, pineapple, carrots, lettuce, egg plants, pawpaw, citrus, peppers, yam, rice, sweet potato and Celosia (Fademi and Fawole, 1992; Fawole and Claudius-Cole, 2000). They are probably the major obstacle to the production of sufficient food and fibre crops in Nigeria and many other nations (Ogunfowora, 1977). They cause huge losses on susceptible crops wherever there is intensive planting of such crops and precautionary measures are not taken against population build-up (Jonathan and Hedwig, 1991).

Root-knot nematodes are ubiquitous and over 126 species have been described (Moens *et al*., 2009), but only about five or six of these are important in agriculture. The most economically important ones are *M. incognita*, *M. javanica*, *M. arenaria*, *M. hapla*, *M. graminicola*, *M. triiticoryzae* and *M. indica* (Mangala and Mauria, 2006). These species can be adapted either to the cooler or the warmer regions of the world. *M. incognita* (Kofoid and White, 1919; Chitwood, 1949), *M. javanica* (Chitwood, 1949) and *M. arenaria* (Chitwood, 1949) are predominant in the warmer regions between

latitudes 35ºS and 35ºN, while *M. hapla* (Chitwood, 1949) is more common north of 35ºN (Taylor and Sasser, 1978).

The root-knot nematodes, *Meloidogyne spp*. were first reported in Nigeria on cowpea in 1959 (Anon, 1961). Three species namely, *M. incognita*, *M .javanica* and *M. arenaria* have been found to be widely distributed in irrigated and upland conditions in the country. *Meloidogyne incognita* is most prevalent in the south forest zone while *M .javanica* is found to be more predominant in the Northern savanna zone. *M. arenaria* is only occasionally found in both the Northern and the Southern States of Nigeria (Ogunfowora, 1976; Olowe, 1976; Babatola, 1984).

*Meloidogyne incognita* and *M. javanica* were found to occur together frequently as mixed populations in the South of Nigeria. In the North however, they often occur as single population (Olowe, 1976). According to Dobson *et al*. (2002), Nigerian soils are highly infested with root-knot nematode. Apart from the presence of knots or galls on roots, other symptoms of root-knot nematode damage on host crops include patchiness in the field, yellowing of leaves (chlorosis), stunting, wilting especially in hot weather and death of plants in severe cases (Adesiyan *et al.*,1990; Ajayi, 1990; Jain, 1992). The effects of these symptoms invariably lead to reduction in crop yield and quality (Adesiyan *et al.*, 1990). In addition to the abilities of root-knot nematodes to cause recognizable diseases, there are ample evidences to show that they also facilitate the entry and establishment of plant- pathogenic fungi, bacteria and viruses (Adeniji, 1992).

Root-knot nematodes adversely affect the symbiotic association between some plants and *Rhizobium* and also predispose the plant to secondary infections (Khan, 1993). An example is the vascular wilt of tomatoes caused by *Ralstonia solanacearum* in which the root-knot nematode acts as a wounding agent. Furthermore, root-knot nematodes have been implicated in the breakdown of resistance e.g. vascular wilt of tobacco caused by *Pseudomonas* being assisted by *Meloidogyne* spp and *Fusarium* wilt of tomato is also assisted by *Meloidogyne incognita acrita* (Adeniji, 1992). Again the overall effect of these would lead to reduction in quality and quantity of farm produce.

### 2.11 Description of the root-knot nematodes.

The root-knot nematodes, *Meloidogyne* species belong to the family Meloidogynidae in the super family Tylenchoidea of the order Tylenchida. The family is characterized by marked sexual dimorphism. The males are vermiform while the females become swollen and flask-shaped with anus virtually terminal in position

(Adesiyan *et al.,* 1990). The root-knot nematode differs from cyst nematodes in that the adult females do not form cyst, and are therefore, not resistant to desiccation. In addition, almost all the eggs are laid in a gelatinous matrix or egg sac (Adesiyan *et al.,* 1990).

## 2.12 Biology of root-knot nematodes: Life cycle of root-knot nematodes

Root-knot nematodes are sedentary endoparasites in that the female nematode is no longer motile once it has established a feeding site in the root. The female deposits single-cell eggs in a gelatinous mass at/or near the root surface. These eggs undergo embryogenesis, first moult takes place inside the eggs and second stage juveniles emerge. This juvenile is about 0.42mm long. These preparasitic second stage juveniles move freely in the soil and are attracted towards roots from as far as 75cm (Prot and Netscher, 1979). After penetrating into the root the juvenile moves between and through cells to the still undifferentiated conductive tissues. Within two or three days, the juvenile becomes settled, with its head embedded in the developing vascular cylinder, and begins feeding, becomes sedentary and enlarges in cross-section. Meanwhile, as the nematode is maturing, it goes through two additional juvenile stages interspersed by moults. The only significant growth is in its diameter.

Continued enlargement of these cells, rapid multiplication of other cells, and growth of the nematode contribute to the developing root gall, which protects the maturing nematode from outside environment. The crop root's conductive tissues no longer function properly, translocation of water and nutrients is impeded and, as a result, top growth is affected adversely. The heavier the infection burden, the more stunting and chlorosis occur above ground (Anon, 1993). Generally, females of root-knot nematodes have a globose body, with a short "neck," containing their stylet, metacorpus and esophageal gland cells (Mitkowski, and Abawi. 2003). The length of the life cycle and rate of population increases depend upon several factors such as soil temperature, host suitability and soil type. At 27$^{o}$C, which is about optimum for most *Meloidogyne* species, one generation on a good host requires approximately 21 to 25 days, whereas at 19$^{o}$C at least 87 days are necessary. Therefore, three to eight generations are possible in one year (Adesiyan *et al*., 1990).

## 2.13 Distribution of the root-knot nematodes (*Meloidogyne* spp.)

According to Adesiyan *et al.* (1990), root-knot nematodes are widely distributed and have an extensive host range. Chitwood (1949) reported that *M. incognita*, *M. arenaria* and *M. javanica* have preference for warm climate and that they are adapted to warm areas lying between latitudes 35$^{o}$N and 35$^{o}$S, while *M. hapla* is often referred to as the northern root-knot nematode because it is common north of latitude $35^{0}$ in the Northern hemisphere. In Nigeria, *M. incognita* is mostly found in Southern States, *M. javanica* in Northern States and *M. arenaria* is dispersed throughout the country. Recently, *M. enterolobii* was discovered in Nigeria (Claudius-Cole *et al*., 2014). It must, however, be stated that although mixed populations of the genus *Meloidogyne* have been reported in the Northern and Southern Nigeria (Ogunfowora, 1982), *M. incognita* predominates over other species in the South (Adesiyan *et al*., 1990). Also looking at a wider distribution, *M. arenaria* is found in the tropical, subtropical and temperate soils, *M. incognita* and *M. javanica* are typically tropical and subtropical and *M. hapla* is limited to temperate soils (Eisenback *et al.*, 1991). The genus has a worldwide distribution and is the most recognized plant-parasitic nematode because it causes the characteristic galling symptoms (Wyss, 2004).

## 2.14 Survival, dispersal and influence of environmental factors on root-knot nematodes

The survival and dispersal of root-knot nematodes depend upon a number of abiotic (temperature, moisture, soil texture, aeration, osmotic potential, etc.), and biotic factors. Relatively higher thermal requirement of *M. javanica* and *M. incognita* explains their prevalence in tropics and sub-tropics than *M. hapla* which has low heat requirement for survival. The survival and infectivity of *Meloidogyne* species in soil are related to the conservation of food and physiological conditions of the juveniles which in turn, are influenced by temperature, moisture and aeration (Van Gundy *et al.*, 1967). However, they survive mostly in the soil as egg masses. The gelatinous layer around the egg masses provides protection against desiccation and chemicals (Jilian, 1996).

The root-knot nematodes are obligate parasites; therefore, the absence of suitable host plants for prolonged periods ultimately leads to their disappearance

(Eisenback *et al.*, 1991). In the absence of susceptible crops, however, they often survive on weed hosts. In general, conditions favourable for plant growth will also be favourable for *Meloidogyne* reproduction. According to Van Gundy (1985), nematodes are active in soils with moisture levels 40 –60 % of field capacity and move through the soil in water films.

As the soil either dries or increases in moisture, nematode activity decreases. Guiran and Demeure (1978) found that the optimum moisture level for emergence of juveniles from egg mass in both sandy and clay soils is slightly above field capacity. If under conditions optimum for emergence are present but host plants are absent, juveniles will deplete their energy reserves in the soil and eventually die. Although nematode populations rapidly decline, a proportion of eggs in the egg mass are in diapause and assure perpetuation of the species (Guiran and Villemin, 1980). Survival is mainly influenced by moisture content in the soil and to a less extent by temperature (Eisenback *et al.*, 1991). However, the survival of some eggs due to inhibition of hatching while juveniles survive periods of moisture stress in a state of anhydrobiosis (Van Gundy, 1985). In field soil, the number of juveniles decreased from an initial infestation of approximately 10,000 nematodes/$dm^3$ of soil to zero after 12 weeks, when soil was gradually dried (Guiran, 1979). Similar effects were found in the dry season in Senegal (Demeure, 1977). Nematodes could not be detected in the top 20cm of the soil at the end of the dry season.

Dissemination takes place when juveniles or eggs are transported from infested to uninfested area by the farm equipment. Spread with irrigation water has been demonstrated in the U.S.A. (Faulkner and Bolandar, 1970) and in Spain (Tobar and Palacios, 1974). Dispersal through run-off water produced during rainstorms, soil adhering to animals, footwear and agricultural implements. Dispersal over long distances might be through accidental importation of infected plants to other countries. The major environmental factor affecting generation time is temperature as it affects egg hatch, nematode mobility, penetration, growth, reproduction and survival. The optimum temperatures range from 15$^o$C to 25$^o$ C for cool climate root-knot species such as *M. hapla*; and from 25$^o$C – 30$^o$C for warm climate species such as *M. incognita* and *M. javanica* (Adesiyan *et al*., 1990; Olowe 1992). Optimum temperatures for nematode development correspond to those found in tropical vegetable growing regions, a factor ensuring heavy root-knot infestation.

Survival of eggs and juveniles of *M. javanica* decreased significantly when subjected to a temperature of 45ºC for three hours (Demeure, 1978). Temperature optimal for *M. hapla* is at least 5ºC lower than for the other major species in the tropics. *M. hapla* is therefore, limited to the upland tropics and temperate regions. *M. incognita, M. javanica and M. arenaria* occur in areas with an average temperature of $36^0$C or lower in the warmest north. *M. hapla* conversely occurs in areas having a temperature as low as - $15^0$C during the coldest month, but is limited to regions with an average temperature of less than $27^0$C during the warmest month (Taylor *et al.,* 1982).

## 2.15 Host range for root-knot nematodes

Root-knot disease is the best known nematode disease with over 2000 plant species susceptible to one or more species of *Meloidogyne* (Horst, 1990). They are obligate plant parasites that parasitize thousands of different plant species, including monocotyledons, dicotyledons, herbaceous and woody plants (Eisenback *et al.*, 1991). Species of *Meloidogyne* are pests of major food crops, vegetables, fruits, and ornamental plants grown in tropical, subtropical and temperate climates. They reduce yield as well as the quality of the produce. Populations of certain species occurred as physiological races e.g. *Meloidogne incognita* with varied abilities to reproduce on key differential host plants (Sasser, 1972; Hartman and Sasser, 1985)

Root-knot nematodes adversely affect the symbiotic association between some plants and *Rhizobium* and also predispose the plant to secondary infections (Khan, 1993). Adesiyan *et al.* (1990) reported that root-knot nematodes are widely distributed, have extensive host range and interact with other microorganisms such as fungi, bacteria and viruses which induce diseases and the overall effect is drastic reduction in quality and quantity of crop yield. Favoured hosts of the root-knot nematodes in Nigeria and other places include cowpea, tomato, sugarcane, tobacco, cotton, pineapple, carrots, lettuce, African spinach, okra, brassica, egg plants, cucumber, pawpaw, citrus, peppers, yam, rice, sweet potato and Celosia (Caveness, 1978; Nwauzor, 1982, Fademi and Fawole, 1992; Fawole and Claudius-Cole, 2000). Available data on the effect of *Meloidogyne* spp. on agricultural crops have shown that both the direct and indirect effects of its infection on food crops lead to yield losses (Nwauzor, 1982, Fademi and Fawole, 1992; Fawole and Claudius-Cole, 2000).

## 2.16 Symptoms of damage by root-knot nematodes

Basically, root-knot nematodes are parasites of roots or underground stems. The disease caused by root-knot nematode is not epidemic in nature but rather of slow decline in yields spreading very gradually and steadily year after year. Some areas within a field may be severely affected, whereas, plants in other parts may not show any sign of the disease (Jain, 1992). However, the above-ground symptoms are similar to those of mineral deficiency or drought injury, even with the presence of adequate fertilizer and moisture. Other symptoms may include dieback, yellowing, wilting and premature shedding of the foliage with severe stunting, depending upon the initial nematode population in the field. Chlorosis of the foliage lowers the quality of vegetable crops resulting in severe losses (Ajayi, 1990; Jain, 1992). Below-ground symptoms include root necrosis, galls, reduction in lateral roots and internal rotting of storage organs such as in onion and yam tubers (Ajayi, 1990). Earlier workers (Viglierchio, 1971; Sasser, 1972; Hartman and Sasser, 1985) have shown that growth and yield in vegetables were related to the biological stress occasioned by nematode attack.

The galls vary in size from pin-head to a large size and, in case of heavy infestation, may coalesce to form large secondary galls. They are nearly round, long and irregular but they are part of the root and cannot be broken-off. This differentiates them from beneficial nodules formed on legume roots by nitrogen-fixing bacteria (Horst, 1990). The size of galls also depends upon the host plant and nematode species. The galls produced on cucurbits are much larger than the ones produced on chillies or cotton by the same nematode species (Horst, 1990). *M. hapla* usually produces small galls as compared to *M. incognita* or *M. javanica* on potato. Besides galling, some other typical symptoms in the form of forking of tap roots as in carrots or beet and pimples like tubercules on tuber (potato) and pods (groundnut) are also manifested. Root-knot nematodes usually cause the formation of knots or galls on roots of susceptible host plants (Eisenback *et al.,* 1991). Second stage juveniles (J2) are attracted to the root tip in the zone of elongation. They are also attracted to areas of lateral root emergence. They are attracted by carbon dioxide and apparently by small molecules that are dialyzable, perhaps amino acids (Wyss, 2004).

The motile and vermiform second stage juvenile is the infective stage which migrates through the soil and penetrates the root of suitable hosts above the root cap in

the meristematic region of the young root (Nwauzor, 1979; Fademi and Fawole 1992). After penetration, it migrates intercellularly and intracellularly until it stops with the head in the developing stele and its body in the root cortex (Adesiyan *et al.*, 1990; Osunlola and Fawole, 2014). The cell walls are pierced with the stylet and then the nematode injects substances from the oesophageal gland. These cause the enlargement of cells in the vascular cylinder and increase rates of cell division in the pericycle. This leads to formation of giant cells (syncitia) caused by enlargement of cells (hypertrophy), dissolution of cell walls, enlargement of nuclei and dissolution of cell content. There is also an intense cell multiplication (hyperplasia) around the head of the juveniles. The nematodes derive nourishment from these specialized nutritive cells and cannot continue their development to adulthood without them (Eisenback *et al.*, 1991).

### 2.17 Estimated yield losses due to root-knot nematodes

In spite of their importance, no accurate data on the extent of losses is available in Nigeria. But on the basis of general survey, average yield losses in the world are believed to be higher in developing countries in the tropics and subtropics (Taylor and Sasser, 1978). According to Sasser (1979), the annual loss due to nematode on carrot was estimated at 38% in USA. Complete failure of cucumber crop due to *Meloidogyne incognita* has been observed in some localities (Darekar *et al*., 1988). *M. incognita* has also been reported to cause an estimated loss of 14% in economically important vegetable and fruit crops (Agrios, 2004)

The root-knot nematodes are reported to cause 40% reduction in market value of infected yam (Nwauzor and Fawole, 1992; Fawole, 1988). Nodulation in cowpea is adversely affected by *M. incognita* at low populations. However, under high soil populations, nodulation was completely prevented in cowpea plants (Babatola and Omotade, 1991). This was apparently due to the hypertrophy and hyperplasia taking place in the infected roots thereby preventing normal activities in such roots (Babatola and Adalemo, 1988). In a study of the relationship between root-knot nematode population density and yield of yam in Nigeria, Nwauzor and Fawole (1992), using population levels 0; 100; 1,000; and 10,000 eggs per plant, obtained a negative correlation between the nematode's population level and yield of yam.

## 2.18 Disease complexes

All interactions of plant- parasitic nematodes with other plant pathogens have three components: the nematode, host and other pathogens. Viruses, bacteria and fungi are plant pathogens known to interact with nematodes (Khan, 1993). Apart from directly causing yield reduction, the root-knot nematode often interacts with other soil-inhabiting pathogens to form disease complexes in which the resulting disease is much more severe than components of the complex would cause alone (Anon, 1993). *Meloidogyne* species interact with both *Verticillium* and *Fusarium* fungi. Moreso some fungi, for example, *Pythium*, *Fusarium* and *Rhizoctonia*, grow and reproduce much faster in the galls than in other areas of the root, thus inducing an earlier breakdown of the root tissues (Anon, 1993). For example, *M. javanica* predisposes sugarcane to *Curvularia lunata* root-rot (Whitehead, 1988). The nematode, while moving between the leaf sheaths to reach the growing point, carries the fungal conidia and deposits them at the growing point (Khan, 1993). Khan (1993) also reported that the feeding process of all plant- parasitic nematodes produces wound in the host plant, either by simple micropuncture or by rupturing or separating cells. Futhermore, second-stage juveniles of sedentary endoparasites migrate intercellularly through the cortex and establishing within the vascular tissue to induce syncytia or giant cells through which secondary infection can be initiated by other pathogens. Syncytium, according to Holtmann *et al.* (2000) is the formation of a mass of cytoplasm containing several nuclei enclosed within a plasma membrane. *Pratylenchus penetrans,* a migratory endoparasite, has been implicated in synergistic interactions with *Verticillium dahliae*, showing distinct lesions on egg plant roots (Francl and Wheeler, 1993). Similarly, some species of root- rot fungi such as *Pythium, Rhizoctonia*, *Phytophthora*, *Sclerotium* and *Collectotrichum* are known to interact with plant-parasitic nematodes, the role of nematode in root-rot diseases is to assist the fungal pathogen in its pathogenesis and increasing host susceptibility (Evans and Haydock, 1993). The lesions caused by lesion or burrowing nematodes or invasion tracks formed by penetrating juveniles of root-knot or cyst nematodes provides a better substratum for establishment and colonization by the fungal pathogens (Khan, 1993). Nordmeyer and Sikora (1983) demonstrated that inoculation of *Heterodera daverti* and *Fusarium avenaceum* on *Trifolium subterraneum* cv Clare, increased the disease index significantly when the nematode was inoculated one or

two weeks after the fungus. Also, there were more dead plants in the same treatments. Also severe damage was observed above ground when pathogens where inoculated simultaneously on olive seedlings. Moorman *et al.* (1980) reported that *Meloidogyne* spp. increased wilt severity on tobacco when inoculated on the same portion of the root system with *F. oxysporum* fsp *nicotianae*. Starr and Martyn (1991) observed that cotton cultivars reported to be highly resistant to Fusarium wilt disease had greater incidence of vascular browning and plant mortality in the presence of *Meloidogyne incognita* than when the nematodes were absent. In chickpea (*Cicer arietinum*) with high level of resistance to Fusarium wilt, co-infection by *M. artiellia* overcame the plant's resistance to wilt (Castillo *et al*., 2003). Moderate to high population densities of *M. incognita* increased both the incidence and severity of cotton seedling disease caused by *Phytophthora parasitica* (Powell and Nusbaum, 1960; Fichtner *et al*., 2005). In a study on tobacco, Powell *et al.* (1971) demonstrated that species of *Trichoderma* and *Penicillium* that were not recognized as pathogens caused substantial root rot disease when the plants were infected by *M. incognita.* Findings revealed that there was an increase in free amino acid in the giant cells of *M. incognita* infected tomatoes, which is a suitable substrate for *Fusarium oxysporum* growth (Sidhu and Webster 1977). The amount of root rot caused by *Pythium aphanidermatum* and *Rhizoctonia solani* on Chrysanthemum was further increased in the presence of *Pratylenchus coffeae* (Evans and Haydock, 1993). Similarly, *Pratylenchus penetrans* increased the infection levels of *Colletotrichum coccodes* and *R. solani* on Russet Burbank potato roots (Evans and Haydock, 1993).

## 2.19 Root-knot nematodes as constraints to production of vegetables and other food crops.

The root-knot nematode species are the most important plant-parasitic nematode affecting vegetables (Netscher and Sikora, 1990). This is because the genus *Meloidogyne* consists of many species which attack many vegetable crops in the tropics and sub-tropics. The losses caused by *Meloidogyne* on roots and tuber crops like carrot are both quantitative and qualitative, because nematode galling affects marketability (Netscher and Sikora, 1990).

Estimations of vegetable crop losses in the tropics range from 17-20% on egg plant, 18-33% on melon, 11% -20% on cucumber and 24-38% on tomato (Sasser, 1979), a yield reduction of 61% in sorghum (Swarup and Sosa-Moss, 1990). The total

crop loss caused by *Meloidogyne* species is difficult to ascertain in cases where crops are suffering from simultaneous attack by fungi, viruses, insects and other nematodes (Lamberti, 1979).

On okra plant, Khan and Saxena (1969) reported growth reductions following the inoculation of Red Wonder line with 0, 10, 100, 1000 or 10,000 *Meloidogyne incognita* eggs in pot trials. Plant growth was significantly reduced when inoculated with 1,000 larvae per plant and above, with the highest reduction in growth (35.7%) occurring with 10,000 larvae per plant. The relationship between soil and root nematode populations, as well as root galls induced by *Meloidogyne inc*ognita and growth of *Abelmoschus* species was investigated in the field by Nwanguma (2002a). He reported that induced galls, soil and root nematode populations correlated negatively with shoot but positively with root weights. Correlation with plant height was also negative. Positive correlation was also observed between fruit weight and number as well as height with fresh shoot weight. In another study Nwanguma (2002b) screened for resistance/susceptibility of sixteen lines of okra to the root-knot nematode *Meloidogyne incognita*. Nine out of the tested lines were highly susceptible. All the 49 cucumber cultivars/lines that were screened for their reaction to *Meloidogyne incognita* were susceptible (Darekar and Bele, 1990)

## 2.20 Use of synthetic nematicides in controlling nematodes

The control of root-knot nematodes poses a major problem because of their extensive host range for most of the species (Adesiyan *et al.*, 1990). However, several control options exist for the control of *M. incognita* and these include; chemical control, use of resistant crop varieties, crop rotation, fallowing and use of botanicals. There is also an integrated pest management strategy involving a combination of any of the above-mentioned methods which is perhaps, a better approach to nematode control. The use of nematicides on the field for the control of plant-parasitic nematodes was not practised until the early 1900 when an effective and economic soil fumigant (chloropicrin) was discovered. Prior to this development, only greenhouse and nursery soils were treated by steam, chloropicrin or methyl bromide (Sasser and Carter, 1985). The discovery of the nematicidal effect of D-D, a mixture of 1,3-dichloropropene (Telone) and 1,2-dichloropropane and a by-product of petroleum refining by Carter in 1943 led to the development of various volatile halogenated

hydrocarbons such as ethylene dibromide (EDB) and 1,2-dibromo-3-chloropropane (DBCP) or nemagon for nematode control (Dropkin, 1988).

The dynamic changes of nematode population in the soil require constant vigilance and updated management decisions in order to avoid major yield losses. According to Adesiyan *et al.,* (1990), aldicarb, carbofuran, miral and oxamyl applied as post-planting treatments in yam mounds two weeks after planting at the rate of 2kg a.i/ha reduced soil population of yam nematode, *Scutellonema bradys* to very low levels with a remarkable yield increase recorded but there was an accumulation of toxic residues in harvested tubers.

Akinlade and Adesiyan (1982) tested the efficacy of carbofuran in controlling *M. incognita* on okra and found that carbofuran was effective at 2.0kg a.i/ha. Adegbite and Adesiyan (2001) reported that carbofuran applied at the rate of 1.5 and 2.5 kg a.i/ha on *M. incognita* infecting soybean resulted in highest grain yield of 2.5 t/ha, and the lowest root galling and nematode population in the soil at harvest. According to Fazal *et al*. (2001a), carbofuran at 0.5 and 1.0kg a.i/ha resulted in lower nematode (*M. incognita*) population on soybean cultivar Bragg. Also, carbofuran at 1 and 2g a.i/ha as seed dressing in controlling *M. incognita* infecting soybean resulted in lower number of galls/ plant, and nematode population in the soil and roots compared to the control (Fazal *et al.*, 2001b).

## 2.21 The use of botanicals in the control of root-knot nematodes

According to Fuglie (1998) and Stoll (2000), more than 2,400 plant species around the world are currently known to possess pest control properties. Several plants have been identified with nematicidal or nematostatic properties either in their seeds, fruits, leaves, barks, and roots or in their root exudates. Treatment of *M. incognita* in soil with extracts of dholkalmi (*Ipomea fistulosa)* alone and in combination with furadan 3G (carbofuran) gave an effective control of root-knot nematodes in eggplant and improved plant growth (Alam *et al.,* 1995). When the efficacy of karanj (*Pongania glabra*) leaves at 20,000ppm, 40,000ppm and 80,000ppm was compared with carbofuran at 2.0kg a.i/ha for the control of *Aphelenchoides composticola*, all concentrations of karanj leaves reduced populations of nematodes by 50-80% with the 80,000ppm concentration being at par with carbofuran (Rao and Pandey, 1991).

Addition of chopped portions of Egyptian clover (*Trifolium alexandrium*), rice (*Oryza sativa* L) , marigold (*Tagetes erecta*), thevetia (*Thevetia nerifolia*) and wheat (*Triticum aestivum*) at 0.5, 1.0 and 1.5% w/w reduced the number of egg masses by 70.5-80.2% and galls of *M. incognita* by 52.5- 59% on roots of sunflower (Abadir *et al*., 1996). Soil amendment with leaves of neem (*Azadiractha indica*), datura (*Datura fastuosa*) and calotropis (*Calotropis procera*) reduced the population of *Helicotylenchus dihysteria* by 52-62% and improved growth of tomato (Firoza and Maqbool, 1996b). Use of datura and calotropis at 3% w/w was however found to be phyto-toxic. The population of the nematode decreased with increase in the rate of amendments. Datura was found most effective in reducing the nematode population build-up followed by calotropis and neem. Onifade and Fawole (1996) reported the efficacy of extracts of some selected plants in the control of *M. incognita* and the growth and yield of Ife Brown cowpea were evaluated both under greenhouse and field conditions. All extracts inhibited nematode egg hatch and larval survival at varying percentages. Extracts from *Anacardium occidentale* and *Gmelina arborea* were the most nematicidal.

The extracts were not toxic to cowpea; rather, they induced increased growth, vigor and yield by suppressing the populations of *M. incognita* both in the soil as well as in infected cowpea roots. As in the *in-vitro* tests, the highest and the lowest reduction in nematode number were associated with cashew and *Gmelina* extract, respectively, in the greenhouse. Compared with the inoculated control, the extracts caused marked reduction in the number of nodules in treated plants. According to Firoza and Maqbool (1996a), leaf extracts of neem (*Azadirachta indica*), datura (*Datura. metel*) and calotropis (*Calotropis procera*) were found to be toxic to *Helicotylenchus dihysteria*. Datura leaf extract gave maximum nematode mortality (80%) followed by calotropis (70%) and neem (40.66%), respectively. The toxicity of extracts increased with increase in concentration as well as the exposure time. Use of the leaf extracts showed a significant increase in growth of tomato plants which was found to be associated with the increasing concentration of extracts and subsequent decrease in nematode population.

Marigold treatment (grown and incorporated into soil before planting rice) suppressed nematode root galling and increased rice grain yield by 46% over the untreated check. The increase in yield was attributed to a reduction of nematode

population in the soil. In addition, marigold plant materials served as organic matter and provided nutrients for rice growth (Polthanee and Yamazaki, 1996). Aqueous extracts of *Chromolaena odorata* have been shown to be toxic to plant-parasitic nematodes (Subramaniyan, 1985,). Similarly, application of 15t/ha of its chopped green leaves effectively reduced *M. incognita* on okra and cowpea also crop yield also increased by 94-135% in okra and 45-50% in cowpea during rainy and summer seasons (Ajith and Sheela, 1996) while 30t/ha of *C. odorata* as soil organic amendment improved the yield of *Musa* species and reduced the population of *Meloidogyne incognita* (Kashaja *et al.*, 1999). Study on the comparative effects of the incorporation of leaves of *Brassica campestris*, *Catharanthus roseus, Pedilanthus tithymaloides, Ricinus communis, Azadirachta. indica* and *Calotropis procera* at 80g/kg soil with carbofuran application at 2kg a.i/ha for the control of *M. incognita* on tomato showed that *B. campestris, P. tithymaloides, R. communis, A. indica* and *C. procera* were as effective as carbofuran for control of *M. incognita* (Rao and Reddy, 1991).

Root exudates of marigold, aloe (*Aloe arboresens*) and lemon grass (*Cymbopogon citratrus*) in comparison with fenamiphos effectively reduced nematode populations by 69.5%, 64.8% and 55.4% respectively while 87.9% reduction was observed in fenamiphos treatment (Sweelam, 1989). The nematicidal action of chopped *C. odorata* leaves persisted for a long period (Ajith and Sheela, 1996) and exerted a residual nematicidal effect (Kashaja *et al*., 1999; Fatoki and Fawole, 1999) by effectively reducing hatching of *M. incognita* eggs and causing the death of larvae after they have hatched (Amosu, 1982). Oyedunmade and Olabiyi (2006) reported that aqueous extracts and leaf powder of Siam weed, red acalypha and bitter leaf reduced populations of root-knot nematode, *M. incognita*, both in soil and root of sesame. According to Chitwood (2002), chemicals produced by plants are a potential source for development of new pesticidal compounds and these nematicidal phytochemicals are generally safe for the environment and humans.

A nematicidal compound may be obtained from Chinese herbal remedies (Ferris and Zheng, 1999; Zasada *et al*., 2002). Many nematicidal phytochemicals from a great variety of chemical structures have been isolated from numerous plant families (Gommers and Bakker, 1988; Chitwood, 2002). Majority of these nematicidal phytochemicals isolated have been from the plant family Asteraceae (Gommers and

Bakker, 1988). *In vitro* α-Terthienyl and related compounds were isolated from *Tagetes* spp. and have been shown to be nematicidal at low concentrations (Uhlenbroek and Bijloo, 1958, 1959). Meanwhile these phytochemicals were not effective in nematode control in soil (Gommers and Bakker, 1988). Polyacetylenes are another chemical group from the Asteraceae with nematicidal activity, nematicidal polyacetylenes have been isolated from flowers of *Carthamus tinctorius* and roots of *Cirsium japonicum* (Kogiso *et al*., 1976; Kawazu *et al.*, 1980) and dithioacetylenes have been isolated from *Milleria quinqueflora*, *Iva xanthiifolia*, *Ambrosia artemisiifolia*, *Ambrosia trifida*, *Schkuhria pinnata*, and *Eriophyllym caespitosum* (Gommers and voor in't Holt, 1976). Thiarubrine C isolated from the roots of *Rudbeckia hirta* has been shown to have nematicidal activity against *M. incognita* and *Pratylenchus penetrans* (Sánchez de Viala *et al.*, 1998).

Plant essential oils, mainly monoterpenes, have been evaluated for their nematicidal activity, and some were highly effective in nematode suppression (Oka *et al*., 2000; Oka, 2001). However, use of natural essential oils as nematicides is not cost effective. Various neem (*Azadirachta indica* A. Juss.) tree preparations are well known as commercially available nematode control products derived from plants (Mojumdar, 1995). Recently, elecampane (*Inula viscosa*, syn. *Cupularia viscosa*, *Dittrichia viscosa*) (Asteraceae), a widespread plant in Mediterranean countries, has been found to have nematicidal activity in the shoot (Oka *et al*., 2000). This plant has antifungal activity as well, and several foliar fungal diseases have been controlled by the plant extracts (Cohen, 1998; Wang *et al*., 2004). Another species, *Inula helenium*, has been known to have anthelmintic activity, probably due to sesquiterpenoid lactones such as alantolactone (Mahajan *et al*., 1986; Bourrel *et al.*, 1993). Sesquiterpenic acids (costic acid and isocostic acid) from *I. viscosa* leaf extracts were found to be the nematicidal phytochemicals (Oka *et al*., 2000).

## 2.22 The use of crop rotation in the management of root-knot nematodes

Damage by nematodes was significantly reduced in tomato planted in rotation with sweetcorn or in sweetcorn with *Tagetes patula*, *Crotalaria juncea*, *Sorghum bicolor* and *Asparagus sp*. in the field (Otipa *et al*., 2003). Bahiagrass rotations provide excellent suppression of peanut (*Meloidogyne arenaria*) and southern root-knot nematodes (*Meloidogyne incognita*). Long-term bahiagrass rotations provide the

additional benefit of suppressing soil-borne diseases (white mold and vascular wilts) and improving soil tilth. All bahiagrass varieties are easily established from seed (Hagan *et al*., 1998). One alternative to using nematicides is to intercrop and rotate vegetables with marigold. Most cultivars of African marigold (*Tagetes erecta)* and French marigold (*Tagetes patula*) are effective in reducing the most common root-knot populations - *M. incognita* and *M. javanica*. The roots of these attractive flowering plants contain chemicals that kill nematodes. As a method of biocontrol, growing marigolds is not only pleasing to the eye but economical and environmentally sound as well. Marigolds act as trap crops. The nematodes enter the plants and are killed because they can not set up successful feeding sites.

Some crops like Asparagus, corn, onions, garlic are good rotation crops for reducing root-knot nematode populations. Crotalaria, velvet bean, and grasses like rye are usually resistant to root-knot nematodes. (Yepsen, 1984; Wang, *et al*., 2004). Crop rotation with non-host will not only help prevent nematode populations from reaching economic threshold levels, but also help control other plant diseases and insect pests (Yepsen, 1984; Wang, *et al*., 2004). Research shows that sesame may be an effective rotation crop to control peanut root- knot nematode (*Meloidogyne arenaria*) and southern root-knot nematode (*Meloidogyne incognita*). Sesame rotation is not effective, however, for *Meloidogyne javanica* (Starr and Black, 1995).

In South Texas, soybean varieties were shown as possible alternatives to grain sorghum in cotton cropping sequences. Eighteen soybean varieties were tested in *Rotylenchulus reniformis*-infested soil either nonfumigated or fumigated with 1,3-dichloropropene. Reproductive rates of *R. reniformis* were compared in the first year. Both experiments were planted with cotton in the second year to measure the rotational effects of soybean on cotton yield compared with grain sorghum and fallow. The high-yielding soybean cultivars with potential to suppress reniform nematode were “HY574,” “Padre,” “DP7375RR,” and “NK83-30” (Westphal and Scott, 2005). The best rotation to control the *Meloidogyne chitwoodi* (Columbia root-knot nematode) in potatoes involves planting a summer non-host crop, followed by a winter cover crop (rapeseed) incorporated as a green manure. Non-host crops include super sweet corn (Crisp and Sweet 710/711), pepper, lima bean, turnip, cowpea, muskmelon, watermelon, squash, rapeseed, canola, mustard, and sudangrass (Ingham, 1990). For root lesion nematode control on potatoes, researchers found that forage

pearl millet (Canadian Hybrid 101) and marigold (Crakerjack) as rotation crops with potatoes resulted in fewer root lesion nematodes and increased potato yields than rotation with rye (Ball-Coelho *et al*., 2003). Tomatoes planted two weeks after African marigolds (*Tagetes erecta*) were disked into soil as a result there was a reduction in root-lesion nematode damage by 99% as compared to tomato-tomato or fallow-tomato rotation. (Grossman, 1999).

## 2.23 Histopathological studies of root-knot-nematode on infected roots and tubers

Consequent upon feeding by root-knot nematode second stage juveniles after penetration, susceptible host plants respond by undergoing pronounced morphological and physiological changes. The most important of these is the development of elaborate feeding sites called giant cells (Bird, 1962). Further nematode development cannot take place without this unique response. Giant cell formation is therefore very essential for a successful host/parasite relationship (Hussey, 1985). Giant cells are highly specialized cellular adaptations induced and maintained in susceptible host plants by feeding nematode. Cytological investigations that giant cells are formed by repeated endomitoses without subsequent cytokinesis (Huang and Maggenti, 1969; Jones and Dropkin, 1975).

Giant cells are transfer cells passing nutrients to the nematode (Huang, 1985). Hussey (1985) reported that the tissues preferred for giant cell formation are the primary phloem and adjacent parenchyma. However, Fawole (1988) observed that giant cells were always closely associated with xylem tissues in yam. Fademi and Fawole (1992) reported that *M. incognita* induced the development of many oval-shaped giant cells in a susceptible rice cultivar within 14 days. It has been reported that one nematode feeds on 5 - 6 giant cells (Hussey, 1985) but Fawole (1988) observed between 1 - 3 giant cells surrounding a nematode head. However, one giant cell per nematode was most common. The thickness of giant cell walls appears to vary from one host crop to another. Furthermore, Fawole (1988) observed strikingly thin-walled giant cells in white yams compared with those formed in tomato roots, although they have dense cytoplasm and are as multinucleate as those observed in tomato. Giant cells formed in yam tubers also contained smaller and fewer nuclei when compared to those in tomato roots.

Thorne (1961) reported that the position of the female root-knot nematodes in potato tubers is usually indicated by brown spots formed by cells that are in contact with the egg mass. He also reported that the gelatinous matrix of egg mass is toxic to cells which turn brown but do not decay. Nwauzor (1982) also reported the presence of brownish dark spots in the cortical areas of the yam tubers infected with root-knot nematodes. He also concluded that the spots correspond to the location of the nematode and the extent to which the yam has to be peeled off. Fawole (1988) also observed a necrotic ring around the female root-knot nematode in yam tissue. He noticed this after the production of gelatinous matrix. According to him the necrotic reaction did not affect the normal nematode growth and development as second stage juveniles were seen in cells adjacent to the necrotic ring indicating that second stage juveniles were able to hatch and cause further attack in the tuber (Fawole, 1988).

Thorne (1961) concluded that a similar toxicity may also occur in sweet potato and the affected sweet potato cells may not perform adequately the functions of storing starch. Nwauzor (1982) reported that adult female nematodes occupy large space in the infected yam. Yam cells that should have been engorged with starch are disrupted, compressed and displaced by enlarging adult root knot nematode. As a result there is less food content in the infected tuber. In their studies. Adesiyan *et al.* (1975) reported that the yam nematode, *Scutellonema bradys* caused an extensive disintegration of the yam epidermis. They reported that the nematodes activities were limited to the tissue lying within the periderm layer and those immediately beneath the periderm. Nematodes activities according to them disrupted the yam tissue by emptying the cell contents and breaking of cell walls. Cavities which may serve as infection sites for other invading micro-organisms were also observed in both the epidermis and cortex.

Adeniji (1970) had earlier observed that careless cuts during harvests or pre-harvest nematode infestation on yam tubers predisposes such tubers to attack by decay organisms such as *Penicillium oxalicum, Aspergillus niger, A. tamarri, Botryodipledia theobromae, Fusarium sp, Cylindrocarpon radicicola, Cladosporium herbarium* and *Rhizopus nigricans.* Nematode infection also leads to conversion of starch to simple sugars which facilitates fungal and bacterial growth. Fasahat (1992) carried out a screenhouse experiment to study the rates of multiplication of *Pratylenchus brachyurus* (Godfrey, 1929) Filip. & Sch. Stek., 1941 on local cultivars

of 30 vegetable crops, commonly grown in northern Nigeria, seven were resistant, five were poor, 12 were susceptible and six were highly susceptible hosts, based on multiplication of the nematode in and around the roots of these crops. A direct relationship between the increasing susceptibility of the plant species and root-population of the nematode was observed. Histopathological studies revealed severe damage to the cortex as well as to the vascular tissue of the highly susceptible plants. Occlusion of xylem vessels was noticed in some cases.

*Meloidogyne incognita* also induced the formation of giant cells as was observed by Osunlola (2011) in sweet potato roots. They are large and multinucleate and are formed close to the vascular cells. Their formation brought about the disruption and disorganization of xylem cells. An average of four giant cells comprising 7-8 nuclei each were found around the head of an adult female nematode. One adult female usually occupied a cavity but multiple infections were also encountered in this investigation as more than one nematode was observed in some galls of sweetpotatoes roots (Osunlola, 2011). However, there is very little or no information about the histopathological changes induced by *Meloidogyne incognita* on cucumber roots.

## 2.24 Description of some selected plants screened for *Meloidogyne incognita* infection

### 2.24.1 Marigold

The ever-popular marigold, *Tagetes erecta* (Plate 2.2 a & b) is the most familiar of the plants known to actively suppress plant-parasitic nematodes. In trials, marigolds grown throughout the summer suppressed common garden nematodes such as root-knot *(Meloidogyne),* lesion *(Pratylenchus),* and stunt *(Tylenchorhynchus)* nematodes. Available evidence indicates that all marigolds act generally as trap crops and also contain chemicals toxic to nematodes. The roots of these attractive flowering plants contain chemicals that kill nematodes. As a method of biocontrol, growing marigolds is not only pleasing to the eye but economical and environmentally sound as well. Plant-parasitic nematodes are controlled when *Tagetes* spp. are planted as intercrops or in rotation with other crops (Alexander and Waldenmaier, 2002). The population levels of root lesion nematodes (*Pratylenchus penetrans*) was found to be reduced by 98% when *T. erecta* grown in rotation with *Lycopersicum esculentum.*

(Alexander and Waldenmaier, 2002). Ploeg (2002) demonstrated that *L. esculentum* fruit yields were consistently higher when grown after *Tagetes* spp. compared to leaving land fallow, and gave yields comparable to those obtained with *L. esculentum* grown in fumigated soils. Similar results were obtained when *T. erecta* was raised as a cover crop and the residues incorporated into the soil before growing taro, *Colocastia esculenta* (Sipes and Arakaki, 1997) or intercropped with soybean, *Glychines max* (El-Hamawi *et al*., 2004).

### 2.24.2 Sesame

Sesame *(Sesamum indicum),* (Plate 2.3) a crop valued for its oil and seed, has suppressive activity against the peanut root-knot nematode (*Meloidogyne arenaria*). When grown as a summer annual, this crop has proven equally if not more effective than bahiagrass and cotton in reducing the carryover of peanut root-knot nematode juveniles in the soil in a peanut or soybean production system. The status of sesame as a host of other species of root-knot nematode commonly found in Nigeria has not been determined. Sesame may be rotated with peanut, soybean, and possibly cotton. A single crop of sesame in a field heavily infested with the peanut root-knot nematode will not suppress nematode populations sufficiently to eliminate the need for a nematicide treatment on the following year's peanut crop.

Research shows that sesame may be an effective rotation crop to control peanut root- knot nematode (*Meloidogyne arenaria*) and southern root knot nematode (*Meloidogyne incognita*). Sesame rotation is not effective, however, for the Javanese root knot nematode (*Meloidogyne javanica*) (Starr and Black, 1995). Sesame is used in flower gardens because they provide flowers over a 30-40 day period. Gardeners use sesame as a companionate plant because they inhibit root knot nematodes (ASGA, 2012). Decorators use sesame stems in dry arrangements. Sesame contains natural oil-soluble and water-soluble antioxidants: sesamin, sesamolin, sesaminol, and sesaminol glucosides. In heating additional lignans are formed: sesamol (ASGA, 2012).

**(a)** **(b)**

**Plate 2.2: *Tagetes erecta* plant in the pot (a) and Tagetes erecta seeds (b)**

(a)

(b)

**Plate 2.3: Sesame plant (a) and Sesame seeds (b)**

### 2.24.3 Sorghum (*Sorghum bicolor*)

Sorghum (Plate 2.3) is a warm season annual grass that is best known in the U.S. as a forage crop. Estimated of Nigeria area planted to sorghum for forage use is 1.5 million acres. New varieties of sorghum, however, are being developed for use as a grain crop. These new hybrid types of sorghum are shorter in stature for easier combining, and higher in seed yield. Use of sorghum grain on a commercial basis only began in the U.S. in the early 1990s, but has led to production on several thousand acres in Georgia and Florida. Most of this initial sorghum production has been for poultry feed, although the crop shows good feed potential for other types of livestock as well. Some sorghum has been grown for birdseed. Sorghum was domesticated as a food crop in the tropical region of East Africa at least 4,000 years ago. Its use as a food grain has grown over the centuries, with an estimated 64 million acres of sorghum being grown in Africa and India (this acreage is equivalent to the total corn crop). The crop is used for a variety of food products, and is even made into a type of beer (Emadi *et al*., 2011).

### 2.24.5 Maize

Maize (Plate 2.4) is commonly known as corn in some countries. The growing of corn first began in Mesoamerica and has since spread throughout the American continents. Today, maize is the largest crop in the Americas. There has been much disagreement about the origin of maize in Mesoamerica. There are some reports that the Spanish first grew maize in southern Mexico. The domestication of maize has been dated back as far back as 12,000 years ago. The United States produces the largest amount of maize throughout the world, but there are other countries that also produce high quantities of maize as well as China, Brazil and South Africa. In 2003 there was six hundred metric tons of maize produced in the world. Maize can only be produced in areas that do not have extreme cold temperature, as it is a cold-intolerant crop.

(a) (b)

**Plate 2.4: Sorghum plant (a) and Sorghum seeds (b)**

**(a)** **(b)**

**Plate 2.5: Maize plant (a) and Maize seed (b)**

### 2.24.6: Turmeric

The genus *Curcuma* belonging to the family Zingiberaceae. *Curcuma longa* Linn. syn. *C. domestica* Valeton. (Plate 2.6) is a perennial herb, 60-90cm in height, with a short stem and tufts of erect leaves. Rhizome is cylindric, ovoid, orange coloured and branched. Leaves are simple, very large, petiole as long as the blade, oblong-lanceolate, tapering to the base up to 45cm long. Flowers are pale yellow, arranged in spikes concealed by the sheathing petioles and flowering bracts are pale green (Warrier *et al*, 1994). Curcumin, the colouring agent and major constituent of *C. longa*, is said to possess local as well as systemic antiinflammatory property which has been found to compare favourably with phenylbutazone (Srimal and Dhawan, 1973). Rhizome is antiprotozoal, spasmolytic, CNS-active, antiparasitic, antispasmodic, antibacterial, antiarthritic (Husain *et al*, 1992). Warrier *et al.* (1994) reported that rhizomes of *C. Longa* are anthelmintic, carminative, antiperiodic, emollient, anodyne, laxative, diruretic, expectorant, alterative, alexertive, febrifuge, opthalmic and tonic. Rhizome is externally effective as insect repellent against houseflies is found to inhibit *Clostridium botulinum*. Essential oil from rhizome showed fungitoxicity (Asolkar *et al*, 1992).

**(a)** **(b)**

**Plate 2.6: Turmeric plant (a) and Turmeric rhizomes (b)**

### 2.24.7 Sunflower

Sunflower (*Helianthus annuus L*.) (Plate 2.7), is one of the main crops for the oil production, following soy, cotton and rape seed (FAO, 2010). It is the common name for any of the plants of the genus *Helianthus* of the flowering plant family *Asteraceae*. It is also commonly used specifically in reference to the annual plant *Helianthus annuus*, the common sunflower, which is characterized by a long stem and a large flowering head (inflorescence) with large seeds. The term sunflower also refers to this plant's seed-like fruit (commonly but incorrectly called the seeds) or the encased, edible, true seeds. The adaptation that allows sunflowers to advance their own individual purpose of reproduction, the "flower head," also contributes larger value for the ecosystem and for humans. Ecologically, sunflowers are copious nectar producers, providing food for pollinating bees, while the seeds and leaves provide food for such animals as birds, insects, and squirrels. For humans, all parts of the plant are used. The seeds of the common sunflower are eaten and are a source of valuable oil (one of the most important vegetable oils). The leaves and stalks are used as fodder for livestock. Overall, the common sunflower, with its flowers of various colors (yellow, maroon, orange, etc.), is a popular ornamental plant for gardens (Judd, *et al*., 1999).

(a)

(b)

**Plate 2.7: Sunflower plant (a) and Sunflower seeds (b)**

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1 Nematode inoculum extraction and population estimation procedures

Eggs of the root-knot nematode, *M. incognita*, used as inoculum in this experiment were extracted using the method of Hussey and Barker (1973) from the galled roots of *Celosia argentea* (L.). Roots of three-month old *M. incognita*-infected *C. argentea* were collected from the vegetable field of National Horticultural Research Institute (NIHORT), Ibadan. The roots were washed to remove adhering soil particles and then cut into 1-2 cm pieces in the Laboratory. The root pieces were vigorously shaken in 0.5% sodium hypochlorite (NaOCl) solution (100ml JIK +600ml water) for four minutes. The NaOCl solution was quickly passed through a 45µm aperture size sieve nested over a 25µm aperture size sieve to collect the root-knot nematode eggs. The 25µm aperture size sieve with the eggs was then quickly placed under a stream of tap water to remove residual NaOCl, and collected in a beaker. The egg suspension in the beaker was thoroughly homogenized using a magnetic stirrer. The number of eggs in 1 ml of suspension was counted under the dissecting microscope. The average of three counts was taken to estimate the egg population per ml of egg suspension.

## 3.2 Extraction of nematodes from soil samples using extraction tray method

This method was described by Whitehead and Hemming (1965). The set-up consists of two sieves separated by a double-ply facial tissue placed on a collection tray. After thorough mixing, 250 ml of soil was poured on the upper sieve and water was added to the collection tray to field capacity. The second stage juveniles ($J_2$) were collected in beakers after 48 hours and counted under the dissecting microscope.

### 3.3 Soil sterilization

A 200 litre metal drum was filled half- way with soil. The drum was placed on three big stones arranged in a triangular pattern. Water was added to the soil inside the drum to moisten it. Fire was then made with firewood arranged under the drum. A spade was used to stir the soil for even distribution of heat. The steaming process was continued for several hours until the soil was very hot to touch (around $90^0$C). The procedure was repeated until sufficient amount of sterilized soil was collected. Sterilized soil was filled into 15 litre pots and stored in the laboratory until needed.

### 3.4 Experiment one: Survey of plant-parasitic nematodes associated with cucumber in major production States in Southwestern and Northern Zones of Nigeria

The survey of plant-parasitic nematodes associated with cucumber was carried out in the cucumber production areas of the Southwest comprising Oyo, Ogun, Osun, and Lagos States while Northern zone included Jos and Kaduna States (Fig 3.1). The survey was carried out in each State through the seeds seller outlet (Agrictropic Nigeria Limited, Ibadan). In each state, four cucumber production Local Government Areas were selected and six farms per local Government were visited for the survey during 2013/2014 and 2014/2015 growing seasons from October to March each year, making a total of 24 farms from each State and 144 farms nationwide. Soil samples of 1 kg constiting of 10 to 15 soil cores were taken in a zig-zag pattern from 0 to 30 cm deep. The cores were combined per farm to form a single composite sample. About 150 g of lateral roots were sampled from 10 individual plants randomly selected per farm. The soil and root samples from each farm were sealed in plastic bags, bulked seperately, properly labelled, and were taken to the Nematology Research Laboratory, National Horticultural Research Institute (NIHORT), Ibadan for extraction while nematode identification was carried out at the Nematology Laboratory of International Institute, Tropical Agriculture (IITA), Ibadan.

Nematodes from the soil samples were extracted using the pie-pan modification of Baermann funnel method (Whitehead and Hemming, 1965) (section 3.2). Each composite sample was thoroughly mixed and nematode was extracted from 250 ml sub-sample. Each extraction set up was left undisturbed for 48 hours after which the nematode suspension was decanted into a beaker. Ten extraction trays were set up per sample.

Nematodes in each beaker were killed by adding an equal volume of boiling water to the nematode suspension in each beaker. Nematode suspension from each sample was adjusted to 10 ml. A 2 ml aliquot of each sample was drawn into a counting dish with a hypodermic syringe (without needle) after it had been thoroughly mixed with magnetic stirrer.

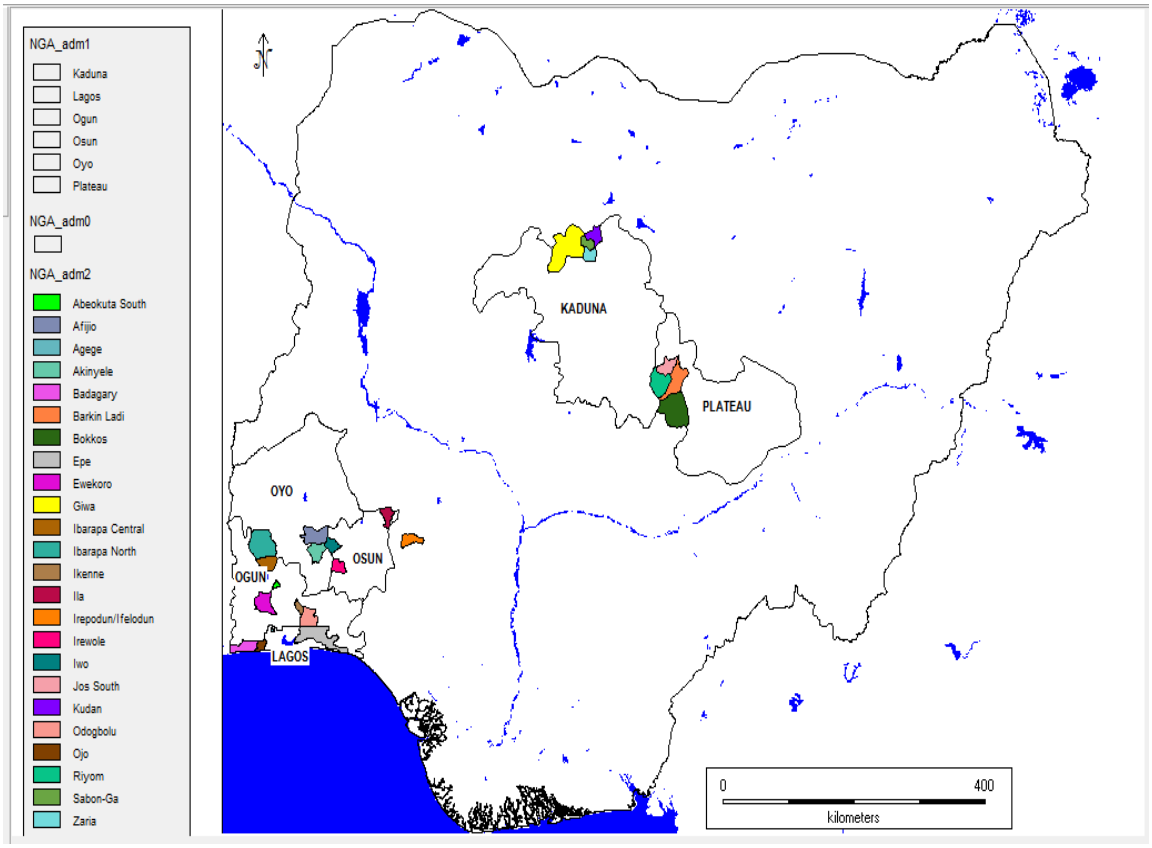



**Fig. 3.1 Map of Nigeria showing the States and Local Government Areas where survey took place.**

Root samples were washed and cut into 2 cm pieces and 10 g sub-samples were taken. The root sub-samples were chopped finely with a knife as macerated cucumber roots quickly deteriorate (Coyne *et al*., 2003*)*. Samples were then processed in the same way as for the soil samples. Nematodes extracted were identified from adult females and counted under a stereomicroscope. Identification of plant-parasitic nematodes to generic level was done using the simplified nematode key (Mai and Lyon 1975; Makete *et al*., 2012)

For each of the surveyed farms, the cropping system, crops grown and irrigation system employed were noticed. Farm location and altitude were documented with GPS coordinates.

### 3.4.1. Statistical analyses

Micfosoft excel was used to calculate the percentage frequency of occurrence (i.e. frequency rating) and percentage nematode population by using the formulae below:

$$\text{Percentage frequency of occurrence} = \frac{n}{N} \times \frac{100}{1}$$

Where, n is the number of times an individual nematode occurred in all the samples and N is sample size.

$$\text{Percentage nematode population} = \frac{In}{TN} \times \frac{100}{1}$$

Where In is the individual nematode population in all the samples, while TN is the total population of all the nematodes extracted in all the samples.

Similarly, the mean and percentage population of each nematode species for each State and Local Government Area were calculated using the same statistical tool.

## 3.5 Experiment Two: Pathogenicity of root-knot nematode (*Meloidogyne incognita*) on cucumber (*Cucumis sativus* L.).

Two experiments were carried out in this study. The first was a pot experiment which was carried out at the screen house of the National Horticultural Research Institute, Ibadan. The second was a field study which was carried out at Teaching and Research Farm of Faculty of Agriculture and Forestry, University of Ibadan.

### 3.5.1 Pot experiment

The *M. incognita* eggs (inoculum) used were extracted from roots of three-month old *M incognita*-galled *Celosia* using 0.5% sodium hypochlorite (Hussey and Barker, 1973) as described in Section 3.1 .The eggs were collected in a 500 ml breaker and then thoroughly mixed using magnetic stirrer and the population per ml of the egg suspension was estimated under the stereoscope in a counting dish. The average of five counts was taken and diluted to about 1,000 eggs/ml of suspension. Two seeds of each of the four varieties of cucumber were sown in a 25- cm- diameter, 15-litre plastic bucket containing steam-sterilized soil. One week after planting, the seedlings were thinned to one uniformly vigorous plant per pot and each seedling was inoculated with *M. incognita* eggs at different levels (0, 10,000, 20,000 or 40,000). 0 eggs served as control.

The experiment was arranged in 4 x 4 factorial design arranged in a randomized complete block design with four replicates for each treatment. The treatments were four varieties of cucumber namely Marketer, Ashley, Tokyo and Marketmore and four different initial population densities (Pi) of *M. incognita* eggs (0, 10,000, 20,000 and 40,000 eggs) (Table 3.1). Temperature and relative humidity of the screen house were properly monitored throughout the period of study. The experiment was carried out twice without any modification

#### 3.5.1.1 Data collection

Immediately after inoculation and subsequently at one week intervals, the following data were collected: Vine length, number of leaves and days to 1$^{st}$ flower. At each harvest, the following data were also recorded: number of marketable fruits, fruit weight (g) and fruit length (cm). The vegetative data was taken for six weeks after which the experiment was terminated eight weeks after inoculation and the following data were recorded: fresh shoot weight, dry shoot weight, fresh root weight, each plant was carefully uprooted and washed to remove adhering soil particles. Galling was quantified using the scale of 0-5 according to Makete, 2000 as shown below:

0 = No gall

1 = 1-10% of the root system galled

2 = 11-35% of the root system galled

3 = 36-65% of the root system galled

4 = 66-90% of the root system galled

5 = More than 90% of the root system galled

Nematode soil populations from 250 ml of soil in each pot and entire cucumber roots for eggs population were also estimated.

In the second trial, the experiment was repeated as described above without any modification.

### 3.5.2 Field experiment

A piece of land naturally infested with *M. incognita* in the National Horticultural Research Institute, Ibadan. Forest savannah zone of located at $3^{o}5^{1}$ E, $7^{o}$ $3^{1}$ N and 168m above mean sea level and Teaching and Research Farm, University of Ibadan (Latitude $7^{o}$ 26'57.27" N, Longitude $3^{o}$ 58' 8.93" E) were used for this study. Nematode identity was confirmed by preparing the perineal pattern of the adult female root- knot nematodes obtained from the roots of infected *C. argentea* plants. In order to increase the nematode population in the field, a susceptible crop, *C. argentea* was planted on the piece of land three months before the commencement of the experiment. Four beds, 22 m long and 2 m wide each with spacing of 0.5 m, were made. Each bed was then divided into two, each having a length of 11m which gave rise to eight beds. Four beds served as control while the other four served as treatment. After this, the control plots were denematized with carbofuran at 3 kg ai/ha.

**Table 3.1: Treatment combinations and layout of the pathogenicity trial in the pot experiment**

| Block One | Block Two | Block Three | Block Four |
|---|---|---|---|
| V1P1 | V2P1 | V3P1 | V4P1 |
| V2P1 | V3P1 | V4P1 | V1P1 |
| V3P1 | V4P1 | V1P1 | V2P1 |
| V4P1 | V1P1 | V2P1 | V3P1 |
| V1P2 | V2P2 | V3P2 | V4P2 |
| V2P2 | V3P2 | V4P2 | V1P2 |
| V3P2 | V4P2 | V1P2 | V2P2 |
| V4P2 | V1P2 | V2P2 | V3P2 |
| V1P3 | V2P3 | V3P3 | V4P3 |
| V2P3 | V3P3 | V4P3 | V1P3 |
| V3P3 | V4P3 | V1P3 | V2P3 |
| V4P3 | V1P3 | V2P3 | V3P3 |
| V1P4 | V2P4 | V3P4 | V4P4 |
| V2P4 | V3P4 | V4P4 | V1P4 |
| V3P4 | V4P4 | V1P4 | V2P4 |
| V4P4 | V1P4 | V2P4 | V3P4 |

Variety 1 (V1) = Marketer
Variety 2 (V2) = Ashley
Variety 3 (V3) = Tokyo
Variety 4 (V4) = Marketmore

Population level 1 (P1) = 0 egg
Population level 2 (P2) = 10,000 eggs
Population level 3 (P3) = 20,000 eggs
Population level 4 (P4) = 40,000 eggs

Each bed was further subdivided into four equal sub-plots. One week after denematization, 250 ml of soil was then collected from each sub-plot for the extraction of the second stage juveniles of the root- knot nematode to determine the initial nematode population ($P_i$) prior to planting using the extraction tray method (Whitehead and Hemming, 1965) as described in Section 3.2. The extraction set- up was left for 48hours. *M. incognita* population was estimated by counting under the compound microscope.

The four cucumber cultivars that were susceptible to *M. incognita* namely Marketer, Ashley, Tokyo and Markermoore were used for this experiment. The seeds of each of the cultivars were randomly planted in each plot such that each plot had the four cultivars planted to it, thus a cultivar of cucumber was planted to each sub-plot. The experimental design was a split–plot design arranged in a completely randomized block design of four blocks. The main plot was nematode treatment while sub-plots were cucumber varieties. The field layout was shown in Fig 3.2.

**3.5.2.1 Data collection**: One week after germination, four plants from each sub-plot were tagged and the following parameters were taken from each plant: vine length, number of leaves and leaf area at 50% flowering. At each harvest, the following parameters were also recorded number of marketable and non-marketable fruit(s) and fruit(s) weight (g). Eight weeks after germination, the experiment was terminated and the following data were recorded; gall index (see section 3.5.1.1), fresh shoot weight, fresh root weight and yields per hectare were also calculated. The final nematode population was taken from the soil and root system as described in section 3.3.1

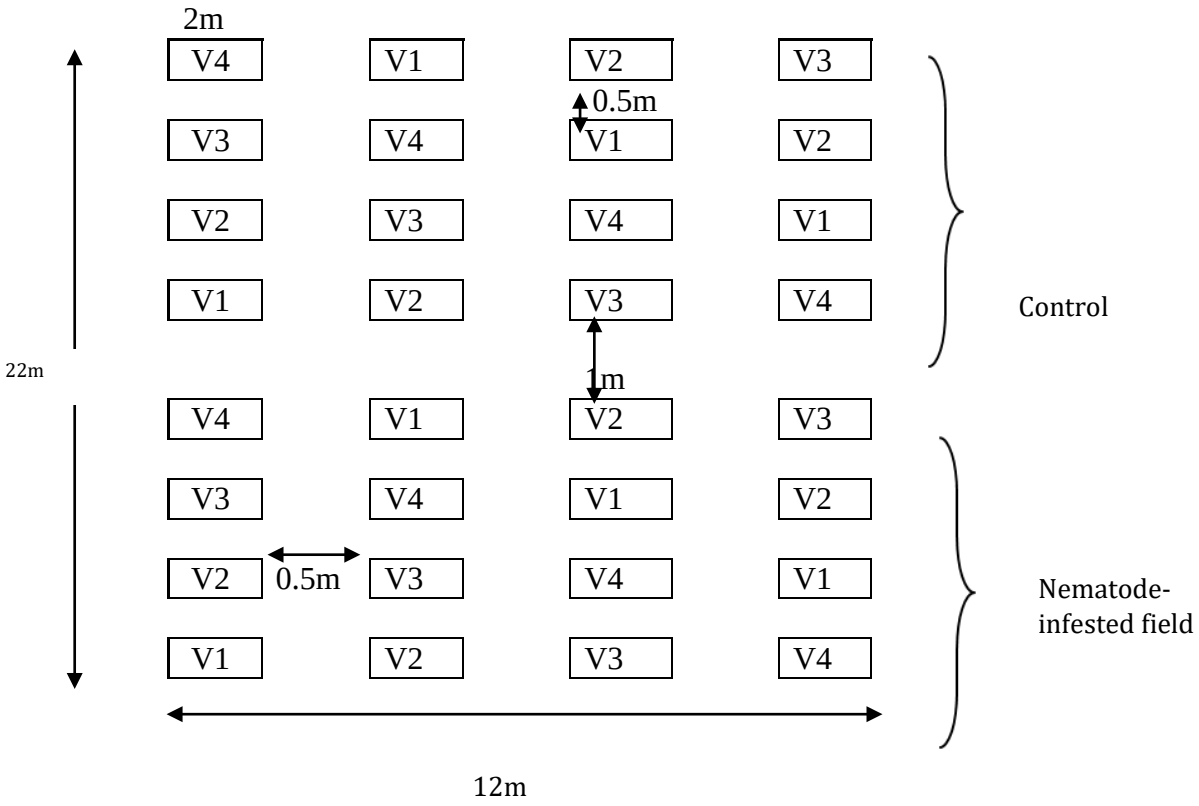



**Fig 3.2: Layout of pathogenicity trial on the field.**

V1-4= Varieties 1= Markerter, 2 = Ashley, 3= Tokyo and 4 = Marketmore

### 3.6 Experiment Three: Histopathology of cucumber roots infected by root-knot nematode *(Meloidogyne incognita*)

One hundred and seventy-five 5- litre polyethylene bags were filled with steam- sterilized sandy- loam soil arranged on the concrete floor at the screen house of National Horticultural Research Institute, Ibadan. Four seeds of cucumber (Marketer) were planted in each pot. Three days after germination, plants were thinned down to two plants per pot. One week after germination the cucumber seedlings in one hundred and forty pots were inoculated with 5,000 freshly hatched second stage juveniles of *M. incognita* while the remaining thirty-five pots were not inoculated these served as control. Twenty-four hours after inoculation, and subsequently daily for 35 days, two inoculated and two uninoculated cucumber plants were randomly and carefully uprooted, washed in a gentle stream of water and dried between paper towels. The roots were then cut into approximately 4 - 5 cm pieces and wrapped in muslin cloth and tied with a piece of cotton strings. The materials were immediately fixed in formalin-aceto-alcohol (FAA), in which the preparation was as follows: 90ml of 50% ethanol + 5ml of glacial acetic acid + 5ml of 37% formaldehyde) in separate Klilner jars securely corked and appropriately labelled. The root tissues remained in the fixative for six weeks prior to further processing. The fixed root segments were dehydrated in a graded ethanol series viz: 70%, 95%, and absolute ethanol for thirty minutes in each solution (Daykin and Hussey, 1985).

The dehydrated tissues were infiltrated by passing them through three different containers of xylene and then through three different changes of paraffin wax, the last change was made in an oven set at a temperature of $60^0$C. On completion of the infiltration process, the tissues were embedded in molten paraffin wax so that they can be sectioned after hardening. After the wax had cooled and solidified, tissue blocks were made and trimmed. Transverse and longitudinal sections 14 µm thick were cut with a rotary microtome (Minot- Microtome Type 1212, Ernest Leitz GMBH, WETZLAR, Germany). The sections were floated out in a water bath and the sections were picked up with a pre-coated microscope slide, labeled and taken to the oven for drying. The sections were rehydrated by passing them through ethanol series (absolute, absolute, 95%, 75%, 70%) stained in safranin and counterstained in fast green (Daykin and Hussey, 1985). All sections were mounted in DPX (a colourless synthetic resin mounting media containing Distyrene, Plasticizer and Xylene) prior to examination with a compound microscope.

### 3.7 Experiment four: Life cycle of the root-knot nematode (*M. incognita*) on cucumber roots.

Ninety-six 5- litre plastic pots were filled with steam-sterilized sandy loam soil. The pots were arranged in 24 x 4 rows on concrete floor in the screen house at National Horticultural Research Institute, Ibadan and spaced one meter within and between the rows. Two seeds of cucumber (Marketer) were planted in each pot. Three days after germination, it was thinned down to one plant per pot. One week after germination the cucumber seedlings in all the pots were inoculated with 5,000 freshly hatched second stage juveniles of *M. incognita.* The inoculation was accomplished by exposing the roots and smearing on the roots with the aid of a plastic hypodermic syringe (without the needle). The roots were covered up with the soil inside the pots immediately after inoculation. On a daily basis three plants were randomly and carefully harvested and the roots were washed in a gentle stream of water and then dry between paper towels and stained for observation. The staining method used for this investigation was Lactoglycerol method described by Bridge *et al*. (1982). The roots were cut into smaller pieces, approximately 4 to 5 centimeters, and wrapped in muslin cloth and tied with a piece of cotton strings. The staining solution consisted of equal amounts of glycerol, lactic acid, and distilled water plus 0.05% acid fuchsin, while the clearing solution was made up of equal volumes of glycerol and distilled water acidified with a few drops of lactic acid. The choped root pieces were boiled in staining solution for three minutes and allowed to cool and then washed in water and allow to clear overnight in the clearing solution. The roots were examined on a 24-hourly basis for nematode penetration and stage of development after penetration. The relative humidity (R.H), soil and atmospheric temperatures of the experimental environment were also monitored throughout the period of study. Photomicrographs of the major life stages of the nematode were taken and the measurements of the length and breath of these stages were made by the use of eye-piece graticule calibrated against stage micrometer. The observation and measurements were continued on a daily basis until second stage juveniles were once more observed.

### 3.8 Experiment Five: Screening of different plants for resistance to *Meloidogyne incognita*

Twenty-one (21) plants including five varieties of sesame and two varieties of *Corchorus olitorius* (Table 3.2) were selected and evaluated to determine their reactions to root-knot nematode (*Meloidogyne incognita*) under screen house conditions. *Tagetes erecta* and 'Marketer' cucumber were used as positive and negative controls, respectively. Pots measuring 21 cm in diameter and 5 litres in volume were filled with 5 kg steam-sterilized top soil. Two seeds of each test plant were sown in each pot and thinned to one seedling per pot after emergence. Ten days after seedling emergence, approximately 5,000 eggs or juveniles suspension in water were inoculated around the base of the seedling in each pot. The treatments were arranged in a completely randomized design with four replications. The experiment was terminated eight weeks after inoculation. At the end of the experiment, each plant was carefully uprooted and washed to remove adhering soil particles. Galling index was quantified using the scale of 0-5 as described in Section 3.5.1.1.

The entire root system of each plant was then cut into 2 cm pieces and shaken vigorously in 0.5% sodium hypochlorite (NaOCl) solution to extract the eggs (Hussey and Barker, 1973). The eggs extracted from each plant were estimated as described in Section 3.1. The soil nematode population was also estimated from 250 ml soil from each pot using the extraction tray method described in Section 3.2 (Whitehead and Hemming, 1965). The total number of nematodes ($J_2$) in the soil was extrapolated from the number of second stage juveniles counted. Thereafter, the number of nematodes in the soil was added to the number of eggs extracted from the roots to obtain the final nematode population (Pf).

The host efficiency, determined by the Reproductive Factor (RF) = $\frac{Pf}{Pi}$, was then Calculated;

where, Pf (final population) was the average total eggs and juveniles populations and Pi = 5,000 eggs, the initial population density. A reproductive factor of >1 indicates an increase in nematode reproduction where an RF of <1 implies no increase in reproduction. The final assessments of resistance of various varieties were based on Canto-Saenz's host designation scheme (Sasser *et al.*, 1984). Plants with GI (Gall index) > 2 are defined as either susceptible (RF >1) or hypersusceptible (RF ≤ 1); plants with GI ≤ 2 are classified either resistant (RF ≤ 1) or tolerant (RF > 1) (Table 3.3). This experiment was repeated without any modification.

**Table 3.2: Lists of the crops/plants that were screened against *Meloidogyne incognita*.**

| Common Name | Scientific Name | Variety |
|---|---|---|
| Marigold –ve Control | *Tagete erecta* | |
| Sunflower | *Helianthus annuus* | Black seeded |
| Sorghum | *Sorghum bicolor* | Local |
| Maize | *Zea mays* | Oba super1 |
| Sweet maize | *Zea mays saccharata* | |
| Leafy Vegetable | Amarathus *cruentus* | |
| Jute mallow | *Corchorus olitorius* | Angbadu |
| Jute mallow | *Corchorus olitorius* | Oniyaya |
| Pigeon peas | *Cajanus cajan* | |
| Garden Eggs | *Solanum aethiopicum* | Kotobi |
| Exotic Eggplant | *Solanum melongena* | Aubergine |
| Sesame | *Sesamum indicum* | E8 |
| Sesame | *Sesamum indicum* | NC 03L |
| Sesame | *Sesamum indicum* | NCR 01M |
| Sesame | *Sesamum indicum* | 530-6-1 |
| Sesame | *Sesamum indicum* | 02M |
| Hot pepper | *Capsicum annuum* | Safi |
| Sweet pepper | *Capsicum annuum* | Yoyo wonder |
| Garlic | *Allium sativum* | |
| Water Melon | *Citrullus lanatus* | F1 Koloss |
| Lettuce | *Lactuca sativa* | Great lakes |
| Turmeric | *Curcuma longa* | Yellow |
| Moringa | *Moringa oleifera* | |
| Tree basil | *Occimum grattissimum* | Local basil |
| Velvet beans | *Mucuna pruriens* | |
| Cucumber ( +ve control) | *Cucumis sativus* | Marketer |

**Table 3.3: Quantitative Scheme for Assignment of Canto–Saenz's Host Suitability Designations**

| Root Damage (GI) | Gall Host Efficiency Index $\frac{Pf}{Pi}$ (R Factor) | Degree of Resistance Designation |
|---|---|---|
| ≤ 2 | ≤ 1 | Resistant |
| ≤ 2 | ≥ 1 | Tolerant |
| ≥2 | ≤1 | Hypersusceptible |
| ≥2 | ≥1 | Susceptible |

GI Rating Scale 1 = 1-10%, 2 = 11-35%, 3 = 36-65%, 4 = 66-90%, 5 = > 90% of the roots system galled

**Sasser *et al.*, 1984**

### 3.8.1 Data Analysis

All data were transformed using square root transformation and statistically analyzed using the SAS version 9.0 (SAS, 2000) statistical package and the means were separated using the Least Significant Difference (LSD) at a probability level of 5%.

## 3.9 Experiment six: Growing cucumber in rotation with resistant crops in a natural *Meloidogyne incognita*- infested field

A field naturally infested with *M. incognita* at National Horticultural Research Institute, Ibadan and crop garden Department of Crop protection and Environmental Biology, University of Ibadan were used for the 1st and 2nd trials respectively. In order to increase the inoculum, the fields were planted to *C. argentea* three months before the commencement of the experiments. The land measuring 16 m x 20 m was marked out. The land was subdivided into six equal blocks of 2 m x 20 m. Each block was further divided into 4 equal sub-plots of 2 m x 1.5 m with 1m within and between rows. The soil nematode population for each sub-plot was estimated from 250 ml soil taken from each sub-plot. The seeds of cucumber (Marketer) were planted in each plot at a spacing of 75 cm x 50 cm. The experimental layout was randomized complete block design with six replications. One week after germination four plants from each plot were tagged and data were recorded weekly on vine length and number of leaves. Forty five days after germination, fruits harvesting started and frequently done as required wherein number of marketable fruits, number of non-marketable fruits and fruits weight were recorded. Two months after planting, experiment was terminated and the following data were recorded: fresh shoot weight (g), fresh root weight (g), Gall index (Section 3.5.1.1) nematode population in 250ml soil and eggs/juveniles in 10g of the root system. Each plot was tilled using simple implement and the plots were planted with Marigold, Sessame "NCR-01M, Maize "Oba super 1 and cucumber "Marketer" as a control. Weeds were regularly controlled and the plants watered when necessary. The experimental design was randomized complete block design with six replications. Three months after planting, four of each of the plants were randomly selected uprooted and roots washed free of soil.

Data on fresh root weights, gall index (Section 3.5.1.1) and nematodes population in the 250 ml of soil were recorded. Immediately after termination of the experiment, the soils were tilled using simple implement and planted to cucumber “Marketer” the following planting season as described above (Fig. 3.3)

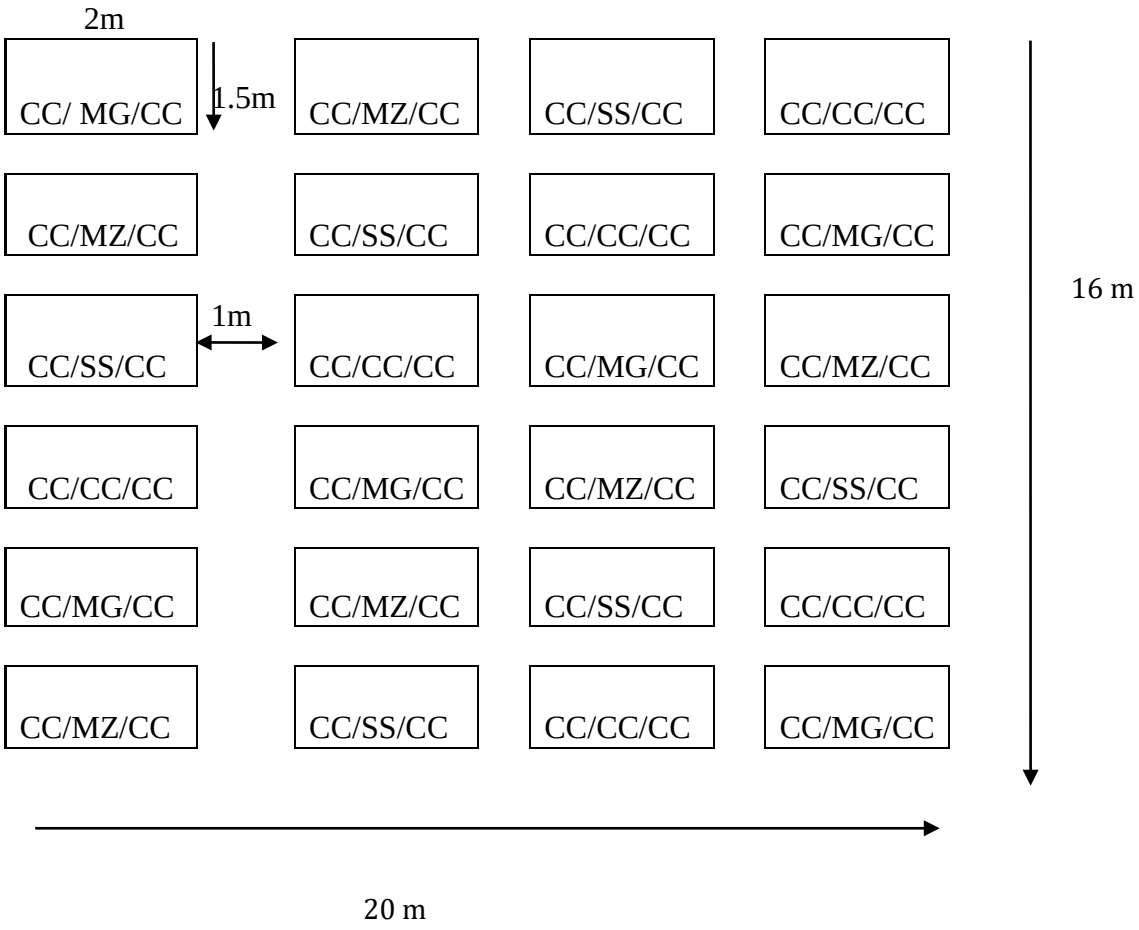



**Fig 3.3: Field layout of rotation sequence of Cucumber with Marigold, Sesame, Maize or Cucumber.**

MG = Marigold, MZ = Maize (Oba super 1)

SS = Sessame (cv NCR-01M), CC = Cucumber (cv Marketer)

### 3.9.1 Statistical Analysis

All data were statistically analyzed using the SAS version 9.0 (SAS, 2000) statistical package and the means were separated using the Least Significant Difference (LSD) at a probability level of 5%.

# CHAPTER FOUR

# RESULTS

## 4.1 Plant-parasitic nematodes associated with cucumber production areas in Southwestern and part of the Northern zones in Nigeria

The population densities of the various plant- parasitic nematodes encountered in soil and roots of cucumber at various Local Government Areas (LGAs) of Lagos, Ogun, Osun, Oyo, Kaduna and Plateau States are presented below (Tables 4.1 – 4.24). The summary tables showing the percentage frequency of occurrence and population of the various nematodes encountered in soil samples and roots in each of the States are also presented.

Eleven and five genera of plant-parasitic nematodes were identified in the soil and root samples collected from Lagos State, respectively. In the soil, plant-parasitic nematodes encountered were *Meloidogyne* spp., *Pratylenchus* spp., *Scutellonema* spp., *Helicotylenchus* spp., *Aphelenchoides* spp., *Longidorus* spp., *Xiphinema* spp., *Criconemoides* spp., *Tylenchus* spp, *Rotylenchus* spp. and *Belonolaimus* spp (Tables 4.1 and 4.2). *Meloidogyne* spp. were the most frequently occurring species in the soil (100%) while the population was 2849 per 250 ml soil (Table 4.1). *Pratylenchus, Helicotylenchus, Criconema, Xiphinema, and Belonolaimus* Spp. also had frequency rating of 100% with population of 1234, 894, 515, 249 and 187 per 250 ml soil respectively. *Scutellonema, Tylenchus* and *Rotylenchus* spp. had the frequency rating of 50% with population of 781, 384 and 64 per 250 ml soil respectively. *Aphenlenchoides* spp had the least frequency rating of 33.3% with the population density of 61 per 250 ml soil (Table 4.1). Agege LGA had the highest population density of *Meloidogyne* spp with the average of 416/250 ml soil (Table 4.2). This was followed by Epe LGA with the mean population of 225.8/250 ml soil. The least *Meloidogyne* spp density was encoun tered in Ojoo LGA with the mean population of 148/250 ml soil (Table 4.2). Next to *Meloidogyne* was *Pratylenchus* spp. with a mean population density of

121/250ml soil with Ojoo LGA having the highest population density (Table 4.2). *Rotylenchus* spp was absent in Epe and Ojoo LGAs while *Tylenchus* spp and *Longidorus* spp were also absent in Badagry LGA (Table 4.2). The most encountered plant parasitic nematodes was *Meloidogyne* spp. (38%) this followed by *Pratylenchus* spp. (17%) and the least encountered was Rotylenchus spp and Aphenlenchoides spp. with 1% each, respectively across the LGAs in Lagos State (Fig 4.1). In the root samples, five out of the eight plant parasitic nematodes encountered in the soil were found in the roots (Table 4.3).

*Meloidogyne* spp., *Helicotylenchus* spp,, *Pratylenchus* spp., and *Belonolaimus* spp had frequency ratings of 100% each with population densities of 23794/10 g, 4320/10 g, 11978/10 g and 1025/10 g of roots respectively (Table 4.3). All the plant parasitic nematodes found in the roots were present in all the four LGA of Lagos State except *Tylenchus* spp., which was absent in Badagry LGA (Table 4.4).

**Table 4.1: Plant-parasitic nematodes extracted from soil around the roots of Cucumber in Lagos State**

| Nematode genus | Frequency of occurrence | % Frequency Rating* | Nematode population** | % Nematode Population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 2849±153.0 | 38.7 |
| *Pratylenchus* spp. | 24 | 100 | 1234±28.8 | 16.8 |
| *Helicotylenchus* spp. | 24 | 100 | 894±30.5 | 12.1 |
| *Criconema* spp. | 24 | 100 | 515±23.9 | 7.0 |
| *Xiphinema* spp. | 24 | 100 | 249±14.2 | 3.4 |
| *Belonolaimus* spp | 24 | 100 | 187±4.7 | 2.5 |
| *Longidorus* spp. | 15 | 62.5 | 138±25.0 | 1.9 |
| *Scutellonema* spp. | 12 | 50 | 781±65.1 | 10.6 |
| *Tylenchus* spp. | 12 | 50 | 384±39.0 | 5.2 |
| *Rotylenchus* spp. | 12 | 50 | 64±5.7 | 3.4 |
| *Aphelenchoides* spp. | 8 | 33.3 | 61±8.5 | 0.8 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24)
** Nematode population per 250 ml soil.
***In/TN x100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples.

**Table 4.2: Population densities of plant-parasitic nematodes associated with Cucumber in various Local Government Areas (LGAs) of Lagos State**

| Locations (LGA) | *Meloidogyne* | *Helicotylenchus* | *Tylenchus* | *Pratylenchus* | *Criconemoides* | *Aphelenchoides* | *Longidorus* | *Rotylenchus* | *Xiphinema* | *Scutello* | *Belonolaimus* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Agege | 416.0*±60.8 (46.7)** | 80.0±8.1 (9.0) | 77.3±8.6 (8.7) | 73.7±6.5 (8.3) | 63.3±8.2 (4.1) | 5.3±2.2 (0.6) | 15.3±1.3 (1.7) | 11.0±0.9 (1.2) | 13.7±1.4 (1.5) | 114.3±7.1 (12.8) | 20.2±0.7 (2.3) |
| Epe | 225.8±9.9 (39.1) | 60.7±7.9 (10.5) | 20.0±5.7 (3.5) | 116.0±2.3 (20.1) | 31.7±4.4 (5.5) | 7.0±2.7 (1.2) | 14.7±14.0 (2.5) | - | 38.0±5.4 (6.6) | 51.7±2.6 (8.9) | 12.7±1.2 (2.2) |
| Ojoo | 148.0±8.2 (30.5) | 85.7±9.5 (17.6) | 30.7±11.6 (6.3) | 121.7±10.8 (25.0) | 26.6±4.3 (5.5) | 3.7±2.1 (0.8) | 16.2±1.5 (3.3) | - | 15.2±1.3 (3.1) | 26.3±4.0 (5.4) | 11.8±0.7 (2.4) |
| Badagry | 160±13.3 (32.1) | 71.7±8.2 (14.4) | - | 100.0±0.0 (20.0) | 50.3±4.1 (10.1) | 4.3±2.7 (0.9) | - | 10.5±0.6 (2.1) | 16.3±1.4 (3.3) | 68±11.5 (13.6) | 17.8±0.7 (3.6) |

*Mean population ±SE (% population); SE= Standard Error

**Nematode population per 250 ml soil

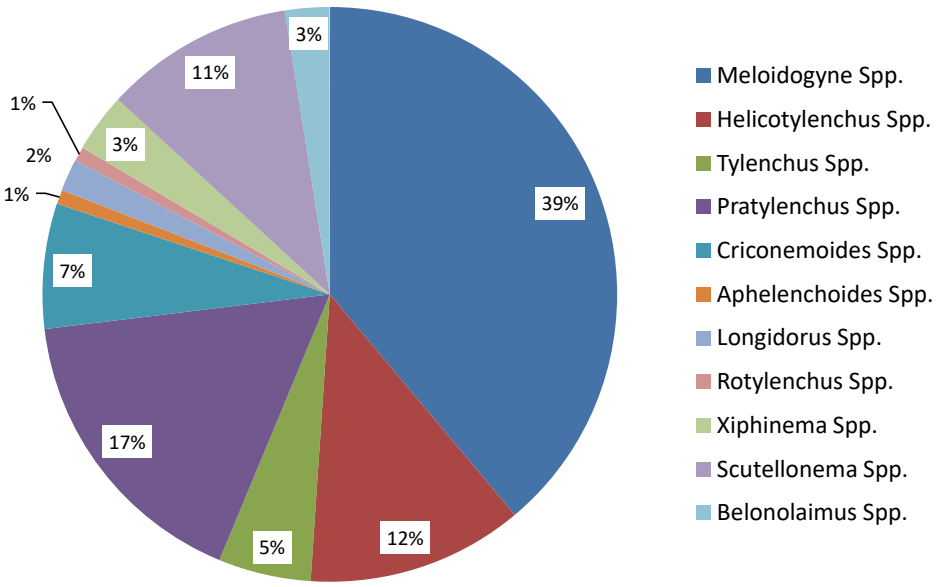



**Fig 4.1: The percentage representation of plant-parasitic nematodes extracted from 250 ml soil around the roots of Cucumber across the LGAs in Lagos State.**

**Table 4.3: Plant-parasitic nematodes extracted from the roots of Cucumber in Lagos State**

| Nematode genus | Frequency of occurence | % frequency Rating* | Nematode Population** | % Nematode Population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 23794±90.7 | 54.5 |
| *Helicotylenchus* spp. | 24 | 100 | 4320±14.9 | 9.9 |
| *Pratylenchus* spp. | 24 | 100 | 11978±51.1 | 27.4 |
| *Belonolaimus* spp. | 24 | 100 | 1025±4.0 | 2.3 |
| *Tylenchus* spp. | 18 | 75 | 2580±18.9 | 5.9 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24)
** Nematode population per 10 g root
***In/TN x100/1 (In = individual nematode in all the samples and TN =Total Population of all nematodes extracted in all the samples)

**Table 4.4: Population densities of plant-parasitic nematodes extracted from the roots of Cucumber in various Local Government Areas (LGAs) of Lagos State****

| LGAs | *Meloidogyne* | *Helicotylenchus* | *Tylenchus* | *Pratylenchus* | *Belololaimus* |
|---|---|---|---|---|---|
| Agege | 151.7±16.5 (61.7)* | 33.7±4.3 (13.7) | 37.7±3.8 (14.5) | 19.0±2.6 (7.7) | 6.0±0.6 (2.4) |
| Epe | 225.8±29.2 (39.1) | 60.7±4.1 (10.5) | 20.0±4.4 (3.5) | 116±8.4 (20.1) | 12.7±0.7 (2.2) |
| Ojoo | 155.0±9.7 (45.7) | 31.0±4.2 (9.1) | 25.3±5.4 (7.4) | 121.3±4.0 (35.6) | 2.2±0.7 (2.2) |
| Badagry | 160.0±21.4 (32.1) | 71.7±4.1 (14.4) | - | 100.0±6.0 (20.0) | 17.8±1.0 (3.6) |

*Mean population ±SE (% population); SE= Standard Error

**Nematode population per 10 g of root

In Ogun State, nine and six genera of plant-parasitic nematodes were encountered in the soil and root samples respectively (Table 4.5-4.8). The plant-parasitic nematodes identified in the soil were *Meloidogyne* spp., *Xiphinema* spp., *Scutellonema spp*., *Pratylenchus* spp., *Tylenchus* spp., *Helicotylenchus* spp., *Criconemoides* spp., *Aphlenchoides* spp., and *Longidorus* spp. (Table 4.5 and 4.6). *Meloidogyne* spp. was the most frequently occurring species in the soil (100%) with a population of 1589 per 250 ml of soil mostly of which came from Abeokuta South LGA. This was followed by *Xiphinema* spp. with 58.3% frequency rating and a population of 271 per 250 ml soils. Ikenne LGA had the largest mean population density of *Xiphinema* spp. (64.3) but was absent in Odogbolu LGA (Table 4.6). The third most frequently occurring species was *Scutellonema* spp. (54.2%) and its population was highest in Ikenne LGA (Table 4.6). *Pratylenchus* spp. and *Tylenchus* spp. had the frequency rating of 41.7% each with population density of 492 and 130 per 250 ml soil respectively and were both absent in Ikenne LGA (Table 4.6). The least frequently occurring was *Longidorus* spp.(4.2%) with population density of 16 in 250 ml soil and this was absent in the three LGAs out of the four LGAs surveyed (Table 4.6). The most encountered plant-parasitic nematodes across the LGAs in Ogun State was *Meloidogyne* spp (33%) and the least was *Longidorus* spp (2%) (Fig 4.2). In the roots, *Meloidogyne* spp and *Pratylenchus* spp. were the most frequently occurring (100%) with population densities of 16890 and 1862 per 10 g of roots respectively (Table 4.7) Ikenne LGA had the highest population densities of *Meloidogyne* spp. and *Pratylenchus* spp. (Table 4.8). followed by *Scutellonema* spp. with frequently rating of 75% and population density of 4405 (Table 4.7). *Xiphinema* spp. and *Helicotylenchus* spp. had frequency rating of 62.5% each with a population density of 2890 and 2120 per 10 g of the roots system respectively (Table 4.7). Tylenchus spp. had the least frequency rating of 4.2% and the population density of 140 per 250 ml soil (Table 4.7). However, *Tylenchus* spp. was absent in Ikenne and Odogbolu LGAs (Table 4.8).

**Table 4.5: Plant-parasitic nematodes extracted from soils around the roots of Cucumber in Ogun State**

| Nematode genus | Frequency of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 1589±59.0 | 43.2 |
| *Xiphinema* spp. | 14 | 58.3 | 271±34.7 | 7.4 |
| *Scutellonema* spp. | 13 | 54.2 | 388±43.1 | 10.5 |
| *Pratylenchus* spp. | 10 | 41.7 | 492±53.3 | 13.4 |
| *Tylenchus* spp. | 10 | 41.7 | 130±9.9 | 3.6 |
| *Helicotylenchus* spp. | 6 | 25 | 517±39.3 | 14.1 |
| *Criconemoides* spp. | 8 | 33.3 | 142±13.1 | 3.9 |
| *Aphelenchoides* spp. | 8 | 33.3 | 132±16.7 | 3.6 |
| *Longidorous* spp. | 1 | 4.2 | 16±0.9 | 0.4 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24)
** Nematode population per 250 ml of soil
***In/TN x100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples)

**Table 4.6: Population densities of plant-parasitic nematodes associated with Cucumber in various Local Government Areas (LGAs) of Ogun State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Scutellonema* | *Pratylenchus* | *Aphelenchoides* | *Tylenchus* | *Criconemoides* | *Xiphinema* | *Longidorus* |
|---|---|---|---|---|---|---|---|---|---|
| Abeokuta South | 160.7±17.7 (39.3)* | - | 85.3±8.8 (20.8) | 104±8.1 (25.4) | 25.7±3.8 (2.9) | 26.7±5.0 (6.5) | - | 20.7±2.4 (5.05) | - |
| Ikenne | 155±20.5 (36.0) | 68.0±14.2 (15.8) | 101.7±11.7 (23.6) | - | 36.9±7.6 (7.4) | - | 25.0±3.7 (2.3) | 64.3±16.5 (14.3) | - |
| Yelwa South | 107.3±26.9 (37.3) | 48.7±16.8 (16.9) | 72.9±15.2 (18.4) | 67.7±21.0 (17.1) | - | 27.7±4.1 (4.2) | 27.8±5.0 (7.0) | 11.4±1.5 (3.0) | 32.0±7.3 (8.1) |
| Ilaro | | | | | | | | | |
| Odogbolu | 106.7±21.7 (42.8) | 55.7±10.6 (22.3) | - | 78.0±15.0 (31.3) | - | 29.8±4.1 (2.0) | 40.0±8.2 (1.6) | - | - |

*Mean population ±SE (% population); SE= Standard Error

**Nematode population per 250 ml soil

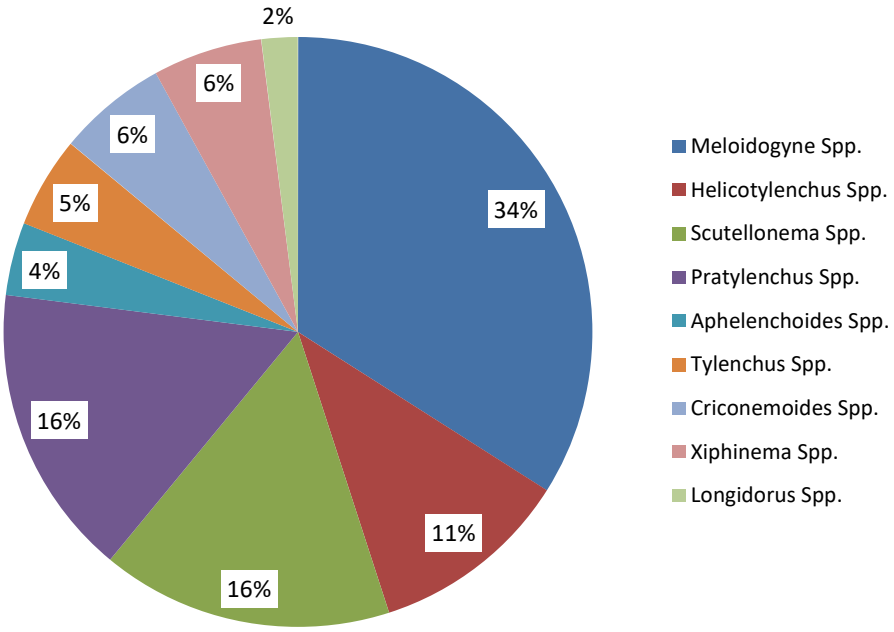



**Fig 4.2: The percentage representation of plant-parasitic nematodes extracted from 250 ml soil around the roots of Cucumber across the LGAs in Ogun State.**

**Table 4.7: Plant-parasitic nematodes extracted from the roots of Cucumber in Ogun State**

| Nematode genus | Frequency of occurrence | % Frequency Rating* | Nematode population** | % Nematode Population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 16890±57.5 | 60.3 |
| *Pratylenchus* spp. | 24 | 100 | 1562±10.9 | 5.6 |
| *Scutellonema* spp. | 18 | 75 | 4405±31.1 | 15.7 |
| *Xiphinema* spp. | 15 | 62.5 | 2890±4.9 | 10.3 |
| *Helicotylenchus* spp. | 15 | 62.5 | 2120±19.2 | 7.6 |
| *Tylenchus* spp. | 1 | 4.2 | 140±12.2 | 0.5 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24)

**Nematode population per 10 g of root

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples)

**Table 4.8: Population densities of plant-parasitic nematodes associated with roots of Cucumber in various Local Government Areas (LGAs) of Ogun State****

| LGA | *Meloidogyne* | *Helicotylenchus* | *Scutellonema* | *Pratylenchus* | *Xiphinema* | *Tylenchus* |
|---|---|---|---|---|---|---|
| Abeokuta South | 145.7±1 (61.1)* | - | 55.7±10.6 (23.4) | 14.7±4.0 (6.2) | 18.2±1.0 (7.6) | 40.0±8.2 (1.7) |
| Ikenne | 179.3±26.7 (63.7) | 20.8±10.2 (7.4) | 56.0±11.4 (19.9) | - | 25.3±1.9 (9.0) | - |
| Yewa South Ilaro | 106±23.3 (49.1) | 26.2±3.4 (12.1) | 35.2±7.7 (16.3) | 19.2±4.3 (8.9) | 28.5±1.0 (13.2) | 10.3±0.0 (0.3) |
| Odogbolu | 132.0±14.9 (66.6) | 23.7±3.7 (11.9) | - | 18.2±2.5 (9.2) | 24.3±1.0 (12.3) | |

*Mean population ±SE (% population); SE= Standard Error

**Nematode population per 10 g of root

Eight and five genera of plant-parasitic nematodes were identified in soil and root samples collected from Osun State. In the soil, plant-parasitic nematodes encountered were *Meloidogyne* spp., *Pratylenchus* spp., *Scutellonema* spp., *Helicotylenchus* spp., *Aphelenchoides* spp., *Xiphinema* spp., *Criconemoides* spp., and *Tylenchus* spp. (Table 4.9). *Meloidogyne* and *Helicotylenchus* spp. were the most frequently occurring species in the soil (100%) with the population densities of 1332 and 576 per 250 ml soil, respectively (Table 4.9). Next to these nematodes were *Scutellonema* spp. and *Pratylenchus* spp. with frequency rating of 16.7% each and population of 392 and 227 per 250 ml soil respectively. *Aphenlenchoides* spp. and *Tylenchus* spp. had the frequency rating of 8.3% each with populations 110 and 35 per 250 ml soil sample respectively. The lowest frequency rating was *Criconemoides spp* with frequency rating of 4.2% and population of 14 per 250 ml soil sample (Table 4.9).

In Osun State, out of the four LGAs surveyed, Irepodun LGA had the highest population density of all the species of plant parasitic nematodes encountered except *Tylenchus* spp which was absent (Table 4.10). Iwo LGA had the highest population density of *Scutellonema* spp. (Table 4.10) *Criconemoides* spp and *Tylenchus* spp were absent in Irepodun and Ila LGAs, also *Aphelenchoides* spp was absent in Iwo and Ila LGAs (Table 4.10). The most encountered plant parasitic nematodes across the LGAs in Osun State was *Meloidogyne* spp. (37%) while the least were *Tylenchus* spp and *Criconemoides* spp with 5% of the population each, respectively (Fig. 4.3). In the root samples, *Meloidogyne* spp and *Helicotylenchus*. had the highest frequency rating of 100% each with population density of 3460 and 1825 per 10 g of root sample and the highest population came from Irepodun LGA (Table 4.11 and 4.12) to this was *Pratylenchus* spp. which had the frequency rating of 41.7% (Table 4.12) *Xiphinema* spp had the least frequency rating of 8.3% and population density of 370 per 10 g of root which was most encountered in Irepodun LGA and was present in all the LGAs surveyed (Table 4.12)

**Table 4.9: Plant-parasitic nematodes extracted from the soil around the roots of Cucumber Osun State**

| Nematode genus | Frequency of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 1332±67.0 | 47.6 |
| *Helicotylenchus* spp. | 24 | 100 | 576±25.3 | 20.6 |
| *Scutellonema* spp. | 4 | 16.7 | 392±24.3 | 14.0 |
| *Pratylenchus* spp. | 4 | 16.7 | 227±16.2 | 8.1 |
| *Xiphinema* spp. | 3 | 12.5 | 113±10.2 | 4.0 |
| *Aphelenchoides* spp. | 2 | 8.3 | 110±18.3 | 3.9 |
| *Tylenchus* spp. | 2 | 8.3 | 35±16.4 | 1.3 |
| *Criconemoides* spp. | 1 | 4.2 | 14±2.5 | 0.5 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24).

** Mean Nematode population per 250 ml soil

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total Population of all nematodes extracted in all the samples).

**Table 4.10: Population densities of plant-parasitic nematodes associated with cucumber in various Local Government Areas (LGAs) of Osun State**

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Scutellonema* | *Pratylenchus* | *Aphelenchoides* | *Tylenchus* | *Criconemoides* | *Xiphinema* |
|---|---|---|---|---|---|---|---|---|
| Iwo | 106.7±21.7 (42.7) | 55.7±10.6 (22.3) | 48.0±9.7 (19.2) | 28.0±3.9 (7.5) | - | 30.0±4.1 (2.0) | 40.0±8.2 (1.6) | 25.7±9.7 (4.8) |
| Irepodun | 179.3±26.7 (56.2) | 56.0±11.4 (17.5) | 42.1±9.1 (6.2) | 42.1±8.5 (6.2) | 36.3±7.5 (10.2) | - | | 30.0±4.9 (3.8) |
| Irewole | 83.3±23.9 (48.4) | 32.0±9.2 (18.6) | 25.3±11.3 (14.7) | 37.3±9.1 (10.8) | 40.0±8.2 (2.3) | 25.0±5.8 (3.9) | 20.0±0.0 (0.4) | 16.7±1.1 (1.0) |
| Ila | 74.7±12.3 (39.0) | 48.3±6.6 (25.3) | 37.7±7.6 (19.7) | 28.0±3.9 (9.8) | - | - | - | 25.7±3.8 (6.3) |

*Means population ±SE (% population); SE = Standard Error

**Nematode population per 250 ml of soil

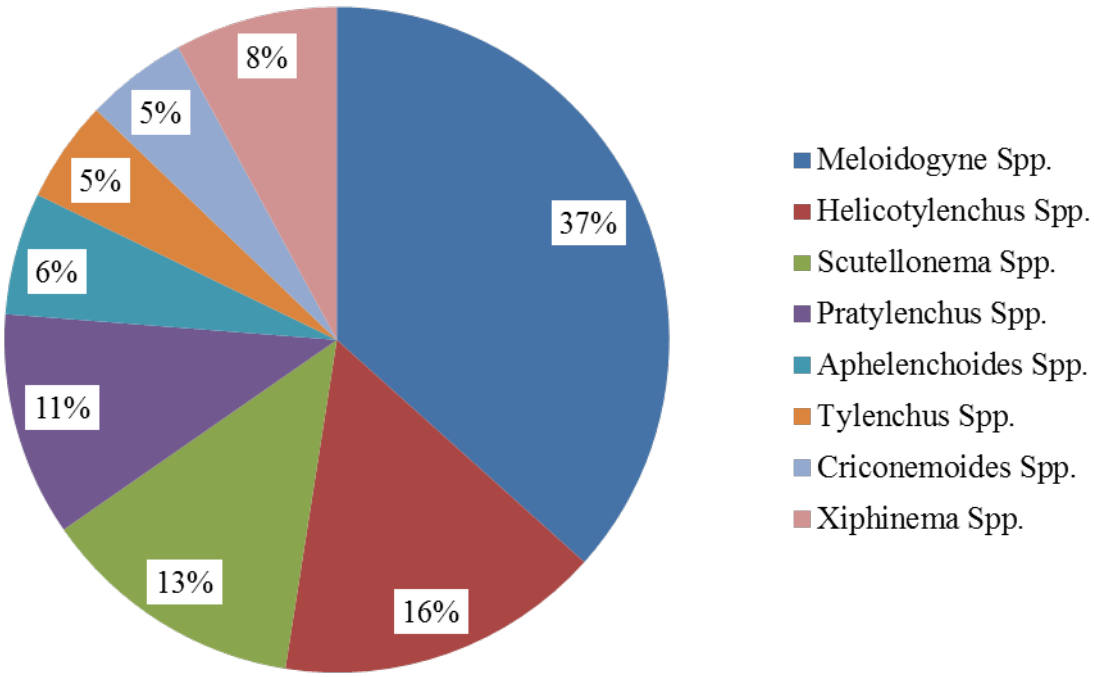



**Fig 4.3: The percentage representation of plant-parasitic nematodes extracted from 250 ml soil around the roots of Cucumber across the LGAs in Osun State.**

**Table 4.11: Plant-parasitic nematodes extracted from the roots of Cucumber in Osun State**

| Nematode genus | Frequency of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 3460±18.5 | 47.3 |
| *Helicotylenchus* spp. | 24 | 100 | 1825±9.1 | 24.9 |
| *Pratylenchus* spp. | 10 | 41.7 | 560±3.6 | 7.7 |
| *Aphelenchoides* spp. | 5 | 20.8 | 1100±18.3 | 5.0 |
| *Xiphinema* spp. | 2 | 8.3 | 370±3.4 | 15.0 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24).

** Mean Nematode population per 10 g of root

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples).

**Table 4.12: Population densities of plant-parasitic nematodes associated with Cucumber roots in various Local Government Areas (LGAs) of Osun State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Pratylenchus* | *Aphelenchoides* | *Xiphinema* |
|---|---|---|---|---|---|
| Iwo | 52.0±7.6 (63.7)* | 20.0±3.9 (24.5) | 8.0±1.5 (6.5) | - | 8.1±1.5 (5.3) |
| Irepodun | 179.3±26.7 (56.2) | 56.0±11.4 (17.5) | 42.1±8.5 (6.2) | 36.3±7.5 (10.2) | 30.0±4.9 (3.8) |
| Irewole | 83.3±23.9 (48.4) | 32.0±9.2 (18.6) | 37.3±9.1 (10.8) | 40.0±8.2 (2.3) | 16.7±1.1 (1.0) |
| Ila | 74.7±12.3 (39.0) | 48.3±6.6 (25.3) | 28.0±3.9 (9.8) | - | 25.7±3.8 (6.3) |

*Mean population ±SE (% population); SE= Standard Error

**Nematode population per 10 g of root

In Oyo State, nine and seven plant-parasitic nematodes were encountered in soil and root samples respectively (Tables 4.13-4.16). The plant-parasitic nematodes identified in the soil were: *Meloidogyne* spp., *Helicotylenchus* spp, *Pratylenchus* spp., *Xiphinema* spp., *Scutellonema* spp., *Aphelenchoides* spp., *Tylenchus* spp., *Criconema* spp., and *Longidorus* spp., (Table 4.13) *Meloidogyne* spp. was the most frequently occurring species (100%) while the population was 1197 per 250 ml soil in Oyo State. Akinyele LGA had the highest mean population density of 155/250ml soil (Tables 4.13 and 4.14). This was followed by *Helicotylenchus* spp.with the population density of 679 per 250ml in which the highest population came from Ibarapa North LGA with the mean population density of 85.7/250 ml soil. However, *Pratylenchus* spp. and *Xiphinema* spp each having the frequently rating of 10 % each with population density of 180 and 44/250 ml soil respectively (Table 4.13). It was most abundant in Akinyele and Ibarapa LGAs respectively (Table 4.14). *Criconemoides* spp. and *Longidorus* spp. had the least frequency occurrence of 2.0% each and population density of 360 and 160 per 250 ml soil respectively (Table 4.14). It was most abundant in Afijio LGA with average population of 40/250 ml soil and was absent in Akinyele LGA (Table 4.14). In the roots, *Meloidogyne* sppalso had the highest population of 7920 per 10 g root, followed by *Pratylenchus* spp with frequency rating of 62.5% and a population of 1140 per 10 g of root (Table 4.15). *Melodogyne* spp was most abundant in Afijio LGA with an average density of 169 per 10 g of roots while the highest population density for *Helicotylenchus* spp came also from Afijio LGA with an average population density of 58 per 10 g of root (Table 4.16). *Scutellonema* spp was most abundant in Ibarapa North with average population density of 68.3 per 10 g of root and was absent in Akinyele LGA. *Pratylenchus* spp was the most abundant in Ibarapa North with population density of 93.3 per 10 g of root and was absent in Akinyele and Ibarapa North LGAs. Afijio LGA had the highest population density of *Aphelenchoides* spp (39) per 10 g of root and was absent in Akinyele and Ibarapa North LGAs (Table 4.16). *Criconemoides* spp had the highest average population density of 32.5 per 10 g of root in Ibarapa North LGA and was absent in Akinyele and Afijio LGAs. *Tylenchus* spp had the least average population density of 29.8 per 10 g of root in Afijio LGA and was absent in the remaining three LGAs surveyed (Table 4.16).

**Table 4.13: Plant-parasitic nematodes extracted from soils around the roots of Cucumber in Oyo State**

| Nematode Genus | Number of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 1197±64.1 | 34.8 |
| *Helicotylenchus* spp. | 12 | 50 | 679±51.5 | 19.7 |
| *Pratylenchus* spp. | 10 | 41.7 | 473±36.7 | 13.8 |
| *Xiphinema* spp. | 10 | 41.7 | 198±40.9 | 5.8 |
| *Scutellonema* spp. | 8 | 33.3 | 615±48.2 | 17.9 |
| *Aphelenchoides* spp. | 5 | 20.8 | 180±3.2 | 5.2 |
| *Tylenchus* spp. | 5 | 20.8 | 44±15.9 | 1.3 |
| *Criconemoides* spp. | 2 | 8.3 | 36±21.7 | 1.0 |
| *Longidorus* spp. | 2 | 8.3 | 16±0.9 | 0.5 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24)
** Nematode population per 250 ml of soil
***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total Population of all nematodes extracted in all the samples).

**Table 4.14: Population densities of plant-parasitic nematodes associated with Cucumber in various Local Government Areas (LGAs) of Oyo State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Scutellonema* | *Pratylenchus* | *Aphelenchoides* | *Tylenchus* | *Criconemoides* | *Xiphinema* |
|---|---|---|---|---|---|---|---|---|
| Akinyele | 155.0±20.5 (31.1)* | 68.0±14.2 (13.6) | 101.7±11.7 (20.4) | 78.0±15.0 (15.6) | 36.9±7.6 (6.4) | - | - | 64.3±16.5 (12.9) |
| Ibarapa North | 85.7±30.7 (30.2) | 83.7±29.8 (29.5) | 66.0±21.9 (23.3) | 22.0±9.1 (7.8) | 10.7±7.3 (3.8) | 9.3±5.5 (3.3) | 3.3±2.2 (1.2) | 2.7±2.4 (0.9) |
| Ibarapa Central | 60.7±12.6 (41.3) | 29.3±11.5 (20.2) | 60.0±21.7 (6.8) | 34.6±11.8 (20.6) | - | 7.7±3.8 (2.3) | 15.7±6.1 (5.0) | 32.0±5.2 (3.6) |
| Afijio | 106.7±21.7 (38.8) | 55.7±10.6 (20.3) | 48.0±9.7 (17.5) | 31.0±5.5 (11.3) | 23.6±4.0 (8.9) | 29.8±4.1 (1.8) | 40.0±8.2 (1.5) | - |

*Mean population ±SE (% population); SE = Standard Error

**Nematode population per 250 ml of soil

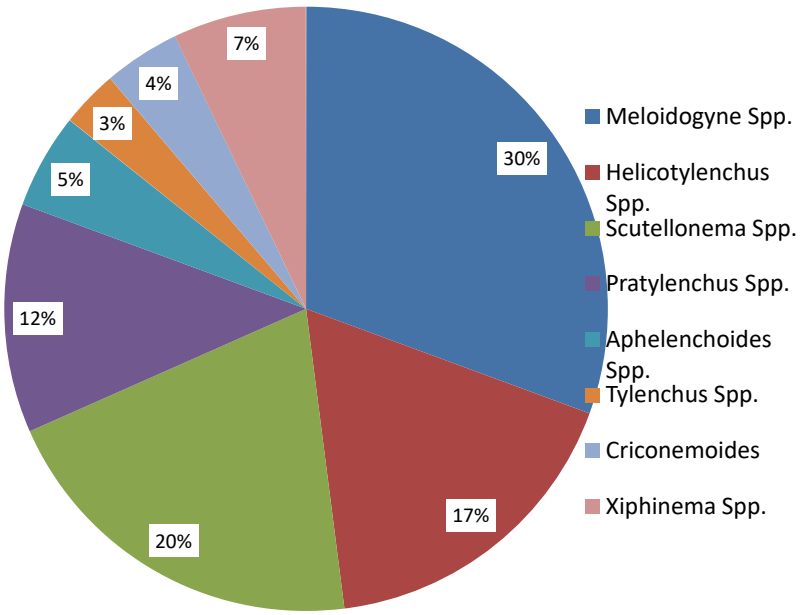



**Fig 4.4: The percentage representation of plant-parasitic nematodes extracted from 250 ml soil around the roots of Cucumber across the LGAs in Oyo State.**

**Table 4.15: Plant-parasitic nematodes extracted from the roots of Cucumber in Oyo State**

| Nematode genus | Number of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 7920±75.9 | 55.3 |
| *Pratylenchus spp* | 15 | 62.5 | 1140±28.9 | 8.0 |
| *Helicotylenchus* spp. | 12 | 50 | 1800±33.8 | 12.6 |
| *Aphelenchoidies* spp. | 10 | 41.7 | 960±18.50 | 6.7 |
| *Xiphinema spp* | 8 | 33.3 | 360±12.1 | 2.5 |
| *Scutellonema* spp. | 6 | 25 | 1990±27.3 | 14.0 |
| *Criconemoides* spp. | 5 | 20.8 | 150.0±2.9 | 1.0 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24).
** Nematode population per 10 g of root
***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total Population of all nematodes extracted in all the samples).

**Table 4.16: Population densities of plant-parasitic nematodes associated with Cucumber roots in various Local Government Areas (LGAs) of Oyo State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Scutellonema* | *Pratylenchus* | *Aphelenchoides* | *Tylenchus* | *Criconemoides* | *Xiphinema* |
|---|---|---|---|---|---|---|---|---|
| Akinyele | 22.0*±12.1 (65.3) | 6.0±1.9 (17.6) | - | 2.2±1.4 (6.4) | - | - | - | 3.5±1.8 (10.4) |
| Ibarapa North | 99.0±12.9 (60.9) | 20.0±0.0 (1.2) | 68.3±5.5 (25.2) | 93.3±10.3 (8.6) | - | | 32.5±2.1 (16.7) | |
| Ibarapa Central | 128.0±27.2 (56.1) | 32.9±9.6 (12.3) | 54.7±8.3 (18.0) | 44.3±6.2 (6.8) | 26.0±3.0 (3.5) | | 20.0±0.0 (0.4) | 26.0±4.4 (2.9) |
| Afijio | 169.0±15.9(53.4) | 58.0±7.9 (18.3) | 43.3±6.5 (6.2) | 41.1±5.5 (5.8) | 39.0±5.8 (12.3) | 29.8±4.5 (2.3) | - | 31.3±2.9 (3.9) |

*Mean population ±SE (% population); SE = Standard Error,

**Nematode population per 10 g of root

In Kaduna State, seven and five plant-parasitic nematodes were encountered in soil and root samples of cucumber, respectively (Tables 4.17-4.20). The plant- parasitic nematodes identified in the soil were: *Meloidogyne* spp, *Tylenchus* spp, *Pratylenchus* spp, *Helicotylenchus* spp, *Criconemoides* spp, *Aphelenchoides* spp and *Longidorus* spp (Tables 4.17and 4.18). *Meloidogyne* spp was the most frequently occurring species (100%) while the population was 2819 per 250 ml soil in the Kaduna State. Zaria LGA had the highest mean population density of 39/250 ml soil (Tables 4.17 and 4.18). This was followed by *Tylenchus* spp and *Pratylenchus* spp. having a frequency rating of 83.3% each and *Tylenchus* spp. and *Pratylenchus* spp. both had the same population of 617/250 ml soil (66.7%) (Table 4.17). *Tylenchus* spp. (72.3/250 ml) and *Pratylenchu*s spp (53.7/250 ml) were the most abundant in Giwa and Kudah LGAs respectively (Table 4.18). *Longidorus* spp. had the least frequency rating of 16.7% with population density of 890/250 ml soil and was not identified in two of the LGAs sampled (Table 4.18). The most encountered plant-parasitic nematodes in the soil across LGAs in Kaduna State was *Meloidogyne* spp (53%) and the least encountered were *Aphelenchoides* spp. and *Longidorus* spp. 1% each (Fig 4.5). In the root samples, the plant- parasitic nematodes present were *Meloidogyne* spp., *Helicotylenchus* spp., *Tylenchus* spp., *Pratylenchus* spp. and *Criconemoides* spp. with frequency rating of 100% each and population densities of 8430, 4320, 3240, 6319 and 1025 per10 g root respectively (Table 4.19). However, *Meloidogyne* spp. had the highest population density and was most prevalent in Sabo LGA with an average population of 96.7/10 g root, while the highest population of *Pratylenchus* spp. was observed in Giwa LGA with an average population of 68 per10 g root (Table 4.20). *Tylenchus* spp. was the most prevalent in Zaria LGA with an average population density of 35.7 per 10 g root. *Criconemoides* spp. with the least population density of 13.7 per 10 g root was the most prevalent in Kudah LGA (Table 4.20).

**Table 4.17: Plant-parasitic nematodes extracted from soils around the roots of Cucumber in Kaduna State**

| Nematode genus | Frequencyof occurrence | % Frequency of rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp | 24 | 100 | 2819±146.0 | 49.8 |
| *Tylenchus* spp. | 20 | 83.3 | 617±41.1 | 10.9 |
| *Pratylenchus* spp. | 16 | 66.7 | 619±48.3 | 10.9 |
| *Helicotylenchus* spp. | 15 | 62.5 | 1044±57.9 | 18.4 |
| *Criconemoides* spp. | 12 | 50 | 412±35.5 | 7.3 |
| *Aphelenchoides* spp. | 10 | 41.7 | 61±8.5 | 1.1 |
| *Longidorus* spp. | 4 | 16.7 | 89±29.7 | 1.6 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24).

** Nematode population per 250 ml soil,

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples).

**Table 4.18: Population densities of plant-parasitic nematodes associated with soil around the roots of Cucumber of various Local Government Areas (LGAs) of Kaduna State**

| Location (LGAs) | *Meloidogyne* | *Tylenchus* | *Pratylenchus* | *Helicotylenchus* | *Criconemoides* | *Aphelenchoides* | *Longidorus* |
|---|---|---|---|---|---|---|---|
| Sabo | 118.0±30.5 (45.2) | 18.0±9.6 (6.9) | 44.0±20.2 (16.9) | 40.7±13.7 (15.6) | 18.7±15.9 (7.2) | 7.0±3.9 (2.7) | 14.7±9.7 (5.6) |
| Kudah | 228.3±20.7 (51.0) | 38.0±5.5 (8.5) | 53.7±7.1 (12.0) | 88.3±9.2 (19.7) | 35.0±6.1 (7.8) | 4.3±1.7 (1.0) | - |
| Giwa | 203.3±16.6 (44.4) | 72.3±8.4 (15.8) | 45.3±6.3 (9.9) | 105.7±9.7 (23.1) | 7.2±1.0 (13.6) | 3.7±1.3 (0.8) | 5.0±3.7 (1.1) |
| Zaria | 390.0±28.1 (53.3) | 63.3±11.8 (8.7) | 5.3±1.4 (1.4) | 77.3±5.5 (10.6) | 60.7±5.9 (8.3) | 10.0±5.0 (1.4) | |

*Mean population ±SE (% population); SE = Standard Error
**Nematode population per 250 ml of soil

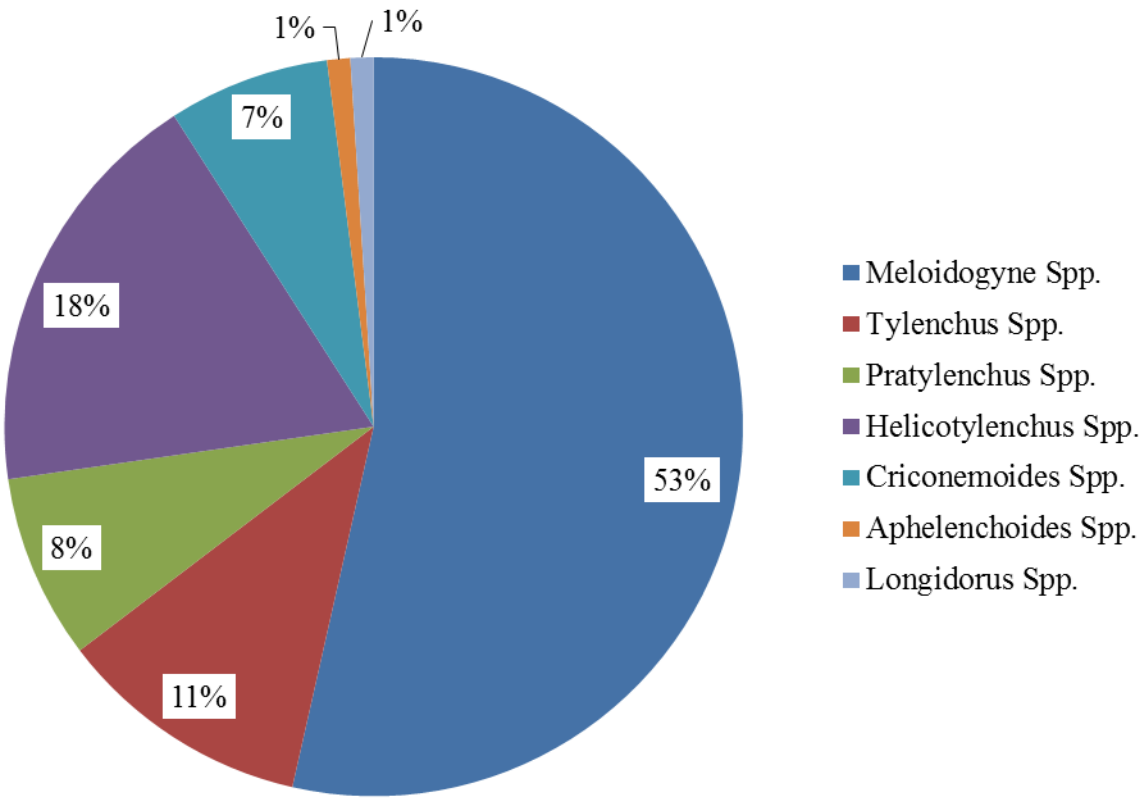



**Fig 4.5: The percentage representation of plant-parasitic nematodes extracted from 250 ml soil around the roots of Cucumber across the LGAs in Kaduna State.**

**Table 4.19: Plant-parasitic nematodes extracted from the roots of Cucumber in Kaduna State**

| Nematode genus | Frequencyof occurence | % Frequency of rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 8430±34.1 | 36.1 |
| *Helicotylenchus* | 24 | 100 | 4320±14.9 | 18.5 |
| *Tylenchus* spp. | 24 | 100 | 3240±18.9 | 13.9 |
| *Pratylenchus* spp. | 24 | 100 | 6319±35.9 | 27.1 |
| *Criconemoides* spp. | 24 | 100 | 1025±4.0 | 4.4 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (18)

** Nematode population per 10 g of root

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples

**Table 4.20: Population densities of plant-parasitic nematodes associated with the roots of Cucumber of various Local Government Areas (LGAs) of Kaduna State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Pratylenchus* | *Tylenchus* | *Criconemoides* |
|---|---|---|---|---|---|
| Sabo | 96.7*±13.7 (50.0) | 39.7±5.8 (20.5) | 25.0±4.6 (12.9) | 25.0±6.2 (12.9) | 7.0±1.0 (3.6) |
| Kudah | 66.7±9.8 (39.9) | 25.0±4.6 (15.0) | 39.7±5.8 (23.8) | 22.0±2.2 (13.2) | 13.7±1.4 (8.2) |
| Giwa | 92.0±5.1 (41.1) | 31.0±6.0 (13.8) | 68.0±10.0 (30.4) | 25.3±7.6 (11.3) | 7.5±1.0 (3.4) |
| Zaria | 91.3±6.7 (49.2) | 33.7±6.1 (18.1) | 19.0±3.7 (10.2) | 35.7±5.4 (19.2) | 6.0±0.8 (3.2) |

*Mean population ±SE (% population); SE = Standard Error,

**Nematode population per 10 g of root

In Plateau State, eight and four plant- parasitic nematodes were encounterd in soil and root samples, respectively (Table 4.21 – 4,24). The plant parasitic nematodes identified in the soil were: *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchus* spp.,*Criconemoides* spp., *Belonolaimus* spp., *Aphelenchoides* spp. and *Longidorus* spp. (Table 4.21). *Meloidogyne* spp., *Helicotylenchus* spp, *Tylenchus* spp. and *Pratylenchus* spp. were the most frequently occurring species (100%) with population densities of 1814, 413, 199, and 130 per 250 ml soil respectively. Riyom LGA had the highest mean population density of *Meloidogyne* spp with 158 per 250 ml soil (Table 4.22). This was followed by *Criconemoides* spp and *Belonolaimus* spp. each having a frequency rating of 83.3% and 58.3% respectively; and populations of 262 and 97/250 ml soil respectively (Table 4.21). *Criconemoides* spp. and *Belonolaimus* spp. were the most abundant in Riyom LGA with average population of 28 and 9.7 /250 ml soil (Table 4.22). *Longidorus* spp. had the lowest frequency rating (41.7%) and lowest population of 380/250 ml soil (Table 4.21) and was absent in Riyom LGA (Table 4.22). The most encountered was also *Meloidogyne* spp. (60%) while the least were *Aphelenchiodes* spp and *Longidorus* spp. 2% each across LGAs in Plateau State (Fig 4.6). In the root samples, *Meloidogyne* spp., *Helicotylenchus* spp, and *Cricomemoides* spp. were the most frequently occurring with 100% each and population densities of 2720, 1144 and 813/10 g root respectively (Table 4.23). *Meloidogyne* spp. was the most abundant in Biriladi LGA with mean population of 28.7/10 g root (Table 4.24). *Pratylenchus* spp was absent in Riyom and Bokkos LGAs (Table 4.24). *Criconemoides* spp. was the most abundant in Jos South LGA with average density of 7.8/10 g root (Table 4.24) This was followed by *Pratylenchus* spp with frequency rating of 50% and population densitiy of 280/10 g root (Table 4.23) and was absent in Riyom and Bokkos LGAs. (Table 4.24)

**Table 4.21: Plant-parasitic nematodes extracted from the soil around the roots of Cucumber in Plateau State**

| Nematode genus | Number of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 1814±45.2 | 60.0 |
| *Helicotylenchus* spp. | 24 | 100 | 413±16.6 | 13.7 |
| *Tylenchus* spp. | 24 | 100 | 199±4.8 | 16.6 |
| *Pratylenchus spp* | 24 | 100 | 130±6.9 | 4.3 |
| *Criconemoides Spp* | 20 | 83.3 | 26±1.5 | 8.7 |
| *Belonolaimus Spp.* | 14 | 58.3 | 97±8.5 | 3.2 |
| *Aphenelenchoides* | 12 | 50 | 69±8.1 | 2.3 |
| *Longidorus Spp*. | 10 | 41.7 | 38±0.7 | 1.3 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (24).

** Nematode population per 250 ml soil,

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples).

**Table 4.22: Population densities of plant-parasitic nematodes associated with Cucumber in various Local Government Areas (LGAs) of Plateau State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Pratylenchus* | *Aphelenchoides* | *Tylenchus* | *Criconemoides* | *Belonolaimus* | *Longidorus* |
|---|---|---|---|---|---|---|---|---|
| Jos South | 158.3±17.7 (61.2)* | 35.0±7.6 (13.7) | 8.3±1.5 (3.2) | 9.7±2.7 (3.7) | 17.3±1.8 (6.7) | 18.7±15.9 (7.2) | 7.2±3.5 (2.8) | 4.0±3.0 (1.5) |
| Riyom | 153.0±19.8 (61.6) | 31.7±7.3 (12.7) | 7.3±4.0 | 4.3±3.8 (1.7) | 15.0±2.1 (6.0) | 28.0±6.9 (11.3) | 9.7±3.4 (3.7) | - |
| Biriladi | 140.3±15.7 (57.5) | 37.0±6.6 (15.2) | 17.7±1.8 (7.2) | 3.7±2.9 (1.5) | 16.0±2.0 (6.6) | 20.7±2.4 (8.5) | 6.7±3.4 (2.7) | 5.0±3.8 (0.8) |
| Bokkos | 153.0±20.4 (59.7) | 33.7±5.5 (13.1) | 10±0.0 (3.9) | 5.3±3.2 (2.1) | 18.0±1.7 (7.0) | 20±0.0 (7.8) | 9.4±3.5 (3.7) | 6.7±7.1 (2.6) |

*Mean population ±SE (% Population); SE = Standard Error

** Nematode population per 250 ml soil

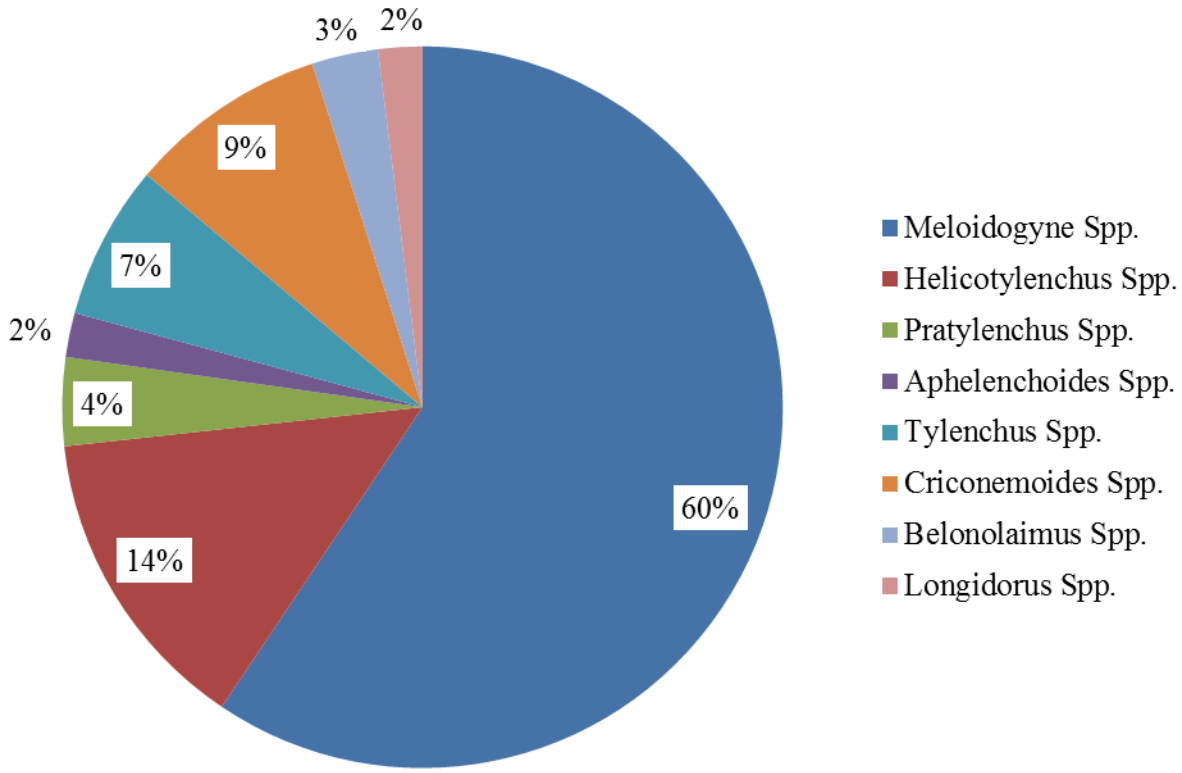



**Fig 4.6: The percentage representation of plant-parasitic nematodes extracted from 250 ml soil around the roots of Cucumber across the LGAs in Plateau State.**

**Table 4.23: Plant-parasitic nematodes extracted from the roots of Cucumber in Plateau State**

| Nematode genus | Number of occurrence | % Frequency rating* | Nematode population** | % Nematode population*** |
|---|---|---|---|---|
| *Meloidogyne* spp. | 24 | 100 | 2720±11.4 | 54.9 |
| *Helicotylenchus* spp. | 24 | 100 | 1144±4.9 | 23.1 |
| *Criconemoides* spp. | 24 | 100 | 813±2.4 | 16.4 |
| *Pratylenchus* spp | 12 | 50 | 280±2.6 | 5.7 |

*n/N x 100 (number of times individual nematodes occurred and N = Sample size (45)

** Nematode population per 10 g of root

***In/TN x/100/1 (In = individual nematode in all the samples and TN =Total population of all nematodes extracted in all the samples)

**Table 4.24: Population densities of plant-parasitic nematodes associated with cucumber roots various Local Government Areas (LGAs) of Plateau State****

| Locations (LGAs) | *Meloidogyne* | *Helicotylenchus* | *Pratylenchus* | *Criconemoides* |
|---|---|---|---|---|
| Jos South | 20.3±4.2 (47.1) | 9.5±1.8 (21.9) | 5.6±0.7 (12.9) | 7.8±0.8 (18.0) |
| Riyom | 16.0±6.2 (52.7) | 6.7±2.4 (22.0) | - | 7.7±2.5 (25.4) |
| Biriladi | 28.7±6.3 (54.3) | 13.2±2.0 (25.0) | 3.8±0.6 (7.1) | 7.2±1.0 (13.6) |
| Bokkos | 25.7±3.0 (65.9) | 8.8±2.1 (22.7) | - | 4.5±0.2 (11.5) |

*Mean population ±SE (% population); SE= Standard Error

** Nematode population per 10 g of root

## 4.2: Pathogenicity of *Meloidogyne incognita* on cucumber (Screen house Trials)

### 4.2.1 Effects of different inoculum densities of *Meloidogyne incognita* on fresh and dry shoot weight, gall index and nematode reproduction in cucumber

The highest fresh shoot weight was recorded from uninoculated plants which was not significantly different from plant inoculated with 20,000 eggs. The least fresh shoot weight was recorded from plant inoculated with 40,000 eggs which was statistical different from other treatments ($p \leq 0.05$). The uninoculated plants had the highest significant mean dry shoot weight for both trials (Table 4.25) followed cucumber plants infected with 20,000 eggs. This was significantly higher than the dry shoot weight values observed from plants inoculated with 10,000 and 40,000 eggs in the first trial. In the second trial the dry shoot weight was not statistically different from plants infected with 10,000 eggs and 20,000 eggs. The least significant dry shoot weight was observed from plants inoculated with 40,000 eggs in the two trials (Table 4.25).

The uninoculated plants had the lowest fresh root weight and it was statistically lower than fresh root weight from other treatments. The highest significant mean fresh root weight was observed in cucumber plants that received the 20,000 inoculum density which was not significantly different from the plants that was inoculated with 10,000 and 40,000 eggs. The trend was the same in both trials (Table 4.25). The mean gall indices differed statistically ($P \leq 0.05$) from each other in both trials. The plants inoculated with 40,000 eggs had the highest mean gall index which did not differ significantly from the values obtained from those inoculated with 10,000 and 20,000 eggs. However, in the second trial the plants inoculated with 10,000 eggs had the highest mean gall index which was not significantly different from other treatments. The lowest significant mean gall index of 0 was recorded in the control plants for both trials (Fig 4.7).

The mean total population of *M. incognita* differed significantly ($P \leq 0.05$) from each other. The highest value was obtained from plants inoculated with 10,000 eggs which did not differ from the values recorded from the plants infected with 20,000 and 40,000 eggs in both trials. The lowest significant mean value came from the uninoculated plants (Fig 4.8). The mean nematode reproductive factor also differed significantly from each other. The highest mean reproductive factor came from the plants inoculated with 10,000 eggs and decreased as inoculum densities increased in

both trials. The lowest significant reproductive factor value of 0 was obtained from the uninoculated plants for the two trials (Fig 4.9).

### 4.2.2 Effects of *M. incognita* populations on root damage, nematode reproduction and growth parameters of different cucumber cultivars

The highest mean fresh shoot weight was produced by cultivar Tokyo and it was not significantly different from the value obtained from Cucumber Ashley in the two trials (Table 4.26). The lowest mean value came from Marketer variety which was not significantly lower than the value from Marketmore in the two trials (Table 4.26). Tokyo variety had the highest significant mean dry shoot weight which was not significantly higher than value from cultivar Ashley, but it was significantly higher than values from Marketer and Marketmore in both trials (Table 4.26).

The highest mean fresh root weight was recorded from Marketer variety and was not significantly higher than values from cultivar Ashley and Tokyo varieties in both trials. The least mean value was obtained from Marketmore variety which was not significantly lower than values for Marketer and Ashley varieties in both trials (Table 4.26). The highest mean gall index was recorded from Marketer and cultivar Ashley but they were not significantly higher than values from the remaining two varieties (Tokyo and Marketmore) in the two trials (Fig 4.10). The mean final nematode population was not significantly different from each other. Although the highest mean population was recorded from Marketer varieties while the least was recorded for Marketmore variety (Fig 4.11). The highest mean significant mean reproductive factor was observed from Marketer and this did not differ significantly from the value obtained from cultivar Ashley and Tokyo. The least significant value came from Marketmore in both trials (Fig 4.12).

**Table 4.25: Effects of different *M.incognita* populations on fresh shoot weight, dry shoot weight and fresh root weight of cucumber in 1st and 2nd trials.**

| | 1st Trial | | | 2nd Trial | | |
|---|---|---|---|---|---|---|
| Treatments | Fresh sht wt (g)/plant | Dry sht wt (g) | Fresh rt wt (g) | Fresh Sht wt (g)/plant | Dry sht wt (g) | Fresh rt wt (g) |
| Control | 135.4 | 15.1 | 18.9 | 130.5 | 13.8 | 15.8 |
| 10,000 eggs | 113.5 | 14.3 | 35.6 | 112.6 | 11.5 | 30.3 |
| 20,000 eggs | 129.7 | 15.0 | 38.0 | 125.6 | 12.9 | 32.0 |
| 40,000 eggs | 94.7 | 11.9 | 33.2 | 80.5 | 9.3 | 29.2 |
| LSD≤0.05 | 26.6 | 2.4 | 10.7 | 20.5 | 2.7 | 11.3 |

Sht = shoot; wt = weight; rt = root

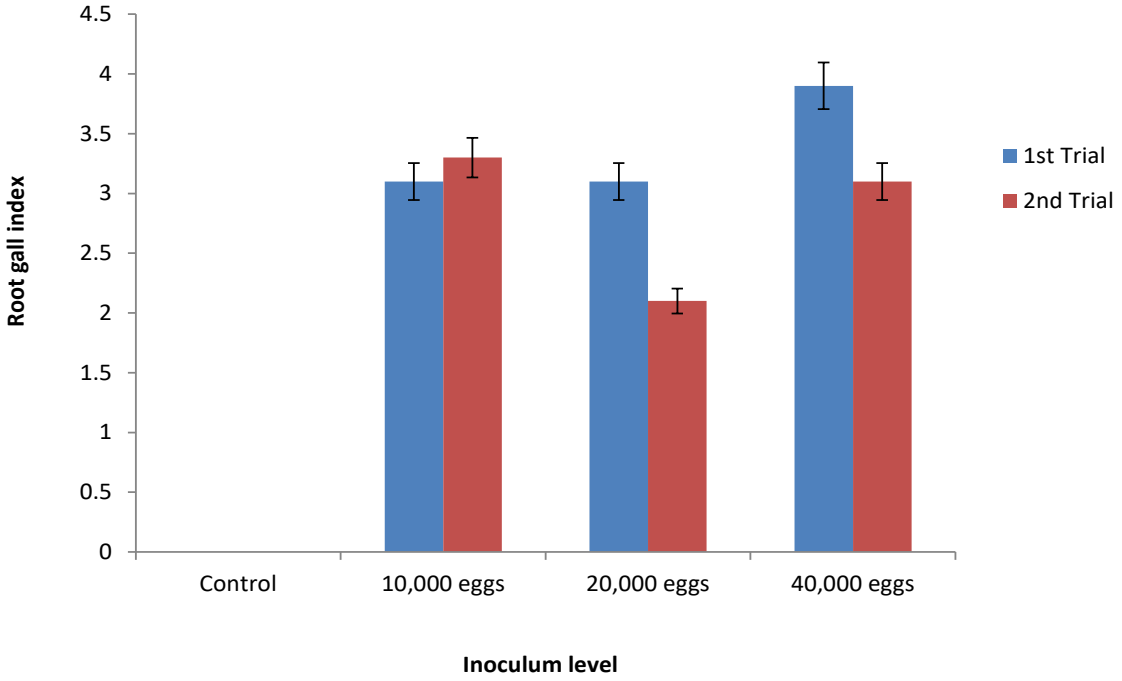



**Fig 4.7: Root gall indices of cucumber roots as influenced by various inoculum levels of *Meloidogyne incognita* (1st and 2nd trials).**

Galling index (GI) 0-5 scale according to Makete (2000).

| | | |
|---|---|---|
| 0 | = | No gall |
| 1 | = | 1-10% of the root system galled |
| 2 | = | 11-35% of the root system galled |
| 3 | = | 36-65% of the root system galled |
| 4 | = | 66-90% of the root system galled |
| 5 | = | More than 90% of the root system galled |

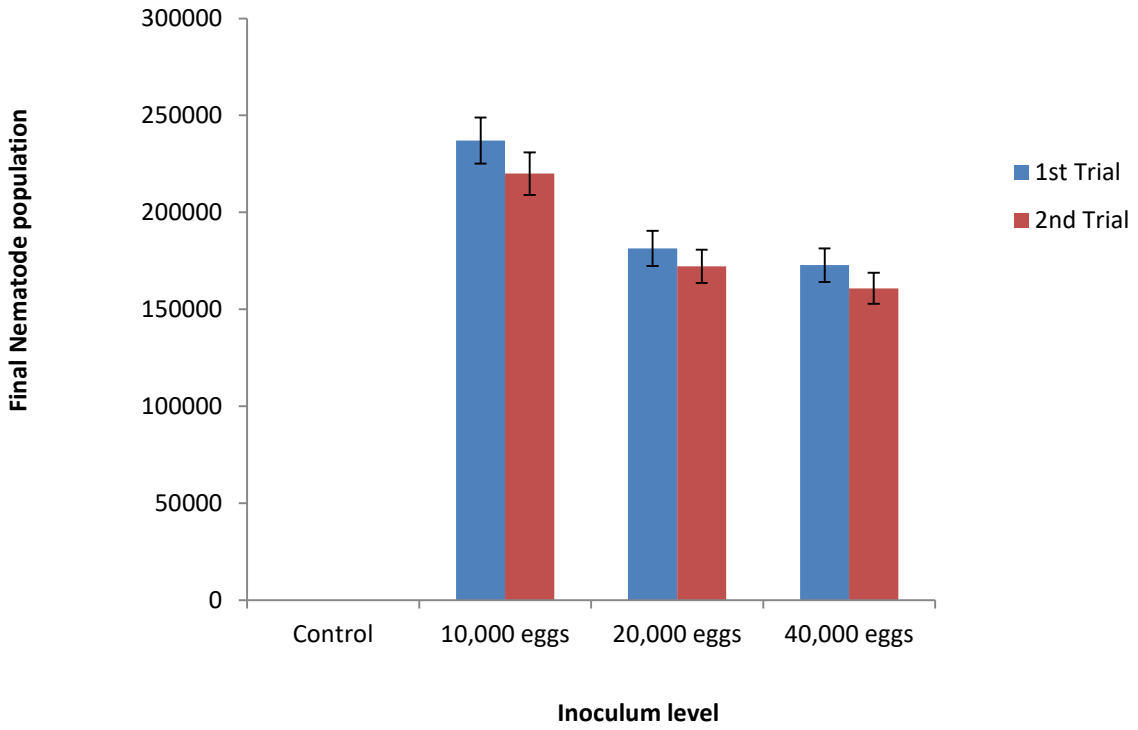



**Fig 4.8: Final nematode population of *Meloidogyne incognita* on cucumber as inflenced by the various inoculum densities (1st and 2nd trials).**

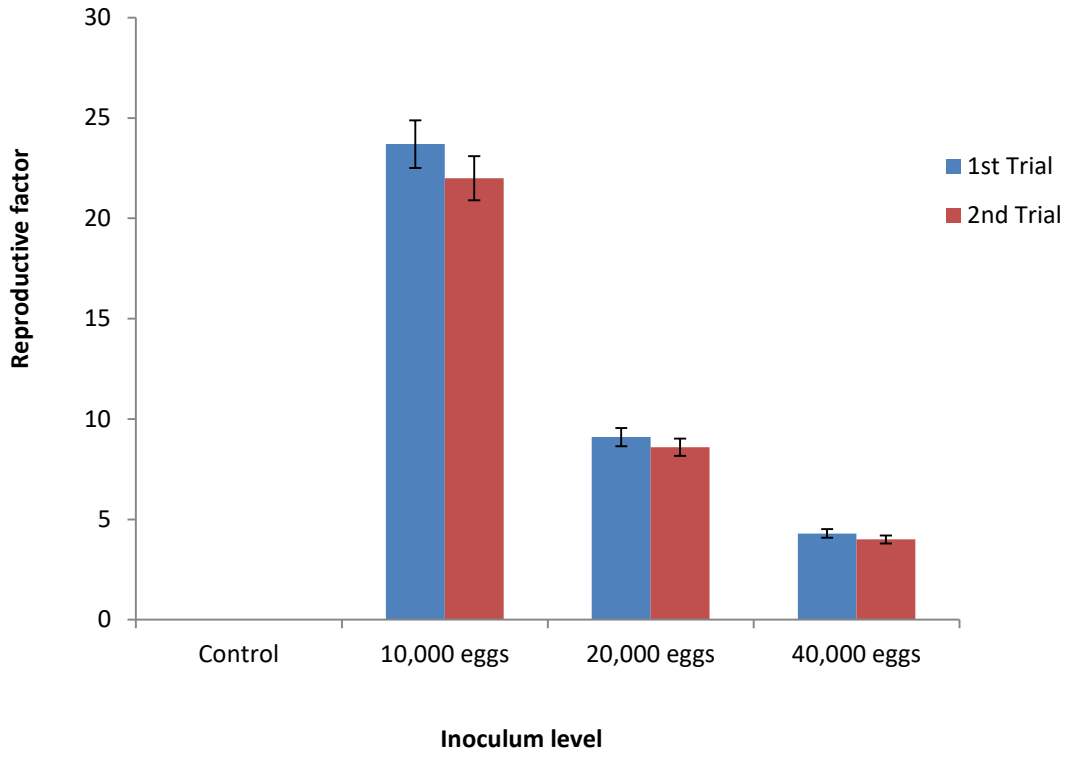



**Fig 4.9: Effects of different inoculum densities on reproductive factor of M. incognita in Cucumber (1st and 2nd trials).**

Reproductive factor ( Rf) = $\frac{\text{Final nematode population } (P_f)}{\text{Initial nematode population } (P_i)}$

$P_i$= 10,000 eggs

**Table 4.26: The fresh shoot weight, dry shoot weight and fresh root weight of different cucumber varieties as influenced by different inoculum density of *Meloidogyne incognita*.**

| | 1st Trial | | | 2nd Trial | | |
|---|---|---|---|---|---|---|
| Variety | Fresh sht wt (g) | Dry sht wt (g) | Fresh rt wt (g) | Fresh sht wt (g) | Dry sht wt (g) | Fresh rt wt (g) |
| Tokyo | 145.1 | 15.6 | 29.8 | 140.2 | 14.3 | 20.5 |
| Marketer | 96.1 | 13.2 | 38.7 | 87.5 | 12.2 | 35.7 |
| Cucumber Ashley | 129.2 | 15.7 | 30.5 | 124.4 | 15.0 | 28.9 |
| Marketmore | 102.8 | 11.9 | 26.7 | 98.8 | 11.9 | 25.8 |
| LSD≤0.05 | 26.55 | 2.44 | 10.74 | 24.54 | 2.38 | 9.24 |

Sht= Shoot; wt = weight; rt= root

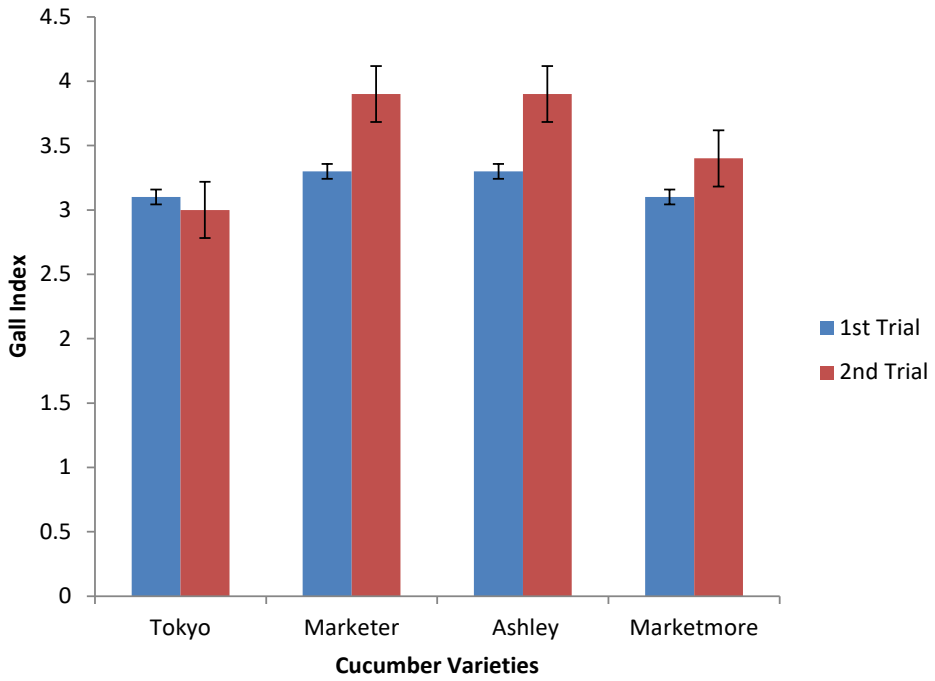



**Fig 4.10: Gall index induced on various cucumber varieties by *M. incognita* (1st and 2nd trials).**

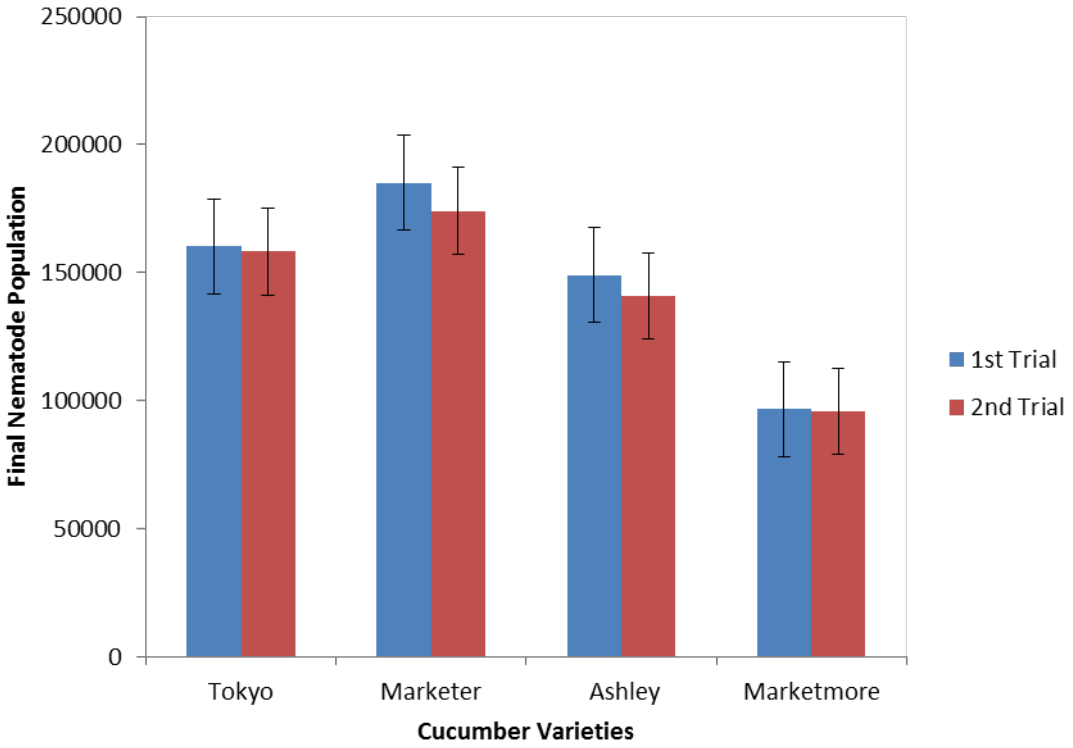



**Fig 4.11: Nematode Reproduction on various cucumber varieties (1st and 2nd trials).**

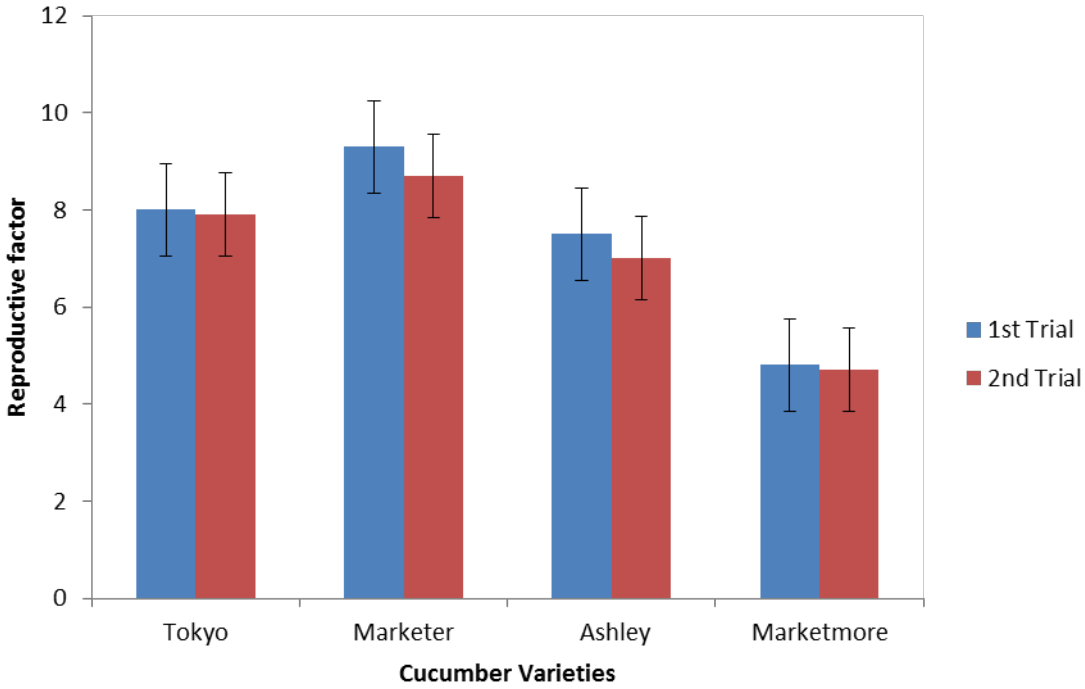



**Fig 4.12: Reproductive factor of *M. incognita* on various cucumber varieties (1st and 2nd trials).**

### 4.2.3. Effects of different inoculum densities of *M incognita* on vine length, number of leaves, number of branches and stem girth of cucumber

The mean vine length of cucumber did not differ significantly from each other throughout the period of study. However, the plants that received 40,000 eggs had lowest mean vine length at 5 and 6 weeks after inoculation (WAI). The trend was the same for both trials (Fig 4.13). There was no significant difference in the mean number of leaves, of cucumber varieties at different nematode population levels throughout the period of study (Fig 4.14).

### 4.2.4 Effects of *M. incognita* populations on vine length and number of leaves for various cucumber varieties

Tokyo variety had the highest significant mean vine length which was not higher than value from Marketmore but higher statistically from other two varieties in both trials (Fig 4.15). Tokyo had the highest mean number of leaves which was not significantly different from Ashley but different from other varieties in the 1$^{st}$ trial. In the 2$^{nd}$ trial Ashley recorded highest mean number of leaves and this was not significantly higher than other varieties (Fig 4.16).

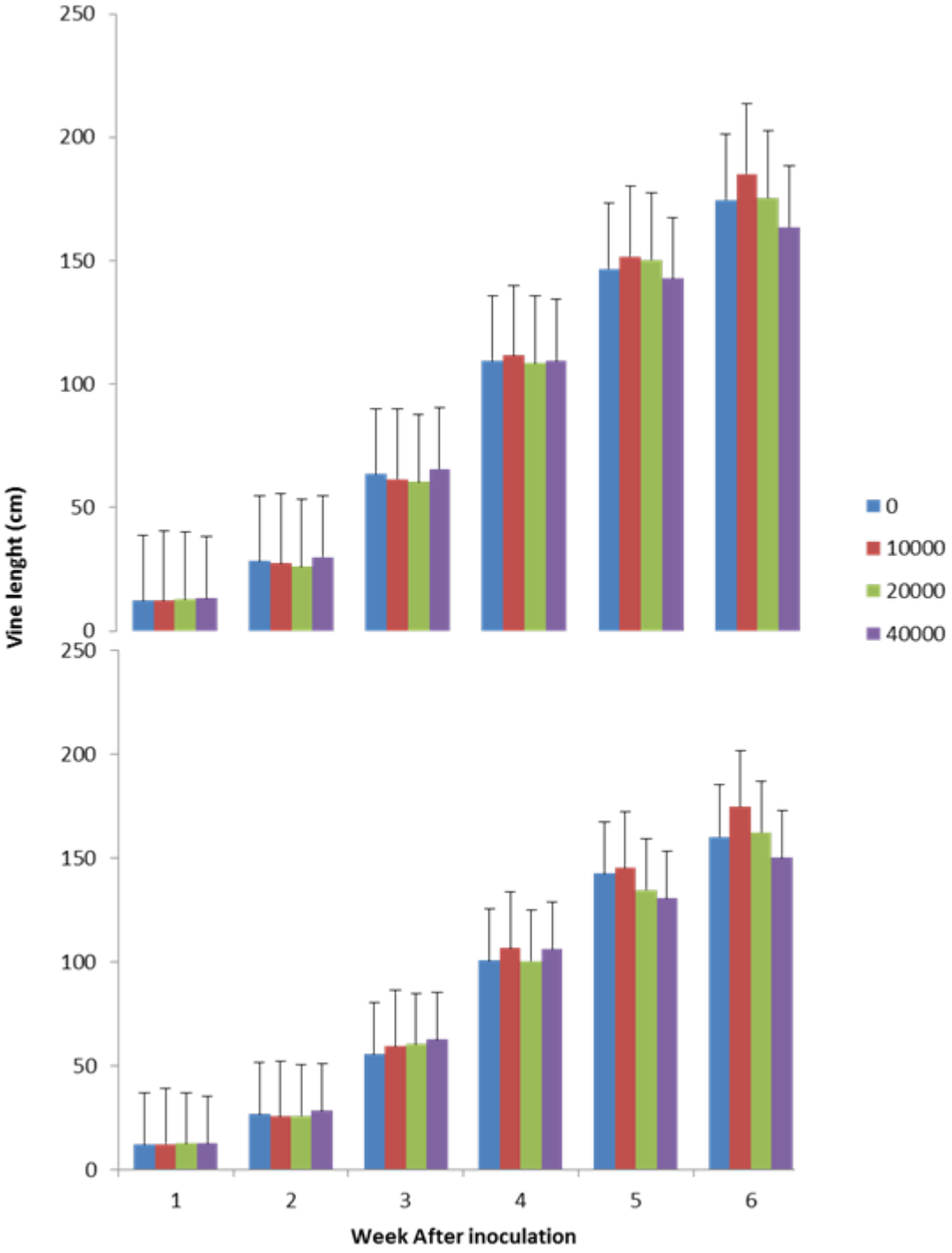



**Fig 4.13: Effect of different *M. incognita* population densities on vine length of cucumber at different weeks (1st & 2nd trials).**

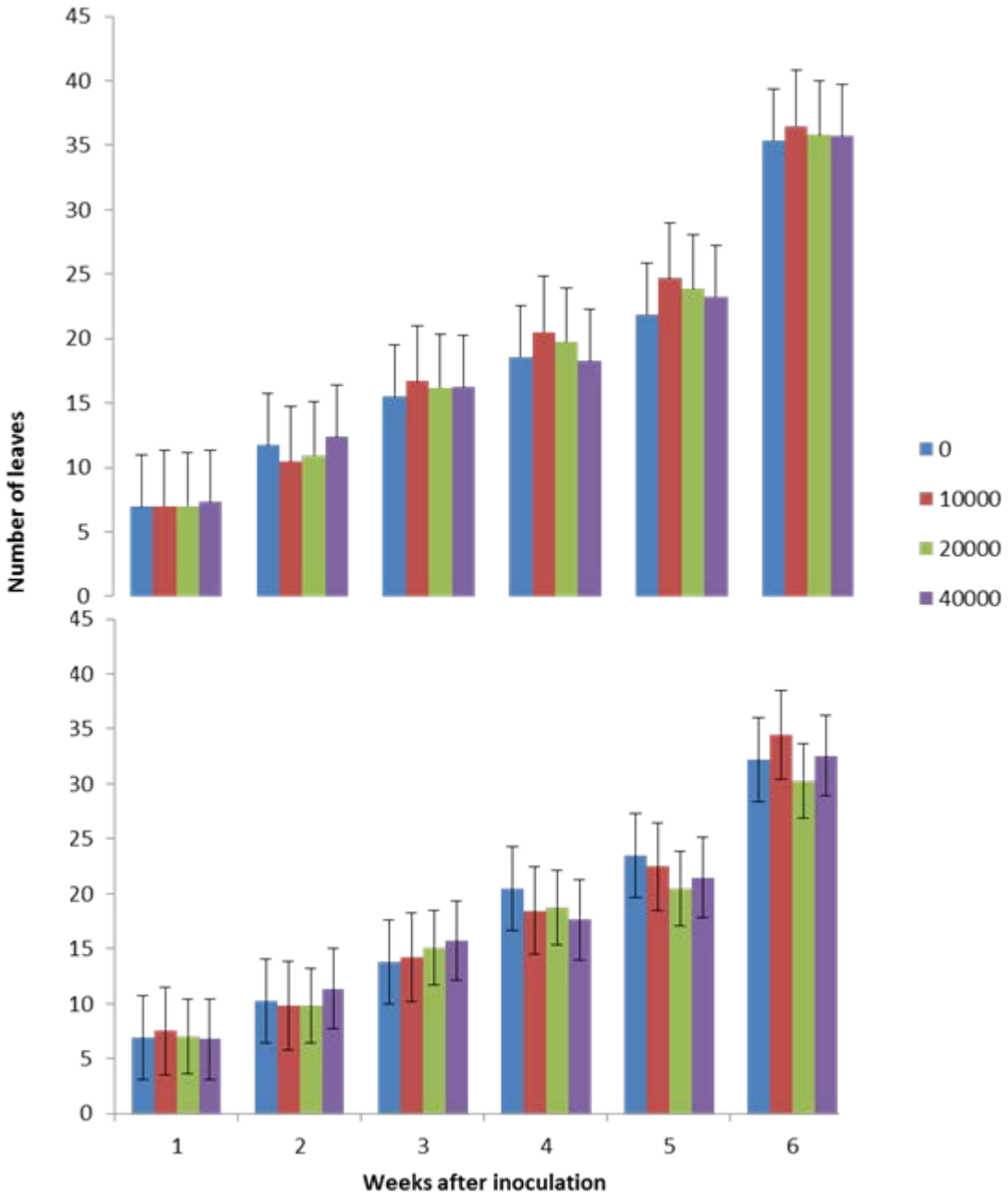



**Fig 4.14: Effect of different *M. incognita* population densities on the number of leaves of cucumber at diferent weeks (1st & 2nd trials).**

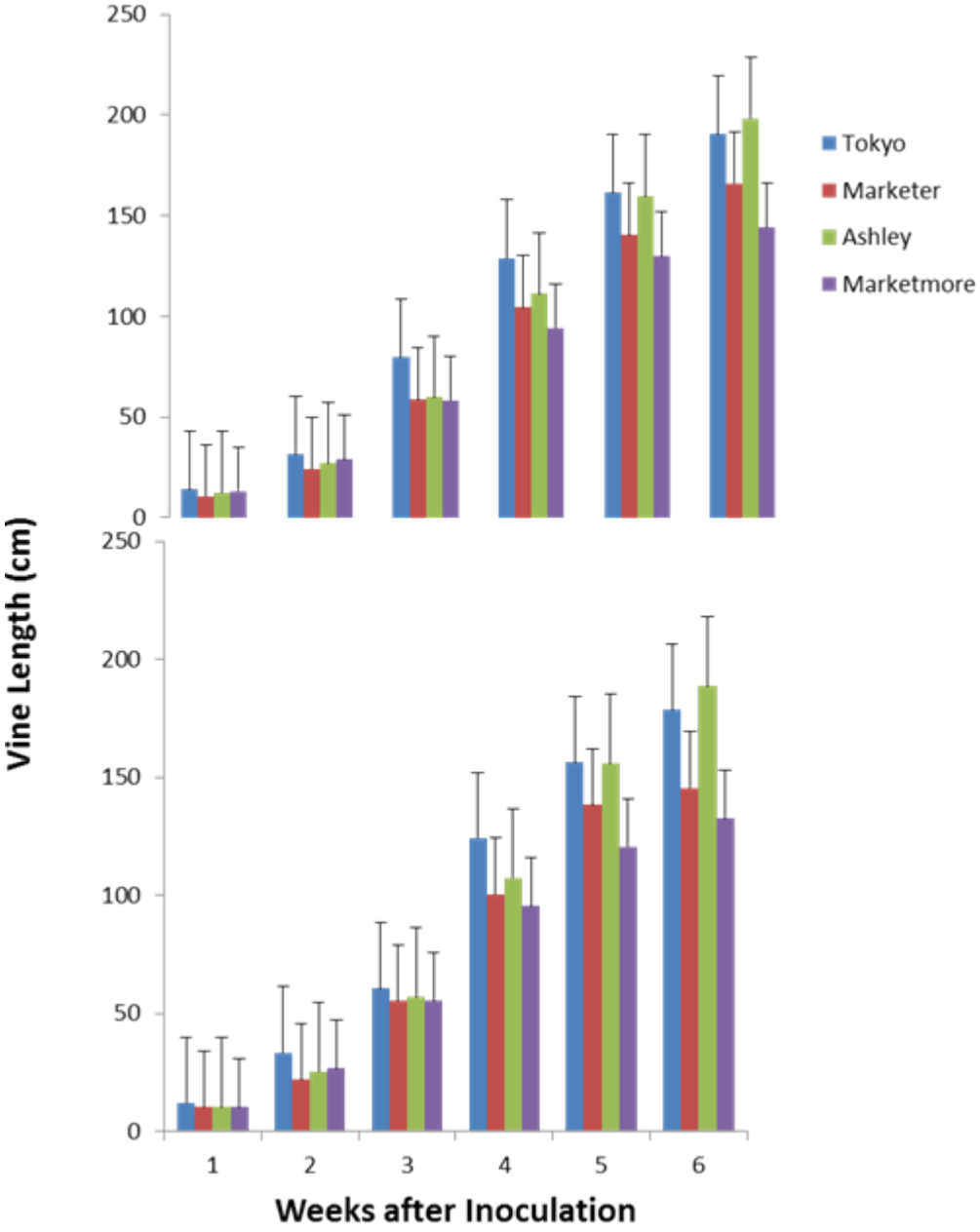



**Fig 4.15: Effect of population densities of *M. incognita* on the vine length of various cucumber varieties at different weeks (1st & 2nd trials).**

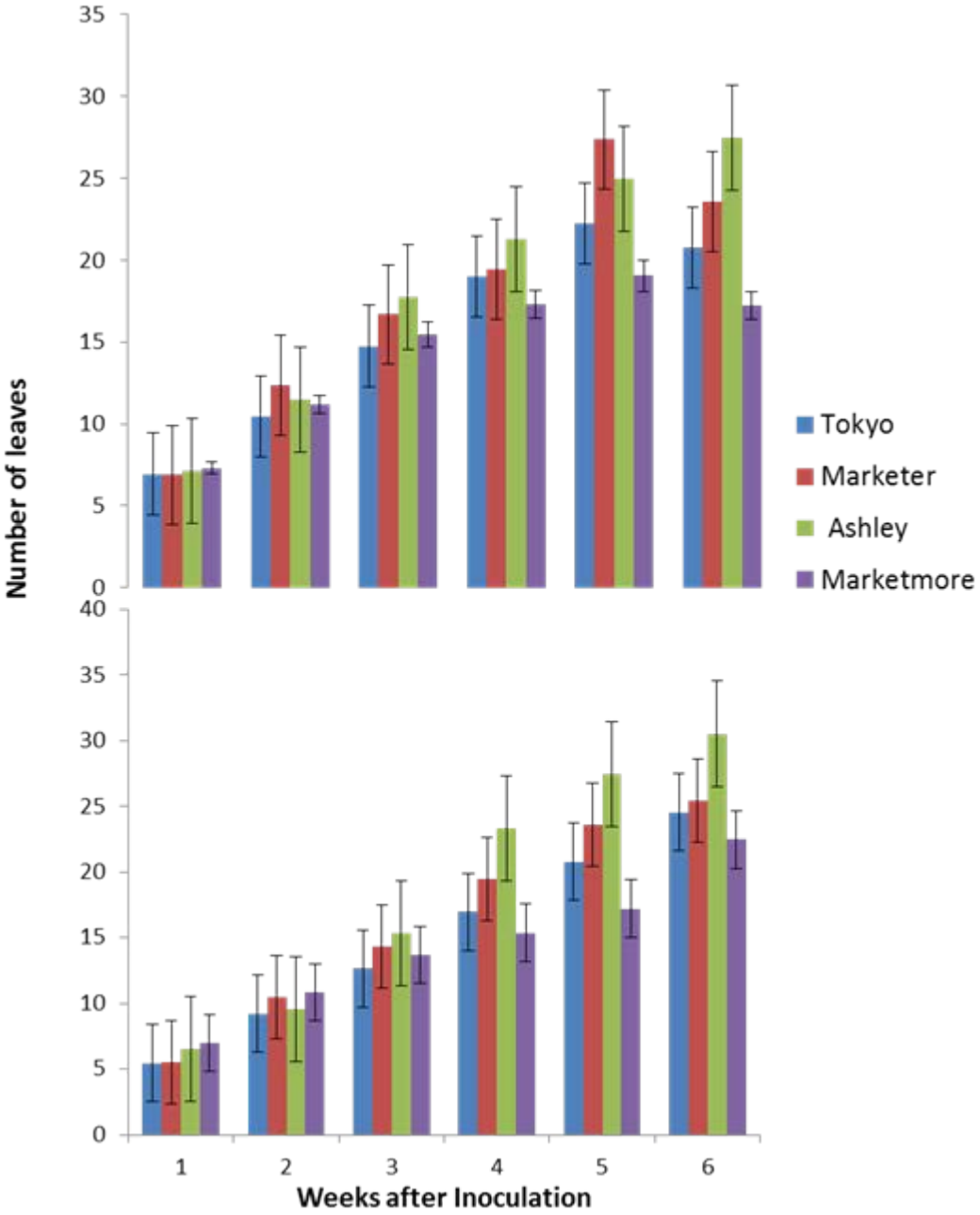



**Fig 4.16: Effect of population densities of *M. incognita* on number of leaves of various cucumber varieties at different weeks (1st Trial & 2nd Trials).**

### 4.2.5 Interaction effect of cucumber varieties and populations of root-knot nematode on fresh and dry shoot weight, fresh root weight, galling index, nematode population in soil and roots.

Result from this study showed that interaction of cucumber variety and nematode populations had no significant effect on fresh and dry shoot weight, fresh root weight, galling index, nematode population in the roots and the soil (Tables 4.27 -4.30). All the cucumber varieties used in this study without nematode inoculation except Tokyo produced significantly higher number of fruits and fruit yield than those inoculated with nematodes at varying populations (Table 4.30).

**Table 4.27: Interaction effect of cucumber varieties and populations of *M. incognita* on fresh and dry shoot weight of cucumber**

| | Shoot weight | | | | Dry Shoot Weight | | | |
|---|---|---|---|---|---|---|---|---|
| Variety X Population | 0 | 10000 | 20000 | 40000 | 0 | 10000 | 20000 | 40000 |
| Tokyo | 139.5 | 73.6 | 103.9 | 67.5 | 12.9 | 13.1 | 16.4 | 10.6 |
| Marketer | 165.9 | 129.1 | 151.3 | 134.1 | 18.2 | 14.3 | 16.4 | 13.6 |
| Ashley | 142.1 | 144.7 | 138.7 | 91.1 | 16.4 | 16.8 | 16.8 | 12.6 |
| Marketmore | 93.7 | 106.6 | 124.8 | 85.9 | 12.8 | 13.1 | 11.1 | 10.7 |
| LSD>0.05 | | | 60.1 | | | | 5.5 | |

**Table 4.28: Fresh root weight and Galling index of cucumber root as affected by the interaction of cucumber variety and population of *M. incognita***

| | Fresh Root weight (g/plant) | | | | Galling Index | | | |
|---|---|---|---|---|---|---|---|---|
| Variety X Population | 0 | 10000 | 20000 | 40000 | 0 | 10000 | 20000 | 40000 |
| Tokyo | 16.6 | 36.3 | 37.0 | 29.4 | 0 | 2.3 | 3.0 | 2.5 |
| Marketer | 21.2 | 33.7 | 53.3 | 46.8 | 0 | 3.0 | 4.3 | 3.8 |
| Ashley | 21.3 | 39.5 | 35.7 | 25.5 | 0 | 3.5 | 3.0 | 2.5 |
| Marketmore | 16.4 | 33.0 | 26.1 | 31.1 | 0 | 3.0 | 2.3 | 3.0 |
| LSD>0.05 | 21.3 | | | | 1.7 | | | |

**Table 4.29: Interaction effect of cucumber varieties and various *M. incognita* population on number of fruits, fruit weight and yield of cucumber**

| | Number of Fruit/plant | | | | Fruit weight (g/plant) | | | | Yield t/ha | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variety X Population | 0 | 10000 | 20000 | 40000 | 0 | 10000 | 20000 | 40000 | 0 | 10000 | 20000 | 40000 |
| Tokyo | 1.5 | 1.3 | 0.8 | 0.3 | 262.6 | 235 | 91.3 | 7.5 | 7.0 | 6.3 | 2.4 | 0.2 |
| Marketer | 3.0 | 0 | 0 | 0 | 1112.2 | 0 | 0 | 0 | 29.0 | 0.0 | 0.0 | 0.0 |
| Ashley | 2.5 | 0.5 | 0.3 | 0.5 | 762.5 | 122.5 | 62.5 | 107.5 | 20.3 | 3.3 | 1.7 | 2.9 |
| Marketmore | 3.8 | 0.3 | 0.3 | 0.3 | 1220. | 60 | 27.5 | 37 | 32.5 | 1.6 | 0.7 | 1.0 |
| LSD>0.05 | 1.0 | | | | 419.9 | | | | 11.2 | | | |

**Table 4.30: Interaction effect of cucumber varieties and population levels of *M. incognita* on roots and soil populations**

| **Nematode population in the roots of cucumber** | | | | | **Nematode population in the soil** | | | |
|---|---|---|---|---|---|---|---|---|
| **Variety X Population** | **0** | **10000** | **20000** | **40000** | **0** | **10000** | **20000** | **40000** |
| Tokyo | 0 | 313000 | 34000 | 48000 | 0 | 134000 | 60000 | 52000 |
| Marketer | 0 | 131500 | 166000 | 103000 | 0 | 73000 | 73000 | 194000 |
| Ashley | 0 | 52000 | 132000 | 54000 | 0 | 69000 | 118000 | 171000 |
| Marketmore | 0 | 44500 | 39000 | 47500 | 0 | 73000 | 69000 | 56000 |
| LSD>0.05 | 250978 | | | | 115885 | | | |

### 4.2.6: Effect of *M. incognita* infection on yield and root gall index of cucumber

Number of marketable fruits and marketable fruit weight (yield) were significantly different between the treated (denematized) and untreated (nematode-infested) plots of cucumber (Table 4.31). The shoot weight of cucumber planted on treated plots, irrespective of variety were significantly higher than cucumber planted on untreated plots (Table 4.31).There was no significant difference in the number of leaves, vine length and leaf area of cucumber at 50% flowering between treated and untreated plots (Table 4.31). The same trends were observed from the 2nd trial (Table 4.32). The mean number of marketable cucumber fruits produced in the treated soil was significantly higher than what was harvested in the untreated soil in both trials (Table 4.33). The untreated plots had less marketable fruit weight (3.28t/ha) compared with treated cucumber plots that had marketable fruit weight of 16.31t/ha irrespective of the variety planted (Table 4.33).

For both trials, the mean gall indices differed significantly between the treated (denematized) and untreated (nematode-infested) soils. The untreated soil produced cucumber with mean gall index that was significantly higher than that of treated soil (Table 4.34). In both trials, the mean gall indices of the various varieties were not significantly different from each other (Table 4.34). There was a significant difference between the mean fresh root weight of cucumber plants grown on treated and untreated soils for both trials. The higher significant mean value came from the treated soil (Table 4.34). The mean final soil nematode population was significantly higher in the untreated soil than in treated soil in both trials (Table 4.35). In both trials, there were no significant differences in the mean soil nematode population of all the varieties used (Table 4.35). The untreated plots produced the higher significant mean final root egg population in the two trials (Table 4.35). There was no significant difference in the mean egg population among the four varieties in the treated plots for both trials (Table 4.35).

**Table 4.31: ANOVA of growth and yield parameters of cucumber as affected by treatments, varieties and their interaction (1st Trial)**

| Source of variation | Df | MS | | | | | |
|---|---|---|---|---|---|---|---|
| | | Number of leaves 6WAP | Vine length (cm) 6WAP | NMF | MFW (yield) (t/ha)) | Shoot weight (g) | Leaf area at 50% Flowering |
| Rep | 3 | 162.28 | 42.10 | 8.37 | 11.23 | 708 | 31.34 |
| Trt | 1 | 915.06$^{ns}$ | 1357.60$^{ns}$ | 488.28** | 1359.31** | 310472** | 594.95$^{ns}$ |
| Error (t) | 3 | 61.56 | 186.90 | 4.37 | 7.54 | 111 | 46.39 |
| Var. | 3 | 215.92$^{ns}$ | 317.80$^{ns}$ | 10.12$^{ns}$ | 9.89$^{ns}$ | 19043** | 189.13$^{ns}$ |
| Trt x Var. | 3 | 412.66$^{ns}$ | 239.40 | 4.28$^{ns}$ | 15.99$^{ns}$ | 14285 | 201.78$^{ns}$ |
| Error (V) | 18 | 69.11 | 115.40 | 5.50 | 9.12 | 1863 | 57.18 |
| Total | 31 | | | | | | |

NMF= Number of Marketable Fruits
MFW= Marketable fruit weight
WAP= Weeks after planting

Trt=Treatment
Var= Varieties
Rep=Replicates

**Table 4.32: ANOVA of growth and yield parameters as affected by treatments, varieties and their interaction (2nd Trial).**

| Source of variation | Df | MS | | | | | |
|---|---|---|---|---|---|---|---|
| | | Number of leaves 6WAP* | Vine length (cm) 6WAP | NMF** | MFW*** (t/ha)) | Shoot weight (g) | Leaf area at 50% Flowering |
| Rep | 3 | 100.85 | 328.00 | 7.25 | 6.78 | 1136 | 37.80 |
| Trt | 1 | $33.52^{ns}$ | $65.10^{ns}$ | 465.13** | 1122.93** | 273105** | $40.40^{ns}$ |
| Error (t) | 3 | 89.05 | 402.20 | 3.38 | 5.53 | 270 | 87.90 |
| Var. | 3 | $299.13^{ns}$ | $68.00^{ns}$ | $36.08^{ns}$ | $57.83^{ns}$ | 175663** | $104.60^{ns}$ |
| Trt x Var. | 3 | $116.18^{ns}$ | $270.00^{ns}$ | 16.71 | $42.99^{ns}$ | 9939 | $97.7^{ns}$ |
| Error (V) | 18 | 56.92 | 233.80 | 9.67 | 23.95 | 1901 | 112.50 |
| Total | 31 | | | | | | |

NMF= Number of Marketable Fruits
MFW= Marketable fruit weight
WAP= Weeks after planting
Trt=Treatment
Var= Varieties
Rep=Replicates

**Table 4.33: Means of the shoot weight and yield of cucumber as affected by treatments and varieties (1st and 2nd trials)**

| | 1st Trial | | | 2nd Trial | | |
|---|---|---|---|---|---|---|
| Treatment | NMF | MFW Yield (t/ha) | Shoot weight (g) | NMF | MFW Yield (t/ha) | Shoot weight (g) |
| Nematicides | | | | | | |
| Treated | 11.81 | 16.31 | 311.30 | 11.94 | 15.63 | 303.60 |
| Untreated | 4.00 | 3.28 | 114.30 | 4.31 | 3.84 | 118.90 |
| LSD> 0.05 | 2.35 | 3.09 | 11.85 | 2.07 | 2.65 | 18.48 |
| Variety | | | | | | |
| 1 | 7.00 | 8.77 | 248.70 | 6.88 | 8.16 | 251.30 |
| 2 | 6.88 | 9.08 | 259.50 | 5.88 | 7.29 | 250.80 |
| 3 | 9.00 | 10.10 | 160.20 | 9.25 | 10.27 | 160.20 |
| 4 | 8.75 | 11.23 | 182.8 | 10.50 | 13.33 | 182.8 |
| LSD>0.05 | 2.46 | 3.17 | 45.35 | 3.27 | 5.14 | 45.81 |

NMF= Number of Marketable Fruits
MFW= Marketable fruit weight
Variety 1 = Tokyo

Variety 2 = Marketer

Variety 3 = Cucumber Ashley

Variety 4 = Marketmore

**Table 4.34: ANOVA of root damage, soil nematode population and eggs population as affected by treatments, varieties and interaction between 1st and 2nd Trials**

| Source of variation | Df | MS | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1st Trial | | | | 2nd Trial | | | |
| | | Gall index | Root weight (g) | Root population | Soil population | Gall index | Root weight (g) | Root population | Soil population |
| Rep | 3 | 0.54 | 0.35 | 14344 | 3225.00 | 0.71 | 0.47 | 53533.00 | 2312.00 |
| Trt | 1 | 136.13** | 648.36** | 6490804** | 292612.00** | 128.00** | 697.70** | 5678450.00** | 94612.00** |
| Error (t) | 3 | 0.04 | 0.90 | 13044 | 2638.00 | 0.08 | 1.57 | 54585.00 | 9912.00 |
| Var. | 3 | 0.13$^{ns}$ | 1.61 | 20991 | 725.00$^{ns}$ | 0.13 | 2.77 | 27433.00 | 3212.00 |
| Trt x Var. | 3 | 0.46$^{ns}$ | 0.51 | 26558 | 1821.00$^{ns}$ | 1.00 | 2.44 | 36950.00 | 7412.00 |
| Error (V) | 18 | 0.29 | 1.40 | 44781 | 4540.00 | 0.40 | 0.83 | 63058.00 | 5712.00 |
| Total | 31 | | | | | | | | |

**Significant ($p \leq 0.05$)
NS = Not significant
Trt=Treatment
Var= Varieties
Rep=Replicates

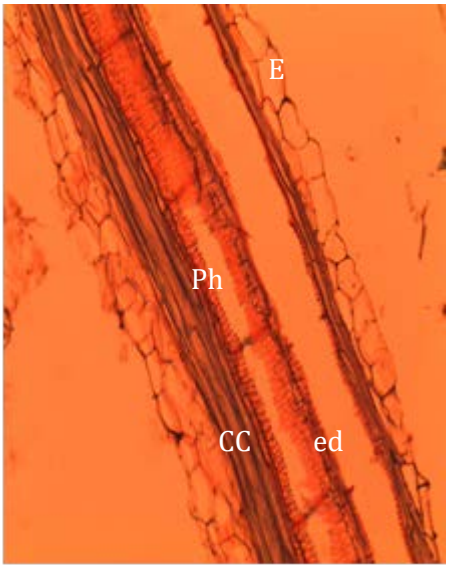
**Table 4.35: Means of the Gall index, root weight, root and soil nematode population as affected by treatments and varieties (1st and 2nd Trials).**

| | 1st Trial | | | | 2nd Trial | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | GI | Root weight (g) | Root population | Soil population | GI | Root weight (g) | Root population | Soil population |
| Nematicides | | | | | | | | |
| Treated | 0.25 | 5.57 | 6.00 | 28.00 | 0.31 | 5.13 | 6.00 | 46.00 |
| Untreated | 4.38 | 14.57 | 906.00 | 219.00 | 4.31 | 14.46 | 849.00 | 155.00 |
| LSD> 0.05 | 0.23 | 1.07 | 128.50 | 57.80 | 0.33 | 1.41 | 262.90 | 112.00 |
| Variety | | | | | | | | |
| Tokyo | 2.50 | 10.47 | 408.00 | 130.00 | 2.55 | 10.28 | 345.00 | 115.00 |
| Marketer | 2.25 | 10.17 | 428.00 | 115.00 | 2.25 | 10.17 | 428.00 | 100.00 |
| Ashley | 2.25 | 9.42 | 462.00 | 116.00 | 2.25 | 8.99 | 462.00 | 115.00 |
| Marketmore | 2.25 | 10.20 | 525.00 | 134.00 | 2.25 | 9.71 | 475.00 | 72.00 |
| LSD>0.05 | 0.57 | 1.24 | 222.30 | 70.80 | 3.27 | 0.96 | 263.80 | 79.40 |

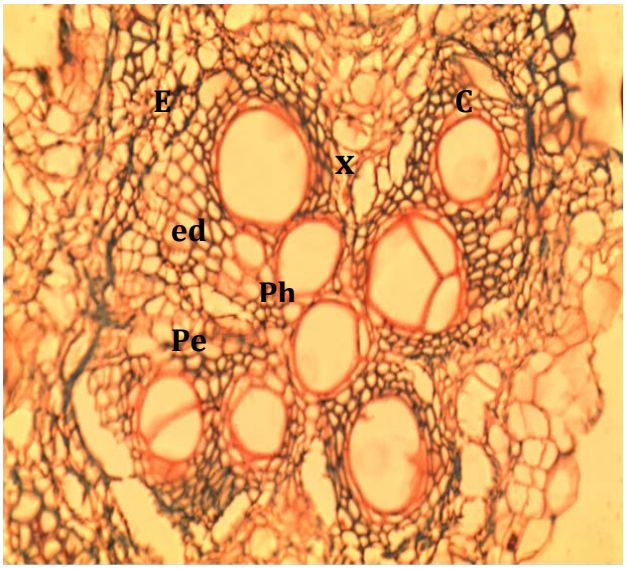
## 4.3 Histopathology studies of cucumber roots infected by *Meloidogyne incognita*

Longitudinal and tranverse sections of uninoculated cucmber root revealed the anatonomical structures typical of dicotyledonous root (Plates 4.3a and 4.3b). Examination of roots stained with acid fuchsin in lactoglycerol at 48 hours after inoculation revealed that the second-stage juvenile (J2) had penetrated the root (Plate 4.3c). A longitudinal section of the root at the meristematic zone revealed that the J2 moved intercellularly within the root cortex until it reached the vascular tissues at four days after inoculation (DAI) (Plate 4.3c). There was little or no visible damage observed in the root cells penetrated by *Meloidogyne incognita* second-stage larvae at this stage. Sections of cucumber roots at 10 days after inoculation (DAI) revealed the presence of an enlarged third-stage juvenile (J3) completely embedded in a well-established giant cells induced by the nematode which are multinucleate and contained dense cytoplasm (Plates 4.3.1e and 4.3.1f). The number of giant cells that were observed near the head of each nematode ranged from four to six. The root cells surrounding the nematodes were usually compressed and disorganized creating cavity around the nematode. It was also noted that the nematode increased in size as the diameter of the infected roots increased (4.3.1.h). Young female nematode was embedded withing the cytoplasm 24 DAI (Plate 4.3.1 f). Comparative histological observation of healthy (Plates 4.3a and 4.3b) and infected roots showed cellular disorganization in the cortex, endodermis and vascular cells highly compressed cortical and endodermal cells had occurred at this stage. *M. incognita* induced the formation of giant cells (Plate 4.4i-l). They are large and multinucleate and are formed close to the vascular cells. Their formation brought about the disruption and disorganization of xylem cells. An average of four giant cells comprising 4-6 nuclei each were found around the head of an adult female nematode. One adult female usually occupied a cavity at 28 DAI (Plate 4.4l)

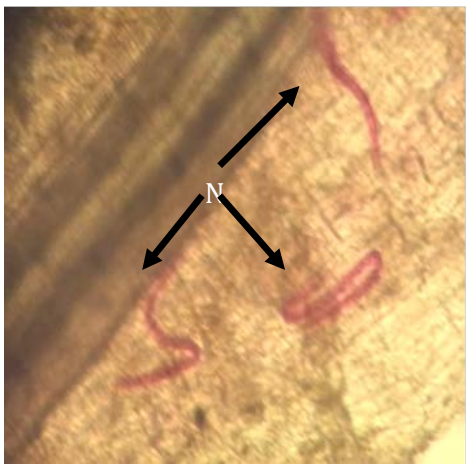
Adult females of *M.incognita* were observed from sections made from infected roots. The nematodes displaced the root cells and occupied large cavities (Plates 4.4j and 4.4k). The histopathology study also showed that nematode eggs were deposited inside the cortical cells but more eggs were observed near the root surface (Plate 4.4.1l). At 30 DAI matured female *M. incognita* was observed with eggs, giant cells and disorganized compressed cell (Plate 4.4l).




a. Mag. X 100




b. Mag X 250

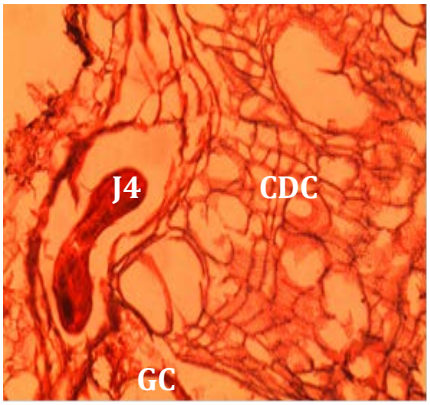



c. Mag X 100

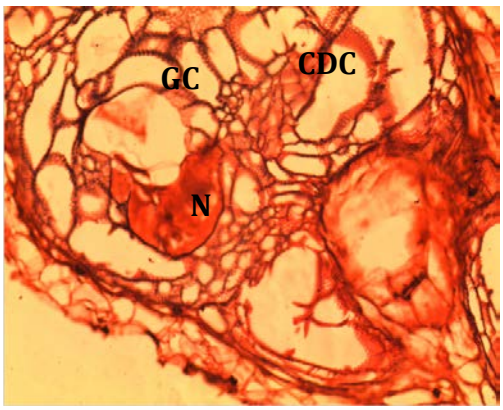
**Plate 4.3: Healthy and infected root sections of Marketer Cucumber.**

a = Longitudinal Section (LS) of healthy cucumber root, b = Tranverse Section (TS) of healthy cucumber root,

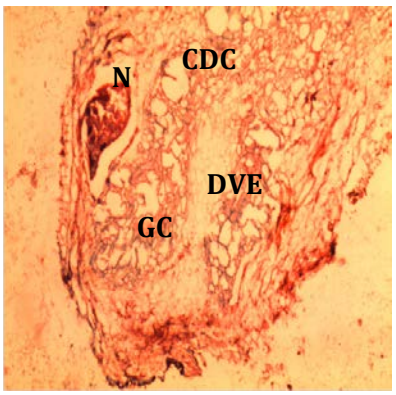
c = LS of cucumber root with second-stage juvenile (J2) of *Meloidogyne incognita* (N) migrating within the root cells at 48hrs after inoculation. Epidermis (E), cortical cell (CC), endodermis (ed), vascular elements (VE), phloem (ph), Xylem (x), cortex (C), pericycle (pe).



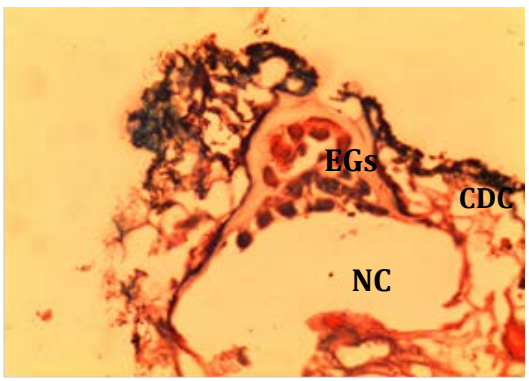

**d** Mag. X 100




**e** Mag X 100



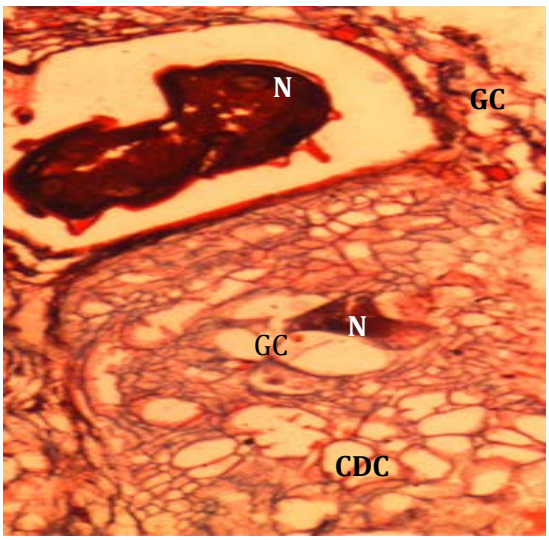

**f** Mag. X 100



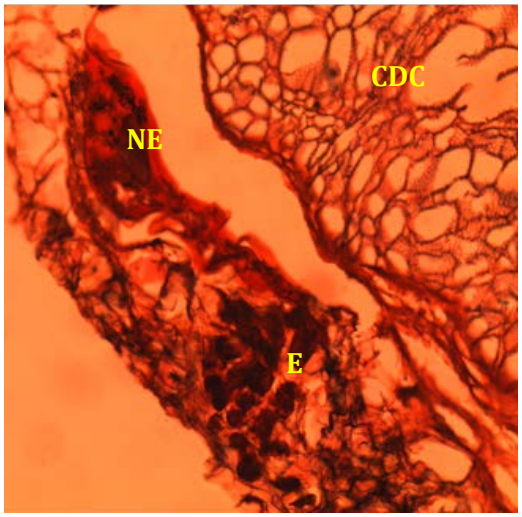

**g** Mag. X 100

**Plate 4.3.1: TS of Marketer cucumber root with compressed and disorganized cellular integrity**.

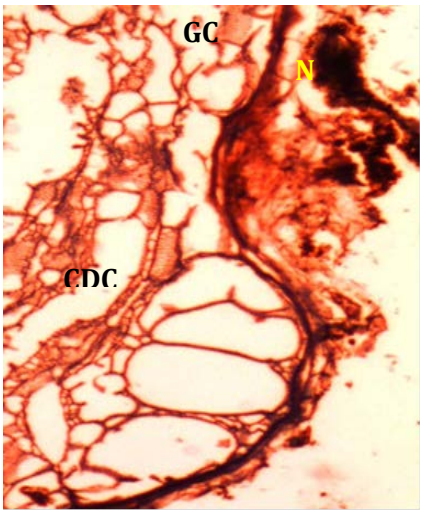
d =Third-stage juvenile (J3) of Meloidogyne incognita inside cucumber root and the giant cells (GC) distrupting the efficiency integrity of vascular element (VE) (10 DAI), e = Pre-adult stage of *Meloidogyne incognita* within the cortical layer of infected cucumber root and the giant cells (GC) distrupting the efficiency integrity of vascular element (VE) (20 DAI), f = Tranverse section (TS) of Marketer cucumber root with young female adult of *Meloidogyne incognita* (N) giant cells (GC) and disorganized, compressed and degenerated vascular elements (DVE) and cortical cells (24 DAI), g = Transverse section (TS) of marketer cucumber root with a nematode cavity (NC) and eggs (EGs) (28 DAI), compressed and disorganized cells (CDC).



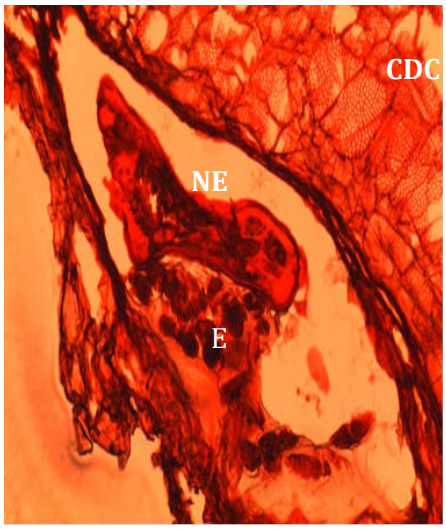

h Mag X 100




i. Mag X 100



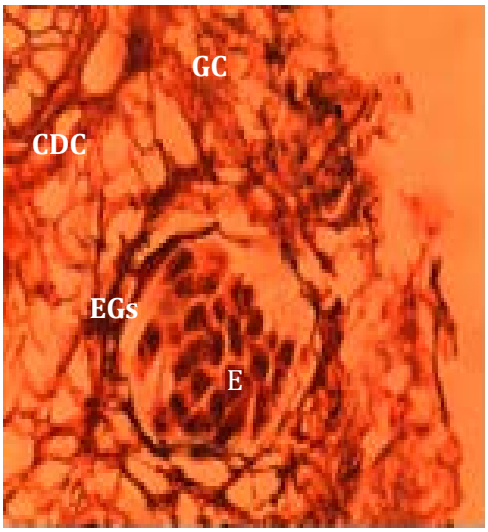

j. Mag X 250




k. Mag. X 100

**Plate 4.4. Longitudinal Sections (LS) of Marketer cucumber roots infected by *Meloidogyne incognita***

h = LS of cucumber root with J3, giant cells (GC) around the nematode (N) head distrupting the efficiency and cellular integrity of root tissue (8 DAI), i = Cucumber root with matured female nematode with eggs (28 DAI) j = Cucumber root with matured female nematode (N), giant cells (GC) compressed disorganized and degenerated cells (20 DAI), k = LS of cucumber root showing matured female nematode (Ne) with eggs, compressed disorganized cells (CDC) distrupting the proper functions of the root at 30 DAI..




**l.** Mag X 250

**Plate 4.4.1: Longitudinal Sections (LS) of Marketer cucumber roots infected by *Meloidogyne incognita***

l = LS of cucmber root with egg (EGs), compressed disorganized and degenerated cells (CDC) (30 DAI

## 4.4 Life Cycle and Development of Root-knot nematode (*M incognita*) in the roots of cucumber (Marketer)

The environmental conditions of the screen house of National Horticultural Research Institute, Ibadan where the experiment was carried out are given below.
The soil temperature (at 15cm depth) at 9.00am ranged between 26.06 and 30.18ºC with 27.53ºC as mean. At 3.00 pm., it ranged between 29.04 and 35.53ºC with 34.09ºC as mean.

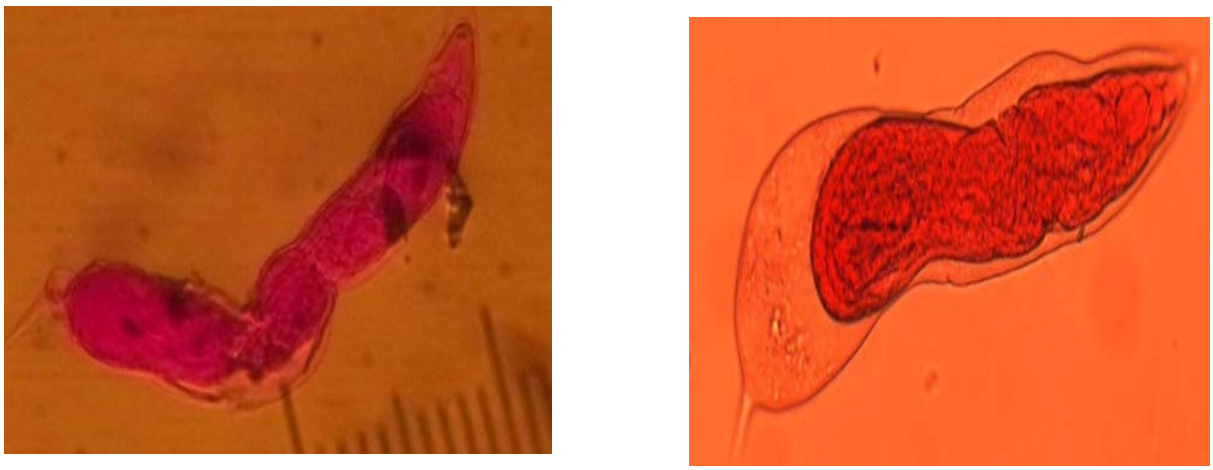
The minimum atmospheric temperature at 9.00 am ranged between 27.5 and 36.1ºC with 30.32±2.30$^0$C as mean while the maximum temperature ranged between 27.6 and 39.30$^0$C with 31.12±2.96$^0$C. At 3.00pm the minimum temperature ranged between 31.30 and 41.30$^0$C with 38.24±2.96 as mean while maximum temperature ranged 31.60 and 43.80$^0$C with 39.07±2.64 as mean. The minimum RH of the study environment at 9.00 am ranged between 44 and 86% with 65.58±9.56% as mean. The maximum RH ranged between 72 and 97% with 88.06±5.23% as mean. At 3.00 pm the minimum ranged between 30 and 57% with 37.15±4.72 while maximum ranged between 72 and 98% with 88.58±4.77 as mean.

The mean length and width of the second stage juveniles which were used to inoculate cucumber seedlings were 405.0 µ and13.51 µ respectively from the eggs with 93.75±2.7µ length and 37.83±1.6 µ width. The Juveniles were seen in the roots of cucumber 48 hours after inoculation (Plate 4.5a). At 72 hours (3days) after inoculation, second stage juveniles were still penetrating the roots behind the root cap. Some were already inside the roots but many had not fully entered the roots. They resembled those that hatched from the eggs that were used for inoculation. Their mean length and broadest width were 405.6 µ and 13.50 µ respectively.

From the third day after inoculation most of the juveniles were observed in the stele, though some were still entering the roots. At this stage, the juveniles had started to increase in size and this was very noticeable on the fifth day (Plate 4.5b). The developing juvenile, now fusiform (swollen) in shape measured an average of 475.0 µm in length. Increase in size continued and by the eighth day, the developing juvenile measured 486.50 µm. (Plate 4.5c). Moulting was first noticed at 10 days after inoculation (Plate 4.5d). This gave rise to the third stage juvenile. The third stage juvenile had no stylet and was enclosed in the old cuticle. The old stylet was observed on the anterior part of the old cuticle (4.5e).

A second moult occurred 12 days after inoculation i.e two days after the first moult. This gave rise to the fourth stage juveniles. Again, the developing nematode did not drop the old cuticles but were worn like protective clothing. Two types of developing juveniles were seen at this stage, one type with two gonads which will eventually become females and the other with one gonad which will develop into adult male. Throughout the period of study adult female was seen with two gonads. A third moulting was observed 16 days after inoculation and this produced a young female nematode. The young female nematode was embedded inside three old cuticles. By 17 days after inoculation young female nematodes that had shed the old cuticles were observed. The vulva and the perineal patterns were observed in the posterior parts of the nematodes. The young females now possessed stylet with knobs and large median bulb. The head of the nematode was observed to the attached to the stele of the root and other parts of the body were observed in the cortex. The female measured a mean length of 527.0 µ. The young females continue to increase in size and number by 24 days after inoculation, the developing females had started producing gelatinous matrix or egg sac which had no eggs in them. Eggs were observed for the first time in the egg sac at 28 days after inoculation. The mature and egg producing females measured an average of 608.10 µ in length and 405.40 µ respectively. The mean length and width of the eggs observed were 93.75 µ and 37.83 µ at their broadest width. The second stage juveniles observed measured a mean of 405.0 µ in length and 13.51 µ in width (Plate 4.6).

**a.** **b** **c**



**d** MAG X 100 **e**

**Plate 4.5:** (a) Second stage juveniles (J2) penetrating the root 48 hours after inoculation, (b) J2 viewed under microscope, (c) J2 increased in size 3 days after inoculation (DAI), (d) J2 eight days after inoculation, (e) Third stage juvenile enclosed in an old cuticle (10 DAI).

C = cortex, RS = root stele, J2 = second stage juveniles

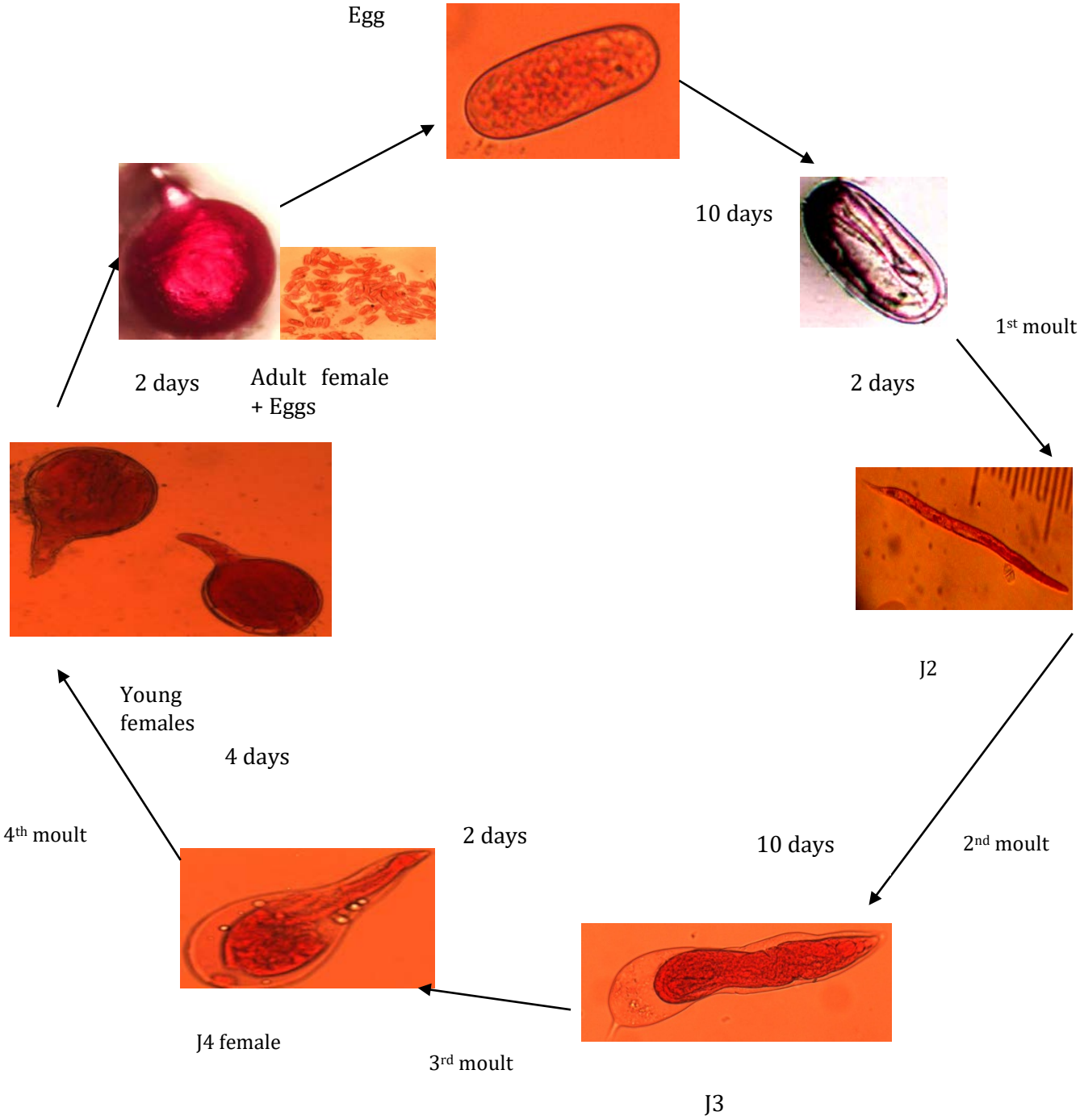



**Plate 4.6: Life cycle of *M. incognita* in the root of Cucumber (Marketer) Mag: X 100**

J1 = First stage juvenile

J2 = Second stage juvenile

J3= Third stage juvenile

J4 = Fourth stage juvenile

## 4.5 Screening of some crops for Resistance to Root-knot Nematode (*Meloidogyne incognita*)

The twenty six crops evaluated for resistance to root-knot nematode exhibited differences in their abilities to support the reproduction of *M. incognita* (Table 4.36*).* In addition, the degrees of root damage, measured by gall index, varied among the crops. These were the two criteria upon which rating for resistance to root-knot nematodes was based.

Eight plants namely Jute mallow cv angbadun, Jute mallow cv awoyaya cucumber, garden eggs cv Kotobi, water melon cv koloss, turmeric (*Curcuma longa*), amaranth (*Amaranthus cruentus*), Sunflower (*Helianthus annuus*), Sweet corn and pigeon peas had significantly higher mean galling index than other plants. Fifteen of the 26 plants screened, marigold (*Tagetes erecta*), sorghum (*Sorghum bicolor*), Oba super 1 maize (*Zea mays*), sesame (*Sesame indicum*), garlic (*Allium sativum*),, moringa (*Moringa oleifera*), tree basil (*Occimum grattissimum*), velvet beans (*Mucuna pruriens*) and hot pepper safi (*Capsicum annuum*) were poor hosts with mean gall index value <2) (Table 4.36). The same trend was observed for the second trial. The highest mean reproductive factor of root-knot nematode was from sunflower (10.54) which was significantly different from other plants. (Table 4.36)

The highest soil population of root-knot nematode was observed from garden egg but was not significantly different from jute mallow cv awoyaya (Table 4.37). The lowest soil population was observed from all accessions of sesame. maize cv oba super 1 and sorghum cv local and were not significantly different from marigold, tree basil, moringa, velvet beans, garlic, and sesame cv 02M which had no root-knot nematode in the soil (Table 4.37).

The highest total population of root knot nematode was observed from sunflower (*Helianthus annuus* ) sweet corn (*Zea mays saccharata),* jute mallow (*Cochorus olitorus*), pigeon peas (*Cajanus cajan*), garden egg (*Solanum aethiopicum*), exotic egg plant (*Solanum melongena),* watermelon (*Citrullus lanatus*) and cucumber (*Cucumis sativus*) (Table 4.37).

**Table 4.36: Host status of some selected crops to *Meloidogyne incognita* two months after infestation based on gall index (GI) and reproductive factor (RF)**

| Crop | 1st Trial | | 2nd Trial | | |
|---|---|---|---|---|---|
| | **Galling Index** | **RF** | **Galling Index** | **RF** | **Host Rating** |
| Sunflower | 2.5 | 3.3 | 2.5 | 3.3 | Susceptible |
| Jute mallow cv angbadu | 5.0 | 3.6 | 5.5 | 3.8 | Susceptible |
| Cucumber | 5.0 | 7.0 | 5.0 | 7.1 | Susceptible |
| Garden eggs cv kotobi | 5.0 | 9.1 | 5.0 | 10.9 | Susceptible |
| Jute mallow cv awoyaya | 5.0 | 10.9 | 5.0 | 10.4 | Susceptible |
| Water melon | 5.0 | 9.0 | 5.0 | 9.2 | Susceptible |
| Pigeon peas | 5.0 | 9.2 | 5.0 | 9.1 | Susceptible |
| Sweet corn | 3.0 | 5.5 | 3.0 | 5.8 | Susceptible |
| Lettuce | 5.0 | 8.5 | 5.0 | 9.0 | Susceptible |
| Turmeric | 1.0 | 1.6 | 1.0 | 1.2 | Tolerant |
| Amaranth | 1.0 | 9.2 | 1.0 | 9.1 | Tolerant |
| Sweet pepper Yolo wonder | 1.2 | 3.0 | 1.2 | 3.0 | Tolerant |
| Pea nut | 1.0 | 0.4 | 1.0 | 0.2 | Resistant |
| Sesame cv E8 | 1.0 | 0.0 | 1.0 | 0.0 | Resistant |
| Maize cv Oba super 1 | 1.0 | 0.2 | 1.0 | 0.2 | Resistant |
| Sesame cv 02M | 1.0 | 0.0 | 1.0 | 0.0 | Resistant |
| Sesame cv NCR 01M | 1.0 | 0.0 | 1.0 | 0.0 | Resistant |
| Sesame cv 530-6-1 | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Sesame cv NC 03L | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Sorghum Local variety | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Local basil | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Marigold | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Hot Peppercv safi | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Garlic | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Velvet beans | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| Moringa | 0.0 | 0.0 | 0.0 | 0.0 | Resistant |
| LSD | 0.2 | 0.9 | 0.2 | 0.8 | |

Canto-Seanz's host designation scheme (Sasser *et al*., 1984)

GI ≥2 and RF ≥ 1 = Susceptible, GI≤ 2 and RF ≥ 1 = Tolerant. GI≤ 2 and RF ≤ 1 = Resistant.

**Table 4.37: Population of *Meloidogyne incognita* on some selected crops two months after infestation**

| | First Trial | | | Second Trial | | |
|---|---|---|---|---|---|---|
| Crop /Variety | Soil Population/unit | Root Population | Total Population | Soil Population | Root Population | Total Population |
| Marigold (+ve Control) | 0.00 (0.71)* | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) |
| Sunflower | 28000 (166.46) | 509000 (694.96) | 537000.00 (715.55) | 16875.00 (127.59) | 55760.00 (730.79) | 574475.00 (743.57) |
| Sorghum | 5.63 (38.00) | 481.25 (20.39) | 519.25 (21.28) | 275.00 (16.42) | 1562.50 (39.17) | 1837.50 (42.74) |
| Maize | 27.50 (4.68) | 1112.50 (30.82) | 1140.00 (31.24) | 1062.50 (32.56) | 267.25 (16.03) | 1329.75 (36.88) |
| Sweet maize | 26560 (53.71) | 1750.00 (40.69) | 28310.00 (60.13) | 26560.00 (48.61) | 1750.50 (245.43) | 28310.00 (251.32) |
| Amaranth | 2400 (48.91) | 57.50 (7.44) | 2457.50 (49.97) | 2450.00 (49.30) | 360.63 (17.29) | 2810.62 (53.29) |
| Peanut | 178 (13.12) | 1008 (31.69) | 1198 (34.55) | 195 (13.74) | 1400 (37.03) | 1609 (39.84) |
| Jute mallow Angbadu | 121.06 (14700) | 3602.50 (59.72) | 231.20 (135.53) | 135.45 (18625.00) | 59.72 (3602.50) | 148.77 (22227.50) |
| Jute mallow awoyaya | 52625 (226.19) | 2040.00 (44.61) | 54665.00 (231.20) | 52625.00 (226.19) | 3690.00 (58.99) | 56315.00 (234.60) |
| Pigeon peas | 39075 (196.74) | 3687.50 (59.99) | 42762.50 (206.35) | 34950.00 (184.60) | 4125.00 (63.72) | 39075.00 (195.84) |
| Garden Eggs Kotobi | 67313 (254.12) | 4135.00 (60.11) | 71447.50 (263.06) | 51162.50 (221.72) | 9925.00 (94.05) | 61087.50 (243.64) |
| Sessame *E8* | 35.00 (4.95) | 40.00 (6.14) | 75.00 (8.34) | 35.00 (4.95) | 57.50 (7.36) | 92.50 (9.38) |
| Sessame *NC 03L* | 17.50 (3.26) | 130.00 (11.38) | 147.50 (12.03) | 17.50 (3.26) | 190.00 (13.58) | 207.50 (39.45) |

Pi = 5000 eggs,. Numbers parenthesis is means of the transformed data √x+0.5, which was used in ANOVA. LSD values are for transformed means.

**Table 4.37 Contd.: Population of *Meloidogyne incognita* on some selected crops two months after infestation**

| Crop Variety | Soil Population (Juveniles) (J) | Root Population (Eggs) (E) | Total Population of M. incognita (J+E) | Soil Population (Juveniles) (J) | Root Population (Eggs) (E) | Total Population of M. incognita (J+E) |
|---|---|---|---|---|---|---|
| Sessame *NCR 01M* | 5.00 (1.66) | 41.50 (6.06) | 46.50 (6.43) | 2.50 (1.34) | 45.75 (6.73) | 48.25 (6.96) |
| Sessame *530-6-1* | 10.00 (2.62) | 535.00 (19.81) | 545.00 (20.13) | 10.00 (2.62) | 535.00 (22.78) | 545.00 (23.09) |
| Sessame *02M* | 0.00 (0.71) | 253.50 (14.96) | 253.50 (14.98) | 0.00 (0.71) | 262.50 (15.60) | 320.00 (17.68) |
| Hot pepper Safi | 0.00 (0.71) | 0.00 (0.71) | (0.00 (0.71) | 0.00 (0.71) | 0.71 (0.00) | 0.71 (0.00) |
| Sweet pepper | 4250 (64.86) | 3785.00 (194.23) | 6460.00 (205.00) | 7000.00 (82.54) | 35165.00 (186.25) | 42165.00 (204.74) |
| Garlic | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) |
| Water Melon | 33800 (170.05) | 47000 (212.35) | 80800.00 (272.64) | 41800.00 (198.26) | 31075.00 (174.25) | 72875.00 (268.31) |
| Turmeric | 163 (12.63) | 3250.00 (56.38) | 3412.50 (57.88) | 310.00 (17.56) | 14590.00 (120.12) | 14900.00 (121.47) |
| Moringa | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) |
| Tree basil | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) |
| Velvet beans | 0.71 (0.00) | 0.71(0.00) | 0.71 (0.00) | 0.00 (0.71) | 0.00 (0.71) | 0.00 (0.71) |
| Cucumber (-ve control) | 5750 (75.18) | 24425.00 (153.53) | 30175.00 (172.68) | 8150.00 (89.83) | 23150.00 (151.58) | 31300.00 (176.50) |
| LSD | 15481 (36.83) | 72383 (54.79) | 75504 (63.16) | 12271 (29.59) | 72073 (52.85) | 71453 (54.79) |

Pi = 5000 eggs,. Numbers parenthesis is means of the transformed data √x+0.5, which was used in ANOVA. LSD values are from transformed means.

## 4.6 Growing cucumber in rotation with resistant crops in a natural *Meloidogyne incognita* infested field

The yield data obtained from planting cucumber in rotation after resisitant crops differed significantly ($p<0.05$) among the treatments (Fig 4.17). The cucumber vine lengths for various treatments recorded from one to four weeks after germination (WAG) were not significantly different ($P\leq0.05$) from one another (Fig 4.17). However from four weeks after germination, the highest mean vine length was produced by cucumber grown after Marigold which was not statistically higher than the one grown after sessame and maize (Fig 4.17). The least vine length was recorded from the cucumber grown after cucumber (Fig 4.17). The Second trial followed the same trend, however at 6WAG the cucumber grown after Marigold also had the highest vine length which was not significantly different from the one grown after Sessame. The least vine length was also recorded from the cucumber grown after cucumber but not significantly different from the one grown after maize (Fig. 4.17).

The number of leaves produced by cucumber grown after resisistant crops also differed significantly ($p\leq0.05$) (Fig. 4.18). There was no significant different from the the leaves produced by cucumber grown after resisitant crops from one to three WAG (Fig 4.18). The highest mean number of leaves was recorded from cucumber grown after Marigold and this was not significantly different from the number of leaves produced by cucumber grown after sesame and maize (Fig. 4.18). However, the least number of leaves was recorded from the cucumber grown after cucumber at 6WAG (Fig. 4.18). The second trial shown significant diferent at 6WAG the highest number of leaves was recorded from cucumber grown after Marigold which was significantly different from the one grown after maize and sesame (Fig. 4.18). The least number of leaves was also recorded from cucumber grown after cucumber (Fig. 4.18).

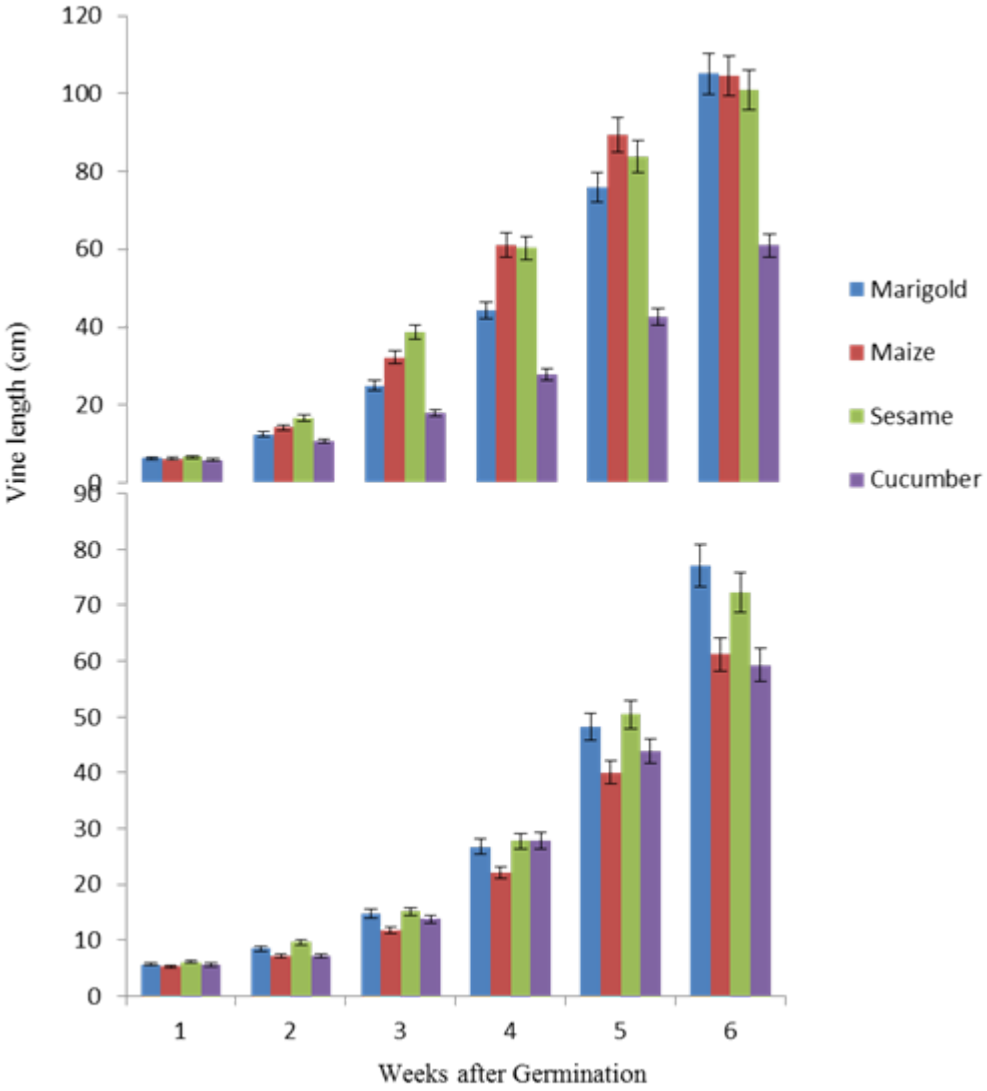



**Fig 4.17: The Vine length of cucumber grown after Marigold, Maize, Sesame or Cucumber in rotation at different weeks (1st and 2nd trials).**

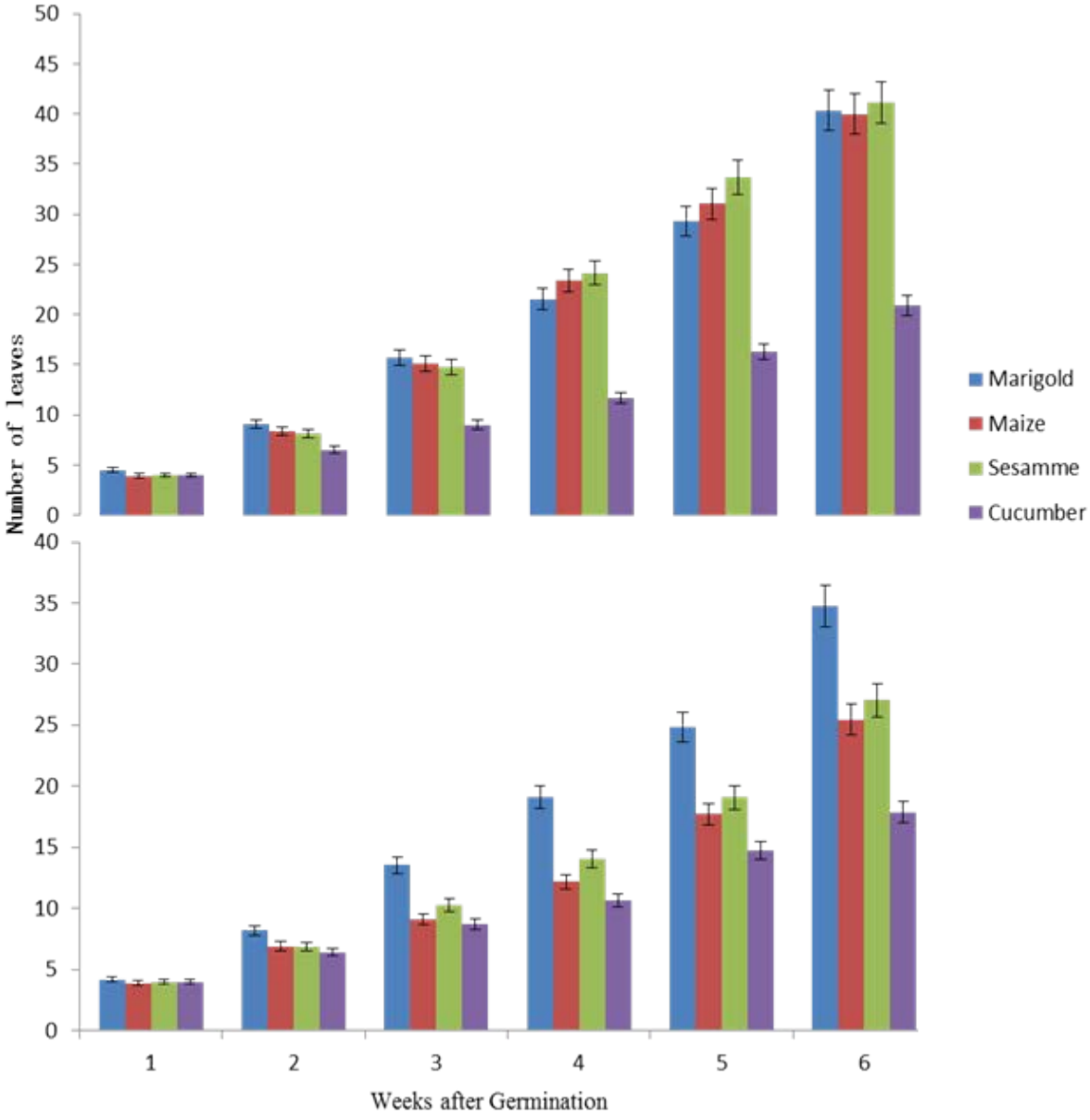



**Fig 4.18: The Number of leaves of cucumber grown after Marigold, Maize, Sesame or Cucumber at different weeks (1st and 2nd trials).**

The lowest significant mean gall index came from the cucumber grown after marigold, sesame and maize while the highest significant mean gall index was recorded from the cucumber grown after cucumber in rotation (Table 4.38). The highest fresh root weight was recorded from the cucumber root grown after cucumber which was significantly different from the cucumber root grown after Marigold, sesame and maize (Table 4.38). The highest number of eggs of root knot nematode extracted was recorded from the cucumber roots grown after cucumber while the least was recorded from the cucumber root grown after Marigold which was not significantly different from the cucumber grown after sesame and maize (Table 4.38), Second stage juveniles (J2) and total nematode population (TN) in the soil followed the same trend that is the the highest J2 and TN were recorded from the plot that was grown with cucumber after cucumber in rotation while the least were recorded from the plot grown with cucumber after marigold in rotation which was not significantly different from the plots that maize and sesame were planted (Table 4.38). The Second trial also followed the same trend (Table 4.38).

The highest shoot weight was recorded from the cucumber grown after Marigold but not significantly different from the cucumber grown after Sessame and maize (Table 4.39) while the least shoot weight of cucumber was recorded from the cucumber grown after cucumber in rotation (Table 4.39). The highest marketable fruit yield in tonnes per hectare (t/ha) was recorded from cucumber grown after maize (10.8t/ha) which was not significantly different from the cucumber grown after sessame (8.5 t/ha) and Marigold (6.5 t/ha). Meanwhile, the least marketable fruit yield of cucumber was recorded from the plot grown with cucumber in rotation (0.9 t/ha). The highest non-marketable fruit yield of cucumber was recorded from the plot grown to cucumber in rotation (1.87 t/ha). The highest marketable fruit yield was recorded from the cucumber grown after sessame in the second trial which was not significantly different from the cucumber grown after maize and marigold (Table 4.39). The least marketable fruits yield was also recorded from the cucumber grown after cucumber in rotation in the second trial (Table4.39).

**Table 4.38: Galling indices, Fresh root weight, Eggs in the root, J2 in the soil and Total nematode population of *Meloidogyne incognita* of Cucumber grown in rotation with marigold, Maize, Sessame and Cucumber in 1st and 2nd Trials.**

| | Treatment | | Gall Index | Fresh Root weight (g) | Eggs in Root (10 g) | J2 in Soil (100 ml) | Total Nematode |
|---|---|---|---|---|---|---|---|
| Session 1 | Session II | Session III | | | | | |
| Cucumber | Marigold | Cucumber | 1±0.8 | 22±4.2 | 228±61.7 | 245±43.0 | 473.3±40.6 |
| Cucumber | Maize | Cucumber | 1±0.8 | 22.2±5.6 | 348±106.0 | 241.67±61.9 | 590±111.5 |
| Cucumber | Sessame | Cucumber | 1±0.5 | 21.5±3.5 | 323±128.7 | 306.67±92.7 | 630±130.7 |
| Cucumber | Cucumber | Cucumber | 4±1.0 | 36.8±4.6 | 7300±1392.6 | 1700±85.6 | 9000±1372.1 |
| | LSD ≤0.05 | | 0.4 | 4.7 | 2174.5 | 196.8 | 2129.2 |
| 2nd Trial | | | | | | | |
| Cucumber | Marigold | Cucumber | 1±0 | 19.667±0.8 | 167±61.5 | 181.7±33.8 | 348±62.8 |
| Cucumber | Maize | Cucumber | 1±0 | 17.67±1.4 | 267±97.2 | 491.7±141.1 | 758±149.1 |
| Cucumber | Sessame | Cucumber | 1±0 | 19.167±0.7 | 325±151.0 | 343.3±112.3 | 668±177.5 |
| Cucumber | Cucumber | Cucumber | 3.5±0.22 | 30.33±2.8 | 6325±1383.1 | 1333.3±95.5 | 7658±1360.4 |
| | LSD ≤0.05 | | 0.3 | 4.9 | 2174.8 | 298.7 | 2139.3 |

**Table 4.39: The Shoot weight, Marketable fruit yield and Non- Marketable fruit yield of Cucumber grown in rotation with Marigold, Maize, Sessame and Cucumber in 1st and 2nd Trials.**

| Treatment | | | Shoot weight (g) | Marketable Fruit yield t/ha | Non-Marketable fruit yield t/ha |
|---|---|---|---|---|---|
| 1st Trial | | | | | |
| Session I | Session II | Session III | 1st Trial | 1st Trial | 1st Trial |
| Cucumber | Marigold | Cucumber | 204±30.75 | 6.5±0.99 | 1.30±0.14 |
| Cucumber | Maize | Cucumber | 187.1±29.56 | 10.8±2.85 | 1.10±0.25 |
| Cucumber | Sessame | Cucumber | 189.2±25.15 | 8.5±1.08 | 0.87±0.11 |
| Cucumber | Cucumber | Cucumber | 80±33.40 | 0.9±0.33 | 1.87±0.31 |
| | | LSD≤0.05 | 28 | 5.1 | 0.5 |
| 2nd Trial | | | | | |
| Cucumber | Marigold | Cucumber | 114.92±16.87 | 4.11±1.05 | 0.72±0.12 |
| Cucumber | Maize | Cucumber | 97.75±9.89 | 4.41±1.05 | 0.62±0.05 |
| Cucumber | Sessame | Cucumber | 88.5±6.09 | 7.05±0.87 | 0.63±0.07 |
| Cucumber | Cucumber | Cucumber | 58.25±3.85 | 0.72±0.16 | 1.31±0.20 |
| | | LSD≤0.05 | 34.03 | 2.78 | 0.39 |

# CHAPTER FIVE

# DISCUSSION

Eleven genera of plant-parasitic nematodes occurring at varying frequencies and populations were identified in this survey. These include *Meloidogyne* spp, *Pratylenchus* spp., *Scutellonema* spp., *Helicotylenchus* spp., *Xiphinema* spp., *Aphelenchoides* spp., *Tylenchus* spp., *Criconemoides* spp., *Longidorus* spp. and *Belonolaimus* spp. Most plant parasitic nematodes identified in this survey can be anticipated to be a major threat to vegetable production and should be considered as serious pests (Anwar *et al*., 2007; Anwar and McKenry, 2012). *Xiphinema* spp., *Tylenchus* spp., and *Longidorous* spp. among the plant- parasitic nematodes identified, they are ectoparasites of epidermal root tissues and they have not been documented as dangerous pests of vegetables (Anwar *et al*., 2013).

*Meloidogyne* spp. a sedentary endoparasites of vascular tissues were identified from the soil and roots of cucumber in all the States surveyed with a frequency and density that was highly variable. *Meloidogyne* spps. were the predominant spp. in all surveyed States, second on the list were *Pratylenchus* spp. a migratory endoparasites and cortical feeders, their infection can result in necrotic brown lesions within rootlets. As a result there is interference with water and nutrient movement within the plant tissues (Dorhout *et al*., 1991). *Pratylenchus* spp is also known to enhance the severity of *Verticillium* wilt of vegetables (Vrain, 1987). *Aphelenchus* spp, *Helicotylenchus* spp, *Tylenchus* spp, and *Xiphinema* spp were also recorded from each of the States but not at each of the Local government Areas.

*Meloidogyne* spp. are common in vegetable soils worldwide where they parasitize vascular root tissues and induce root galls. Root-knot nematodes, *M. incognita,* is among the most common plant-parasitic nematodes (Sasser, 1979; Abawi and Widmer, 2000; Davies *et al*., 2003; Anwar and McKenry, 2010). In addition to extensive root galling leading to malfunctioning of the root systems, its presence is often

associated with increased incidence and severity of *Fusarium* wilts (Anwar and Khan, 1973, Martin *et al*., 1994). The reduction in yield of vegetables crop due to plant parasitic nematodes feeding can range up to more than 40% (Anwar and McKenry, 2012), however, this depend on soil texture and prevailing weather conditions (Starr *et al*., 1993).

The presence of these serious plant-parasitic nematodes in abundance on cucumber production should be taken seriously by the farmers. From the discussions held with a cross- section of farmers in the locations from where samples were collected, it was clear that they were not aware of the presence of nematodes, symptoms of damage and yield reduction potentials of nematodes. More so, majority of the farmers practise mixed farming and all the host plants to root-knot nematode are planted together this lead to rapid multiplication of root-knot nematode population on their field and reduction in yield. There is therefore an urgent need for extension officers in the State Ministries of Agriculture and Agricultural Development Projects (ADPs) to enlighten farmers on the presence of plant-parasitic nematodes in their farms and their implications on crop growth and yield. Also, they may need to conduct farm trials in form of demonstration plots for nematode control. The results of this survey indicate that plant-parasitic nematodes are widely distributed on cucumber production. This information on their occurrence on cucumber will be helpful for growers for planning and administering appropriate management strategies to reduce the populations below their threshold levels.

The four varieties of cucumber used for this trial were all susceptible to the *M. incognita*. The most susceptible variety was marketer which had the highest nematode population, eggs in the root and juvenile population in the soil. As a result the variety performed very low and had crop failure in the screened house. However, on the field the nematode uninfested soil perform better with a very good yield. On the other hand, nematode infested soil had a very low yield, this is as a result of nematode infection on cucumber root that distort the intake of nutrients and water from the soil which lead to poor performance on the field. The results of this investigation indicate that the pathogenic effect of root–knot nematode (*M. incognita)* on cucumber increased with increase in the initial nematode population density but reproduction reduced at the highest inoculum level this might be as a result of competition for the little space, nutrient and water which resulted in starvation and death of some

nematodes. These findings agree with Darekar *et al*. (1988) on cucumber where, as a result of *Meloidogyne* infection, complete crop failure occurred.

The fresh shoot weight was also severely affected by *M. incognita*. This might have resulted from poor absorption of water and mineral salts leading to a decreased growth rate. Taylor and Sasser (1978) found infection with *Meloidogyne* to cause an increased protein synthesis in galls and the consequent disruption of growth regulators and other compounds between roots and stems and these result in profound disturbance of top growth. It was also observed in this study that galling indices increased with increase in inoculum density. These findings agrees with Mekete *et al.*, (2003) that root galling severity in tomato and pepper also increased with increase in inoculum level of *M. javanica.*

The high reproductive rate of *M. incognita* and degree of root damage exhibited by *M. incognita* on cucumber indicates that cucumber is a good host for this nematode. It also demonstrates the pathogenic effect of *M. incognita* and indicates that severe damage could occur if the crop is grown in the field infested with *M. incognita*. The information obtained in this study may prove useful in predicting the effect of different inoculum densities on the growth, development and yield of cucumber. At an inoculum density of 10,000 eggs per pot and above (i.e > 1000eggs/ml soil) cucumber growth and development were reduced. The reproductive factor of *M. incognita* in this study increased with increasing inoculum level from 10,000 to 20,000 eggs per plant and then declined at 40,000 eggs per plant. Similar results were reported by Sasanelli *et al.* (1992); Sasanelli and Di Vito (1992); Di Vito *et al.* (2000); Zahid *et al.* (2001); Osunlola (2011) who observed maximum reproductive rates at lower inoculum densities and this declined as the initial population density increased.

The reproduction rates of *M. exigua* eggs and juveniles at 0.125, 0.25, 0.5, 1, 16, 128, and 512 per $cm^3$ of soil were 422.4, 336.0, 199.0, 161.5, 15.6, 1.0 and 0.2 respectively (Di Vito *et al.*, 2000). The decline in nematode population at higher inoculum densities likely resulted from reduced food supply for nematodes because of poor plant growth.

A nematode management programme is therefore essential to reduce pre plant nematode population in an infested soil as its high potential fecundity will permit population densities to reach an economic threshold and as a results lead to greater damage and loss to cucumber farmers.

The results of the anatomical changes or modifications induced by *M. incognita* in the roots of cucumber (Marketer) revealed a typical susceptible reaction to infection by root-knot nematodes (*Meloidogyne spp*). These changes are similar to the findings of other researchers on different crops (Salawu, 1986; Fawole, 1988; Castillo *et al.*, 1999; Di Vito *et al.*, 2000, Zahid *et al.*, 2001, Osunlola 2011). The longitudinal and traverse sections of healthy and nematode infected cucumber roots showed that the second-stage juveniles invaded the roots using their protractible stylet and releasing secretory proteins that stimulate changes within the parasitized cells. The parasitized cells rapidly become multinucleate (contain many nuclei) has nuclear division occurs in the absence of cell wall formation. They move intracellularly through the cortex until they settled and established a permanent relation with the host. The feeding activities of root-knot nematode, *M. incognita* induced cellular alterations in the cortex, endodermis, pericycle and vascular parenchyma tissues of cucumber roots. In response to feeding activities of root-knot nematodes, some parenchyma cells became hypertrophied and multinucleate giant cells which are of xylem and phloem cells origin. According to Bird 1962 these giant cells are essential for root-knot nematode growth, development and reproduction because they are essentially transfer cells passing nutrients to the nematodes. Since nematodes depend on giant cells for feeding, adult nematodes are usually found concentrated in the vascular tissue of the roots. Fawole (1988) found adult females in the 4-6 mm layer of *Dioscorea rotundata* tuber where the first set of vascular bundles are located. Recent cytological evidence suggests that giant cells are formed by repeated endomitosis without subsequent cytokinesis. The number of giant cells (2-7) observed in this work agrees with the range reported by earlier workers on different crops (Fawole, 1988, Osunlola, 2011 and Akpheokhai 2014). These tissues were extensively damaged by the nematode. Since the nematode traverse the endodermis, it then feeds on parenchymatous phloem and xylem tissues and this will adversely affect supply of nutrient and water to other parts of the plant, consequently, wilting, stuntedness,

chlorosis of the foliage and other above ground symptoms will be observed due to *M. incognita* infection on cucumber.

The life cycle and development of *M. incognita* in the root of cucumber as observed in this investigation corroborates the reports of earlier workers (Taylor and Sasser, 1978; Nwauzor, 1979; Nwauzor and Fawole, 1992; Fatoki 2001; Claudius-Cole, 2005; Osunlola and Fawole, 2014). The point of entry of the second stage juveniles was just behind the root cap. The root cap corresponds to the meristematic regions of plant roots which Nwauzor and Fawole (1992) reported in their studies as the point of entry for *Meloidogyne* juveniles in white yam. In this investigation, second stage juveniles were recovered from cucumber roots 24 hours after exposure. Nwauzor (1979), Nwauzor and Fawole (1992) also observed penetration after 24 hours in their studies. Other workers reported juvenile's penetration within 6-48 hours of exposure. The difference in penetration time might be due to the host crop used, the species of the *Meloidogyne*, the inoculum used and the prevailing environmental conditions. The fusiform shaped juveniles observed on the $4^{th}$ and $6^{th}$ day after inoculation in this study is in line with the observations made by other researchers. Nwauzor and Fawole (1992) also observed six days after inoculation. The rapid growth and development of nematode which started with the appearance of third stage juvenile conforms to the reports of other researchers (Nwauzor, 1979; Adesiyan *et al*, 1990; Nwauzor and Fawole, 1992; Claudius-Cole, 2005; Osunlola and Fawole, 2014). The third and fourth stage juveniles lacked stylet (feeding structure) which had been cast off during the first moult and as such they do not feed. They must have relied on the food and energy reserves during moulting. The moulting process must therefore be completed quickly with regeneration of a new stylet for nematode to resume feeding.

For most species of root-knot nematodes the life cycle is completed between 25 and 40 days at between 25 and $30^0$C (Adesiyan *et al*., 1990). Nwauzor (1979), and Nwauzor and Fawole (1992) observed laying of eggs 22 days after inoculation (DAI) in tomato at a mean minimum temperature of 22.2±0.55 $^o$C and mean maximum temperature of 29.08±0.99 $^o$C and 28 DAI in white yam at a mean minimum temperature of 22.5±1.2 $^o$C and a mean maximum temperature of 37.6±3.2$^o$C respectively using second stage juveniles as inoculum in both cases. Claudius-Cole (2005) using eggs to inoculate observed that eggs were laid in the roots *Centrosema*,

*Stylosanthes* and *Vigna unguiculata* 31, 29 and 22 DAI respectively, Osunlola and Fawole, (2014) observed laying of eggs 30 DAI in sweetpotatoes. The time taken for the development of *M. incognita* in cucumber cv marketer from the time of inoculation as second stage juvenile to second stage juvenile was 30 days at a atmospheric mean minimum temperature of 30.3±2.3$^0$C and a mean maximum temperature of 31.1±3.0$^0$C, relative humidity ranged from 65.6±9.6% and 88.1±5.2%,, soil temperature ranged between 27.5$^o$C and 34.1 $^o$ C.

Therefore, the time for completion of a life cycle as observed in this study was 30days. There is no male nematode observed throughout the period of this study is unusual as Nwauzor and Fawole (1992), Osunlola and Fawole (2014) observed a dearth of male root-knot nematode in their studies on yam and sweetpotato. This is because root-knot nematodes reproduce more commonly by the process of parthenogenesis in which case the spermatozoa produced by adult males are not necessary for egg development (Adesiyan *et al*., 1990).

Furthermore, when food is in abundance, most juveniles develop into females (Taylor and Sasser, 1978). In this case, cucumber roots appear to be serving as a very good source of food for this nematode, all the juveniles developed into adult females. It should be noted that this experiment was conducted between the months of October and November. The prevailing environmental conditions reported above were adequate and suitable for the growth and development of root-knot nematode and that cucumber is a favourable host providing abundant food supply for the nematode. The short generation cycle would lead to a quick population build up of the nematode which would severely attack cucumber causing yield and quality reduction. In addition this would leave large population of nematode for subsequent crops.

From the results presented above, fourteen (14) out of the twenty-six crops screened for resistance to the root-knot nematode, *M. incognita* were found to be resistant. Three were tolerant. The remaining nine were susceptible. Marigold exhibited highest degree of resistance to *M. incognita* out of the crops screened. Marigold is commonly used as an ornamental plant; it has been used in nematode and insect pest management (Khan, *et al*., 1971). The result of this study corroborates the findings of Polthanee and Yamazaki, (1996), in which marigold (*Tagetes patula*) was effective in controlling root-knot nematode (*M. incognita*) on rice. Marigold treatment (grown and incorporated into soil before planting rice) suppressed nematode root

galling and increased rice grain yield by 46% over the untreated check. The resistance of marigold to *M. incognita* might be as a result of the presence of alpha-terthienyl, an allelochemical produced by this plant which has adverse effect on populations of root-knot nematodes and may have activity against other plant pests such as fungi, bacteria, and insects (Hooks *et al*., 2010).

The commonly planted local sorghum used was also resistant to *M. incognita*. Similar results were observed by Carneiro *et al*. (1998) for *M. incognita* and *M. javanica*. Sorghum rotated with soybeans increased productivity of soybean and effectively controlled *M. arenaria* and various other nematodes (Rodriguez-Kabana, *et al*,. 1991). The Velvet beans (*Mucuna pruriens*) used was a poor host of *M. incognita*. Similar results were observed for *M. incognita* (Resende *et al*., 1987), *M. arenaria* (Ritzinger and McSorley, 1998) and *M. ethiopica* (Edriana *et al*., 2009) on mucuna. Velvet bean had a good antagonistic response when incorporated into the soil due to release of toxic substances during decomposition (Asmus and Ferraz, 1988; Moraes *et al*., 2006; Inomoto *et al*., 2006). The five accessions of sesame (*Sesamum indicum*) used were also resistant to *M. incognita.* *S*esame and some grasses can also be used as cover crops to manage populations of root-knot nematodes Sesame valued for its oil and seed, has suppressive activity against the peanut root-knot nematode (*M. javanica*) it has proven equally if not more effective than bahia grass and cotton in reducing the carryover of peanut root-knot nematode juveniles in the soil in a peanut or soybean production system (Starr and Black, 1995). The status of sesame as a host of other species of root-knot nematode commonly found in Nigeria has not been determined. Sesame may be rotated with peanut, soybean, and possibly cotton (Starr and Black, 1995).

The results of this study also showed that the various non-host crops grown before cucumber are effective in reducing the root galls and nematode reproduction compare to the cucumber that was grown after susceptible crop (Cucumber) this is in collaborate with the study conducted by Otipa *et al*., 2003 that damage by nematodes was significantly reduced in tomato planted in rotation with sweetcorn or in sweetcorn with *Tagetes patula, Crotalaria juncea, Sorghum bicolor* and *Asparagus sp*. in the field. Cucumber grown after Marigold in rotation reduced the population of *M. incognita* in the soil and also increased the yield of cucumber this might be as a result of the roots of these attractive flowering plants contain chemicals that kill nematodes

which act as trap crops. For successful establishment of feeding sites in the vascular parenchyma, attraction of infective juveniles to roots, penetration of the epidermis and migration through the cortex are essential. The successful establishment of feeding sites in the vascular parenchyma would ensure uninterrupted supply of enough nutrition for nematodes to develop and produce eggs (Abad *et al*., 2009)

Marigold also produce a bioactive compounds like α–therthienyl which is very toxic to nematodes and other soil pathogens like as fungi, bacteria, and insects (Hethelyi *et al*., 1986; Soule, 1993, Hooks *et al*. 2010). Also once *Meloidogyne* spp. juveniles entered the roots they were unable to fully develop in the roots of *T. erecta* (Ploeg and Maris, 1999). Cucumber grown after sesame in rotation also reduced the gall index and root damaged caused by *M. incognita* this is in collaboration with Starr and Black (1995) that cotton grown after sesame in rotation effectively control peanut root- knot nematode (*Meloidogyne arenaria*) and southern root-knot nematode (*Meloidogyne incognita*).

From the foregoing, it is abundantly clear that nematodes are a major constraint to cucumber production. From the survey results, Eleven genera of plant–parasitic nematodes were identified with cucumber production out of which two species, root-knot nematodes, *Meloidogyne* spp and pin nematode, *Pratylenchus* spp have been reported to be the major cucumber nematodes worldwide. It has also been reported that *Belonolaimus* spp., *Helicotylenchus* spp. and *Xiphinema* spp. encountered in this study could become important when present in large numbers. It has equally been demonstrated that *M. incognita* is pathogenic to cucumber both in pot and field causing stunting, yellowish, fruit quality and yield reduction. This is as a result of root damage, cell disruption and disintegration and formation of giant cells which transfer nutrients meant for plant growth to nematodes as revealed in histopathology studies. The fact that this nematode completed its life cycle within 30 days suggests that two or more generations of *M. incognita* are possible in cucumber farms in a growing season thereby leaving behind a large nematode population.

Therefore, there is the need to work out effective nematode control strategy in order to improve cucumber growth, yield and quality. Although chemicals are effective, they are not within the reach of resource poor African farmers and more so, they are environmentally hazardous. Employment of integrated nematode management strategy is the way out of this problem which involves the use of two or more compatible

nematode control methods which is cost effective and ecologically safe. At this end, the use of non-host or resistant crops in rotation with cucumber is a promising strategy for the management of *Meloidogyne incognita* below economy threshold and there will be increased in yield and quality. Above all, farmers need to be enlightened about nematodes, their negative impacts on crops and crop rotation strategies.

# CHAPTER SIX
# CONCLUSIONS AND RECOMMENDATIONS

This study showed that cucumber supports various genera of plant-parasitic nematodes in their roots and rhizosphere though only two of them, *Meloidogyne* spp and *Belonolaimus* spp have been reported to cause losses. However, *Belonolaimus* spp was only encountered in Plateau and Lagos States. Further, research work is recommended to evaluate the roles of other plant-parasitic nematodes that are presently considered unimportant.

The top growth and yield were reduced. The root damage increased with an increased in nematode inoculum density.

The histopathology study revealed that cucumber exhibited a typical *Meloidogyne* spp susceptible reaction. The nematode caused cell distruption and disorganization in the cortex, endodermis and vascular cells. There is formation of giant cells which is necessary for a successful host/nematode relationship. Eggs were also observed on the root surface and inside the cortical cells.

The study of life cycle and development of *M. incognita* on cucumber revealed that root cap serve as the point of entering. The second stage juveniles were recovered from the roots 24 hours after inoculation. The fusiform shaped juveniles observed on the $4^{th}$ and $6^{th}$ days after inoculation while the $3^{rd}$, $4^{th}$ stage juveniles and young adult females were observed 10, 12 and 16 days after inoculation. Eggs laying adult females were observed 30 days after inoculation. In this study, the life cycle of *M. incognita* in cucumber was completed in 30 days.

Ten (10) out of the twenty-six crops screened for resistance to root-knot nematode, *M. incognita* were found to be resistant. Three were tolerant. The remaining eight were, however, susceptible. The resistant crops hold a good promise

in the management of root-knot nematodes. The study also demostrated that *M. incognita* is highly pathogenic.

The use of non-host plants in rotation with cucumber, were very effective in the management of root-knot nematodes (*M. incognita*). The crops were effective in reducing nematode reproduction and root damage which led to increase in the yield of cucumber.

The results of this study indicate that plant-parasitic nematodes are widely distributed in cucumber soil. This information on their occurrence and distribution will be helpful for growers for planning and administering appropriate management strategies to reduce the populations below their threshold levels. Most importantly, the study shows that the question of the effects of the use of non-host or resistant crops in rotation with cucumber on the root-knot nematode infested soil should now be regarded has answered. Further research may be justified to investigate the relative effects of this strategy compared to chemical and other preventive strategies.

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## APPENDICES

### APPENDIX 1:

**Preparation of 0.5% Sodium hypochlorite (NaOCl) solution from commercial bleach (Jik) which contains 3.5% NaOCl**

$C_1V_1=C_2V_2$

Where $C_1$= Concentration of NaOCl in Jik

$V_1$= Vol. of Jik

$C_2$= Conc. of NaOCl in the the solution of Jik and water

$V_2$= the total volume of Jik and water solution

$V_2= \frac{3.5x100}{0.5}$ =700ml

This means that 100ml of the bleach should be dissolved in 600ml of water to make a total volume of 700ml solution to produce 0.5% Sodium hypochlorite solution.

**APPENDIX II**:

Analysis of variance for the Shoot weight (Pathogenicity on the field)

Variate: SHOOTWT

| Source of variation | d.f. | | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 3 | 2125. | 708. | 6.39 | |
| REP.TRT stratum | | | | | |
| TRT | 1 | 310472. | 310472. | 2798.98 | <.001 |
| Residual | 3 | 333. | 111. | 0.06 | |
| REP.TRT.VAR stratum | | | | | |
| VAR | 3 | 57129. | 19043. | 10.22 | <.001 |
| TRT.VAR | 3 | 42855. | 14285. | 7.67 | 0.002 |
| Residual | 18 | 33541. | 1863. | | |
| Total | 31 | 446455. | | | |

**APPENDIX III**:

Analysis of variance for fresh marketable fruits weight (t/ha)

Variate: FWM_t_ha

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 3 | 33.674 | 11.225 | 1.49 | |
| REP.TRT stratum | | | | | |
| TRT | 1 | 1359.309 | 1359.309 | 180.38 | <.001 |
| Residual | 3 | 22.607 | 7.536 | 0.83 | |
| REP.TRT.VAR stratum | | | | | |
| VAR | 3 | 29.666 | 9.889 | 1.08 | 0.381 |
| TRT.VAR | 3 | 47.958 | 15.986 | 1.75 | 0.192 |
| Residual | 18 | 164.190 | 9.122 | | |
| Total | 31 | 1657.404 | | | |

**APPENDIX IV**:

Analysis of variance for Galling Index

Variate: GI

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 3 | 1.6250 | 0.5417 | 13.00 | |
| REP.TRT stratum | | | | | |
| TRT | 1 | 136.1250 | 136.1250 | 3267.00 | <.001 |
| Residual | 3 | 0.1250 | 0.0417 | 0.14 | |
| REP.TRT.VAR stratum | | | | | |
| VAR | 3 | 0.3750 | 0.1250 | 0.43 | 0.735 |
| TRT.VAR | 3 | 1.3750 | 0.4583 | 1.57 | 0.231 |
| Residual | 18 | 5.2500 | 0.2917 | | |
| Total | 31 | 144.8750 | | | |

**APPENDIX V**:

# Analysis of variance for second stage juveniles of *M. incognita*

Variate: J2M

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 3 | 9675. | 3225. | 1.22 | |
| REP.TRT stratum | | | | | |
| TRT | 1 | 292612. | 292612. | 110.94 | 0.002 |
| Residual | 3 | 7912. | 2638. | 0.58 | |
| REP.TRT.VAR stratum | | | | | |
| VAR | 3 | 2175. | 725. | 0.16 | 0.922 |
| TRT.VAR | 3 | 5462. | 1821. | 0.40 | 0.754 |
| Residual | 18 | 81712. | 4540. | | |
| Total | 31 | 399550. | | | |

**APPENDIX VI**:

Analysis of variance for number of marketable fruits

Variate: NFM

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 3 | 25.094 | 8.365 | 1.92 | |
| REP.TRT stratum | | | | | |
| TRT | 1 | 488.281 | 488.281 | 111.87 | 0.002 |
| Residual | 3 | 13.094 | 4.365 | 0.79 | |
| REP.TRT.VAR stratum | | | | | |
| VAR | 3 | 30.344 | 10.115 | 1.84 | 0.176 |
| TRT.VAR | 3 | 12.844 | 4.281 | 0.78 | 0.522 |
| Residual | 18 | 99.062 | 5.503 | | |
| Total | 31 | 668.719 | | | |

**APPENDIX VII**:

Analysis of variance for nematode population in the roots

Variate: RTPop

| Source of variation | d.f. | s.s. | m.s. | v.r. | F pr. |
|---|---|---|---|---|---|
| REP stratum | 3 | 43034. | 14344. | 1.10 | |
| REP.TRT stratum | | | | | |
| TRT | 1 | 6490804. | 6490804. | 497.59 | <.001 |
| Residual | 3 | 39134. | 13044. | 0.29 | |
| REP.TRT.VAR stratum | | | | | |
| VAR | 3 | 62974. | 20991. | 0.47 | 0.708 |
| TRT.VAR | 3 | 79674. | 26558. | 0.59 | 0.628 |
| Residual | 18 | 806061. | 44781. | | |
| Total | 31 | 7521680. | | | |