INTERACTION OF *Meloidogyne incognita* WITH *Botryodiplodia theobromae* ON *Manihot esculenta* (CASSAVA) AND ITS BIOCONTROL

BY

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ABSTRACT

The Root-Knot Nematode (RKN), *Meloidogyne incognita* and fungus *Botryodiplodia theobromae*, are important pests that cause yield losses in cassava and other crops. Chemicals have been used to manage these pests but with undesirable side effects. Information on pathogenicity of *M. incognita*, its interaction with *B. theobromae* and its biocontrol in Nigeria is very little. Therefore, pathogenicity of *M. incognita* on cassava, its interaction with *B. theobromae* and management with biocontrol agents were investigated.

A split–plot experiment was conducted in two cropping seasons with nematode-infested and denematised treatments (main-plot) on five cassava cultivars (sub-plots) to study pathogenicity in both field and pots. Two-week old potted sproutings were inoculated with 0, 1000, 10000 eggs of *M. incognita* per pot in four replicates in a 5 x 3 factorial experiment. Vegetative Growth (VG), galling index and yield-related traits were assessed using standard procedure. Interaction between *M. incognita* and *B. theobromae* on cassava was also investigated in pot and microplot field experiments. Two-week old sproutings of TMS 30572, TME 1 and Ofege cassava cultivars were each inoculated with 0, 1000 and 10000 *M. incognita* eggs, $5x10^5$ spores mL^{-1} of *B. theobromae* and combined inoculation of *M. incognita+B. theobromae* per pot (r=4). Similar treatments were applied to sproutings in the microplots. Plants were assessed for VG, yield, percentage tuber rot and nematode reproduction. The assessment of *Glomus mosseae* and *Paecilomyces lilacinus* solely and in combination in the management of *M. incognita* was evaluated in pot and field studies following standard procedures. Plants were assessed for VG, yield, galling index and nematode reproduction. Data were analysed using descriptive statistics and ANOVA at p=0.05.

Meloidogyne incognita reduced by 35.0%, 30%, 18.8%, 54.3% and 53.0% for plant height, shoot weight, stem diameter, fresh tuber weight and number of tubers, respectively. Galling index increased with increase in inoculum density. In the interaction studies, sole inoculation with *M. incognita* reduced plant height (15.0%), fresh shoot weight (34.9%), number of tubers (35.6%) and tuber weight (32.0%). Inoculation with *B. theobromae* alone significantly reduced plant height (9.0%), fresh shoot weight (15.7%), number of tubers (22.7%) and tuber weight (25.0%). Combined effects of *M. incognita* and *B. theobromae* reduced plant height (25.6%), fresh shoot weight (44.6%), number of tubers (43.2%), tuber weight (72.2%) and increased tuber

rot by 48.1% across cultivars. *Paecilomyces lilacinus* and *G. mosseae* reduced *M. incognita* population by 85.0% and 86.7% respectively; and, when added together, by 60.0%. Galling index was reduced by 66.6% and 66.5% respectively when *P. lilacinus* and *G. mosseae* were solely applied and when applied together by 35.7%. The use of *P. lilacinus* and *G. mosseae* increased VG by 30.4% and 26.7% and tuber weight by 55.9% and 58.3% respectively.

Meloidogyne incognita and *Botryodiplodia theobromae* reduced the growth, yield and quality of cassava. Applications of *Paecilomyces lilacinus* and *Glomus mosseae* have great potential in the management of *Meloidogyne incognita* in cassava production.

Keywords: *Meloidogyne incognita, Botryodiplodia theobromae, Paecilomyces lilacinus, Glomu mosseae,* Pathogenicity in cassava.

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DEDICATION

This work is dedicated to my loving wife, Dr. Bukunola Bolanle Akinlesi; my children, Praise, Love, and Favour; and to the memory of my late mother, Mrs. Titilayo Oluranti Akinlesi.

CERTIFICATION

I certify that this work was carried out by Mr. Rotimiolu Ayodeji Akinlesi under my supervision in the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan.

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CHAPTER ONE

INTRODUCTION

Cassava (*Manihot esculenta* Crantz*)*, a perennial woody shrub of the Euphorbiaceae family, grown principally for its tuber, originated from Brazil and Central America, the two centers of the *Manihot* species (Jones 1959; Leon 1986). Cassava is the principal source of carbohydrate for man as well as a food reserve during the period of drought. Out of the 123 *Manihot* species reported, cassava is the most economically important. It is the crop with the highest total production in Africa, with 140 million tonnes across the continent in 2012. It contributes significant energy input to the population with an average 196 k cal/capital/day (FAO, 2012; Moyib *et al*., 2012).

Cassava is exclusively a tropical crop grown between latitudes 30^{o}N and 30^{o}S, between altitudes 0-2300 m and between temperatures 15^{o}C and 40^{o}C (Cock, 1985). However, Allem and Hahn (1991) and Omodamiro *et al*. (2007) have reported cassava growing in the semi-arid areas where cassava was not cultivated before. The crop is also potentially highly resilient to future climatic changes and could provide Africa with options for adaptation whilst other major food staples face challenges (Jarvis *et al*., 2012). It can also withstand drought and it yields on poor soils better than other crops (Dorosh, 1989).

Cassava is a major staple food crop in the tropics and is the most important food in Africa (Adekunle *et al*., 2005). In most of these countries, cassava is grown mainly for its starchy tuberous roots, which is a valuable source of cheap calories, particularly for the low-income earners (Allem, 2002; Adenle *et al*., 2012). Cassava is simply the most important staple food grown and consumed in the Western Region of Nigeria and it plays a major role in the effort to alleviate the country's food crisis (Fakoya *et al*., 2010). It accounts for approximately one-third of the staple produced in sub-Saharan Africa (FAO, 1999; Sewando, 2012). It is adapted to various climatic and edaphic

conditions. It can also remain on the field up to two years from where it is harvested when needed; and thus, serves as food for household and food security (Cock, 1985; Dorosh, 1989). Both leaves and storage roots of cassava serve as feed for livestock (Sarma and Kunchai, 1989).

In some African countries, cassava is being more and more perceived, not only as a food security crop, but also as a raw material for various types of industries (Sewando, 2012). Cassava can be converted into a large number of products ranging from traditional and novel food products, to livestock feeds, ethanol and starch and its numerous derivatives. In such countries, there are concerted efforts on cassava development being initiated, sometimes with strong political support at the highest level (Kenyon *et. al*., 2006). For example, special presidential initiatives on cassava exist in Nigeria and Ghana to make cassava the engine for economic growth (Ukpongson *et al*., 2011). The Cassava Initiative of the government of Nigeria has projected to the limelight the multifarious uses of cassava; and as a consequence, it has become a viable export crop (Ukpongson *et al*., 2011).

The New Partnership for African Development (NEPAD) has recognized cassava as a crop which can reduce poverty in Africa (Sewando, 2012). In cassava-growing households, approximately 26% of cash income from all food crops can be derived from sale of cassava. In such areas, cassava frequently forms the basis of cottage industries to produce cassava products for domestic consumption and local/export markets. Export of cassava, mainly through increased private sector awareness of international market opportunities for cassava chips and pellets as animal feed ingredients, is strengthening the value of the crop and provides another tool in the fight against poverty. Cassava production and processing provide employment and income for many rural people, especially women (Sarma and Kunchai, 1989).

The Food and Agricultural Organization's food outlook global market analysis (FAO, 2012) predicted that world cassava output will vigorously increase in 2013. In 2012, 282 million tonnes were estimated to have been produced worldwide (FAO 2012). The expected increased growth is expected to be more rapid in Africa where cassava remains a strategic crop for both food security and poverty alleviation

Cassava is vegetatively cultivated and thus planting materials are derived from stems. Yields in farmers' fields range from 3 t/ha for local varieties (Moyo and Pelletier, 1989) to 40 t/ha for improved clones in Nigeria (Fermont *et al*., 2008; Njoku *et al*., 2010). Despite the importance of cassava as a staple food in many developing

countries, particularly in Africa, several production constraints such as poor soils, use of unimproved local varieties, land tenure and damage by pests and diseases, have kept the production of cassava below its full potential in Africa (FAO, 2000; Fakoya *et al*., 2010). Although a relatively hardy crop, the low average yield of cassava has been attributed to various factors. According to Campo *et al*. (2011), insect pests and plant diseases reduce cassava yields substantially, posing a threat to food security throughout the developing world.

Cassava is attacked by more than 50 bacterial, fungal, viral, mycoplasma and nematode agents (IITA, 1990, Coyne *et al*., 2004; Mohammed *et al*., 2012). These pathogens cause losses in crop establishment, lessen normal plant vigour, reduce photosynthetic efficiency or cause pre- and / or post-harvest stem and root rot (Lozano *et al*., 1981; IITA, 1990; Onyeka *et al*., 2005; Buensanteai and Athinuwat, 2012; Amodu and Akpa, 2012). The particular importance of pathological diseases in cassava lies in the fact that cassava is a long cycle crop (8-24 months), and it is vegetatively propagated. These favour easy multiplication and dissemination of the pathogens (Lozano *et al*., 1981).

Plant-parasitic nematode infection and damage on agricultural crops in Africa are widespread (Bridge and Muller, 1984; Bridge, 1988). The most important and widespread plant-parasitic nematodes with high damaging potential are, perhaps, the root-knot nematodes, *Meloidogyne* spp. which have been shown to cause 31- 87% reduction in top and root yield of cassava (IITA, 1985; Nwauzor, 1990), with 98% loss in extreme cases (Theberge, 1985, Coyne, 1994). Loss on a global basis due to nematodes in general has been put at 10% annually (Whitehead, 1998); a loss the hungry masses of the developing world cannot afford.

Plant-parasitic nematodes are among the pests that have been implicated in yield losses of cassava (McSorley *et al*., 1983; Jatala and Bridge, 1990). Many nematode species have been reported associated with cassava but few are reported to have caused economic damage to the crop (Coyne, 1994). *Meloidogyne* spp. has been identified as the main nematode species affecting cassava (McSorley *et al*., 1983, Jatala and Bridge, 1990). They are cosmopolitan and their worldwide distribution, extensive host ranges and involvement with fungi, bacteria and viruses in disease complexes rank them among the major plant pathogens affecting the world's food supply (McSorley *et al*., 1983). Of the various species, *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood are the most important on cassava (Jatala and Bridge, 1990)

although, *M. arenaria* (Neal) Chitwood and *M. hapla* (Chitwood) have been recorded (Coyne and Talwana 2000) and are of major concern in Uganda

Among the diseases of cassava, root and stem rots are important in different ecozones of West Africa (Banito *et al*., 2010) and various pathogens have been implicated in cassava root rot in different environments and under different conditions. Among the commonly reported organisms are *Phytophthora drechsleri* Tuker (Booth, 1978; Theberge, 1985); *Armillaria mellea* (Vahl : Fr) Kummer and *Rigidoporus lignosus* (Booth, 1978; IITA, 1990); *Rosellinia necatrix* Prill. (Booth, 1978); *Botryodiplodia theobromae* (Akinyele and Ikotun, 1989; Onyeka *et al*., 2005, Banito *et al*., 2010); *Sclerotium rolfsii* (IITA, 1990; Banito *et al*., 2010); *Fusarium* (Bandyopadhyay *et al*., 2006) and *Pythium* (Poltroneiri *et al*., 1997, Banito *et al*., 2010). Studies under controlled conditions and in the field showed that root rot disease incidence was related to susceptibility of varieties and also by the presence of nematode in the soil (Okechukwu *et al*., 2005).

Root-knot nematodes have been the subject of many of the studies on biocontrol combinations (Meyer and Roberts, 2002; Hashem and Abo-Elyousa, 2011). Numerous microbes are antagonistic to plant–parasitic nematodes, and some of the organisms reduce pathogen populations and disease (Siddiqui and Mahmood, 1999). Development of natural resistance to nematicides by nematodes and the tendency to withdraw nematicides from the market led to the search for new methods of control (Brand *et al*., 2010). Biological control of nematodes with fungi is being investigated thoroughly (Brand *et al*., 2010). Of the microorganisms that parasitize or prey on nematodes or reduce nematode populations by their antagonistic behavior, fungi had important positions and some of them have shown great potential as biocontrol agents. Fungi continuously destroy nematodes in virtually all soils because of their constant association with nematodes in the rhizosphere. A large number of fungi are known to trap or prey on nematodes but the most important genera include *Paecilomyces, Verticillium, Hirsutella, Nematophtora, Arthrobotrys, Drechmeria, Fusarium and Monacrosporium* (Hashem and Abo-Elyousar, 2011). Arbuscular mycorrhizal fungi (AMF) are ubiquitous in the soil and are commonly symbiotic with the root systems of many crops, supporting shoot growth and phosphorus nutrition (Bethlenfalvay and Liderman, 1992; Barea and Jefferies, 1995). *Glomus mosseae* has been confirmed as a potential bio-control agent for *M. incognita*. The fungus significantly suppressed nematode reproduction and damage on cowpea (Odeyemi *et al*., 2010).

Effective control of plant pests including plant-parasitic nematodes, especially root-knot nematodes, will be enhanced if some of these biocontrol-fungal agents are used in integrated nematode management programmes. Despite huge losses in cassava production due to root-knot nematodes and tuber rots, very little attempt has been made to evaluate the interaction of root-knot nematodes and *B. theobromae* on cassava fungal rot disease complex. Though *G. mosseae* and *P. lilacinus* have been found to be able to suppress nematode populations in some other crops (Inbar *et al*., 1994; Elsen, 2002), work on cassava is lacking. The objectives of this work were, therefore, to:

1. Investigate the effects of different population densities of *M. incognita* on growth and yield of cassava
2. Study the effect of the interaction between *M. incognita* and root rot-causing fungus-*Botryodiplodia theobromae* on growth and yield of cassava.
3. Study the use of *Glomus mosseae*, and *Paecilomyces lilacinus* as bio-control agents in the protection of cassava against *M. incognita*.

CHAPTER TWO

LITERATURE REVIEW

2.0: Root and Tuber crops

The major root crops – cassava, yam, sweet potato and cocoyam are widely grown and mostly used as subsistence staple in tropical and subtropical Africa. They are the major sources of energy for well over 700 million people on the continent (Njoku *et al*., 2010). Root and tubers are recognized to be important components of food security in Africa. They supply as much as 78% required daily intake of African consumers (Manyong and Oyewole. 1980). They supply 33% of the total food energy and 15% of the protein intake of West Africans (Akoroda, 1999). Root and tuber crops contain 60 – 80% moisture and do not keep as long as do cereals. They are rich in carbohydrates and rated low in protein and vitamins (Okigbo, 1984).

Important root and tuber crops grown in Africa in decreasing order of production are cassava, yam, Irish potato, sweet potato and cocoyam (Akoroda, 1999). These root and tuber crops are well-adapted to diverse soil and environmental conditions, as well as to complex traditional farming systems (Hahn, 1985; Ekanayake *et al*., 1997a). However, production of these crops is constrained by many factors such as pest and diseases, which reduce their productivity.

2.1: The Cassava Plant

Cassava (*Manihot esculenta* Crantz) is a dicotyledonous root crop belonging to the Family Euphorbiaceae, Subfamily Crotnoideae, Tribe Manihotae and Genus *Manihot* (Bonierbale *et al*., 1997). Cassava has been found to be the most nutritionally and economically important species of more than 100 *Manihot* species that have been identified (Theberge, 1985). The crop is referred to as manioc (French), yucca (Spanish), mandioca (Portuguese) and tapioca in different places (Bonierbale *et al*.,

1997). Cassava is botanically known as *Manihot esculenta* Crantz, the name Manihot is derived from the Brazilllian name of the crop (Marafioti, 1970). Cassava is commonly divided into two groups, bitter and sweet cassava, which are sometimes considered as two different species, *Manihot utilissima* and *Manihot dulcis*, respectively (FAO, 2000).

Cassava is the most widely cultivated root crop of economic importance in tropical Africa (FAO, 2000). A report in Centro International de Agricultural Tropical's (CIAT) Cassava Improvement Programme website (CIAT, 2005) indicates that the Consultative Group for International Agricultural Researches (CGIAR) group considers cassava the most important root crop in Sub-Saharan Africa (SSA) with average consumption exceeding 300 kg per person per year in some areas.

2.1.1: Origin

The crop was introduced into Africa through the Congo Basin around 1588, by Portuguese traders, through East Africa and Madagascar around the mid-18th century (Cock, 1985) and through the West African coast during the mid-19th Century by freed slaves from Brazil and West Indies (Ikpi, 1989). From these entry points, cassava slowly spread into the African continent. Cassava was introduced into Nigeria by the Portuguese traders and explorers from Fernando-Po to Warri in the then Mid-Western Nigeria in the late 18th Century. It later spread to Lagos, Badagry, Abeokuta and Ijebu in the early 19th Century by slaves returning from West Indies and Sierra Leone who settled in these towns (Agboola, 1976). These returnees processed cassava into gari, lafun and iwa-panya (roast and eat) for food. Cassava and cassava products were later introduced into Eastern Nigeria along the Coastal towns of Calabar and Yenogoa by traders from Western Nigeria. Thus, cassava may have been introduced in Nigeria to different regions about 330 years ago (Agboola, 1976). However, cassava did not attract much attention in the country until perhaps about the end of the 19th century when processing techniques and facilities were introduced, most probably, by slaves returning to Nigeria from some of the slave colonies in the Americas (Ekandem, 1962).

2.1.2: Adaptation

Cassava is grown over a wide range of environments between 30.0^{o}N and 30.0^{o}S (Latitude). It thrives best at mean temperature of between 20^{o}C and 30^{o}C and high light

intensity. Well-distributed rainfall of 1000mm to 2000mm per annum is required by the crop (Alves, 2002). Well drained sandy loam soil with pH of between 4.5 and 7.5 is best soil for cassava. However, some cassava varieties grow at 2000m altitudes with annual mean temperature as low as 16.0^{o}C (Carter *et al*., 1995; Ekanayake *et al*., 1997a; Alves, 2002).

2.2: Importance of Cassava

Cassava is one of the major sources of carbohydrate throughout Asia and Africa lowland tropics. It is Africa's second most important food staple after maize in terms of calories consumed (Nweke, 2004). It is also an important food security crop for the people of Africa (IITA, 1990; Sewando, 2012). It is an essential part of the diet of more than half a billion people around the globe (FAO, 2000). It has a comparatively high biological efficiency of food-energy production because of rapid and prolonged crop growth. It is an important crop in subsistence farming, as it requires few production skills or inputs. It is drought-tolerant and produces reasonable yields (Theberge, 1985; IITA, 1990; FAO, 2000). Cassava has the ability to recover from severe climate stress or pest attacks when favourable conditions return and yields are also reasonable under marginal soil conditions (Hahn *et al*., 1979).

The virtue of cassava as a human food item is that it is a cheap and abundant source of energy. Also, because the crop can be harvested anytime from 8-12 months after planting, it can be left in the ground as a safeguard against unexpected food shortage (FAO, 2000). Cassava roots are prepared in many ways depending upon local customs and preference, and it forms the basic carbohydrate component of the diet (Hahn *et al*., 1979). With the development in the field of human nutrition, home economists and nutritionists in many countries have developed a number of non-traditional foods by incorporating locally grown cassava into the recipe in place of exotic ingredients. For example, in Jamaica, Bammy bread ("wheatless" or high ration composite bread), made by substituting cassava flour for the usual wheat flour is a popular product, which is packaged, frozen and exported to Europe and North America (Satin, 1989; FAO, 1999). Apart from human consumption of cassava, it has been found to be highly valuable as a source of carbohydrate in animal feed, and starch for various industries (Toro and Atlee, 1980; Ojebiyi *et al*., 2010; Sewando, 2012). Over 100 cassava derivatives (chemically-modified starch) have been developed to provide

products with the physical and chemical properties required for specific use (IITA, 1990).

In Nigeria, various factors have contributed to an expansion in the production and demand for fresh and processed cassava products, including on-going policies by the Federal Government that are aimed at encouraging the export of value-added cassava products such as chips, pellets and modified starches. Recent surveys conducted in different parts of the country and in Ghana have also revealed that cassava flours and starch are increasingly being used as a substitute for imported wheat flour in the production of bread, snacks, pies/pastry and other food items (Eke-Okoro and Njoku, 2012).

Cassava, for example, appears to be one of the most valuable resources in reducing rural poverty by increasing farm incomes and the greatest hope for rural development through the establishment of agro-industrial complexes. In spite of this and the rapid growth in cassava production, the cassava sub-sector in Nigeria is still constrained by a number of factors. If cassava is processed and sold only at the primary level as it is done at the moment with *gari*, *lafun* and *fufu*, the prospects for the crop as a source of income are limited. Given its importance in the farming system of the entire nation to encourage production, the value of cassava must also increase, most effectively by developing a range of products through adoption and expanding secondary processing techniques. Once value is added to it beyond the primary level, cassava has substantial potential as a raw material for several agro-allied industries including those for flours and starch, chips for livestock feed formulation and pellets that can be exported to serve as a formidable source for generating foreign exchange earnings for the country (Adebayo, 2009 ; Ogunleye and Oladeji, 2012).

2.3: Some uses of cassava

2.3.1: Food for Man

Cassava is the chief source of dietary food energy for the majority of the people living in the lowland tropics and much of the sub-humid country of West Africa (Tsegai and Kormawa, 2002). About 90% of cassava produced in Nigeria is used locally for food and as human food, cassava is processed into over 50 food forms – gari, lafun, bread, flakes, flour etc. (Denton *et al*., 2004; Komolafe and Arawande, 2010). Measures to promote domestic cassava flour over imported cereals either for direct consumption,

or through blending, remain active throughout the world and constitute an important determinant in boosting cassava food consumption (FAO, 2012)

2.3.2: Animal feed

Cassava is widely used in feeding pigs, cattle, sheep and poultry. Dried peels of cassava roots are fed to sheep and goats, and raw or boiled roots are mixed into a mash with protein concentrates such as maize, sorghum, groundnut, or oil-palm kernel meals and mineral salts for livestock feeding (FAO, 2000). Okereke (2012) found out that tuber crop yield in terms of dry matter per acre is high when compared to maize and if properly handled will successfully replace maize in animal diet. Utilization of cassava as animal feed in the form of dried chips and pellets is mostly concentrated in Latin America and the Caribbean, especially Brazil. In Asia, the use of roots as a direct animal feeding stuff has also been in decline given the higher returns obtained from processing cassava roots for industrial applications. However, compound feed demand involving cassava in Thailand has risen substantially due to higher poultry production (FAO, 2012).

2.3.3: Pharmaceutical

Starch acts as a binder in pharmaceutical tablets and as a disintegrating agent as well. Special starch is used as dusting powder and surgical glove powder. Cassava is used in Sodium salicylate tablet formulations (Mbah *et al.*, 2010).

2.3.4: Ethanol

With the increasing price of oil in the world market and the threat of global warming as a result of increasing production of greenhouse gasses, including automotive exhaust fumes, governments everywhere are looking for alternative sources of energy, especially renewable energy such as ethanol. The major biofuel in use today is ethanol (NREL, 2012) which can be used for conventional automobiles if blended at less than 10%. Thus, in several countries, such as Brazil, USA and Thailand, most cars are gradually using ‘gasohol’, which is a mixture of 10 – 20% ethanol and 90 – 80% gasoline (Howeler, 2006). In Thailand cassava – both fresh roots and dry chips- is

considered the most promising raw material for production of ethanol. Similarly in China, cassava has been identified as a primary raw material for production of fuel alcohol, opening up a huge new market for both fresh cassava roots and dry chips. (Howeler, 2006; Zhang *et al*., 2012; Akponah and Akpomie, 2012).

2.3.5: Biodegradable plastics

Thailand and most other industrialized countries are increasingly concerned with environmental pollution caused by their production processes and the disposal of their end products. This has forced the plastic industry in these countries to look for alternative raw materials and to make its production more recyclable and biodegradable. Partially degradable films using surface-modified cassava starch has been developed and commercialized in Thailand (Sriroth *et al*., 1999). These films contain about 40% of cassava starch and 60% of low-density polyethylene polymer, and, thus, are partially degradable. However, in Thailand and Japan, using a special technology called annealing which enables starch polymers to be converted into a flat sheet, plastic films have been developed from 100% cassava starch. These products, having strength comparable to a usual polyethylene plastic, are biologically degradable and are therefore very friendly to the environment (Sriroth and Sangseethang, 2006)

2.4: Morphology of Cassava

Cassava belongs to the family Euphorbiaceae which comprises of 7200 species. Plants of Euphorbiaceae are characterized by lactiferous vessels composed of secretory cells. Cassava is a perennial woody shrub (Ekanayake *et al*., 1997b). Stem cuttings begin to sprout and root within one week after planting. Plants propagated by seeds (seedlings) take longer to sprout, and are smaller and weaker than plants produced from stem cuttings. Seedlings are generally diverse, while stem cuttings from the same parent material are generally uniform. Within the first few weeks of emergence or sprouting, the shoot lengthens and the roots spread. The cassava plant propagated from stem cuttings produce adventitious roots at the base of the cuttings within 10 -21 days after planting (IITA, 1990; Mandal, 1993). These adventitious roots develop into a fibrous root system. The fibrous root system may grow to a depth of 2 m or more. Only a few fibrous roots develop into tuberous roots, the rest remain fibrous and continue to

function as water- and nutrient-absorbing roots. The adventitious roots usually arise from the base of the cuttings where callus tissue formation is (Nartey, 1978).

These roots develop into fibrous root system and continue to function in nutrient absorption. The number of roots, which eventually form tuberous roots, is usually less than 10, although in some clones, 15-18 tuberous roots per plant have been recorded (Mandal, 1993). The process of tuberization involves the onset of secondary thickening in fibrous roots due to cambial activity, which starts three weeks after planting. The growth of the tuberous root consists of increase in the girth of a root, and this is promoted by exposing the plant to long day (10-20 hours) conditions (Mandal, 1993). Mature tuberous roots may possess rough or smooth surface and the root pigmentation may vary with respect to variety. The inner tissue varies from light-yellow, brown to pink and intermediate shades to yellow, brown or pink, whereas cortex pigmentation varies from white, yellow to pink. The type of anthocyanins present in the root determines root colouration (Nartey, 1978). Within the first few weeks of emergence or sprouting, the shoot lengthens and the roots spread. Tuberization or root thickening begins at about 8 weeks after planting. The height of a mature cassava plant usually ranges from 1 to 2 metres, although some cultivars reach 4 metres (Ekanayake *et al*., 1997a). Varieties can be classified as early or late branching depending on time of first branching. Branching height can be as low as 20cm, while some varieties never branch. Water deficit stress and cool temperatures reduce branching (Ekanayake *et al*., 1997b).

Cassava leaves are simple, consisting of a lamina and a petiole. Each leaf is subtended by two stipules about 1cm long. The petiole is between 5 and 30cm long and varies from green to purple. The lamina has a smooth margin and is deeply palmate or lobed (Ekayanake *et al*., 1997a). The number of lobes varies from green to purple. Also lobe shapes vary, especially in width. Most cassava varieties grown in Africa have elliptical or lanceolated lobes. The arrangement of cassava leaves on a stem (phyllotaxis) is a 2/5 spiral (Ekanayake *et al*., 1997a).

Cassava is a monoecious plant; having male and female flowers located on the same plant. Inflorescences are produced at the reproductive branches. Male flowers develop near the tip while female flowers develop closer to the base of the inflorescence. Each flower, male or female, has 5 yellow perianths. The female has an ovary, mounted on a 10-lobed glandular disc. Female flowers open 1-2 weeks before male flowers (protogyny). Insects carry out cross-pollination. Self-pollination occurs when female and male flowers located on different branches of the same plant open at

Plate 2.1: A cassava plant

the same time. After pollination and subsequent fertilization, the ovary develops into a fruit. The fruit matures in 70-90 days. The cassava seed is oval and 0.7cm-1.0cm long. It has a brittled testa. Seeds can be light grey, brownish or dark grey, with darker blotches. A large caruncle is located at one end of the seed. There are two types of cassava; sweet (low cyanide) and bitter (high cyanide) cassava (Horton *et al*., 1984). The enlarged root has hydrocyanic glycosides in varying quantities (Jatala and Bridge, 1990).

2.5: Agronomy

For good growth and yield, cassava requires friable, light-textured and well-drained soils containing sufficient moisture and a balanced amount of nutrients. Depending on the moisture conditions of the soil, farmers plant cassava cuttings vertically, at an angle or horizontally. Slow initial development of sprout makes cassava susceptible to weed competition in the first 3 to 4 months (Ekanayake *et al.*, 1997a). Once mature, storage roots can remain in the ground and be harvested between 6 and 48 months after planting (Nweke *et al*., 2002). It is the most widely grown root crop across varying agro-climatic conditions (Flach, 1982).

Cassava is almost always intercropped, except on a few large-scale mechanized farms. Farmers intercrop cassava usually with vegetables, plantation crops (such as coconut, coffee), yam, sweet potato, melon, maize, rice, groundnut and other legumes (Ekanayake *et al*., 1997a). Intercropping pattern depends on environmental conditions and food preference of the region.

2.6: Land Preparation and Planting

Land preparation practices depends on climate, soil type, vegetation, topography, degree of mechanization, availability of labour and traditional cropping system (Ekanayake *et al*., 1997b). Land preparation for planting cassava upland and in valley differs. On upland areas, farmers plant on flat ground, or mounds and ridges. In valleys, farmers prepare ridges or mounds above ground level to control water logging (Adekunle *et al*., 2005). Cassava cuttings for planting should be taken from plants 8 -18 months old. Cuttings taken from older plants are lignified and contain only a small amount of nutrient for sprouting (Adekunle *et al*., 2005). A mature cassava stem has 3

sections: hard wood, semi-hardwood, and shoot tips. The hard and semi-hardwood sections are best for planting. Shoot tips are very fragile and have high mortality rate, especially if they are subjected to moisture stress during the first month after planting. The semi-hard wood section gives the best quality (Ekanayake *et al*., 1997b; Adekunle *et al*., 2005).

The best and commonly used planting material is the stem cuttings of 20-25 cm long with 5 - 6 nodes (Ekanayake *et al*., 1997b; Adekunle *et al*., 2005). The planting operation involves planting or inserting two thirds of the cuttings vertically, at an angle of about 45^{o} or placing the entire cutting horizontally in the soil at a depth of 10cm depending on soil type. Cassava is planted at a spacing of 0.9m – 1.0 intra-row and 0.9 – 1.0m inter-row.

2.7: Cassava Production in Nigeria

Cassava production, as estimated by Food and Agriculture Organization (FAO, 2012) indicated an annual global production of 282 million metric tonnes (mt). The annual production of cassava in Africa is 143 mt and Nigeria, being the largest producer in the world, has an annual production of 58 mt (FAO, 2012). Renewed political support for the utilization of cassava is by and large, behind the increase in Nigeria (FAO, 2012). At the moment cassava is one of the most important crops in Nigeria. It is the most widely cultivated crop in the southern part of the country in terms of area planted and the total number of farmers involved in its cultivation. It serves not only as a food crop but even more also as a major source of income for rural households.

In 2002, cassava gained a leading role among crops in Nigeria following the establishment of the cassava initiative, with the establishment of the National Committees on Cassava Production, Processing and Packaging and Market Development and Export with the aim of making the crop a major non-oil foreign exchange earner because of its comparative advantage in the country (Adebayo, 2009). In 2012, Ogunleye and Oladeji carried out a study on the perception of farmers on the Cassava Initiative Policy in Nigeria among members of the cassava growers association. The initiative was favourably perceived among 55.2% of the respondents. Also the current government through her Agricultural Transformation Agenda (ATA) has picked cassava as an important crop in the ATA by adopting the policy of 10% inclusion of cassava flour into wheat flour for baking (Ogunleye and Oladeji, 2012).

2.8: International Trade in Cassava

International cassava trade is being increasingly driven by industrial demand for cassava, particularly from China. Thailand remains the dominant supplier, after undergoing a difficult year in 2011 in meeting the demand for high quality cassava (a starch content of 30 percent or more) at competitive prices (FAO, 2012). International trade in dried cassava, in form of meal or, more generally pellet, accounts for 15% of world production. Although not much cassava is consumed in Thailand, it has become the country's major export crop after rice (Toro and Atlee, 1980). The total estimated world export of cassava in 2012 was 16.6 million metric tonnes, out of which Thailand exported 11.7million metric tonnes and Nigeria 1.0 million metric tonnes. (FAO, 2012) The amount of cassava exported out of Africa and Latin America is negligible, while it is estimated that about 27% of the total Asian cassava production is exported. Thailand alone accounts for about 90% of all Asian cassava exports and Indonesia about 9%. The largest importer of cassava used to be the European Union, which defines the three conventional commodities in international commerce as:

- Dried cassava chips
- Cassava pellets
- Starch and flour

Since the European market collapsed as the major destination for cassava in 2009, the international market has been largely orientated towards supplying neighbouring destinations in East and Southeast Asia. Prospects for a wider international market involving other continents have remained elusive.

2.9: Nematode Pests of Cassava

There are reports of many nematode species associated with cassava in many different geographical areas. Comprehensive lists of the species found have been compiled, together with their distribution (Hogger, 1971; Mcsorley *et al*., 1983; Bridge *et al*., 1991; Coyne and Talwana, 2000; Coyne *et al*., 2003). Although, the list is extensive, the majority of the nematode species is of limited importance and occurs opportunistically on cassava (Coyne, 1994). Others are of known importance but are often disregarded as possible cassava production constraints.

Many cassava farmers cannot specifically point to nematodes as pests causing reduction in the yield of their cassava. This attitude is in part due to the fact that nematode damage and effects regularly go unnoticed. With cassava, this is almost certainly so, whereby the naturally 'knobbly' and rough texture of the roots can disguise nematode damage to casual observers. The long duration over which cassava can be left in the ground and the common 'piece-meal' method of harvesting, means that nematode-affected root systems may have decomposed in the ground or are not exposed at harvest for observation (Coyne, 1994). Also, aboveground symptoms of reduced vigour and chlorosis of the leaves may resemble effects of poor soil fertility conditions in which cassava is regularly grown.

According to Caveness (1982a) and Coyne (1994), with intensification of cassava production, the trend to monocultures and use of new and higher-yielding cultivars, nematodes pose an increasingly greater threat, with the potential to be limiting factors in production. The nematode pests of cassava include root-knot nematodes (*Meloidogyne* spp.) lesion nematodes, *Pratylenchus* spp., *Scutellonema* spp., *Aphelenchus avenae*, *Tylenchus* spp. Most of these nematodes may interact with other pathogenic organisms in the development of disease complexes. Most data relating to nematodes of cassava relate to diagnostic and distribution studies, with some information for screening studies and a limited amount from pathogenicity work, largely in pots (Bridge *et al*., 2005).

2.9.1: Root-Knot Nematodes (*Meloidogyne* spp.) on Cassava

Evidence indicates that root-knot nematodes (*Meloidogyne* spp.) are, by far, the most important group of nematodes affecting cassava (McSorley *et al*., 1983). They are certainly the most reported nematodes occurring on the crop. They have been reported on cassava across Africa, Asia, the Pacific, and the Americas (Bridge *et al*., 2005) Of the various species *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood are the most important (Jatala and Bridge, 1990), although *M. arenaria* (Neal) Chitwood and *M. hapla* (Chitwood) have been recorded (Coyne and Talwana, 2000) but are not of major concern.

Root-knot nematode infection in cassava may result in suppressed root growth and tuber production (Gapasin 1980; Caveness, 1982b; Talwana *et al*., 1997a, b). Symptoms in many other plants may include decreased root-shoot ratio, nutritional deficiencies particularly chlorosis in the foliage, temporary wilting during periods of

mild water stress or during mid-day when temperatures are high though adequate moisture is available, and reduced quality of produce (Hussey, 1985). Such effects on cassava may be at different stages of crop growth and may be most severe at plant establishment as most of the root tissue will be young and undifferentiated (Taylor and Sasser, 1978). Generally, root-knot nematodes infect the feeder roots of cassava causing small galls to from, which later enlarge and coalesce as the female develop within the root tissue and reproduce (Gapasin, 1980). This disrupts the normal translocation of water and nutrients because where gall are formed the vascular elements are broken and deformed (Gapasin, 1980). This deformation and the physical damage caused by the nematodes to the roots also facilitate entry of secondary pathogens.

Meloidogyne spp. have been recorded on cassava from many of the cassava-growing regions of the world, including Brazil (Ponte *et al*., 1980), Venezuela (Crozzoli and Hidalgo, 1992), USA (McSorley *et al*., 1983) Pacific (Bridge, 1988), Mozambique (Van den Oever and Mangane, 1992), Uganda (Bridge *et al*., 1991), Malawi (Saka, 1982), Nigeria (Caveness, 1982b) and Niger (Sikora *et al*., 1988). Coyne and Namaganda (1994) observed physical evidence of root-knot nematodes causing damage to cassava roots on 94% of 88 fields examined in Uganda. Of the roots damaged, 17% were in the severely galled category, indicating that root-knot nematodes are prevalent pests of cassava in Uganda.

From literature, the response of cassava to *Meloidogyne* varies. Diomande (1982) showed that *M. incognita* and *M. javanica* reproduce poorly on particular cassava cultivars. Makumbi-Kidza *et al*., (2000) found that the production loss caused by *M. incognita* to young cassava cultivar SS_4 plants was due to a reduction of storage-root weight. The typical knotting of the feeder and fine filamentous roots occurs and is the most obvious feature of *Meloidogyne* spp. infection (Bridge *et al*., 1991). Such galling damage is common across cassava-growing areas but can vary considerably in the level of galling observed. However, the natural 'knobbly' and rough texture of the feeder roots can disguise nematode damage (Coyne, 1994).

In comparison with the damage reported on roots, less common and rarely documented is nematode damage to the storage roots themselves. In Kenya, severe damage to a small number of cassava germplasm lines (-1%) was observed in a breeder's selection trial (Coyne *et al*., 2004). *M. incognita, M. javanica* and an unidentified *Meloidogyne* spp. were recovered from the cassava tissue. 'Bubbling' of the storage root surface occurred. In some cases, the surface was flaky in appearance

with high levels of necrosis apparent under the surface, when thin sections were cut away, where the nematodes had infected the tissue. Other reports of extensive storage root deformation have been received from Mozambique.

There is little information on the effects of root-knot nematodes on yield. Some substantial yield losses have, however, been demonstrated by Caveness (1982b), who observed a storage root loss of 87% under the heaviest nematode attack. Losses as much as 98% have also been reported in an experimental plot under heavy infestation (Theberge, 1985). Coyne and Talwana (2000) investigated cassava yield under normal growing conditions at different farm sites. Although results were not significant (P=0.05), yields were consistently lower at sites with greater root-knot damage. For the more susceptible cultivars, Bukalasa 11 and TMS 30337, yield reductions of 38 and 24% per plant, respectively, were recorded.

In addition to the direct losses of both quality and quantity of the cassava crop, there is the added effect of reduced stem height and weight associated with high *Meloidogyne* populations (Gapasin, 1980, 1981; Caveness, 1982b). This decreases the quality of the planting materials available in the following season.

McSorley *et al*. (1983) have proposed the resistant or tolerant cultivars as most promising management of *Meloidogyne* spp. on cassava in most parts of the world. Bridge *et al*. (1991) observed that little or no enlargement of storage roots occurred where soils were severely infested with the nematodes, and Talwana *et al*. (1997a) showed that high root-knot nematode densities at planting could lead to a complete failure of the cassava crop to establish.

2.10: Economic losses due to root-knot nematodes

It has been difficult to ascertain the losses caused by root-knot nematodes in developing countries because crops are rarely grown as monocrops and also plant-parasitic nematodes exist in mixed populations and other pathogenic organisms such as fungi, bacteria and viruses are also found on the crops already invaded by nematodes (Adesiyan *et al.*,1990). However, several workers have carried out yield loss studies due to root-knot nematodes in controlled greenhouse and field experiments and these indicated that root-knot nematodes cause considerable yield reduction (Adesiyan *et al.*, 1990). Cases of crop failure were recorded in some crops such as okra, tomato, soyabean and cowpea (Ogbuji and Okonkwo, 1977; Nwauzor, 1979; Babatola and Omotade, 1991). Bridge (1972)

reported a yield loss of 40% in cowpea and Olowe (1976) observed a 25% yield reduction at inoculum level of 133 eggs of *M. incognita* per kg of soil or a 91% reduction with 13,300 eggs per kg of soil in cowpea. A cowpea grain loss of 69% was recorded by Babatola and Omotade (1991). In addition to yield reduction, heavy infestation of cowpea by *M. incognita* led to early senescence (Olowe, 1978). *M. incognita* caused a yield reduction of 60% in rice when inoculated at 8,000 eggs /juveniles per dm^3 of soil at planting (Babatola, 1984). Root-knot nematode damage and grain yield reduction were more severe in upland than in irrigated rice (Fademi, 1984). Rice root-knot nematode, *Meloidogyne graminicola* has attained wide importance due to its potential to cause major damage in rice-wheat cropping system. It has become an emerging problem in the nurseries and upland rice along with its widespread occurrence in the deep water and irrigated rice in the different countries of Southeast Asia (Dutta *et al*., 2012). *Meloidogyne graminicola* is the most common RKN species infecting rice. It is reported to cause 17 – 30% yield loss due to poor filled kernels (MacGowan, 1989; Jain *et al*., 2007) Infection of maize by root-knot nematodes has been reported to cause stunting, yellowing of leaves, patchy growth in the field and small or large terminal root galls (Olowe, 1992). At inoculum level of 2,000 juveniles of *Meloidogyne* spp, maize growth and yield were reduced (Olowe, 1992). In sugarcane, *M. incognita* caused a 20% yield reduction (Salawu, 1985). Other deleterious effects of the infestation include retardation of tillering and proliferation of lateral roots with phloem tissues being mostly affected. *Meloidogyne* spp have been shown to cause a yield reduction of 61% in sorghum (Swarup and Sosa-Moss, 1990). Similarly, it has been proved that *M. incognita* and *M. javanica* cause economic damage to millet (Swarup and Sosa-Moss, 1990).

Meloidogyne spp infection of yam tubers significantly exacerbated yam tuber weight-loss during storage, particularly during the first 2 months, and moreover reduced and delayed tuber sprouting Mudiope *et al*. (2012). There are also reports by many researchers on yield losses on root and tuber crops due to root-knot nematodes invasion. On cassava for example, IITA (1985) and Nwauzor (1990) reported that *Meloidogyne* spp. caused 31-87% loss in top and root yields. Theberge (1985) also reported a yield loss of 90% on the same crop. In Zimbabwe, Caveness (1988) reported a yield reduction of between 35 and 100% in cassava crop planted in *Meloidogyne*-infested soil.

Caveness (1988) also reported that yam seedlings could be totally destroyed by root-knot nematodes. Infected yam tubers are galled, flaky, deformed and have reduced edible portions. In addition, they cause 40% reduction in the market value of the infected

tubers (Nwauzor 1982; Fawole, 1988). Moreover, root-knot nematode infected yam tubers do not store well. They lose weight rapidly and are susceptible to invasion by secondary pathogens like *Aspergillus* and *Penicillium* (Nwauzor and Fawole, 1990). In India, *M. javanica* is said to cause severe losses in cocoyam production (Anon, 1978). Sasser (1979) put the economic losses in the yield of carrots due to root-knot nematode attack in West Africa at about 38%. For sweetpotato, Gapasin and Validez (1979) reported that *M. incognita* could reduce tuber production by as much as 47.7% depending on the nematode population.

Theberge (1985) also reported that yield reduction of 20-30 % or more may occur in sweetpotato depending on the cultivar grown, soil and environment. In the Philippines, losses of about 50% have been recorded but could reach 100% with three continuous croppings in infested fields (Gapasin 1984, 1986). Yield loss of 18% has also been reported in the Southern Highlands of Papua New Guinea (Lenne, 1991). In Ibadan, Nigeria, Fawole and Claudius-Cole (2000) reported the presence of *M. incognita, Rotylenchulus reniformis* and *Pratylenchus brachyurus* in the roots and rhizosphere of sweetpotato in ten locations. In a survey by Bridge and Page (1982) in the Southern Highlands of Papua New Guinea, *M. incognita* was found to be widespread and serious damage was recorded in some areas. In the Western Highlands of Papua New Guinea however, serious root-galling due to *M. incognita* was recorded in one site only. In East Spik, no damage due to *M. incognita* was recorded on sweetpotato. Nematode damage was found to be rare in places where there were no land shortages and long periods of bush fallows were practised. In Burundi (East Africa), one case of devastation of sweetpotato by *Meloidogyne* spp. was observed (Lenne, 1991).

2.11: Nematode Interactions with other Plant Pathogens

Under natural conditions, a plant is a potential host to various microorganisms and they can influence each other by occupying the same habitat. Infection by one pathogen can alter host response to subsequent infection by another. Interaction of different parasites on the same plant results in disease complexes, and these interactions may lead to susceptibility by predisposition or resistance through pre-induction of resistance against a particular parasite (Sidhu and Webster, 1981).

Roots grow in soil containing a great number of microorganisms, whose action is often combined to induce damage. Plant-parasitic nematodes often play a major role

in disease interactions. Interactions involving nematodes is important because they contribute substantially to variability in crop growth (Zadoks and Schein, 1979).

There is little documented evidence that root knot nematodes form associations with other pests or pathogens on cassava. Galling and mechanical damage of roots by nematodes facilitate the entry and development of secondary pathogens, which will probably lead to increase of root necrosis and consequently reduced root weights compared with uninfected plants as observed in some studies (Gapasin 1980, Crozzoli and Hildago, 1992; Talwana *et al*., 1997a, b; Coyne and Talwana 2000).

2.12: Importance of nematode-fungi interactions

Nematode-fungus interactions represent the greatest number of the reported associations between nematodes and other pathogens (Powell 1971). Amongst these, the interaction between root-knot nematodes and the wilt fungi are those that have been extensively studied (Haseeb *et al*., 2005). Many of the early nematode-fungal interactions sprang from breakdown of resistant varieties (Hunger, 1901; Jenkins and Coursen, 1957; Sasser *et al*., 1955). All interactions of plant- parasitic nematodes with other plant pathogens have three components: Nematode, host and other pathogens. The plant pathogens known to interact with nematodes are mainly viruses, bacteria and fungi (Khan, 1993). *Anguina tritici* has been known to vector spores of *Dilophospora alopecari* which attacks aerial parts of cereals (Khan, 1993). The nematode, while moving between the leaf sheaths to reach the growing point, carries the fungal conidia and deposits them on 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, 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 *Colletotrichum* are known to interact with plant parasitic nematodes, the role of

nematodes in root-rot diseases is to assist 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. Furthermore, cyst production was increased when *H. daverti* was inoculated one week after *F. oxysporum* on Clare. Similarly, Saeedizadeh *et al*. (2003) observed that presence of *M. javanica* prior to fungus caused reduction in the roots. Similarly, presence of fungus prior to the nematode caused reduction in number of galls produced by the nematode. Also severe damage was observed above ground when pathogens where inoculated simultaneously on olive seedling. It has been severally reported that infection by root-knot nematodes, *Meloidogyne* spp. increased the severity of *Fusarium* wilt in cotton, tomato and tobacco as expressed by increased death rates and more severe symptoms development (Powell, 1971).

All plant-parasitic nematodes wound roots when feeding and these wounds become sites through which other microorganisms can enter. Certain nematodes, like *Meloidogyne* spp., predispose plants to attack by other pathogens, or make plants susceptible to microorganisms that normally would not parasitize the plant. Such interactions are considered synergistic when the combined effects of two pathogens on the host plant results in more extensive damage than the sum of the effect when both are acting independently (Powell, 1979). Synergistic interactions between nematodes and fungi have been recognized since 1892 when Atkinson (1892) reported that the infection of cotton by the root-knot nematode increased the severity of Fusarium wilt.

The influence of root-knot nematodes is also more effective if the nematode is present on crops two to four weeks before the fungus (Adesiyan *et al*., 1990). *M. incognita* and *M. javanica* also interact with *Phytophtora parasitica* var. *nicotianae* to increase the severity of black shank disease of tobacco (Powell, 1971, Taylor and Sasser, 1978) In the presence of *M. incognita, Pythium* spp. and *Rhizoctonica* spp. infect and cause extensive decay of tobacco roots. This is particularly so if the nematode had been present on the root three to four weeks earlier. *Botrytis*, *Aspergillus* and *Penicillium* are fungi, which ordinarily cannot establish a parasitic relationship on

plants. But when the plant has been disposed by root-knot nematodes, they all cause major diseases (Powell, 1971).

It has been demonstrated that severity of fungal-induced wilt diseases increased greatly when root-knot nematodes were inoculated three to four weeks prior to fungus inoculation of the host in comparison to simultaneous inoculation of both the pathogens (Bergeson, 1972). Multiple interactions are also known to occur. For example, *M. incognita* predisposed tobacco plants to *Fusarium oxysporum, F. nicotianee* wilt. Plants affected by both pathogens are then predisposed to *Alternaria tenuis* to form a destructive complex (Taylor and Sasser, 1978).

Most nematodes interact with other pathogenic organisms e.g. *Botryodiplodia theobromae* in disease complexes. However, information on such interactions on cassava is rather scarce (Jatala and Bridge, 1990). In summarizing root-knot nematode-fungus interactions, therefore, the nematode is important in that it predisposes plants (i) by acting as a wounding agent thereby creating more infection sites for the various fungi, (ii) by changing chemical constituents such that the host is a better and more nutritious growth susbstrate for the fungi.

2.13: Taxonomy and importance of *Botryodiplodia theobromae*

Taxonomically, *Botryodiplodia theobromae* Pat. belongs to the kingdom Fungi, Phylum Ascomycota, Family Botryosphaeriaceae, Genus *Botryodiplodia* (Kunz, 2007). *B. theobromae* grows and sporulates at 10-40^{o}C although optimum temperature for its development is 25-30^{o}C (Alam *et al.*, 2001). The highest mycelial growth is 78-90mm and sporulation is 27-38 conidia/ 0.01ml was observed on potato dextrose agar (Alam *et al.*, 2001). The characteristic feature of ascomycetes is that they sexually produce spores, and the ascospores are contained within a sac (ascus). In most ascomycetes, the ascus contains eight ascospores and is turgid, ejecting its spores by a squirt mechanism. The explosive release of ascospores follows increased turgor pressure, caused by water uptake by the ascus. The fruiting body of an ascus when totally enclosed (in which the ascocarp has no special opening) is termed cleistothecia (Webster and Weber, 2007). *B. theobromae* is a well known parasite causing both field and storage disease of different crops, fruits and plantation trees (Alam *et al.*, 2001). It is an important pathogen of mango, cocoa and other tropical fruits (Alam *et al.*, 2001) and causes black- band disease of jute, crown rot diseases of banana fruit, fruit rot of coconut, stem-end rot of

mango fruit, soft rot of pawpaw, guava and die- back in lemon plants (Alam *et al.*, 2001). Among the diseases of cassava root and stem rots are important in different ecozones of West Africa. Banito *et al*. (2010) isolated a total of 39 fungal strains from diseased root samples of cassava collected from cassava fields. *B. theobromae, Fusarium* sp., *Sclerotium rolfsii* and *Pythium* sp were the fungi isolated from the rotted cassava roots. *B. theobromae* was the most frequently isolated fungus (51.3% of the isolated root rot pathogens) followed by *Fusarium* sp. (33.3% of the isolates), while *S. rolfsii* and *Pythium* sp., were less frequently found. According to Onyeka (2002), *B. theobromae* is predominantly responsible for cassava root rot disease in the humid forest and transition agroecological zones of Nigeria. Rot pathogens such as *B. theobromae* are wide spread in heavy poorly drained soils with high organic matter content (Onyeka, 2002). The pathogens cause root decay whereby the entire plant becomes wilted, defoliates and eventually dies (Onyeka, 2002). *B. theobromae* has been reported in both Africa and tropical America with estimated yield loss of up to 80% in cassava (Onyeka, 2002). Rot caused by *B. theobromae* affects plants at all growth stages causing serious deterioration of the roots. Affected roots are discoloured and decompose rapidly (Onyeka, 2002). In Nigeria, *B. theobromae* was found to be highly virulent on cassava in cassava growing areas of Nigeria (Onyeka, 2002). This showed the relative importance of *B. theobromae* in disease etiology when compared with other rot fungi such as *Trichoderma* sp., *Fusarium solani, Fusarium oxysporum* and *Apergillus niger* on cassava. Furthermore, Onyeka (2002) also reported that *B. theobromae* is a non-specific pathogen with a wide host spectrum and had been previously reported of cassava mini-stems in Nigeria (Osai and Ikotun, 1993). Akinyele and Ikotun (1989) observed soft rot on cassava with characteristic foul odour and dark-blue discolouration of the rotted root tissue was caused by *B. theobromae* on International Institute of Tropical Agriculture (IITA), Ibadan experimental field. Similarly, Boher *et al*. (1997) observed that *B. theobromae* was responsible for large scale damage on roots and stems of cassava in the Danyi plateau zone of South-west Togo. There is no information about the rot fungus *B. theobromae* and *Meloidogyne incognita* on cassava. Oyetunji (2009) observed severe rot on rice root in a pot trial when rice cultivars namely Nerica 1, LAC 23 and OS6 were inoculated with *B. theobromae* alone or in combination with termite.

2.14: Biological Control of Pests

Plant diseases need to be controlled to maintain the quality and abundance of food, feed, and fiber produced by growers around the world and there are different approaches that can be used to prevent, mitigate or control plant diseases (Pal and Mcspadden Gardener, 2006). Beyond good agronomic and horticultural practices, growers often rely heavily on chemical fertilizers and pesticides. Such inputs to agriculture have contributed significantly to the spectacular improvements in crop productivity and quality. However, the environmental pollution caused by excessive use and misuse of agrochemicals, as well as fear-mongering by some opponents of pesticides, has led to considerable changes in people's attitudes towards the use of pesticides in agriculture (Pal and Mcspadden-Gardener, 2006).

The terms "biological control" and its abbreviated synonym "biocontrol" have been used in different fields of biology, most notably entomology and plant pathology. Biological control is considered ecologically friendly and a possible alternative in pest and disease management. In entomology, it has been used to describe the use of live predatory insects, entomopathogenic nematodes, or microbial pathogens to suppress populations of different pest insects. In plant pathology, the term applies to the use of microbial antagonists to suppress diseases as well as the use of host specific pathogens to control weed populations. In both fields, the organism that suppresses the pest or pathogen is referred to as the biological control agent (BCA). Throughout their lifecycle, plants and pathogens interact with a wide variety of organisms. These interactions can significantly affect plant health in various ways. The types of interactions are mutualism, protocooperation, commensalism, neutralism, competition, amensalism, parasitism, and predation.

Biological control is now a key strategy used for controlling pests worldwide (Moosavi and Zare, 2012). It is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be (Eilenberg *et al*., 2001). Four basic strategies can be used in biological control (i) Introduction: which is considered as a classical technique whereby an exotic helpful organism is introduced into a new region and become fully established. This strategy is usually used against introduced pests that have no indigenous antagonists. (ii) Augmentation: in this method laboratory-bred individuals can be released to compensate the inefficiency of present microbial agents. The

inadequate level of control can be driven by low number of native natural enemies. (iii) Inoculation: when an indigenous antagonist is not present or an introduced one cannot survive permanently, an inoculative release is made at the beginning of planting season. This process may need to be repeated for each following crop. (iv) Inundation: in this technique the mass culture of a pathogen is carried out for urgent use at critical periods when rapid suppression of pest population is necessary (Kerry and Hominick, 2002).

Researchers have attempted to characterize the organisms involved in biological control, because plant diseases may be suppressed by the activities of one or more plant associated microbes (Pal and Mcspadden-Gardener, 2006). Historically, this has been done primarily through isolation, characterization, and application of individual organisms. By design, this approach focuses on specific forms of disease suppression. Specific suppression results from the activities of one or just a few microbial antagonists. This type of suppression is thought to be occurring when inoculation of a bio-control agent results in substantial levels of disease suppressiveness. Its occurrence in natural systems may also occur from time to time. For example, the introduction of *Pseudomonas fluorescens* that produces the antibiotic 2, 4-diacetylphloroglucinol can result in the suppression of various soil borne pathogens (Weller *et al*., 2002). However, specific agents must compete with other soil- and root-associated microbes to survive, propagate, and express their antagonistic potential during those times when the targeted pathogens pose an active threat to plant health. In contrast, general suppression is more frequently invoked to explain the reduced incidence or severity of plant diseases because the activities of multiple organisms can contribute to a reduction in disease pressure. High soil organic matter supports a large and diverse mass of microbes resulting in the availability of fewer ecological niches for which a pathogen competes.
The extent of general suppression will vary substantially depending on the quantity and quality of organic matter present in a soil (Hoitink and Boehm, 1999). Functional redundancy within different microbial communities allows for rapid depletion of the available soil nutrient pool under a large variety of conditions, before the pathogens can utilize them to proliferate and cause disease. For example, diverse seed-colonizing bacteria can consume nutrients that are released into the soil during germination thereby suppressing pathogen germination and growth (McKellar and Nelson, 2003). Manipulation of agricultural systems, through additions of composts, green manures and cover crops is aimed at improving endogenous levels of general suppression. Most pathogens will be susceptible to one or more biocontrol strategies, but practical

implementation on a commercial scale has been constrained by a number of factors. Cost, convenience, efficacy, and reliability of biological controls are important considerations, but only in relation to the alternative disease control strategies. Cultural practices (e.g. good sanitation, soil preparation, and water management) and host resistance can go a long way towards controlling many diseases, so bio-control should be applied only when such agronomic practices are insufficient for effective disease control. In general, though, regulatory and cultural concerns about the health and safety of specific classes of pesticides are the primary economic drivers promoting the adoption of biological control strategies in urban and rural landscapes. Self-perpetuating biological controls (e.g. hypovirulence of the chestnut blight pathogen) are also needed for control of diseases in forested and rangeland ecosystems where high application rates over larger land areas are not economically-feasible (Pal and Mcspadden-Gardener, 2006).

The advantages and limitations of biological control are often expressed by comparisons with pesticides. Thus, predators and parasitoids are naturally occurring organisms and usually fairly specific in the range of prey that they will attack. Natural enemies actively seek out their prey and can increase the level of control over time. It is unlikely that resistance will develop to a control agent, and in many cases, the control can be self-perpetuating over long periods of time

The main limitation of biological control is that it is slower to suppress pest populations than most pesticides as parasitized organisms may take several days to die; and also, predators require a period of time to establish an economic level of pest suppression. Development costs of biological control are sometimes described as ‘high’, but these costs are much lower than for the equivalent synthesis, toxicological evaluation and marketing of a new pesticide, and substantial profits can be achieved from biocontrol with long term, effective natural enemies, The comparative advantages of pesticides are that control is rapid, and in the absence of resistance, a high and predictable level of mortality is normally guaranteed.

There are other reported limitations of biological control where pesticides seem to be the more advantageous approach, but the evidence for this conclusion is limited or absent (van Lenteren 1993*a*,*b*). For instance, the fact that biological control ‘regulates’ whereas pesticides ‘eradicate’ is viewed as disadvantageous, but this is a spurious argument. In biological control, pest populations are usually not eradicated, but maintained at very low densities; long-term suppression of pest species is the desirable

aim. Eradication of a pest population after chemical treatment, even if this is achieved, occurs only on local scales, and the environment is then open to re-invasion, often with a much reduced natural enemy fauna.

Biological control has been described as 'unreliable' when compared with pesticides, but again the information for this view is equivocal. Some biological control schemes have been 'partial' rather than 'complete' successes, and in some instances, there have been periodic fluctuations in the level of control (Bellows & Fisher, 1999). But as a counter argument, pesticides also vary in their effectiveness and over time, the development of resistance can lead to the failure of a previously successful chemical.

The introduction of a biological control scheme against a pest, and the resultant withdrawal of broad-spectrum pesticides, can lead to new pest problems, but there is no evidence for this in several well-studied agro-ecosystems, including glasshouse systems. Research on biological control was undertaken from 1965 to 1975 to combat glasshouse pests (*Phytoseilus persimilis* and *Encarsia formosa*) that had become resistant to the then available pesticides (DDT, malathion and resmethrin), but the wide-scale introduction of natural enemies against these two species did not by itself lead to the occurrence of any new pests. The pest problems that have arisen in the European glasshouse industry since 1975 (*Spodoptera exigua*, *Liriomyza trifolii*, *Liriomyza huidobrensis*, *F. occidentalis* and *Bemisia tabaci*) have all been unintentional introductions that arrived with varying levels of resistance to most pesticides. These newly imported pests have threatened the biological control of existing pests because governments usually respond to pest invasions by initiating extermination programmes based on frequent application of pesticides, thereby killing the natural enemies of the 'old' pests. Therefore, it is crucial to identify effective natural enemies of new pests before or soon after invasion, in order to maintain stability in the commercial biological control of existing pests. Pest control must be cost effective relative to the value of the crop.

Despite this advantage, the main reason that biological control is not used on a larger scale is attributable to problems associated with the production and distribution of parasitoids and predators; particularly, the limited shelf life (days or at most weeks) of most natural enemy species and the impossibility of patenting a naturally occurring, unmodified species.

In terms of efficacy and reliability, the greatest successes in biological control have been achieved in situations where environmental conditions are most controlled or

predictable and where biocontrol agents can preemptively colonize the infection court. Monocyclic, soilborne and postharvest diseases have been controlled effectively by biological control agents that act as bioprotectants (i.e. preventing infections). Specific applications for high value crops targeting specific diseases (e.g. fireblight, downy mildew, and several nematode diseases) have also been adopted. As research unravels the various conditions needed for successful biocontrol of different diseases, the adoption of BCAs in IPM systems is bound to increase (Pal and Mcspadden-Gardener, 2006). Several organisms are known to be antagonistic against plant parasitic nematodes. Fungal biological control is an exciting and rapidly developing research area and there is growing attention in the exploitation of fungi for the control of nematodes. In recent decades, concerns about the environmental hazards of using chemical nematicides and limited alternative crops for rotation have led to the development of biological control agents as a component of crop protection (Moosavi and Zare, 2012). Biological control agents have an important effect in the regulation of plant parasitic nematode populations, and numerous organisms including fungi, bacteria, viruses, nematodes and other invertebrates have antagonistic activity against plant parasitic nematodes (Stirling, 1991). The developmental process of progressing biological agents include the isolation and identification of microbial agents associated with plant parasitic nematodes especially in suppressive soils (a soil that completely suppresses nematode reproduction); examination of their potential ability in controlling nematodes; changing the soil environment in favor of antagonistic agents; understanding the mechanisms of parasitism and pathogenicity; and development of commercial product.

2.15: Biological Control of Nematodes

Environmental and health concerns over the use of chemical pesticides have increased the need for alternative measures in the control of plant-parasitic nematodes. Nematodes are attacked, under natural conditions in the soil, by a wide variety of organisms, such as predators and parasites. Predators include fungi, nematodes, insects and mites. Parasites include viruses, protozoa, bacteria and fungi. Several reports attest to the dramatic increase of research efforts towards biological control of plant parasitic nematodes (Sayre and Starr, 1985; Jatala, 1986, Galper *et al*., 1991; Usman and Siddiqui, 2012). The association results in a biological balance manifested by

attachment and penetration by one or more pathogenic microorganisms in the eggs, juveniles and adult nematodes, causing their death (Walsh *et al.,* 1983). Several control strategies combining non-chemical and chemical methods have been developed to reduce damage caused by nematodes. As a result of the danger associated with the use of nematicides, efforts have been made to study the effects of some fungi as nematode bio-control agents. Some fungi have shown great potential as biocontrol agents among microorganisms that parasitise or prey on nematodes (Siddiqui and Mahmood, 1999; Moozavi and Zare, 2012). Fungi continuously destroy nematodes in virtually all soils because of their constant association with nematodes in the rhizosphere. A large number of fungi are known to trap or prey on nematodes but the most important genera include *Paecilomyces, Verticillium, Hirsutella, Nematophthora, Arthrobotrys, Drechmeria, Fusarium* and *Monacrosporium*. Application of some of these fungi has given very interesting results. Vesicular Arbuscular Mycorrhizal fungi (VAM), *Trichodema* spp. *and Paecilomyces* spp. have been suspected to be able to suppress nematode populations in some other crops (Stirling, 1991; Inbar *et al.*, 1994; Elsen, 2002; Kannan and Veeravel, 2012), but work on them on cassava root-knot nematode interaction is lacking.

Several studies have addressed the interactions between VAM fungi and nematodes. (Bagyaraj 1984; Hemavathi *et al*., 2011; Robab *et al*., 2012; Shuxia *et al*., 2012) In most cases, mycorrhizae reduce the severity of the disease caused by the plant-parasitic nematodes (Bagyaraj, 1984). According to Saleh and Sikora 1984, many mycorrhizal associations are reported to have a suppressive effect on sedentary endoparasitic nematodes. In some crops, this effect is significant enough to consider mycorrhizal colonization as a more or less effective means of biological control.

Vesicular-arbuscular mycorrhizae may limit nematode activity and improve plant growth and because the fungus is ubiquitous in agricultural soils, it has been suggested that it may represent a possible alternative to standard management tactics for nematode parasites of field crops (Hussey and Roncadori, 1982). AMF has been shown to reduce the level of damage caused by root-knot nematodes on plant root systems (Caron, 1989; Jaizme-Vega *et al.* 1997). *Paecilomyces lilacinus* has been developed as a microbial bio-control agent against plant-parasitic nematodes, in particular *Meloidogyne* spp. and this fungus will only cause a short-term disturbance to the soil biota and will not have any long-term effects. The fungus has shown potential as a biological control

agent for sedentary plant parasitic nematodes (Jatala, 1986; Kiewnick, 2004; Krishnamoorthi and Kumar, 2007; Khalil *et al*., 2012). This fungus is an egg parasite of sedentary nematode species; eggs of these nematodes are found either in egg masses or cysts and thus are more vulnerable to fungal attack (Jatala, 1986). *Paecilomyces lilacinus* has been effective in controlling species of *Meloidogyne* (Jatala *et al*., 1980), *Tylenchulus* (Jatala, 1986), *Globodera* (Davide and Zorilla; 1983; Jatala *et al.*, 1985; Oduor-owino, 2003).

2.15.1: Arbuscular Mycorrhizal Fungi

A main component of the soil microbiota in agroecosystems is the arbuscular mycorrhizal fungi (AMF). Mycorrhizal fungi are mutualistic symbionts that colonize the roots of the vast majority of plants, including most crop plants (Smith and Read, 1997; Oehl *et al;* 2003). Mycorrhiza can be ectotrophic or endotrophic, living outside our inside the root cortex respectively. The term mycorhiza simply means "fungus root" and was first introduced by a German Botanist-Albert Bernard Frank in 1885, to describe the association of two different organisms (Powell and Bagyaraji, 1984; Sieverding, 1991). It was reported that more than 85% of tropical plant species are mycorrhizal and that colonization of arbuscular mycorrhizal fungi (AMF) is about 70%, of which maize is one (Trappe, 1989). Mycorrhiza fungi represent an important group because they have a wide distribution; may contribute significantly to microbial biomass and to soil nutrient cycling processes in plants (Harley and Smith, 1983). They improve on nutrient uptake, especially phosphorus, and also micronutrients such as zinc or copper, they stimulate growth substances and may reduce stresses, diseases or pest attack (Smith and Read, 1997).

Furthermore, mycorrhza may decrease or increase nematode penetration, development and reproduction. If nematode reproduction is decreased, the plant and its fungal symbiont can be described as having induced resistance or it can be described as a poorer host to the parasitic nematode than non-mycorrhizal plant. Mycorrhizal fungi also are capable of directly interacting with sedentary states of plant-parasitic nematodes. The reaction of plant growth to these interactions may be positive, negative or neutral. Yield, therefore, is dependent dynamically upon how much damage the nematode is doing and how much benefit the plant is deriving from the fungus. If

growth is enhanced, the plant (again with its fungal symbiont) can be described as more tolerant to the nematode (Hussey and Rocandori, 1982; Smith 1987).

For an appropriate use of this technology, it is necessary to select appropriate inocula adapted to specific limiting environmental factors for crop productivity. Oxisol / Utisol are acidic soils, low in nutrient availability; low in available P which is generally less than 5-10 ppm. They have high aluminium concentration which is highly toxic and may inhibit fungal spore germination and symbiosis, root growth, plant development and yield. Maize forms effective symbiotic association with indigenous arbuscular mycorrhizal fungi. The arbuscular mycorrhizal fungus *Glomus* spp. have been severally reported to increase growth and yield of many plants as a result of enhanced nutrient status especially phosphorus (Rhodes and Gerdermann, 1975; Gianinazzi and Gianinazzi 1983; Huang *et al.*, 1985, Simpson and Daft 1990, Osonubi *et al.*, 1991). Also G. *mosseae* improves water status and transport (Allen and Boosalis 1983; Levey *et al.*, 1983, Osonubi, 1989, Ahmed *et al*., 2009).

Study by Roa *et al.*, (1995) using split root technique indicated that reduced nematode infection on mycorrhizal plants only occurred if the two were together on the same root. Root infections by pathogenic nematodes are generally less on VAM plants than on non-mycorrhizal plants, but the response may vary at times (Sikora, 1979; Roa *et al.*, 1995). AMF can also enhance tolerance of or resistance to root pathogens (Borowicz, 2001) or abiotic stresses, such as drought and metal toxicity (Meharg and Cairney, 2000).

Robab *et al.* (2012) studied the effects of root knot disease on *Solanum nigrum*, in presence or absence of AM fungus (*G. mosseae*). The data revealed that plant height, plant weight, number of flowers, number of fruits and amount of chlorophyll a and b decreased significantly in plants inoculated with nematode alone compared with plants inoculated with nematode and *G. mosseae*. Hemavathi *et al.* (2011) also found *G. fasciculatum* at 200 spores/g of soil to be effective in improving plant growth parameters and reducing soil nematode population by 84 percent with less number of galls per plant. An experiment conducted by ShuXia *et al*. (2012) to study the effects of interaction between fungus Arbuscular mycorrhiza (AM) and root-knot nematode *Meloidogyne incognita* on the growth and physiological characteristics of cucumber showed that fresh and dry weight of fruits increased significantly in the treatment of pre inoculating AM fungi and then inoculating root-knot nematode compared with the treatment of inoculating root-knot nematode only.

Furthermore, AMF plays a vital role in the formation of stable soil aggregates. This is possible through the secretion of glomalin, a glycoprotein having glue-like properties, secreted by AMF. Glomalin activity may lead to better plant production (Wright and Upadhyaya, 1996; 1999).

2.15.2: *Paecilomyces* spp.

Paecilomyces lilacinus (Thom) Samson (Eurotiales: Trichocomaceae) is one of the most widely tested soil Hyphomycetes for the biological control of plant-parasitic nematodes (Atkins *et al*., 2005). They are facultative parasites. They parasitize eggs of *M. incognita* and could be used in the management of some nematodes (Benjamin and Grover, 1987; Stirling, 2001, Sharma and Trivedi 2012). *Paecilomyces* colonies have more or less well developed, colourless, simple or branched conidiophores bearing two to several phialides. The phialides are characteristically swollen at the base and gradually narrowed into a long beak. The spores (conidia), produced in chains from the tips of the phialides, are colourless or brightly pigmented, and are usually rather narrow (Bissett, 1979; Samson, 1974). Many soil-borne fungi are known to produce secondary metabolites of the various soil-inhabiting fungi.

In most species colonies are fast growing, powdery, gold, green-gold, yellow-brown, lilac or tan, but never green or blue-green as in *Penicillium*. Phialides are swollen at their bases, gradually tapering into a rather long and slender neck, and occur solitarily, in pairs, as verticils, and in penicillate heads. Long, dry chains of single-celled, hyaline to dark, smooth or rough, ovoid to fusoid conidia are produced in basipetal succession from the phialides. The genus *Paecilomyces* may be distinguished from the closely related genus *Penicillium* by its having long slender divergent phialides and colonies that are never typically green.

Samson (1974) provides a modern treatment of the genus based on morphology. *Paecilomyces* differs from *Penicillium* in several aspects: (1) *Penicillium* are less well defined; (2) conidia are rarely of the symmetrical, spherical to ellipsoidal shape characteristic of *Penicillium*; (3) conidial mass is bright coloured and rarely green or never blue; (4) phialides are longer than those of *Penicillium*.

The availability of molecular phylogenetic techniques now provides the opportunity to combine morphology and sequences to more fully resolve differences at the species level. With few plastic morphological features available for study, confident identification of many *Paecilomyces* spp. has been problematic. The opportunity was

taken to sequence many of the isolates used in the Samson Monograph (SM) to form a basis for future studies. In Thailand, invertebrate-pathogenic *Paecilomyces* have been collected and isolated over a period. Eight species are currently recognised from natural forest in Thailand. *Paecilomyces tenuipes, P. javanicus and P. cinnamomeus* were the most commonly collected and isolated species while *P. lilacinus and P. amoeneroseus* were rarely encountered. Notably, *P. fumosoroseus* (which was commonly reported from agro-ecosystems) ranked with *P. cicadae* as the rarest encountered in the natural forest. A further 51 isolates were labelled as *Paecilomyces* sp. Many of these are expected to be either *P. farinosus or P. javanicus* but morphological and colony characters were insufficient to make confident identifications. Lara *et al.* (1996) demonstrated that *P. lilacinus* significantly reduced *M. incognita* soil and root populations and increased yield of tomato. Reduction of *M. javanica* infection on tomato by *P. lilacinus* has been reported (Siddiqui *et al*., 2000). Cannayane and Sivakumar (2001) reviewed the biocontrol efficacy of *P. lilacinus* and listed several reports where root-knot nematodes and the potato cyst nematode *Globodera rostochiensis* (Wollenweber) Behrens (Tylenchida: Heteroderidae) were successfully controlled by this egg-pathogenic fungus. However, biocontrol efficacy under glasshouse and field conditions was often inconsistent (Kerry and Evans, 1996). In the past, *P. lilacinus* was applied to soil using various organic materials such as oil cakes, leaf residues, wheat bran and gram seeds as carrier (Cannayane and Sivakumar, 2001 and Siddiqui and Mahmood, 1996). The effects of using these materials were often difficult to reproduce because changes in the substrate used for the production of fungal propagules can significantly affect their biocontrol efficacy (Jenkins *et al*., 1998). Moreover, the amounts of product needed to achieve sufficient control reached up to 3 tons of bulky preparations per ha (Siddiqui and Mahmood, 1996). Usman and Siddiqui (2012) conducted a glass house experiment to control root-knot nematode, *M. incognita* affecting egg plant with two biocontrol fungal strains of *Trichoderma harzianum* and *Paecilomyces lilacinus.* Both fungi were found to be effective in the control of root-knot nematode and enhanced all plant growth characters.

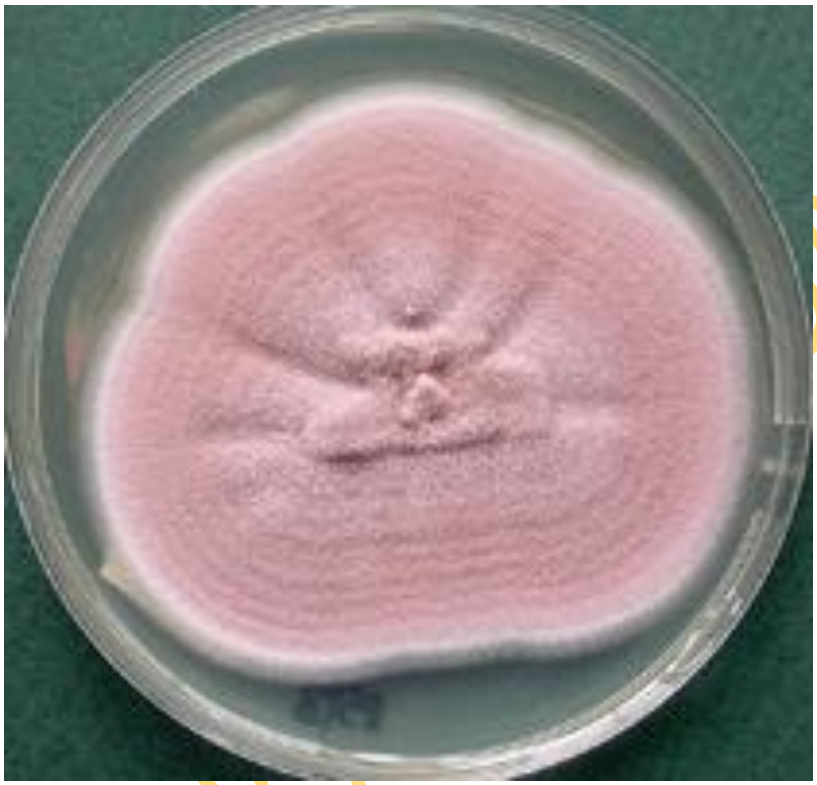


Plate 2.2: Petri dish with *Paecilomyces lilacinus*

Kiewnick and Sikora (2006) evaluated the potential of *Paecilomyces lilacinus* strain 251 (PL 251), for its potential to control the root-knot nematodes *M. incognita* on tomato. Root galling was reduced by 66%, number of egg masses by 74% and the final nematode population in the roots was increased by 71% compared to the uninoculated control.

In India, seven isolates of *P. lilacinus* were obtained from the rhizosphere of nematode infested vegetable fields. Culture filtrates of all seven isolates of *P. lilacinus* showed toxic effect against *M. incognita* of varying degrees. Percentage mortality and hatching inhibition of *M. incognita* were directly proportional to the concentration of culture filtrates and exposure period to each filtrate. (Kumar *et al*., 2008). Castillo *et al*., (2013) found cotton yields from the application of *P. lilacinus* 251 to be similar to those from aldicarb, the chemical nematicide standard in the control of *Rotylenchulus reniformis.*

2.16: Effects of Carbofuran on Plant-parasitic nematodes

The use of nematicides is the most effective and rapid method for combating plant-parasitic nematodes but the main disadvantage is that they are very costly for resource-poor African farmers (Adesiyan *et al*., 1990). Furthermore, they are harzardous to users as well as non-target organisms. Nematicides can be categorised into two groups namely: the fumigants and the non-fumigants. The fumigants are halogenated hydrocarbons with very low vapour pressure. Examples include 1.3-dichloropropene (1,3-D or Telone), 1,3-dichloropropene-1,2 dichloropropane (DD), ethylene dibromide (EDB) and 1,2 dibromo-3-chloropropane (DBCP or Nemagon) (Adesiyan *et al*., 1990). However, DBCP has been withdrawn from the market because it causes sterility in man. Non-fumigants are non-volatile nematicides which include carbofuran (Furadan), Aldicarb (Temik), Fenamiphos (Nemacur) and Oxamyl (Vydate) (Adesiyan *et al*., 1990). Carbofuran (2,3-dihydro-2, 2-dimethylbenofuran-7-ylmethylcarbamate) is a broad spectrum carbamate that kills insects, mites and nematodes on contact or after ingestion. It is available in liquid and granular formulations (Baron, 1991). Akinlade and Adesiyan (1982) tested the efficacy of carbofuran (Furadan) in controlling *M. incognita* on okra. The yields of treated plants were superior to the untreated. Adesiyan

and Badra (1982) evaluated four granular nematicides including carbofuran for controlling the yam nematode, *Scutellonema bradys*, on guinea yam in the field. Tuber yield was increased (90.6%) by carbofuran treatment over untreated plots.

Adesiyan and Badra (1985) evaluated the toxicity of three systemic nematicides, with or without extra ammoniacal fertilization, against root-knot nematodes attacking tomato. These nematicides were Aldicarb, carbofuran and miral. The galling indices showed that all treatments were superior to the control. Babatola (1988) reported reduction in the populations of *Helicotylenchus multicinctus* and *Meloidogyne incognita* in plantain with the application of Furadan 3G and Vydate 10G. Yields in terms of mature arms and fingers and total bunch weight per stand were significantly higher than the control. However, plantain crop treated with Furadan 3G out-yielded those treated with Vydate 10G. Fatoki (2001) found that carbofuran-treated cowpea plants had the least galling compared with other treatments including cassava peels, neem (bark and leaves), Gliricidia (leaves and roots), Siam weed (leaves and roots). Similarly, the least nematode population was recorded in carbofuran-treated soil. Tanimola (2008) compared the nematicidal potentials of carbofuran (2kg a.i/ha) and poultry manure (2t/ha) on *M. incognita*-infected cowpea. He reported that plants treated with carbofuran had the best growth, highest yield and lowest nematode population. Similar reports exist for pineapple (Daramola, 2012) and cocoa (Orisajo *et al*., 2008).

However, the increasing environmental and health concerns over the use of chemical pesticides will continue to make the use of biological control of nematodes a welcome development.

CHAPTER THREE

MATERIALS AND METHODS

3.1: Experimental Site

All experiments were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria ($7^{o}3'$N, $3^{o}5'$E).

3.2: Sources of Cassava Cultivars

Stem cuttings of TMS 326, 4(2)1425, TME 1 and TMS 30572 cultivars were collected from IITA Pathology Unit while stem cuttings of local cultivar OFEGE were collected from a farmer's field at Odo Aye, a suburb of Ijebu-Ode, Ogun State, Nigeria as the cultivar is commonly grown in the locality.

3.3: Nematode inoculum extraction and population estimation procedures

Eggs of the root-knot nematode *Meloidogyne incognita*, used as inoculum in this study were extracted from the roots of *Celosia argentea* (Linn.) that were infected by the nematode using the method of Hussey and Barker (1973) as follows:

a) *Meloidogyne incognita*-infected *C. argentea* roots were collected from the National Horticultural Research Institute, Ibadan and washed to remove adhering soil particles and then cut into 1-2cm segments.

b) The root pieces were vigorously shaken in 0.5% sodium hypochlorite (NaOCl) solution (100ml JIK +600ml water) for four (4) minutes.

c) The NaOCl solution was quickly passed through a 200-mesh sieve nested over a 500-mesh sieve to collect the freed eggs.

d) The 500-mesh sieve with the eggs was then quickly placed under a stream of cold water to remove residual NaOCl, and collected in a beaker.

e) The egg suspension in the beaker was thoroughly mixed using a magnetic stirrer. One ml of the homogenized *M. incognita* suspension was taken during the process

of stiring with the aid of a glass pipette into a counting slide and the nematode eggs counted under the dissecting microscope at x 100 magnification. The average of three counts was taken to estimate the egg population per ml of egg suspension.

3.4: Extraction of nematodes from soil samples using extraction tray method

Nematode extraction from soil was done using the modified Baermann funnel (pie-pan) method (Whitehead and Hemming, 1965). Sub-samples of 100 cm^3 of soil were measured and placed in the extraction trays. The extraction sample of 100 cm^3 was placed in a plastic sieve that was lined on the inside with a double-ply facial tissue. These were then placed on a plastic tray to which water of 250 ml was added and left for 48 hours. The extracts were then poured into a beaker and allowed to settle overnight. Excess water was siphoned off leaving about 20 mls of nematode suspension for counting. Counting was in number per ml and number per 100cm^3 of soil.

3.5: Soil Sterilization

Sandy-loam top soil collected from the west bank area of the International Institute of Tropical Agriculture (IITA), Ibadan was steam-sterilized for two hours thirty minutes at 90^oC at the Nematology Laboratory, IITA, Ibadan. This was done with the aid of the sterilizing machine - Terra Force® (Horticultural Engineers, Maidstone, New York, U.S.A). The sterilized soil was then collected in a large tarpaulin sheet laid on the ground and filled into pots when sufficiently cool. Soil was kept in the glasshouse and covered with tarpaulin until needed. The 40-litre capacity black polythene bags used as pots in this study were purchased from a local market. Holes were made in the bags using a perforator to allow for drainage.

3.6: Counting of Nematodes

With the aid of a counting slide, the number of nematodes in each extract was estimated. The counting slide is made of Perspex and is 4x8 cm with a well that is 0.5 cm deep and has vertical grid lines. The capacity of the counting slide is 2 ml. After the nematode suspension was homogenized with a magnetic stirrer, 1-ml was taken out with a 1-ml capacity Eppenoff® pipette and let out into the counting slide to which 1ml of water was added. Counting was done under a compound microscope at a magnification of 10 x 15 using a hand tally counter. Counts were expressed as number of nematodes

per millilitre and counting was done three times. Estimating the total number of nematodes in the suspension was done, thus:

Total no. of nematodes = No of nematodes per ml x Total volume of suspension

$$\text{No. of nematode in 1g of root} = \frac{\text{No. of nematodes in Extract}}{\text{Wt. of extracted root material}}$$

3.7: Preparation of culture media

Thirty nine grams (39 g) of Potato Dextrose Agar (PDA) was measured into a one litre conical flask and 200 ml of distilled water was added into the conical flask in order to dissolve the PDA. Distilled water was added into the conical flask to make up the PDA solution to 1 litre. The mixture was autoclaved for 15 minutes at a temperature of 121^0C. The prepared agar was allowed to cool to a temperature of about 40^0C and poured into sterile Petri dishes and stored in the refrigerator at the temperature of 4^0C until it was required.

3.8: Isolation of *Botryodiplodia theobromae* from cassava tubers

This activity was carried out in a sterile condition under a lamina flow chamber hand-swabbed with 75% alcohol. *Botryodiplodia theobromae*-infected Ofege cassava root collected from a farmer's field in Odo-Aye in Ogun State was surface-sterilized by washing each root sample in potable running tap water and sections of ~2 mm² were cut from the tissue, using a sterile scalpel, at the interface between healthy and infected portions of the tuber. The pieces of tissue were surface-sterilized with 1% sodium hypochlorite for 2 min, and rinsed in five changes of sterile distilled water. The pieces were tapped dry with a sterile paper towel. Five sections of this root were plated out on PDA. The inoculated Petri dishes were sealed with parafilm to prevent contamination and then incubated in a Gallenkamp incubator at 28 ± 2^oC for 48 hours. Thereafter; the Petri dishes were incubated at 28.1^0C for 48 hours. The different fungi in the Petri dishes were then sub-cultured into newly prepared culture media (PDA) and placed in the incubator for another 48 hours. The isolated subcultures were taken to Pathology Laboratory, IITA, Ibadan for identification with the aid of a compound microscope and fungi identification key. The pure cultures of the isolated *B. theobromae* were then

preserved in a refrigerator set at 4^0C until they were required for future experiments. Plate 3.1 shows the pure culture of *B. theobromae* isolated from the cassava tubers.

3.9: Estimation of *Botryodiplodia theobromae* Spores

Ten milliliters (10 ml) of sterile distilled water was added to 2-week old *B. theobromae* mycelia on agar in the petri-dish and washed into a sterile 500 ml beaker. A volume of 0.2 ml of the fungus suspension was taken after thorough mixing with an Eppendorf pipette and placed into a haemocytometer slide for counting. This was done five times and the average count per milliliter was determined at the pathology laboratory, IITA, Ibadan.

3.10: Isolation and multiplication of *Glomus mosseae*

Arbuscular mycorrhizal fungi (AMF) spores were extracted from a 250 ml core of soil sample collected from maize field (IITA research field, ES 4 Ibadan) using sucrose flotation method (Walker, 1991). The weighed freshly collected soil sample was passed through 2.3 mm mesh Endecotts sieve. The 250 ml soil was poured into a 1000 ml beaker.

Tap water was introduced into the soil in a 1000 ml beaker and wet sieving of soil sample was done through 1000 µm, 500 µm and 32 µm aperture size sieves (Endecotts). *Glomus mosseae* spores were collected on the 32 µm seive and transferred into centrifuge tubes. The centrifuging was done at 1800 revolutions per minute (rpm) for five minutes and the supernatant discarded. The pellets were re-suspended in sucrose solution (440 g/l) and recentifuged at 2000 rpm for two minutes. The supernatant containing the mycorrhizal spores were rapidly sieved and washed on the 32 µm sieve to replace sucrose and reduce osmotic stress on spores. The spores were then counted on a grid-lined minipore petri dish, under a dissecting microscope and were picked out with the aid of fine tip forcep and multiplied in equal volumes of sterile garden soil and sea sand on which Oba Super 1 genotype maize was planted and allowed to grow for 3 months to multiply *G. mosseae* for spore production. The pots were irrigated daily with tap water. The spores were taken to the Soil Microbiology Laboratory, IITA, Ibadan and identified with the aid of a compound microscope and fungi identification key.

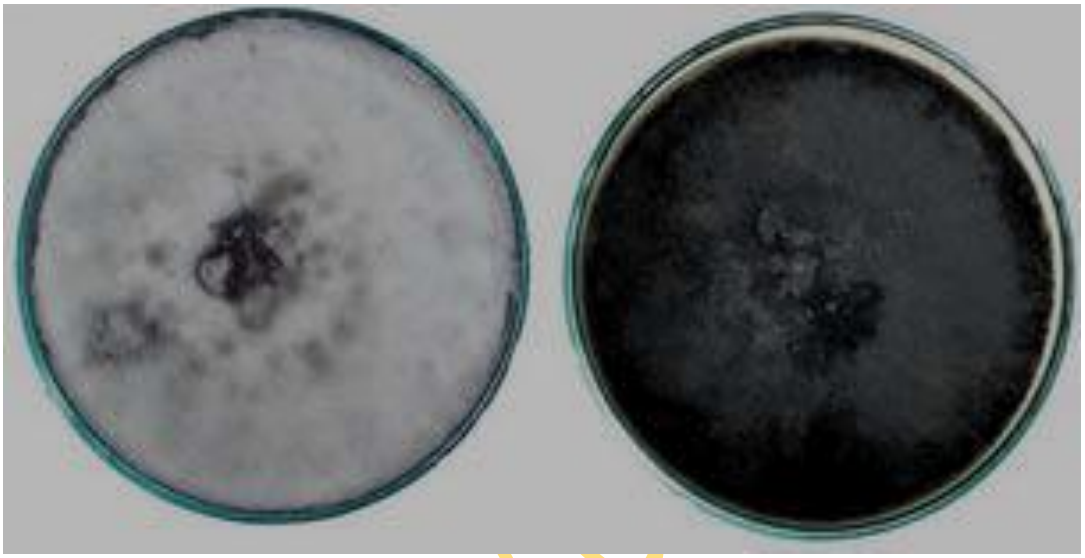


Plate 3.1: Petri-dishes with *Botryodiplodia theobromae* after seven days (left) and after four weeks (right)

3.11: Source of *Paecilomyces lilacinus*

A culture of *Paecilomyces lilacinus was* obtained from the pathology laboratory of IITA, Ibadan, and grown on standard potato dextrose agar (PDA) medium, and was later sub-cultured into newly prepared PDA culture media and placed in the incubator for another 48 hours. The pure cultures of the isolated *P. lilacinus* were then preserved in a refrigerator set at 4^0C until they were required for future experiments.

At the time of usage, 10 ml of sterile distilled water was added to 15 day-old culture on agar in the petri-dish and washed into a sterile 500 ml beaker. A volume of 0.2 ml of the fungus suspension was taken after thorough mixing with an Eppendorf pipette and placed into a haemocyctometer slide for counting. This was done five times and the average count per milliliter was determined at the pathology laboratory, IITA, Ibadan.

3.12: Inoculation of Plants

On the field, plants were inoculated with chopped galled roots of *Celosia argentea* while inoculation in pot experiments was done with RKN egg suspension using a syringe to place the nematode as close to the roots as possible. Nematode egg suspension was obtained as earlier stated (Section 3.3). For field experiments, the galled roots of *C. argentea* were cut into 2-cm pieces and thoroughly mixed. Nematode eggs from roots weighing 5 g each were extracted with sodium hypochlorite as earlier stated. The number of nematodes from each suspension was counted; the mean number of nematode eggs per gram was then calculated and used to estimate the root weight that would contain the required number of nematodes needed for inoculation. Chopped galled roots were used to inoculate field experiments after the number of nematode eggs per gram of root was estimated. Ten ml of sterile distilled water was added to 2-week old *B. theobromae* mycelia on agar in the Petri-dish and washed into a 500ml sterile beaker. The suspension was thoroughly mixed and an Eppendorf pipette was used to measure out 0.2 ml which was placed into a haemocytometer slide for counting. This was done five times and the average count per ml was determined at the Pathology Laboratory, IITA, Ibadan.

Inoculation of *Botryodiplodia theobromae* spores was done by exposing the roots and dispensing the inoculum on the roots with the aid of an Eppendorf pipette. The roots were covered up with sterilized soil inside the pots immediately after inoculation.

However, the exposed roots of the control plants were inoculated with distilled water and the roots were immediately covered with sterilized soil inside the pots and micro-plots. This method was also used in the inoculation of *Paecilomyces lilacinus* while *Glomus mosseae* spores were incorporated into pots by spreading 60 g of soil spore mixture into the centre of the pot at a depth of 3 cm prior to planting. This was also done on the field.

3.13: Soil Sampling

Soil sampling was done before and after planting in field experiments. Samples were taken from plots marked out for each nematode treatment. Random samples were taken from each row with an auger. The samples were placed into polythene sample bags and bulked for each treatment plot. This was properly mixed and 250 ml sub-samples were taken for extraction using the modified Baermann technique, the pie pan method. Sterilized soil was used for pot and microplot experiments.

3.14: Data analyses

Data was processed using Microsoft Excel and Analysis of Variance (ANOVA) was done using SAS 9.1 (2002) statistical package. Means were separated using Least Significant Difference (LSD) at 5 % level of probability.

3.15: Experiment One: Pathogenicity of *Meloidogyne incognita* on cassava (*Manihot esculenta*).

3.15.1: Pot Experiment

Five cultivars of cassava: TMS 30572, TME 1, 4(2) 1425, TMS 326, and Ofege, were used for this study. Five-node cassava stem cuttings of each cultivar were planted singly in polyethylene bags containing 20 litres of steam-sterilized sandy loam soil.

Two weeks after the establishment of cassava seedlings, they were inoculated at three inoculum densities: 0, 1,000 or 10,000 eggs of *Meloidogyne incognita*. This was accomplished by pipeting the egg suspension into four holes, each about 4cm deep, made at the base of the plants. Ordinary water was introduced into the holes for 0 eggs. The *M. incognita* eggs (inoculum) used were extracted from roots of three-month old *M.*

incognita-galled *Celosia argentea* using 0.5% sodium hypochlorite method (Hussey and Barker, 1973) as described in Section 3.3. The eggs were diluted to about 1,000 eggs/ml of suspension. After inoculation, the holes were covered with sterilized sandy-loam soil. Each treatment was replicated four times for each cultivar. The pot experiment was a 5 x 3 factorial (five cultivars of cassava and three levels of nematode inoculum) arranged in four randomized complete blocks in the screen house of the Nematology Unit of the International Institute of Tropical Agriculture (IITA), Ibadan.

The plants were watered daily throughout the period of study. At harvest; 12 months after planting, data were collected on plant height, plant girth, and shoot weight. The root mass from each pot was separated from the soil by gently washing with a jet of tap water and thereafter observed for nematode damage using gall index rating on a scale of 0-5 (Makumbi-Kidza *et al*, 2000)

Where 0 = no galls

1 = 1- 10% of the root system galled;

2 = 11-20% of the root system galled;

3 = 21- 70% of the root system galled;

4 = 71-90% of the root system galled; and

5 = greater than 90% of the root system galled.

Fresh tuber weights and the root and soil nematode populations were also determined. The soil nematode population was determined from 250ml soil sample obtained from each pot after mixing, using the modified Baermann funnel (pie-pan) method (Hooper, 1990). Eggs were extracted from the root system of each plant using the sodium hypochlorite method (Hussey and Barker, 1973). In a second trial, the experiment was repeated as described above without any modification.

3.15.2: Field Experiment

A piece of land naturally infested with *Meloidogyne incognita* at the research field of IITA, Ibadan was identified and used in this study. Nematode identity was confirmed by preparing the perineal pattern of the adult female root-knot nematodes obtained from the roots of infected plants.

In order to effect a nematode population build-up, *Celosia argentea*,was planted on this piece of land three months before the commencement of the experiment. Four

ridges, 34 m long and 2 m wide, each with spacing of 1m, were made. Each ridge was then divided into two, each having a length of 17 m which gave rise to 8 ridges. Four ridges served as control while the other four served as treatment plots. After this, the control plots were denematized with carbofuran at 3 kg a.i/ha while the treatment plots were not denematized. These served as main-plots. Each bed was further subdivided into five equal sub-plots. Two hundred 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 modified Baermann funnel (pie-pan) method (Whitehead and Hemming, 1965). The extraction set-up was left for 48 hours and nematode suspension was concentrated to about 20 ml sample. *M. incognita* population was estimated by counting under the compound microscope.

Five cassava cultivars; namely TMS 30572, TME 1, 4(2)1425, TMS 326, and Ofege were used for this experiment. Stem cuttings of the five cassava cultivars were randomly planted in each plot such that each plot had the five cultivars planted to it. Thus, a cultivar of cassava was planted to each sub-plot of 2m x 2m giving a population of nine plants per sub plot of 2 m x 2 m. The experimental design was a split-plot arranged in a randomized complete block design of four blocks. Nematode infestation is the main plot and the cassava cultivars are the sub-plots. The field layout is as shown in Fig. 3.1.

At harvest, 12 months after planting, data were collected on plant height, stem diameter, and shoot weight (g). The roots were carefully dug out and washed to remove soil particles and the following data were taken: galling index, fresh root weight (g) and number of tubers. The final nematode population was also determined from the soil and root system as described in Sections 3.3 and 3.4. The experiment was repeated the following year as described above. The procedure and data collected were as in the first trial.

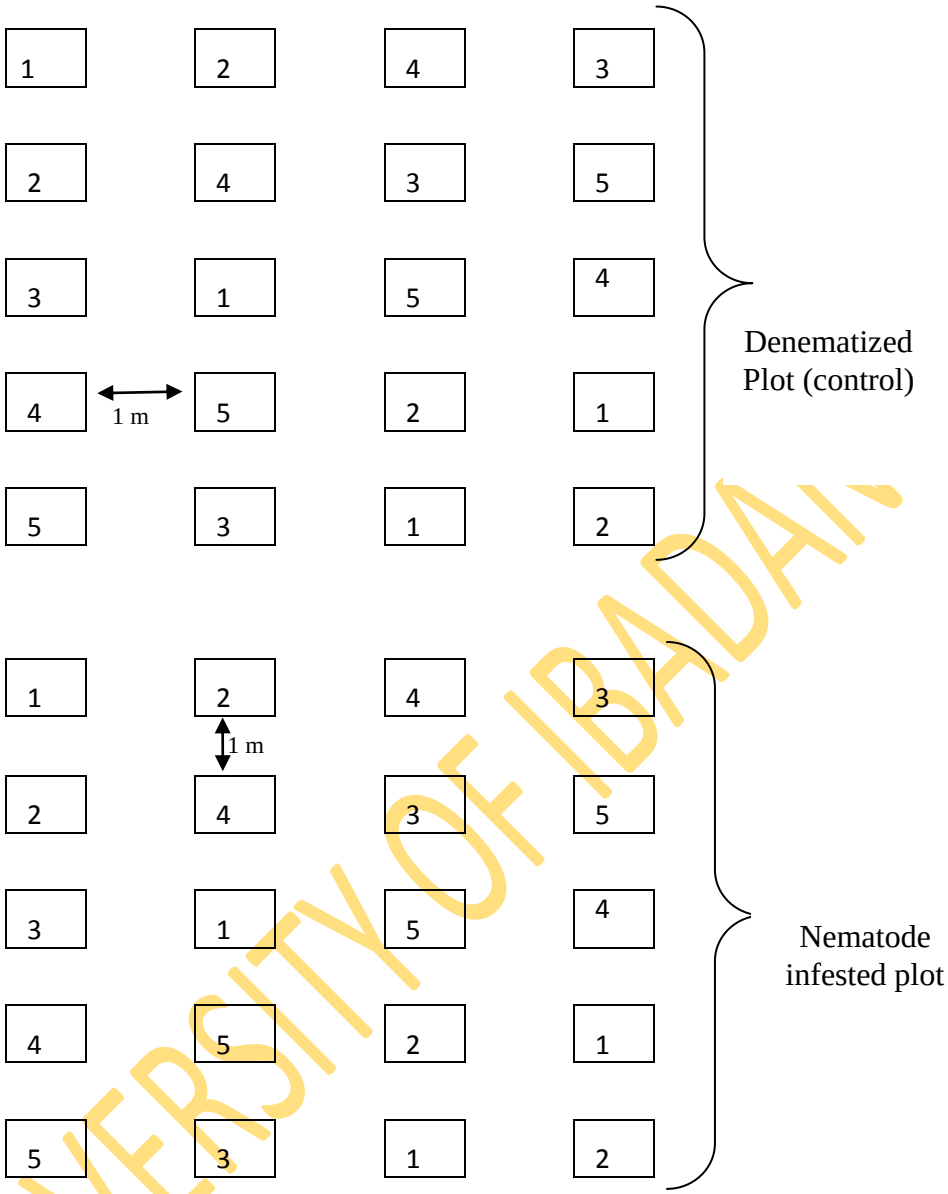


Fig 3.1: The Experimental Layout of the Field Pathogenicity Trial

CULTIVARS

1- TMS 30572

2- TME 1

3- 4(2)1425

4- TMS 326

5- Ofege

3.16: Experiment Two: Effects of the interaction between *Meloidogyne incognita* and *Botryodiplodia theobromae* on growth, yield and quality of cassava

3.16.1: Pot Experiment

Three cultivars of cassava namely; TMS 30572, TME 1 and Ofege were used for this study. Cultivars were selected on the basis of their abilities to support *Meloidogyne incognita* reproduction. The *M. incognita* eggs (inoculum) used were extracted from roots of three-month old *M. incognita*-galled *Celosia argentea* using 0.5% sodium hypochlorite method (Hussey and Barker, 1973) as described in Section 3.3. The eggs were collected in a 500 ml beaker 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 1000 eggs/ml of suspension. Also, the *B. theobromae* spores used were isolated from cassava roots. Spore population per ml was determined as described in Section 3.10. Five-node cassava stem cuttings of each cultivar were planted singly in polyethylene bags containing 20 litres of steam-sterilized sandy loam soil.

Two weeks after planting, they were inoculated as follows:

1. 0 eggs (which served as control),
2. 1,000 eggs of *M. incognita* alone,
3. 10,000 eggs of *M. incognita* alone,
4. $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* alone,
5. 1,000 eggs of *M. incognita* + $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* inoculated simultaneously/plant.
6. 1,000 eggs of *M. incognita* + $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* two weeks later,
7. $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* + 1,000 eggs of *M. incognita* inoculated 2 weeks later.
8. 10,000 eggs of *M. incognita* + $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* inoculated simultaneously/plant.
9. 10,000 eggs of *M. incognita* + $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* 2 weeks later,
10. $5 \text{ X } 10^5$ spores ml^{-1} of *B. theobromae* + 10,000 eggs of *M. incognita* 2 weeks later.

This was accomplished by pipetting 0 or 1 ml or 10 ml *M. incognita* of egg suspension and/or 1 ml of *Botryodiplodia theobromae* spore suspension, respectively, into four holes, each 4cm deep made at the base of the plants. Distilled water was introduced into the holes for 0 eggs. After inoculation, the holes were covered with sterilized sandy-loam soil. The plants were adequately watered throughout the period of the study by adding 100 cl of water to the plant. Each treatment was replicated 16 times in a completely randomized design arranged in the screen house. There were four destructive sampling dates viz: 2, 4, 9 and 12 months after planting. Data collected at 2 and 4 months after planting were to determine the effect of treatments on tuber initiation and formation, while the data taken at 9 and 12 months after planting were taken to determine effects of treatments on the plant at maturity. At each harvest, four replicates of each treatment were randomly selected for data collection. Data were collected on plant height (cm), stem diameter, fresh shoot weight (kg), number of tubers, tuber weight (g), number of rotted tubers, % tuber rot and rot severity, at 2, 4, 9 and 12 months after which the experiment was terminated. The root mass from each pot was separated from the soil by gently washing with tap water and thereafter observed and rated for nematode infection by gall index (Section 3.16). Number of rotted tubers was determined by physical observations made to note the number of decayed tubers in each treatment.

Tuber rot severity was determined by the method of Eze and Maduewesi (1990). The rot severity was determined for each tuber in which rot had developed. Rot severity was recorded using the following rating scale:

0 = No rotting
1 = very mild rotting (Less than 5% of tuber rotted)
2 = Mild rotting (6-10% of tuber rotted)
3 = Moderate rotting (11-25% of tuber rotted)
4 = Severe rotting (26-50% of tuber rotted)
5 = Very severe rotting (more than 50% of tuber rotted)

The entire feeder roots of each plant were weighed and then cut into 2 cm pieces and shaken vigorously in 0.5% sodium hypochlorite (NaOCl) solution to extract the eggs (Hussey and Baker, 1973).

The number of eggs extracted from each plant was estimated as described in Section 3.3. The soil nematode population was also estimated from 250 ml soil from each pot using the extraction tray method described in Section 3.3. 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 (R):

$$R \text{ where } \quad R = \frac{Pf}{Pi}$$

was then calculated; where Pf (final population) was the average total eggs and juveniles populations and Pi = 1,000 eggs or 10,000 eggs, the initial population density. The experiment was repeated the following year as described above. The procedure and data collected were as in the first trial.

3.16.2: Micro-plot Experiment

A micro-plot trial was carried out in the field at IITA, Ibadan. Three cultivars of cassava: TMS 30572, TME 1 and Ofege were used for this study. Cultivars were selected on the basis of their abilities to support *M. incognita* reproduction. 5-node cassava stem cuttings of each cultivar were planted singly in a polyethylene bag containing 40 litres of steam-sterilized sandy loam soil. Two weeks after planting, they were inoculated as follows:

1. 0 eggs (which served as control)
2. 1,000 eggs of *M. incognita* alone
3. 10,000 eggs of *M. incognita* alone
4. 5 X 10^5 spores ml^{-1} of *B. theobromae* alone
5. 1,000 eggs of *M. incognita* + 5 X 10^5 spores ml^{-1} of *B. theobromae* inoculated simultaneously/plant
6. 1,000 eggs of *M. incognita* + 5 X 10^5 spores ml^{-1} of *B. theobromae* two weeks later

7. 5 X 10^5 spores ml^{-1} of *B. theobromae* + 1000 eggs of *M. incognita* inoculated 2 weeks later
8. 10,000 eggs of *M. incognita* + 5 X 10^5 spores ml^{-1} of *B. theobromae* inoculated simultaneously/plant
9. 10,000 eggs of *M. incognita* + 5 X 10^5 spores ml^{-1} of *B. theobromae* 2 weeks later
10. 5 X 10^5 spores ml^{-1} of *B. theobromae* + 10,000 eggs of *M. incognita* 2 weeks later

This was accomplished by pipetting 0 or 1 ml or 10 ml *M. incognita* of egg suspension and or 1 ml of spore suspension respectively into four holes each 4cm deep made at the base of the plants. Distilled water was introduced into the holes for 0 eggs. After inoculation, the holes were covered with sterilized sandy-loam soil. Each treatment was replicated 16 times in a completely randomized design arranged in the screen house. There were four destructive sampling dates viz: 2, 4, 9 and 12 months after planting. Data collected at 2 and 4 months after planting were to determine the effect of treatments on tuber at initiation and formation, while the data taken at 9 and 12 months after planting were taken to determine effects of treatments on the plant at maturity. At each harvest, four replicates of each treatment were randomly selected for data collection. Data were collected on plant height (cm), stem diameter, fresh shoot weight (kg), number of tubers, tuber weight (kg), number of rotted tubers, % tuber rot and rot severity, at 2, 4, 9 and 12 months after which the experiment was terminated. The root mass from each pot was separated from the soil by gently washing with tap water and thereafter observed and rated for nematode infection (Section 3.14.1). Number of rotted tubers was determined by physical observations made to note the number of decayed tubers in each treatment.

Tuber rot severity was determined as previously described (3.17.1). The entire fibrous root system of each plant after weighing 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 number of eggs extracted from each plant was estimated as described in Section 3.3. The soil nematode population was also estimated from 250 ml soil from each pot using the extraction tray method described in Section 3.3 (Whitehead and Hemming, 1965). The total number of nematodes (J_2) in the soil was extrapolated from

the number of second stage juveniles counted. The experiment was repeated the following year as described above.

3.17: Experiment Three: Evaluation of *Glomus mosseae* and *Paecilomyces lilacinus* for the management of *M. incognita* on cassava.

3.17.1: Pot Experiment

TMS 30572, TME 1 and Ofege cassava cultivars were used in this experiment. Five–node stem cuttings of each cultivar were planted in polyethylene pots containing 30 litres of steam-sterilized sandy-loam top soil. Two weeks after planting, the cassava seedlings were given the following treatments:

1 = No *M. incognita*, No *G. mosseae* and No *P.lilacinus*
2 = 5000 eggs of *M. incognita*
3 = 140 spores of *G. mosseae*
4 = 2 x 10^6 spores ml^{-1} of *P. lilacinus*
5 = 5000 eggs of *M. incognita* + 140 spores of *G. mosseae*
6 = 5000 eggs of *M. incognita* +2 x 10^6 spores ml^{-1} of *P. lilacinus*
7 = 5000 eggs of *M. incognita* + 140 spores of *G. mosseae* +2 x 10^6 spores ml^{-1} of *P. lilacinus*
8 = 5000 eggs of *M. incognita* + Carbofuran 3kg a.i./ha

The *M. incognita* eggs (inoculum) used were extracted from roots of three-month old *M. incognita*-galled *Celosia argentea* using 0.5% sodium hypochlorite method (Hussey and Barker, 1973) as described in Section 3.3. The eggs were collected in a 400ml beaker and then thoroughly mixed using magnetic stirrer and the population per ml of the egg suspension was estimated under the stereoscopic microscope in a counting dish. Five counts were taken and diluted to about 1000 eggs/ml of suspension. *Glomus mosseae* spores were incorporated into pots at a rate of approximately 140 spores per pot by spreading 50 g of soil and spore mixture at a depth of 3 cm near the centre of the pots where the cassava cutting were planted. The cassava plants were inoculated with 2 x 10^6 spores ml^{-1} of *P. lilacinus* spores in holes made around each seedling.

Distilled water was introduced into the holes as control (0 eggs, 0 spores of both *P. lilacinus* and *G. mosseae*). After inoculation, the holes were covered with sterilized sandy-loam soil. The treatments were replicated four times and the pots were arranged in a completely randomized design in the screen house. Data on plant height, fresh shoot weight and stem diameter were taken at 2, 4 and 9 months. The plants were watered adequately throughout the period of the experiment. At harvest, 12 months after planting, data were taken on plant height, fresh shoot weight, stem diameter, number of tubers, tuber weight (g).

Root and soil nematode populations were also determined using the methods of Hussey and Barker (1973) and Whitehead and Hemming (1965), respectively. From these data, the final nematode population per 'pot' was estimated and the Reproductive Factor ($R = P_f/P_i$) was calculated, where P_f (final population) is the average total soil and root nematode populations; P_i is the initial nematode population (5,000 eggs).

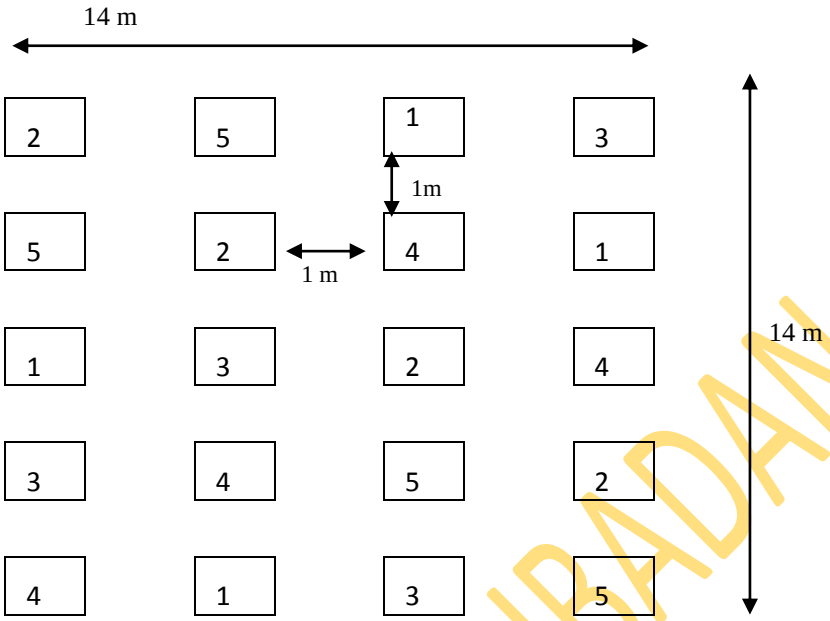
3.17.2: Field experiment

A piece of land measuring 14 m x 14 m and located at the International Institute of Tropical Agriculture, Ibadan was used for this study. Three months before this experiment began; *M. incognita* was multiplied under *Celosia argentea* on the plot. The initial root-knot nematode (*M. incognita*) population was estimated four days before the commencement of the trial. The piece of land was divided into four equal blocks of 14 m x 2 m. Each block was further divided into five equal sub-plots. There was a spacing of 1m between the blocks and 1m between sub-plots. The experiment was laid out in a randomized complete block design with five treatments in four replicates. Five spots were randomly marked out in each block for nematode population estimation. A sample of 250 ml of top soil was collected from each of the marked spots. Nematodes were extracted from the sampled soils using pie-pan extraction trays (Whitehead and Hemming, 1965). The root-knot nematode juveniles (J_2) extracted were counted under a stereo microscope after 48 hours in a counting dish. Ofege cultivar was planted in each sub-plot using 5-node stem cuttings. Two weeks after planting the following treatments were applied.

1. 140 spores of *G. mosseae*
2. $2x10^6$ spores ml^{-1} of *P. lilacinus*,

3. 140 spores of *G. mossae* + $2x10^6$ spores ml^{-1} of *P. lilacinus*,
4. carbofuran at 3kg a.i./ha and
5. nematode-infested sub-plots

The field layout was as shown in Fig 3.2. The plants were rain-fed. The experimental plot was kept weed-free by regular hoeing. Plant height (cm), stem diameter (cm) and fresh root weight (g) were taken at 2, 4, 9 and 12 months after planting. The plants were harvested at 12 months after planting and the following data were taken: plant height (cm), stem diameter (cm) fresh root weight (g), number of tubers, tuber weight (g), root and soil root-knot nematode populations were determined as described in sections 3.3 and 3.4. The experiment was repeated the following year as described above. The procedure and data collected were as in the first trial.



1 = 140 spores of *G. mosseae*
2 = 2 x 10^6 spores ml^{-1} of *P. lilacinus*
3 = 140 spores of *G. mosseae* +2 x 10^6 spores ml^{-1} of *P. lilacinus*
4 = Carbofuran at 3kg a.i./ha
5 = Untreated soil

Fig 3.2: Experimental Layout of Field Assessment of *Glomus mosseae* and *Paecilomyces lilacinus* in the management of *M. incognita*

CHAPTER FOUR

RESULTS

4.1: Pathogenicity of Root-knot nematode on cassava in pots

4.1.1: Effects of various inoculum densities of *Meloidogyne incognita* on vegetative growth of five cassava cultivars from inoculation to the twelveth month.

There were significant differences in the mean height of cassava cultivars at different inoculum levels at the second, fourth, ninth and twelfth month after planting (MAP). At two months after inoculation, the cultivar 4(2)1425 had no significant difference between the height of the uninoculated plants (43.6cm) and plants inoculated with 1,000 eggs/plant (41.2cm). Plants inoculated with 10,000 eggs/plant had the significantly lowest plant height followed by plants inoculated with 1,000 eggs/plant and they were all significantly shorter than the control (0 eggs) (Fig.4.1). This trend was the same in cultivars TMS 326 (Fig. 4.2), Ofege (Fig. 4.3), TME 1 (Fig. 4.4) and TMS 30572 (Fig. 4.5)

The mean fresh shoot weight also differed significantly ($P \leq 0.05$) at different inoculum densities at the second, fourth, ninth and twelfth month after planting. The fresh shoot weight was significantly lowest in plants inoculated with 10,000 eggs/plant followed by plants inoculated with 1,000 eggs/plant compared with the control. In the cultivar 4(2)1425, fresh shoot weight decreased with increasing inoculum density. (Fig. 4.6) and this was the trend in all the other cassava cultivars TMS 326 (Fig. 4.7), Ofege (Fig. 4.8), TME 1 (Fig. 4.9) and TMS 30572 (Fig. 4.10) used in the experiment.

In the cultivar 4 (2)1425, plants inoculated with 10,000 eggs/plant had the lowest significant mean stem diameter at 2 MAP (0.5cm), 4 MAP (0.8 cm), 9 MAP (1.6cm) and 12 MAP (1.8 cm) followed by plants inoculated with 1,000 eggs/plant, while the control (0 eggs) had the highest significant stem diameter (Table 4.11). This was the trend in both trials. No significant difference was shown in the stem diameter between the control (2.3 cm) and the plants inoculated with 1,000 eggs (2.0 cm) in the cultivar 4(2)1425 at 12 months after planting in the two trials (Fig 4.11). In TMS 326, at

2 MAP there were significant differences between the stem diameter of plants inoculated with 10,000 eggs/plant (0.6 cm), 1,000 eggs/plant (0.9 cm) and the control (1.1 cm) (Fig. 4.12), while in Ofege there were no significant differences between the stem diameter of plants inoculated with 1,000 eggs/plant and 10,000 eggs per plant at 4MAP, 9MAP and 12MAP in the first trial and at 4 MAP and 9 MAP in the second trial (Fig.4.13). While there were significant differences between the stem diameter of TME 1 cassava plants inoculated with 0 eggs/plant, 1,000 eggs/plant and 10,000 eggs/plant in the first trial, there was no significant difference between the stem diameter of the cultivar at 4MAP, 9 MAP and 12 MAP in the second trial (Fig. 4.14).

The cultivar TMS 30572 at 12 MAP showed significant differences between the stem diameter of plants inoculated with 0 eggs/plant (3.1 cm), plants inoculated with 1,000 eggs/plant (2.8 cm) and plants inoculated with 10,000 eggs/plant (2.3 cm) (Fig 4.15).

4.1.2: Effects of various inoculum densities of *Meloidogyne incognita* on root galling and yield parameters of cassava.

The uninoculated plants in all the cultivars had the highest mean number of tubers in both trials and it was significantly higher than in other treatments in the two trials (Table 4.1). The least mean number of tubers was obtained from CV TME 1 in both trials while the highest mean number of tubers was obtained from TMS 30572 in both trials (Table 4.1)

In both trials, the fresh tuber weight of plants inoculated with 0 eggs / plant was statistically higher than the fresh tuber weight from other treatments. It was followed in both trials by the values obtained from plants inoculated with 1,000 and 10, 000 eggs and these differed significantly from each other. The lowest significant mean fresh root weight came from plants that received the highest inoculum density (10,000 eggs) for both trials (Table 4.1).

Plants inoculated with 10,000 nematode eggs had the highest significant gall index compared with plants inoculated with 1,000 eggs and the control (0) (Table 4.1). The trend was the same for the two trials (Table 4.1). The highest gall index was recorded on TME 1 (4.0) followed by Ofege (3.0) and then TMS 326 and 4(2)1425 (2.8), while TMS 30572 had 2.5 (Table 4.1) in the first trial. The same trend was observed in the second trial.

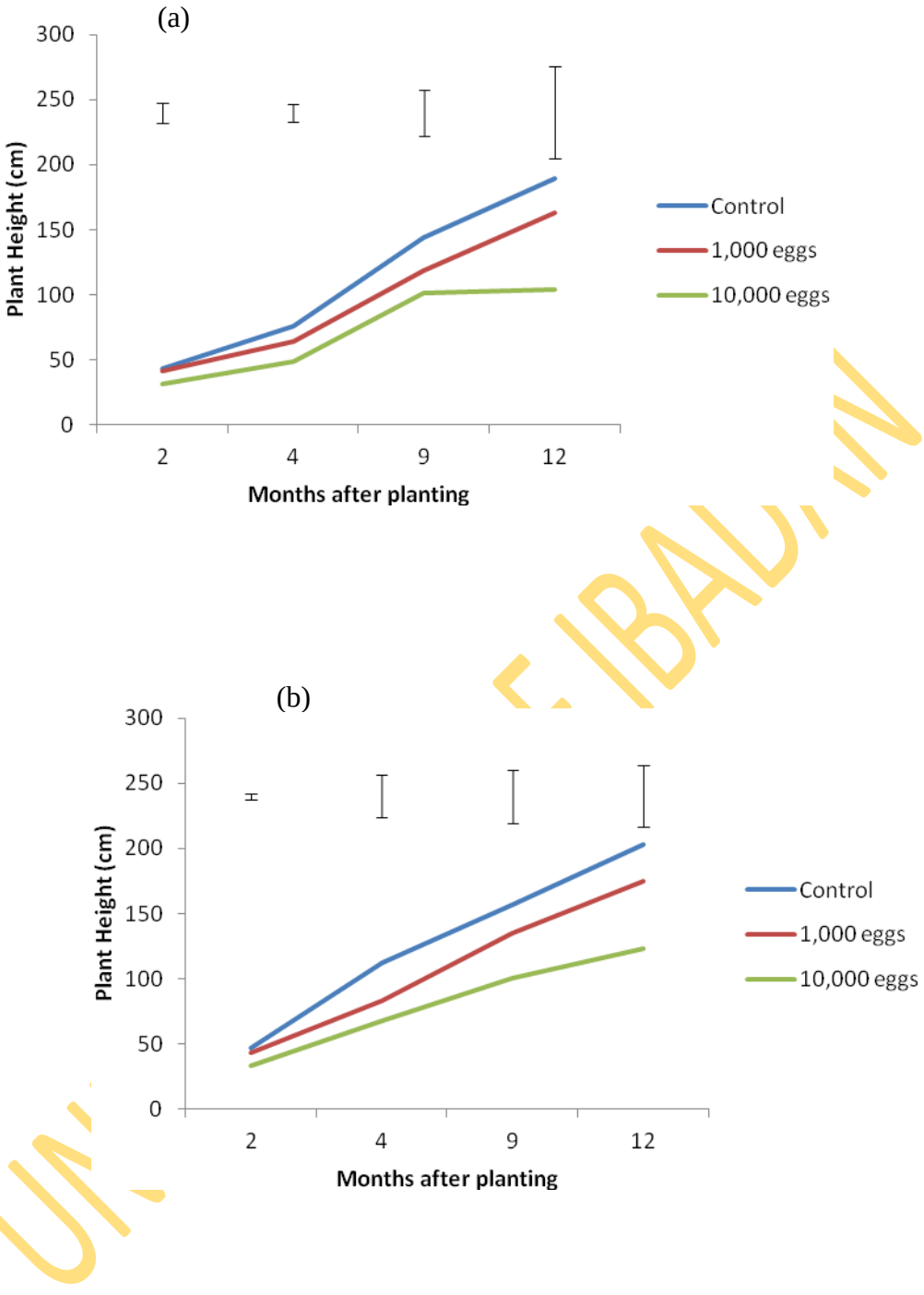


Figure 4.1: Effects of *M. incognita* population on plant heights of 4(2)1425 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

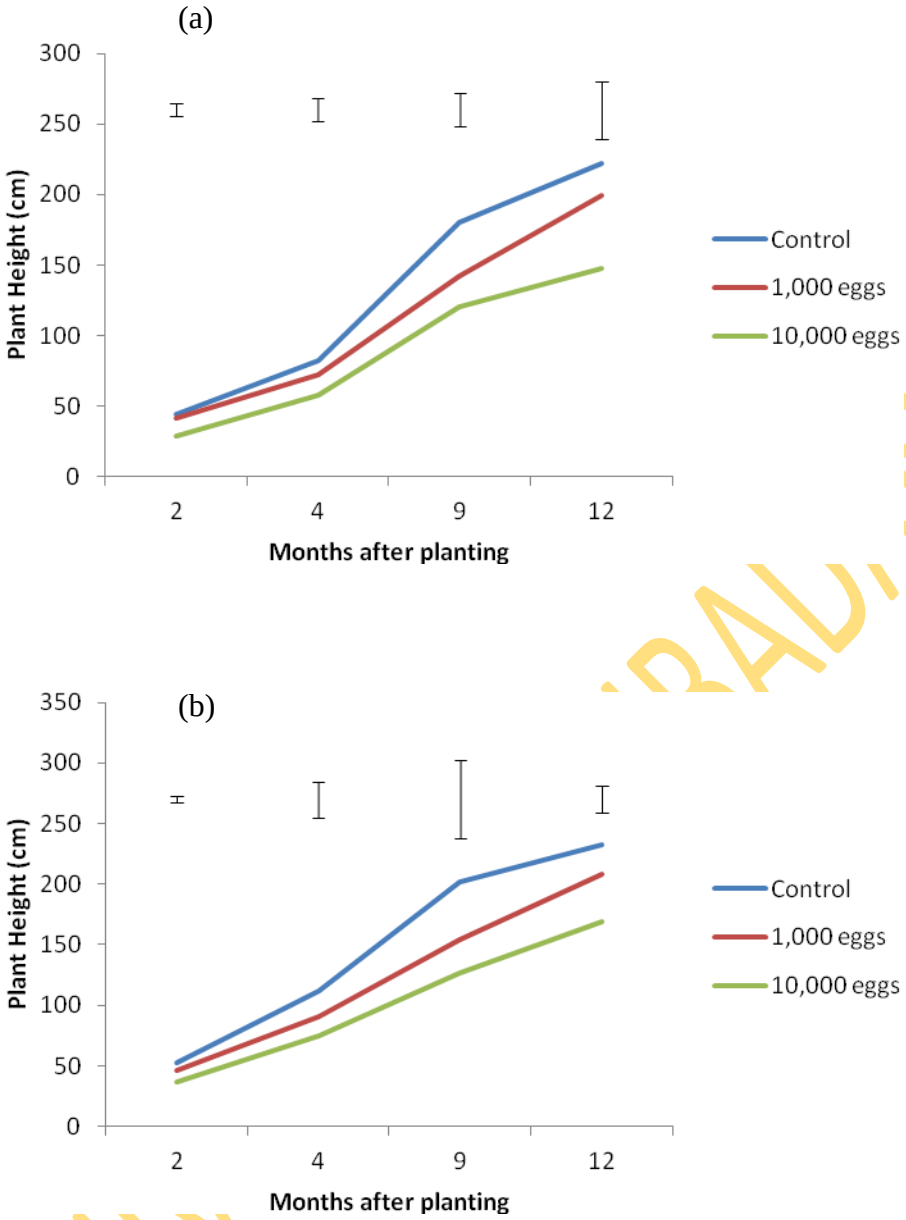


Figure 4.2: Effects of *M. incognita* population on plant heights of TMS 326 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

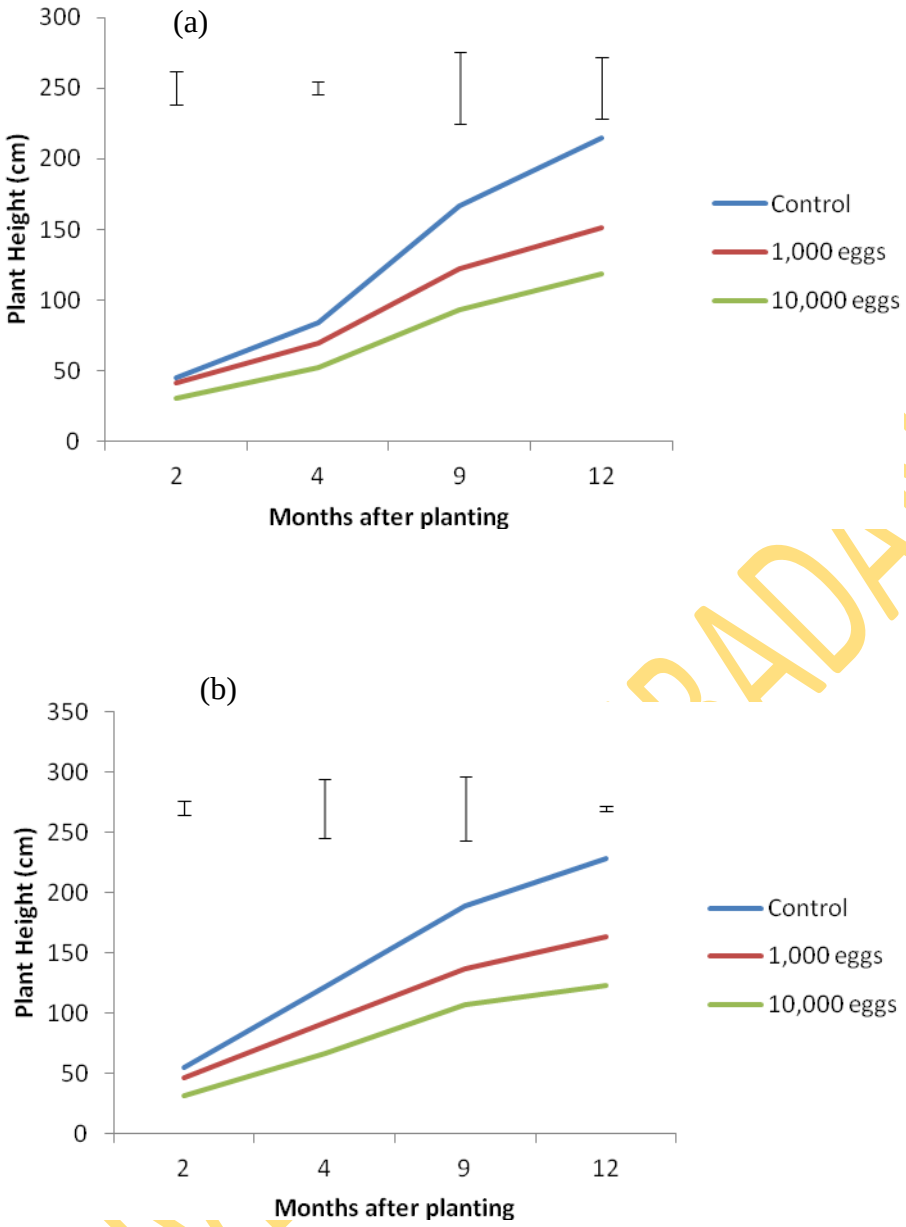


Figure 4.3: Effects of *M. incognita* on plant heights of Ofege cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

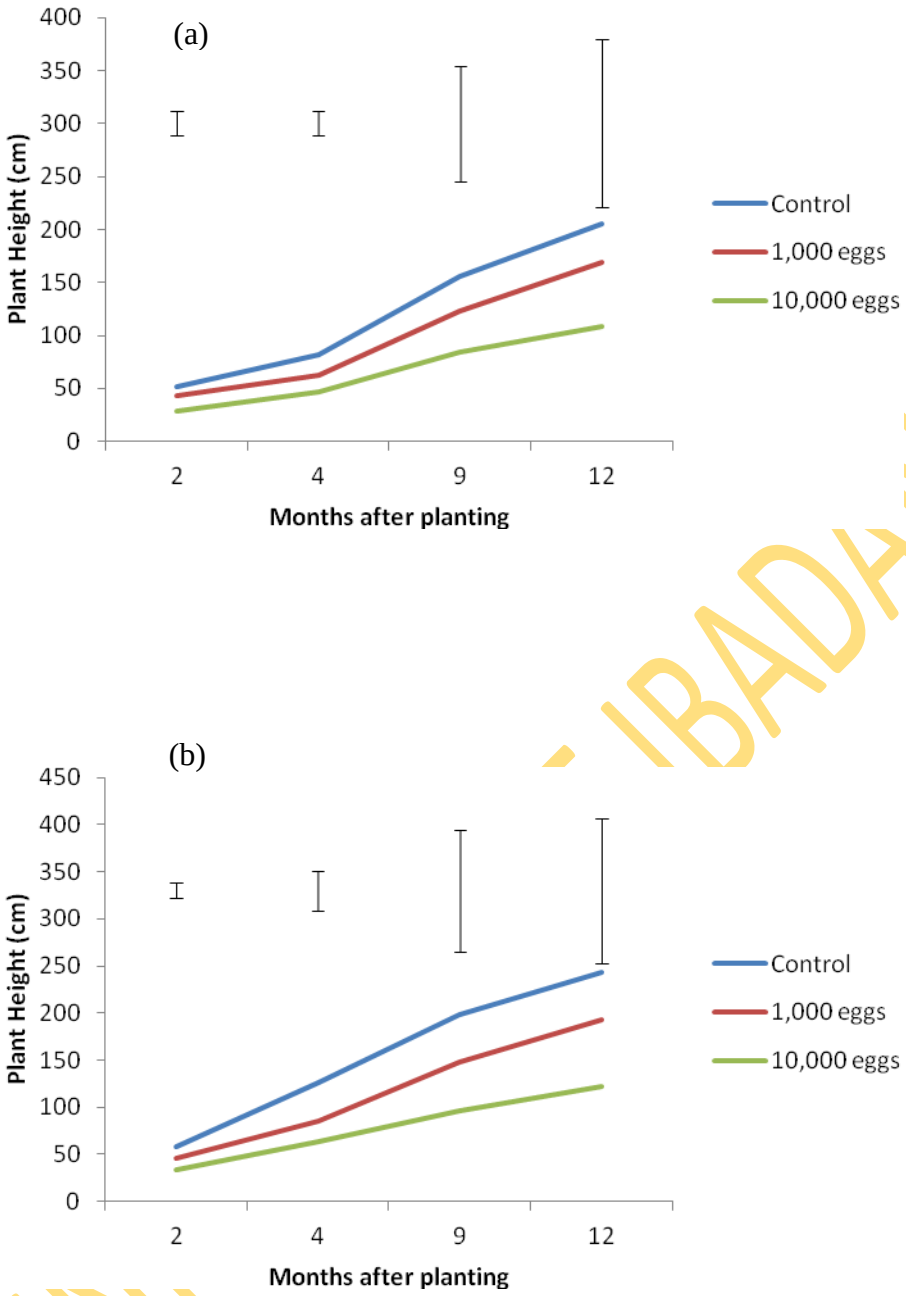


Figure 4.4: Effects of *M. incognita* on plant heights of TME 1 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bar is for comparing treatment means at each specific sampling time.

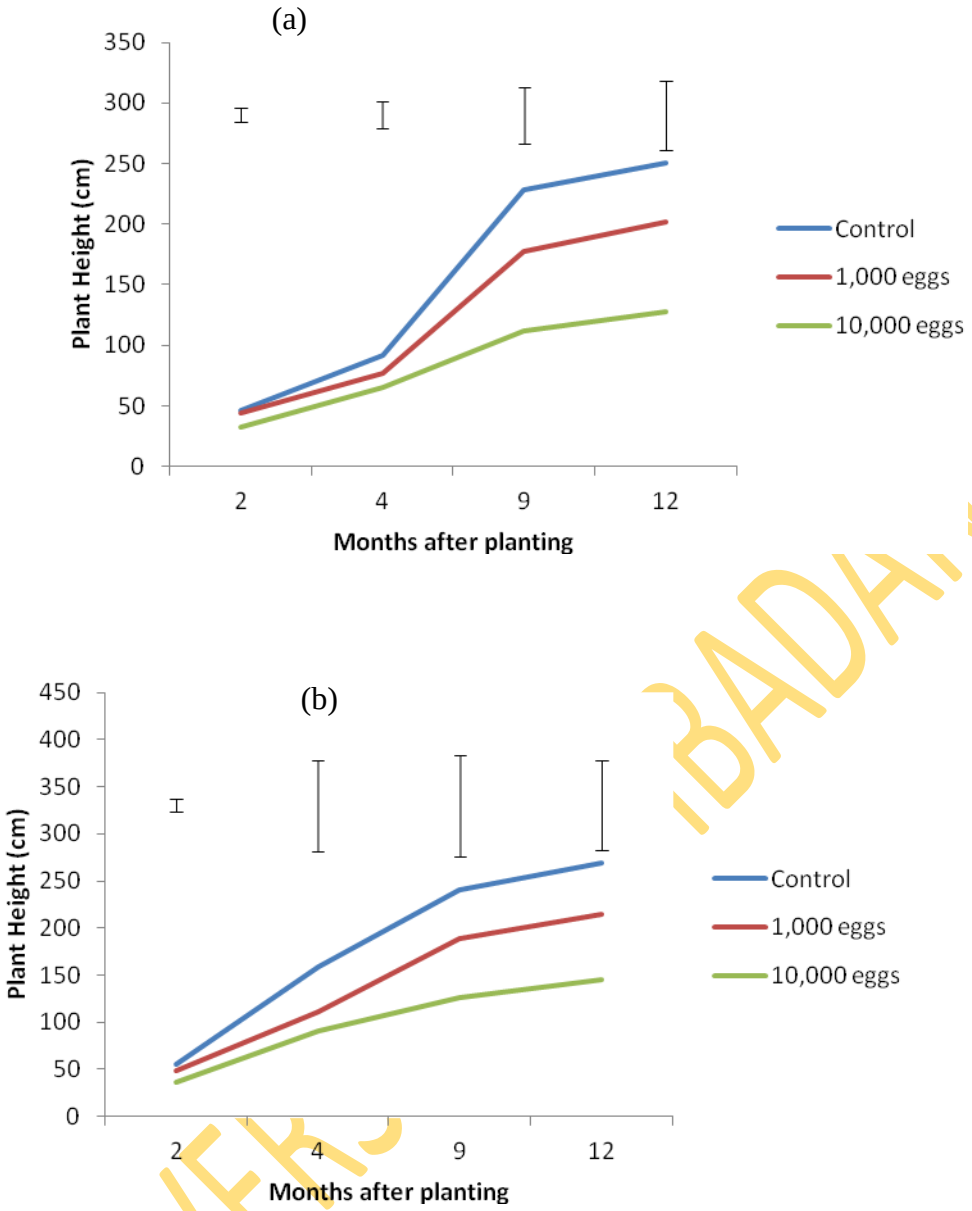
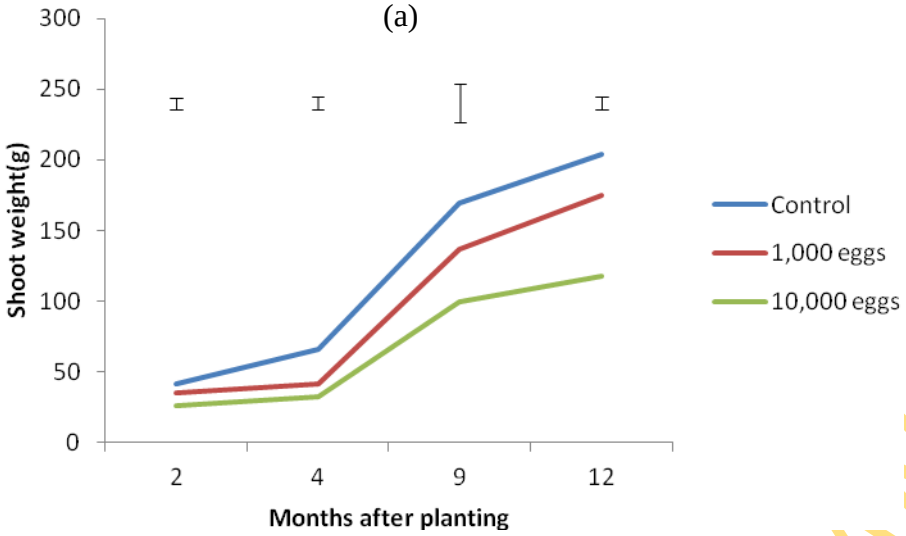


Figure 4.5: Effects of *M. incognita* on plant heights of TMS 30572 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

(a)



(b)

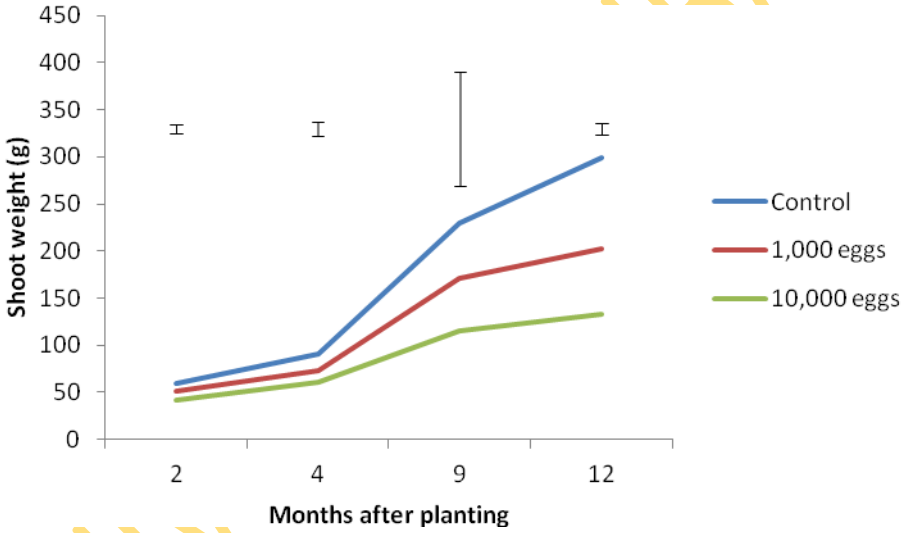


Figure 4.6: Effects of *M. incognita* on fresh shoot weights of 4 (2)1425 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bar is for comparing treatment means at each specific sampling time.

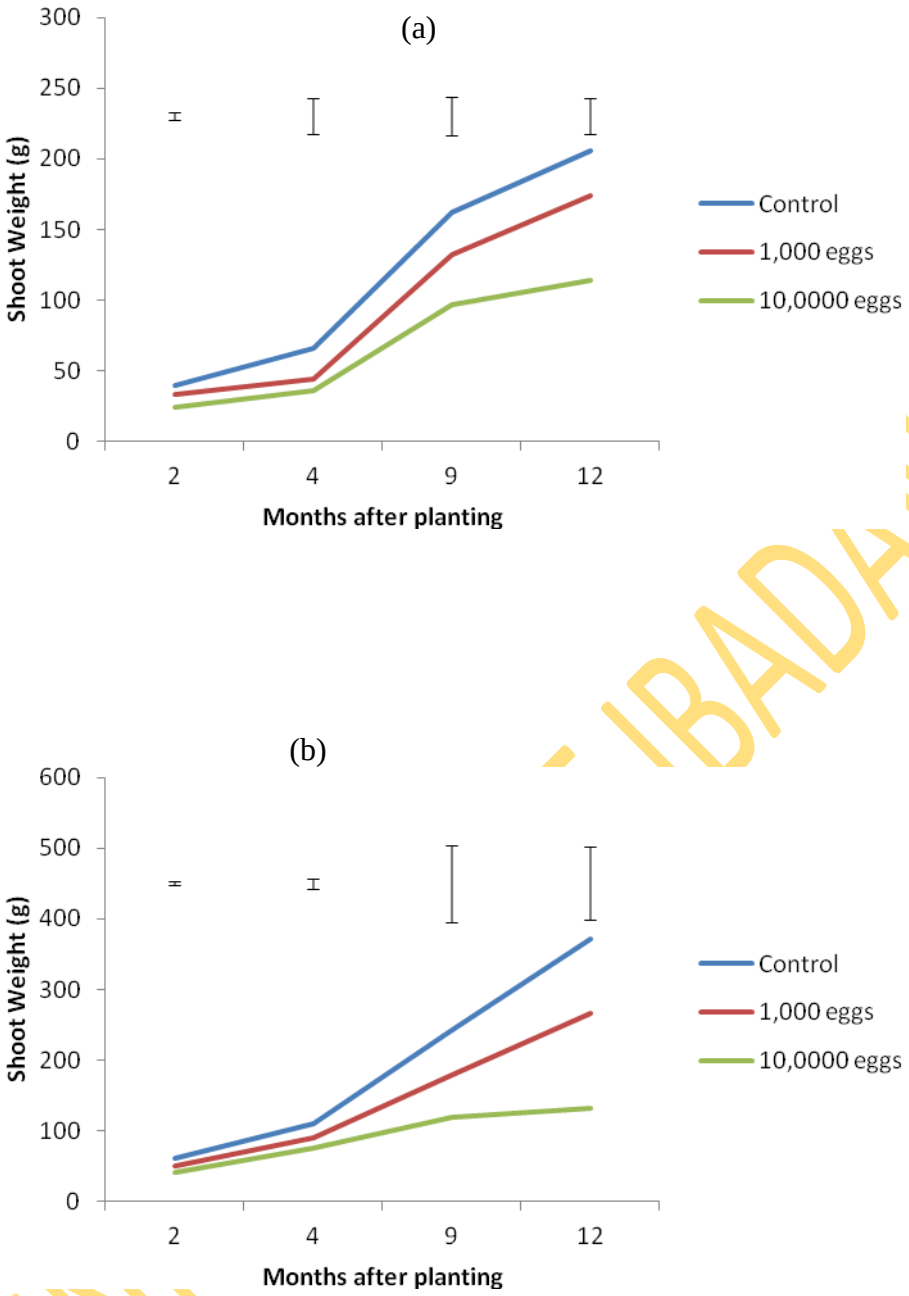
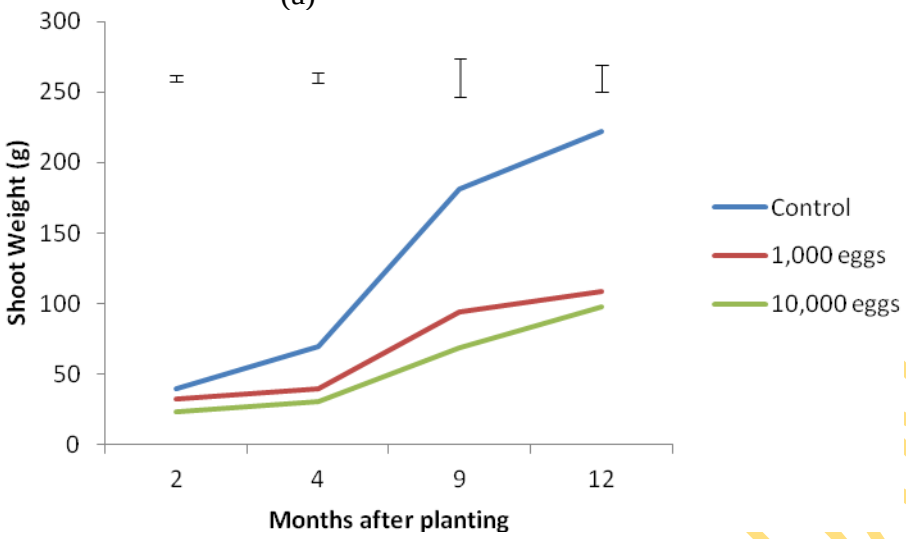


Figure 4.7: Effects of *M. incognita* on fresh shoot weights of TMS 326 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

(a)



(b)

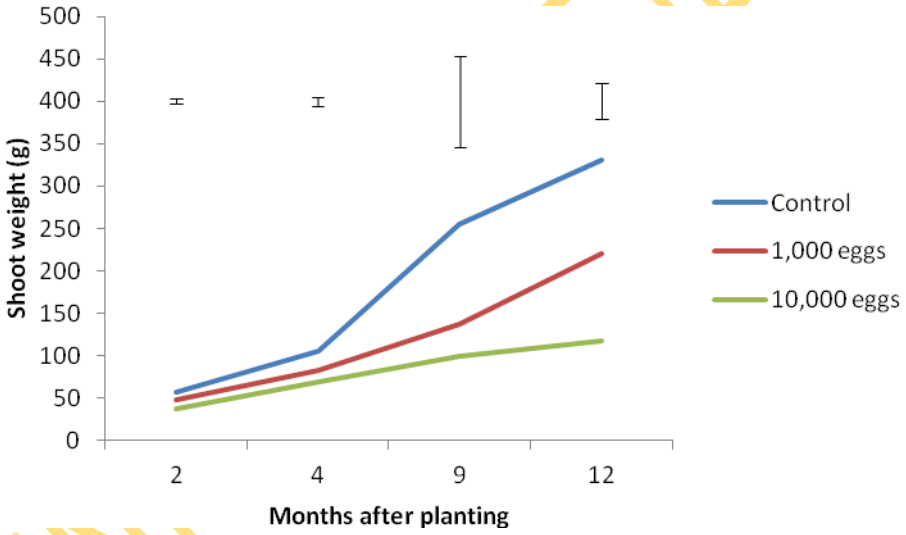


Figure 4.8: Effects of *M. incognita* on plant shoot weights of Ofege cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

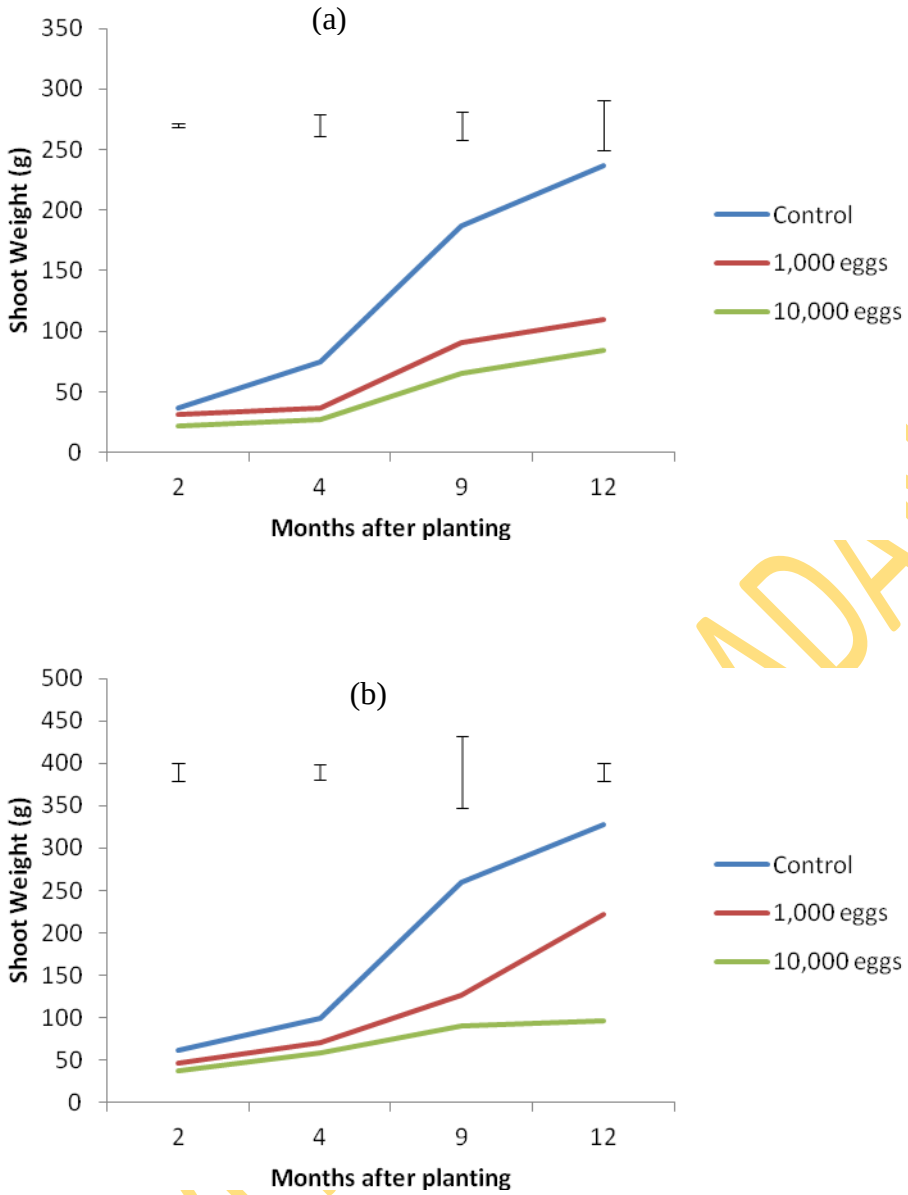


Figure 4.9: Effects of *M. incognita* on shoot weights of TME 1 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

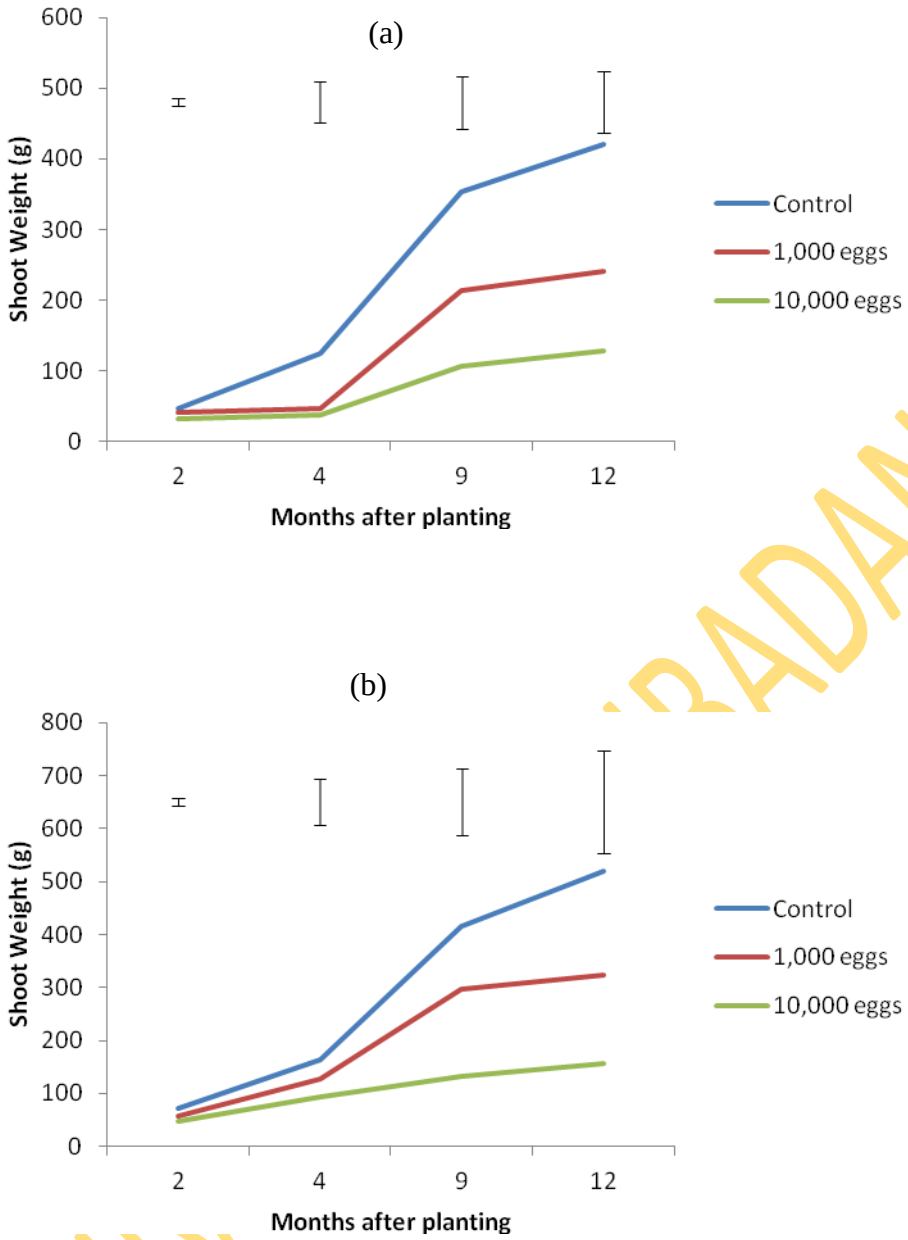


Figure 4.10: Effects of *M. incognita* on fresh shoot weights of TMS 30572 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

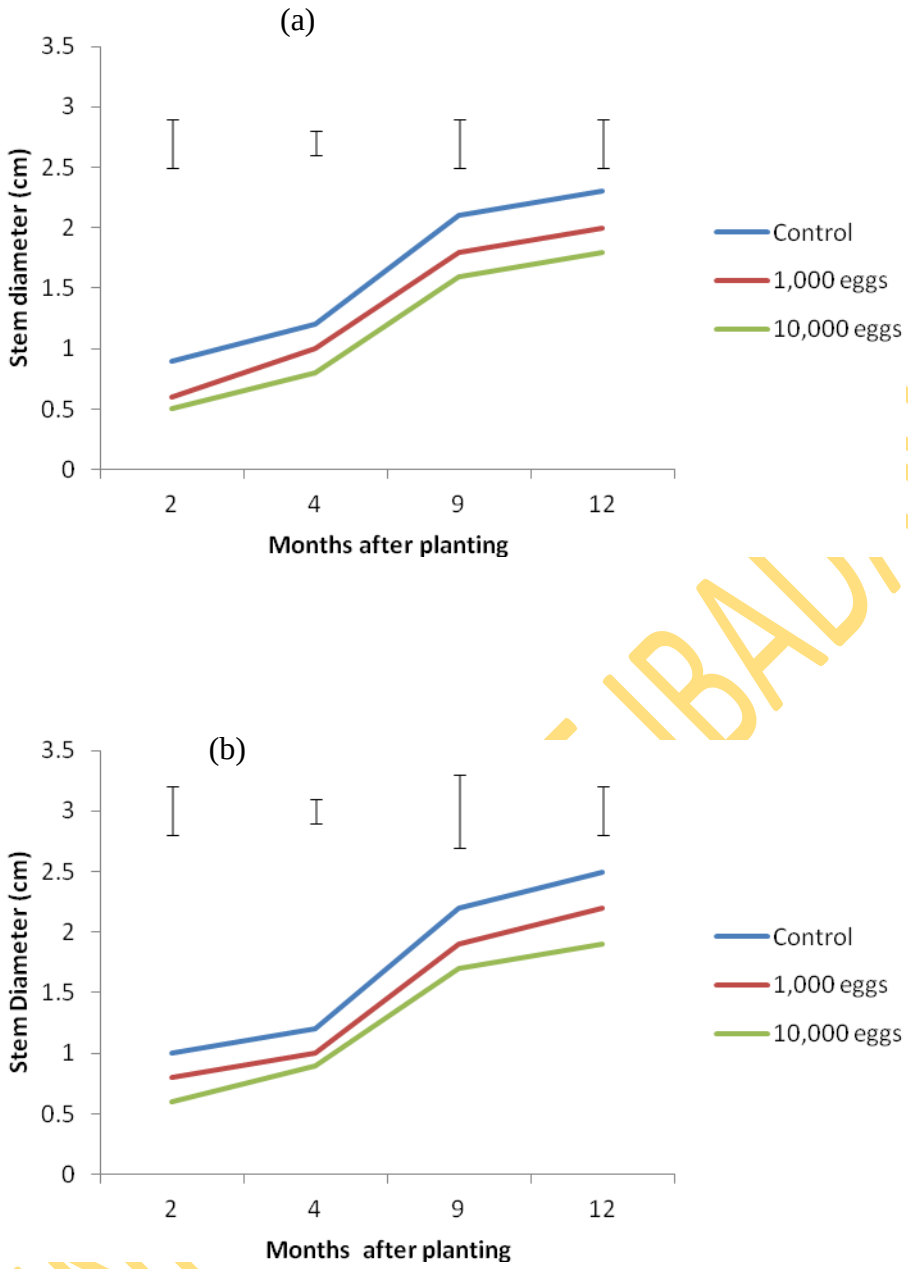


Figure. 4.11: Effects of *M. incognita* on stem diameter of 4(2)1425 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

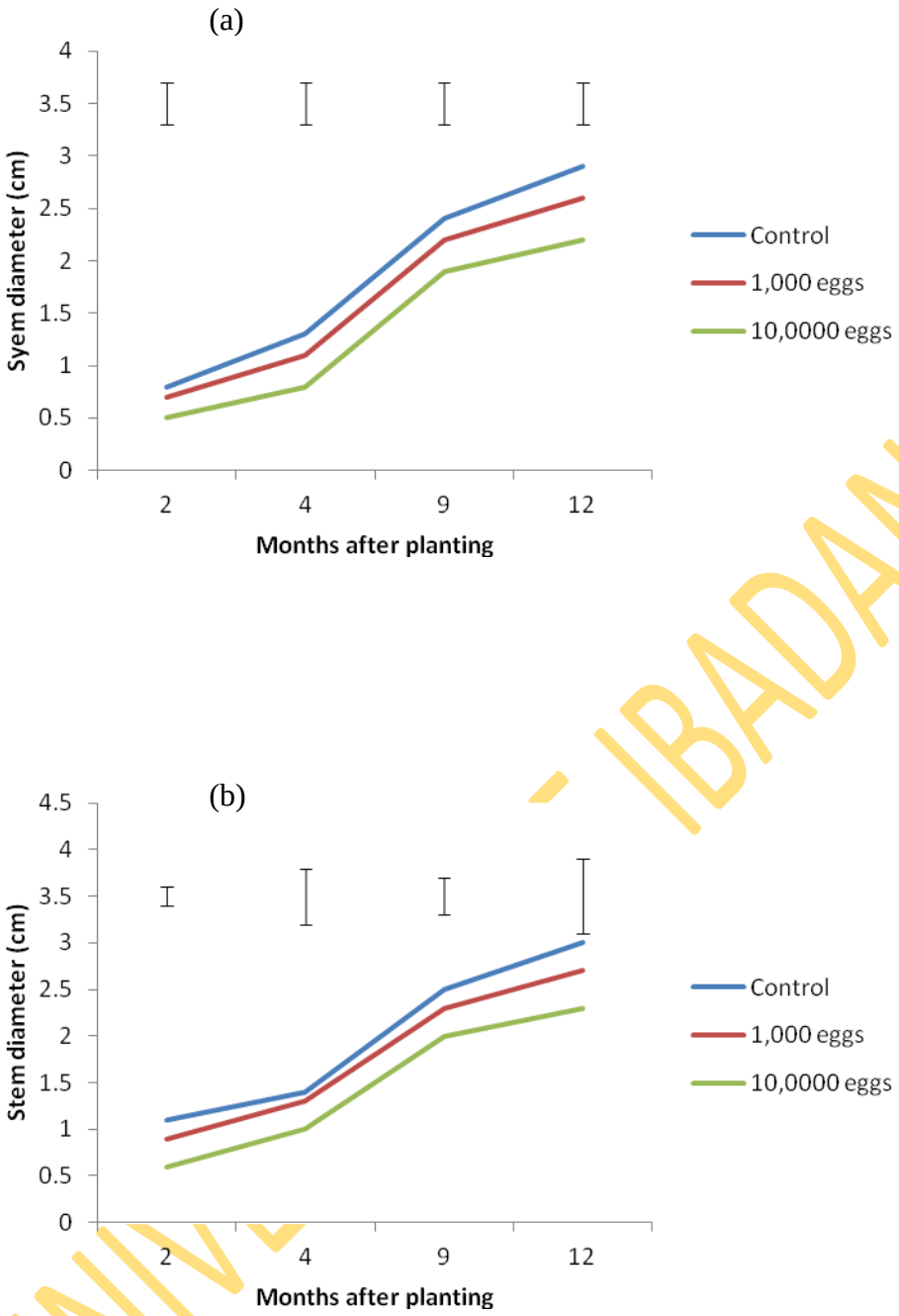


Figure 4.12: Effects of *M. incognita* on stem diameter of TMS 326 cassava cultivar in a pot experiment. (a) = first trial (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

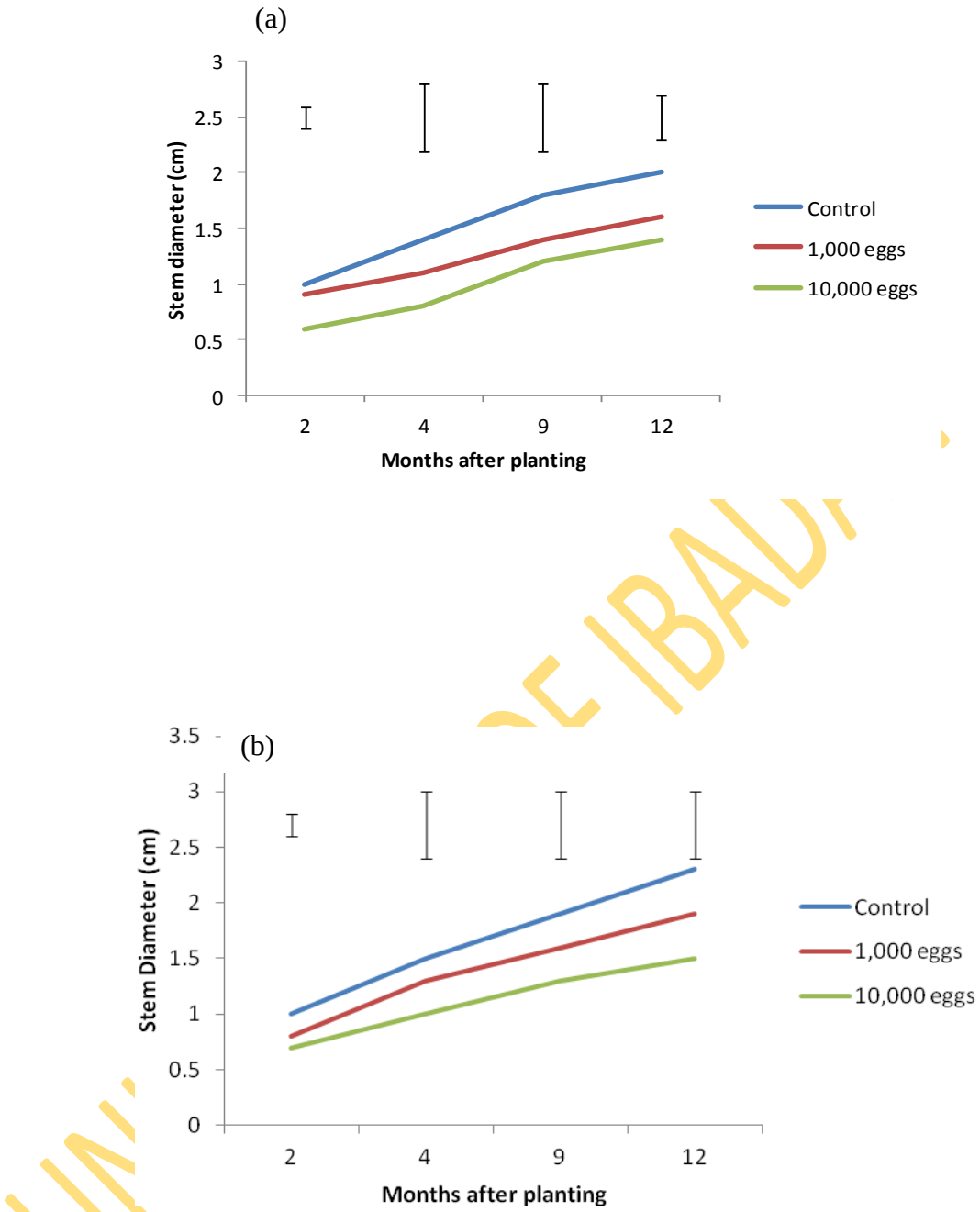


Figure 4.13: Effects of *M. incognita* on stem diameter of Ofege cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bar is for comparing treatment means at each specific sampling time.

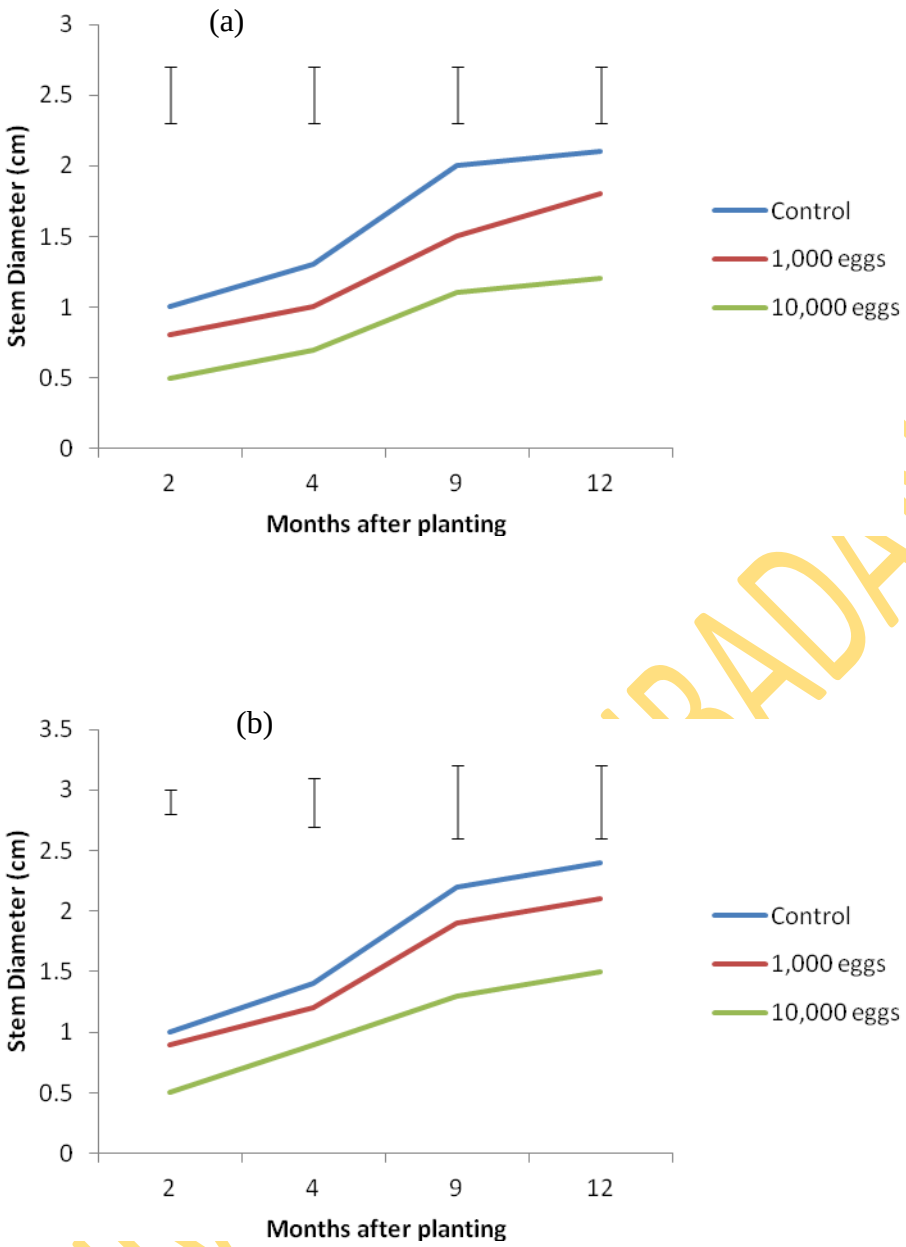


Figure 4.14: Effects of *M. incognita* on stem diameter of TME 1 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

(a)

(b)

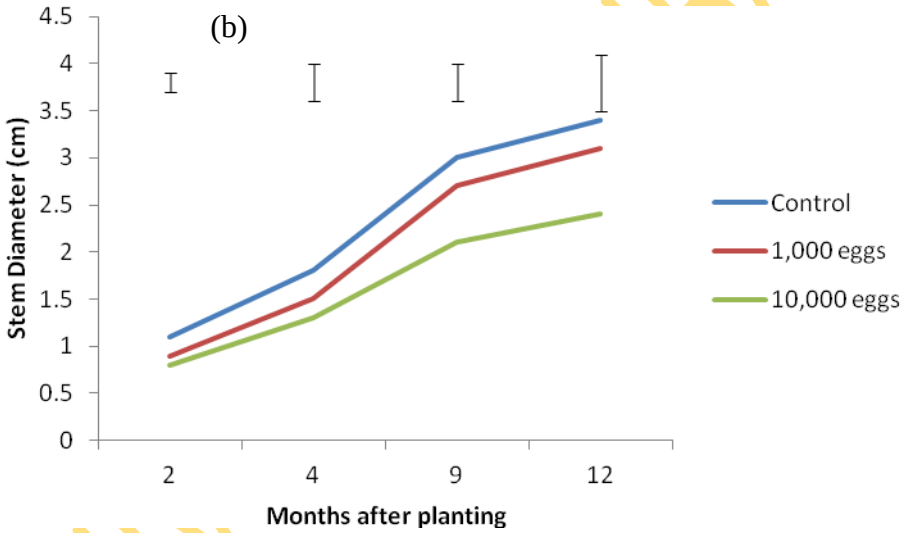


Figure 4.15: Effects of *M. incognita* on stem diameter of TMS 30572 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

The mean final nematode population differed significantly from each other. The highest value was obtained from plants inoculated with 10,000 eggs and this differed ($P \leq 0.05$) from the value recorded from the plants infected with 1,000 eggs and the control in the two trials. The lowest mean value came from the uninoculated plants (0.0) which were statistically lower than values from other treatments in both trials (Table 4.1).

The mean nematode Reproductive Factor (R) also differed significantly from each other (Table 4.1). In both trials, the highest mean R came from the plants inoculated with 10,000 eggs. There was significant difference in the mean nematode R among the inoculated plants in both trials. The lowest value came from control plants for the two trials (Table 4. 1).

4.1. 3: Pathogenicity of *Meloidogyne incognita* on cassava in the field

4.1.3.1: Effect of *M. incognita* infection on Plant height, Stem diameter and fresh shoot weight of five cassava cultivars.

In both trials, plant height differed significantly between the denematized soil and the nematode-infested soil. Plants on the nematode-infested soil were significantly shorter ($P \leq 0.05$) in height than plants on the denematized soil (Table 4.2).

The size of the stem diameter reduced significantly in plants in nematode infested soil than in plants on denematized soil. The highest stem diameter was obtained in TMS 30572 (Table 4.2)

The plants grown on nematode-infested soil had significantly lower mean fresh shoot weights than those planted on denematized soil in both trials (Table 4.2).

There was a significant difference between the mean number of cassava tubers obtained from plants grown on nematode-infested soil and denematized soil for both trials. The higher significant mean number of tubers came from the denematized soil (Table 4.3). While TMS 30572 had the largest number of tubers (12.0), TME 1 had the lowest number of tubers (7.8) (Table 4.3). There was a significant difference between the mean fresh tuber weight of cassava plants grown on denematized soils and nematode-infested soils for both trials (Plate 4.1).

Table 4.1: Effect of *M. incognita* population on yield parameters of five cassava cultivars

Cultivars	Treatments	Number of Tubers	Tuber weight (g)	Galling Index*	Final Nematode population	Reproductive Factor (R)
First Trial						
TMS 326	Control	5.0	123.8	0.0	0.0	0.0
	1,000 eggs	4.0	111.3	1.0	1101.0	1.1
	10,000 eggs	3.8	67.8	2.8	22039.0	2.2
	LSD(P≤0.05)	0.5	16.4	0.5	3656.2	0.4
4(2)1425	Control	5.3	125.7	0.0	0.0	0.0
	1,000 eggs	4.5	106.7	1.3	1028.0	1.0
	10,000 eggs	2.8	56.7	2.8	22771.0	2.3
	LSD(P≤0.05)	1.3	24.3	0.6	2758.0	0.3
TMS 30572	Control	7.8	154.8	0.0	0.0	0.0
	1,000 eggs	5.3	121.9	1.0	786.0	0.8
	10,000 eggs	3.8	80.6	2.5	19490.0	2.0
	LSD(P≤0.05)	1.0	15.0	0.6	4225	0.4
OFEGE	Control	5.0	130.8	0.0	0.0	0.0
	1,000 eggs	3.5	105.2	1.8	1732.0	1.7
	10,000 eggs	2.0	50.4	3.0	35781.0	3.4
	LSD(P≤0.05)	0.8	26.3	0.8	3621.0	0.5
TME 1	Control	5.5	139.5	0.0	0.0	0.0
	1,000 eggs	4.5	110.7	2.3	1620.0	1.6
	10,000 eggs	1.5	28.4	4.0	40687.0	4.0
	LSD(P≤0.05)	0.0	17.7	0.5	4913.1	0.6
Second Trial						
TMS 326	Control	6.0	148.8	0.0	0.0	0.0
	1,000 eggs	4.0	140.6	1.0	1112.0	1.1
	10,000 eggs	4.0	84.9	2.8	26864.0	2.7
	LSD(P≤0.05)	1.4	26.2	0.5	3503.0	0.4
4(2)1425	Control	6.3	148.0	0.0	0.0	0.0
	1,000 eggs	4.3	146.3	1.3	1287.0	1.3
	10,000 eggs	3.0	73.0	3.0	23969.0	2.4
	LSD(P≤0.05)	1.5	33.0	0.4	3033.0	0.4
TMS 30572	Control	9.3	204.5	0.0	0.0	0.0
	1,000 eggs	5.5	174.0	1.0	1183.0	1.2
	10,000 eggs	4.0	96.7	2.3	16843.0	1.7
	LSD(P≤0.05)	0.6	47.1	0.5	3617.1	0.4
OFEGE	Control	5.8	153.6	0.0	0.0	0.0
	1,000 eggs	3.5	133.1	2.0	2240.0	2.2
	10,000 eggs	2.3	57.9	3.5	39769	4.0
	LSD(P≤0.05)	0.6	13.0	0.6	5858.9	0.6
TME 1	Control	5.5	152.0	0.0	0.0	0.0
	1,000 eggs	4.0	128.1	2.3	2218.0	2.1
	10,000 eggs	1.3	41.7	4.5	47560.0	4.7
	LSD(P≤0.05)	0.6	15.6	0.6	7064.3	1.3

Each value is a mean of four replicates.

LSD for comparing treatment means within the same column. Control (no nematode)

* 0 = no galls ; 1 = 1-10% of the root system galled; 2 = 11-20% of the root system galled; 3 = 21- 70% of the root system galled; 4 = 71-90% of the root system galled; 5 = greater than 90% of the root system galled.

The higher significant mean value came from the denematized soil (Table 4.3). For both trials, the mean gall indices differed significantly between the denematized and nematode-infested soils. The nematode-infested soil produced mean gall index that was significantly higher than that of denematized soil (Table 4.4). 4(2)1425 cultivar had a gall index of 3.0 in nematode infested soil and 0.0 in denematised in denematised soil (Table 4.4). In the trial the mean gall indices of the various cultivars differed from each other significantly. TME 1 had the highest significant mean gall index (4.5). Also in the second trial, the nematode-infested soil produced mean gall index that was significantly higher than that of denematized soil (Table 4.4). 4(2)1425 cultivar had a gall index of 3.0 in nematode infested soil and 0.0 in denematised in denematised soil (Table 4.4).TMS 326 cultivar had a gall index of 3.3 in nematode-infested soil and 0.8 in denematised soil. Ofege cultivar had a gall index of 3.5 in nematode infested soil and 0.0 in nematode infested soil. In the trial the mean gall indices of the various cultivars differed from each other significantly. TME 1 had the highest significant mean gall index (5.0).

The mean final soil nematode population was significantly higher in the nematode-infested soil than on denematized soil in both trials (Table 4.4). In the first trial, the highest mean soil nematode population came from the soil planted to TME 1 (31346.0) and was followed by the mean values from Ofege (16220.0) but in the second trial the highest mean nematode population was obtained from TME 1 (48227.0) but was followed by TMS 326 (22640.0) (Table 4.4).

In the second trial, the mean final soil nematode population was significantly higher in the nematode-infested soil than on denematized soil (Table 4.4). The highest mean soil nematode population came from the soil planted to TME 1 (48227.0) and was followed by the mean values from TMS 326 (22640.0). TMS 30572 cultivar had mean soil population of 12059 in nematode infested soil and a population of 15.0 in denematised soil (Table 4.4).

Table 4.2: Effect of *Meloidogyne incognita* on plant height, stem diameter and fresh shoot weight of five cassava cultivars in field.

	Height (cm)					Stem diameter (cm)					Fresh Shoot Weight (g)				
Treatment*	CV1	CV2	CV3	CV4	CV5	CV1	CV2	CV3	CV4	CV5	CV1	CV2	CV3	CV4	CV5
First Trial															
Denematised soil	298.5	280.3	313.0	310.3	270.8	4.4	4.5	4.5	5.0	5.7	6399.3	5437.0	5230.0	5460.0	5250.0
Nematode-infested soil	197.5	230.0	224.0	177.0	118.0	3.8	4.0	3.9	4.0	4.2	3338.3	4375.0	4275.0	1775.0	4377.0
LSD(P≤0.05)	41.7	86.1	61.1	66.2	3.2	1.1	0.1	0.6	0.0	0.3	1114.9	784.8	237.6	428.3	329.4
Second Trial															
Denematised soil	298.0	317.5	266.8	310.8	313.3	4.5	5.5	4.5	5.3	5.7	6602.8	6479.3	6413.8	6671.8	8077.0
Nematode-infested soil	197.5	225.0	251.5	112.5	192.8	4.0	3.9	3.8	3.8	4.2	2154.0	4215.0	4121.3	2162.3	4544.0
LSD(P≤0.05)	41.7	55.2	120.4	21.2	49.8	0.1	0.2	0.5	0.8	0.3	701.8	1475.3	994.0	709.5	2569.4

LSD for comparing treatment means within the same column

*Treatment key: CV1=4(2)1425, CV2=TMS326, CV3=OFEGE, CV4= TME 1, CV5=TMS 30572

Table 4.3: Effect of *Meloidogyne incognita* on number of tubers and fresh tuber weight of five cassava cultivars in field.

	Number of tubers					Fresh tuber weight (g)				
Treatment	4(2)1425	TMS326	OFEGE	TME 1	TMS 30572	4(2)1425	TMS326	OFEGE	TME 1	TMS 30572
First Trial										
Denematised soil	8.0	6.8	7.5	7.0	11.0	7389.0	8492.5	6925.0	7400.0	10212.0
Nematode-infested soil	4.3	3.2	3.8	2.5	4.8	3921.0	2412.5	3150.0	2907.0	3500.0
LSD(P≤0.05)	2.5	1.9	1.4	1.2	1.2	935.4	1854.1	382.0	374.8	715.8
Second Trial										
Denematised soil	8.0	7.8	7.8	7.8	12.0	7355.1	8109.8	7488.5	7910.0	11988.8
Nematode-infested soil	4.0	3.5	3.8	2.0	5.3	3834.7	3683.8	2939.8	2900.0	4242.0
LSD(P≤0.05)	2.3	1.7	1.6	1.2	3.1	901.4	1727.1	1233.4	712.3	1905.4

LSD for comparing treatment means within the same column

Plate 4.1: **A = TME 1 cassava tubers harvested from carbofuran-treated plot 12 months after planting; B = TME 1 cassava tubers harvested from *Meloidogyne incognita*-infested plot 12 months after planting.**

Table 4.4: Effect of *Meloidogyne incognita* on Galling indices and nematode populations of five cassava cultivars in field.

	Galling index*					Total number of nematodes				
Treatment	4(2)1425	TMS326	OFEGE	TME 1	TMS 30572	4(2)1425	TMS326	OFEGE	TME 1	TMS 30572
First Trial										
Denematised soil	0.0	0.3	0.0	0.8	0.3	1.0	10.0	14.0	6.0	88.0
Nematode-infested soil	3.0	3.0	3.5	4.5	2.5	13328.0	13664.0	16220.0	31346.0	9884.0
LSD(P≤0.05)	0.0	1.9	0.7	0.9	0.9	9257.6	15627.0	8084.4	12859.0	2540.7
Second Trial										
Denematised soil	0.3	0.8	0.0	0.5	0.3	9.0	46.0	22.0	10.0	15.0
Nematode-infested soil	3.0	3.3	3.5	5.0	2.0	13300.0	22640.0	14342.0	48227.0	12059
LSD(P≤0.05)	1.9	0.9	0.7	0.7	0.6	9245.0	12486.0	5444.6	37725.0	3489.0

LSD for comparing treatment means within the same column

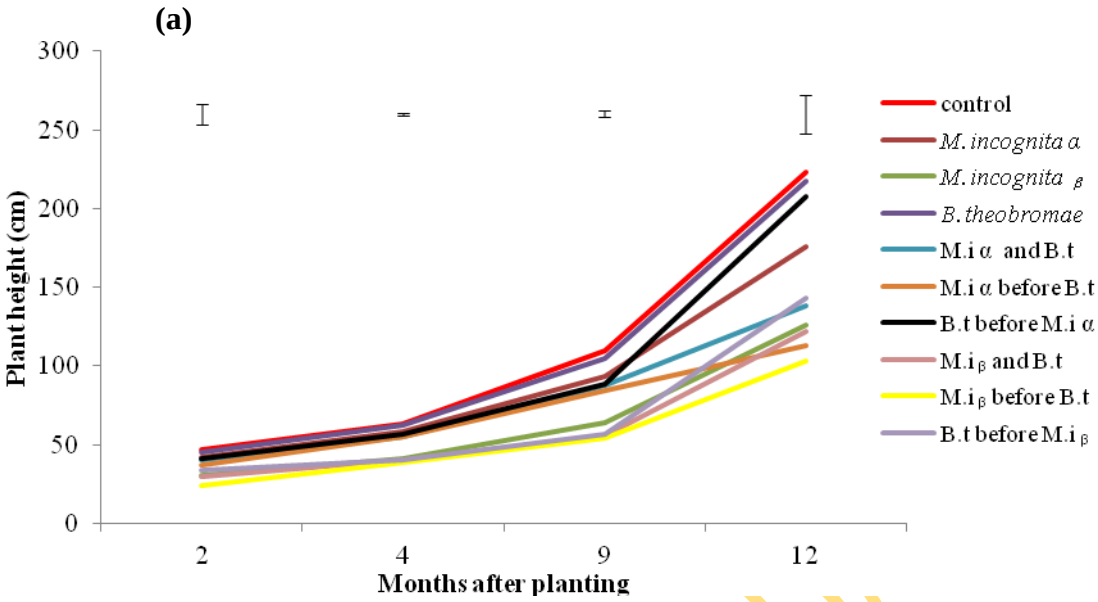
* 0 = no galls; 1 = 1-10 % of the root system galled; 2 = 11 – 20% of the root system galled; 3 = 21 -70% of the root system galled; 4 = 71- 90% of the root system galled; 5 = greater than 90% of the root system galled.

4.2: Interaction *between Meloidogyne incognita* and *Botryodiplodia theobromae* on growth, yield and nematode reproduction on cassava cultivars (Pot)

4.2.1: Effect of *M. incognita* and *B. theobromae* interaction on plant heights of three cassava cultivars.

The mean plant height for various treatments recorded at two, four, nine and twelve months after planting were significantly different (P≤0.05) from one another for the three cultivars used in the experiment. In ofege, at 2 MAP, the heights of plants inoculated with *B. theobromae* alone (44.9cm) and the control (46.6cm) recorded higher mean heights that were not significantly different from each other but significantly higher than treatments inoculated with *M. incognita* alone (30.2cm), *M. incognita* plus *B. theobromae* at two weeks after nematode inoculation (24.2cm) , *B. theobromae* plus *M. incognita* at two weeks after the fungus inoculation (33.3cm) and *B. theobromae* plus *M. incognita* inoculated simultaneously (29.5cm) (Fig. 4.16). The lowest significant plant height was recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (33.3cm) (Fig. 4.16). This trend continued until the end of the experiment 12 MAP, when the control plants recorded the highest mean height (222.5cm), followed by *B. theobromae* alone (201.2cm). These values differed significantly (P≤0.05) from the mean heights of other treatments with the lowest significant plant height recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (102.8cm). This trend was the same for the two trials (Fig. 4.16).

In TME 1, at 2 MAP, the heights of plants inoculated with *B. theobromae* alone (44.8cm) and the control (47.2cm) recorded higher mean heights that were not significantly different from each other but significantly higher than treatments inoculated with *M. incognita* alone (30.0cm), *M. incognita* plus *B. theobromae* at two weeks after nematode inoculation (23.7cm) , *B. theobromae* plus *M. incognita* at two weeks after the fungus inoculation (31.8cm) and *B. theobromae* plus *M. incognita* inoculated simultaneously (30.1cm) (Fig. 4.17).



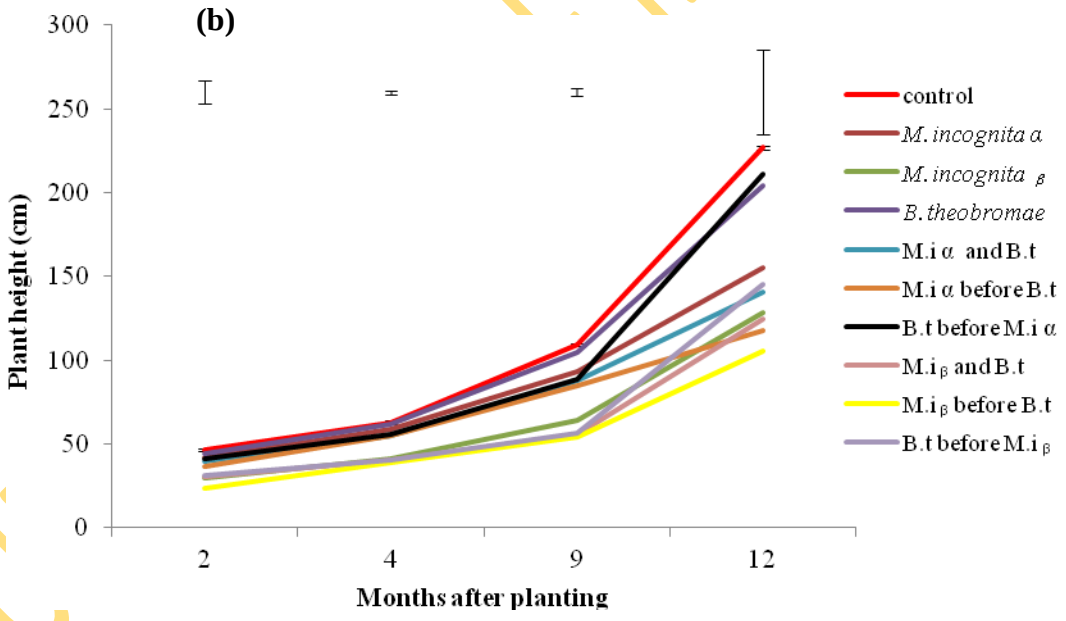


Figure 4.16: Effects of *M. incognita* and *B. theobromae* interaction on height of Ofege cassava cultivar. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

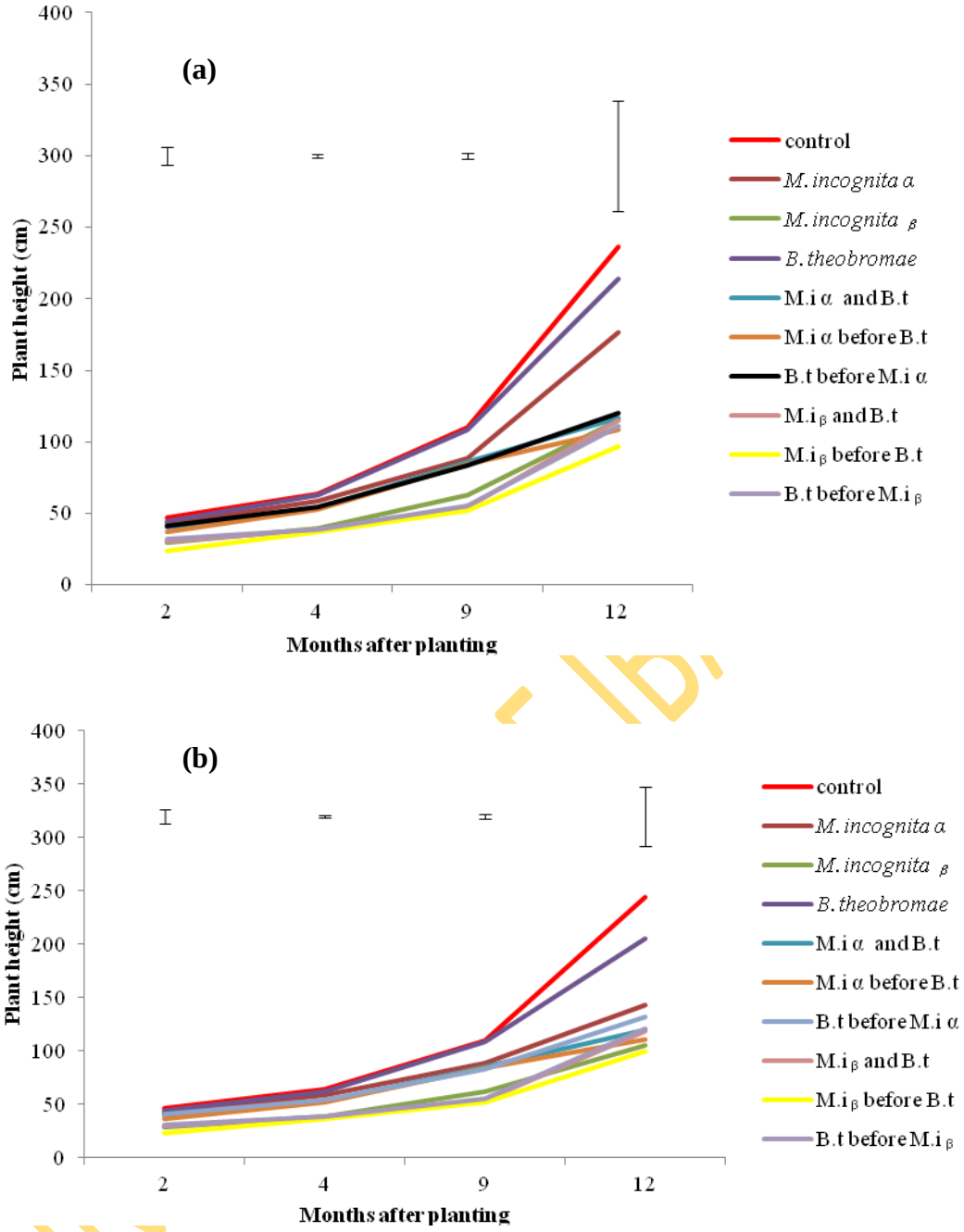


Fig 4.17 : Effects of *M. incognita* and *B. theobromae* interaction on height of TME 1 cassava cultivar. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

. Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

The lowest significant plant height was recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (23.7cm) (Fig. 4.17). At 12 MAP, the control plants recorded the highest mean height (236.3cm), followed by *B. theobromae* alone (214.1 cm). These values differed significantly (P≤0.05) from the mean heights of other treatments with the lowest significant plant height recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (96.8 cm). This trend was the same for the two trials (Figs. 4.17).

In TMS30572, at 2 MAP, the heights of plants inoculated with *B. theobromae* alone (43.9 cm) and the control (44.5 cm) recorded higher mean heights that were not significantly different from each other but significantly higher than treatments inoculated with *M. incognita* alone (32.4 cm), *M. incognita* plus *B. theobromae* at two weeks after nematode inoculation (26.6 cm) , *B. theobromae* plus *M. incognita* at two weeks after the fungus inoculation (32.9 cm) and *B. theobromae* plus *M. incognita* inoculated simultaneously (30.1 cm) (Fig. 4.18). The lowest significant plant height was recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (26.6 cm) (Fig. 4.18). This trend continued until the end of the experiment 12 MAP, when the control plants recorded the highest mean height (260.1 cm), followed by *B. theobromae* alone (243.5 cm). These values differed significantly (P≤0.05) from the mean heights of other treatments with the lowest significant plant height recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (103.8 cm). This trend was the same for the two trials (Fig. 4.18).

4.2.2: Effect of *M. incognita* and *B. theobromae* interaction on fresh shoot weights of three cassava cultivars.

For both trials, the mean fresh shoot weights differed significantly among the treatments. The mean fresh shoot weight for various treatments recorded at two, four, nine and twelve months after planting were significantly different (P≤0.05) from one another in all the cultivars used in the experiment. In ofege, at 2 MAP, the mean shoot weight of plants inoculated with *B. theobromae* alone (27.5 g) and the control (29.7 g) recorded higher mean shoot weight that were not significantly

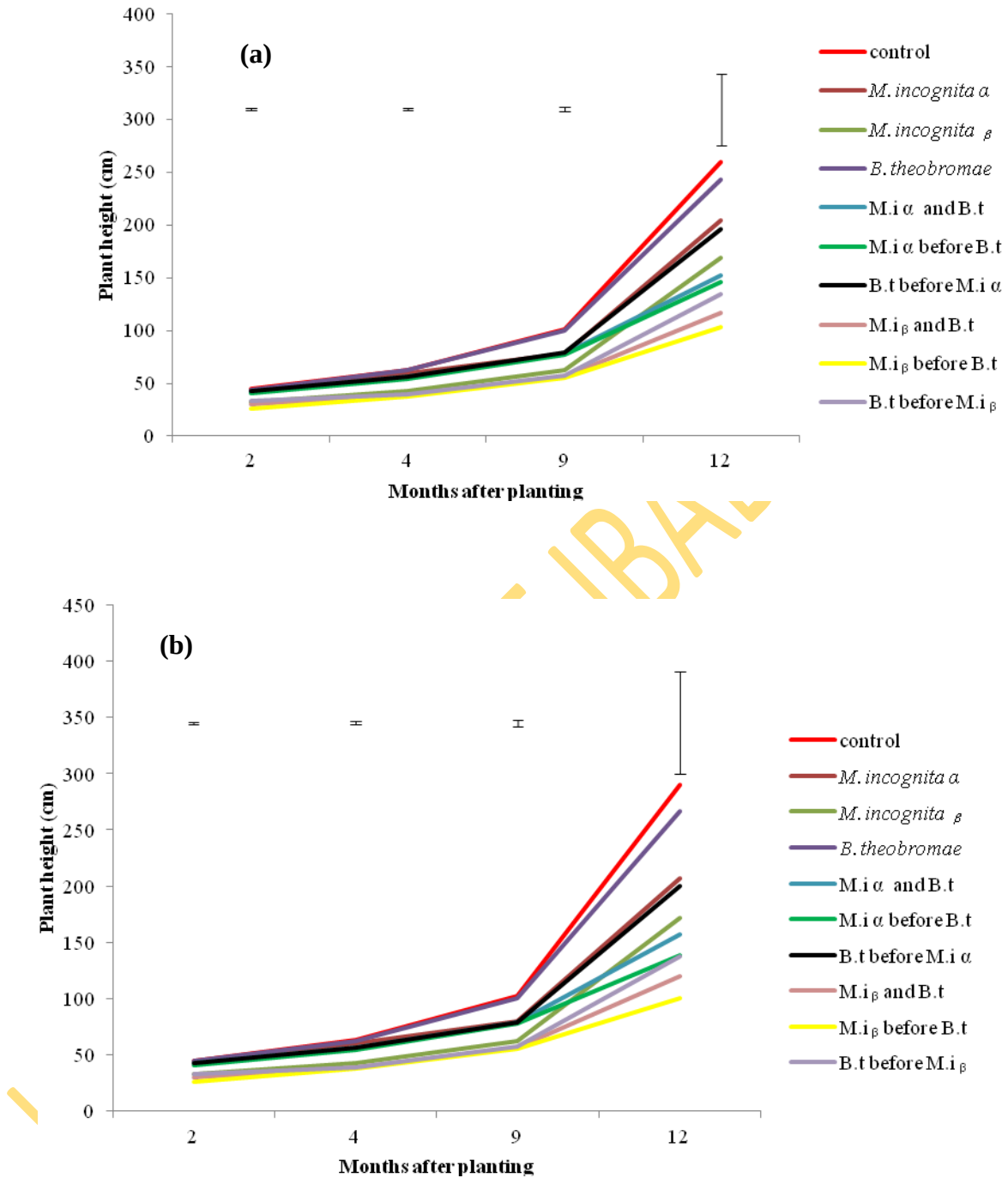


Fig 4.18: Effects of *M. incognita* and *B. theobromae* interaction on height of TMS 30572 cassava cultivar. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

different from each other but significantly higher than treatments inoculated with *M. incognita* alone (18.8 g), *M. incognita* plus *B. theobromae* at two weeks after nematode inoculation (14.9 g) , *B. theobromae* plus *M. incognita* at two weeks after the fungus inoculation (18.5 g) and *B. theobromae* plus *M. incognita* inoculated simultaneously (18.1 g) (Fig. 4.19). The lowest significant mean shoot weight was recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (14.9 g) (Fig. 4.19). This trend continued until the end of the experiment at 12 MAP, when the control plants recorded the highest mean height (203.4 g), followed by *B. theobromae* alone (200.8 g). These values differed significantly (P≤0.05) from the mean shoot weight of other treatments with the lowest significant plant height recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (9 vbbb
9.8 g). This trend was the same for the two trials (Fig. 4.19).

In TME 1, at 2 MAP, the mean shoot weight of plants inoculated with *B. theobromae* alone (27.3 g) and the control (30.0 g) recorded higher mean shoot weight that were significantly different from each other and significantly higher than treatments inoculated with *M. incognita* alone (18.1 g), *M. incognita* plus *B. theobromae* at two weeks after nematode inoculation (14.2 g), *B. theobromae* plus *M. incognita* at two weeks after the fungus inoculation (18.3g) and *B. theobromae* plus *M. incognita* inoculated simultaneously (17.3 g) (Fig. 4.20). The lowest significant mean shoot weight was recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (14.2 g) (Fig. 4.20). This trend continued until the end of the experiment at 12 MAP, when the control plants recorded the highest mean shoot weight (202.8 g), followed by *B. theobromae* alone (196.6 g) and are not significantly different from each other but differed significantly (P≤0.05) from the mean heights of other treatments with the lowest significant plant height recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (76.0 g). This trend was the same for the two trials (Fig. 4.20).

In TMS 30572, at 2 MAP, the mean shoot weight of plants inoculated with *B. theobromae* alone (30.0 g) and the control (28.4 g) recorded higher mean shoot weight that were significantly different from each other and significantly higher than treatments inoculated with *M. incognita* alone (23.0 g), *M. incognita* plus *B.*

theobromae at two weeks after nematode inoculation (19.9 g), *B. theobromae* plus *M. incognita* at two weeks after the fungus inoculation (20.9g) and *B. theobromae* plus *M. incognita* inoculated simultaneously (20.9 g) (Fig. 4.21). The lowest significant mean shoot weight was recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (19.9 g) (Fig. 4.21). This trend continued until the end of the experiment at 12 MAP, when the control plants recorded the highest mean shoot weight (386.4 g), followed by *B. theobromae* alone (184.9 g). These values differed significantly ($P \leq 0.05$) from the mean shoot weight of other treatments with the lowest significant plant height recorded in plants inoculated with 10000 *M. incognita* and *B. theobromae* with the fungus inoculated two weeks after the nematode (102.0g). This trend was the same for the two trials (Fig. 4.21).

4.2.3: Effects of *M. incognita* and *B. theobromae* interaction on stem diameter of three cassava cultivars

The mean stem diameter recorded for various treatments were significantly different from each other ($P \leq 0.05$). In Ofege, the highest mean value was from the uninoculated control throughout at 2 MAP (1.0cm), 9 MAP (2.0cm) and 12 MAP (2.3cm) (Fig 4.22). This was followed by the treatment with *B. theobromae* inoculation alone, and then 1,000 eggs of *M. incognita* alone. However, at 4 MAP the highest significant mean value was from plant inoculated with *B. theobromae* alone. In the second trial the highest mean value was from the uninoculated control throughout the period of study.

In TME 1, the highest mean value was from the uninoculated control throughout at 2 MAP (1.0cm), 4 MAP (1.5cm), 9 MAP (2.1cm) and 12 MAP (2.3cm) (Fig 4.23). This was followed by the treatment with *B. theobromae* inoculation alone, and then 1,000 eggs of *M. incognita* alone.

In TMS 30572, the highest mean value was from the uninoculated control throughout at 2 MAP (1.0cm), 4 MAP (1.6cm), 9 MAP (2.0cm) and 12 MAP (2.5cm) (Fig 4.24). This was followed by the treatment with *B. theobromae* inoculation alone which was not significantly different from the uninocualted control, except at 12 MAP (Fig. 4.24).

At 2 MAP, all three cultivas with the nematode fungus combination had the lowest significant stem diameter size and they were not significantly different from

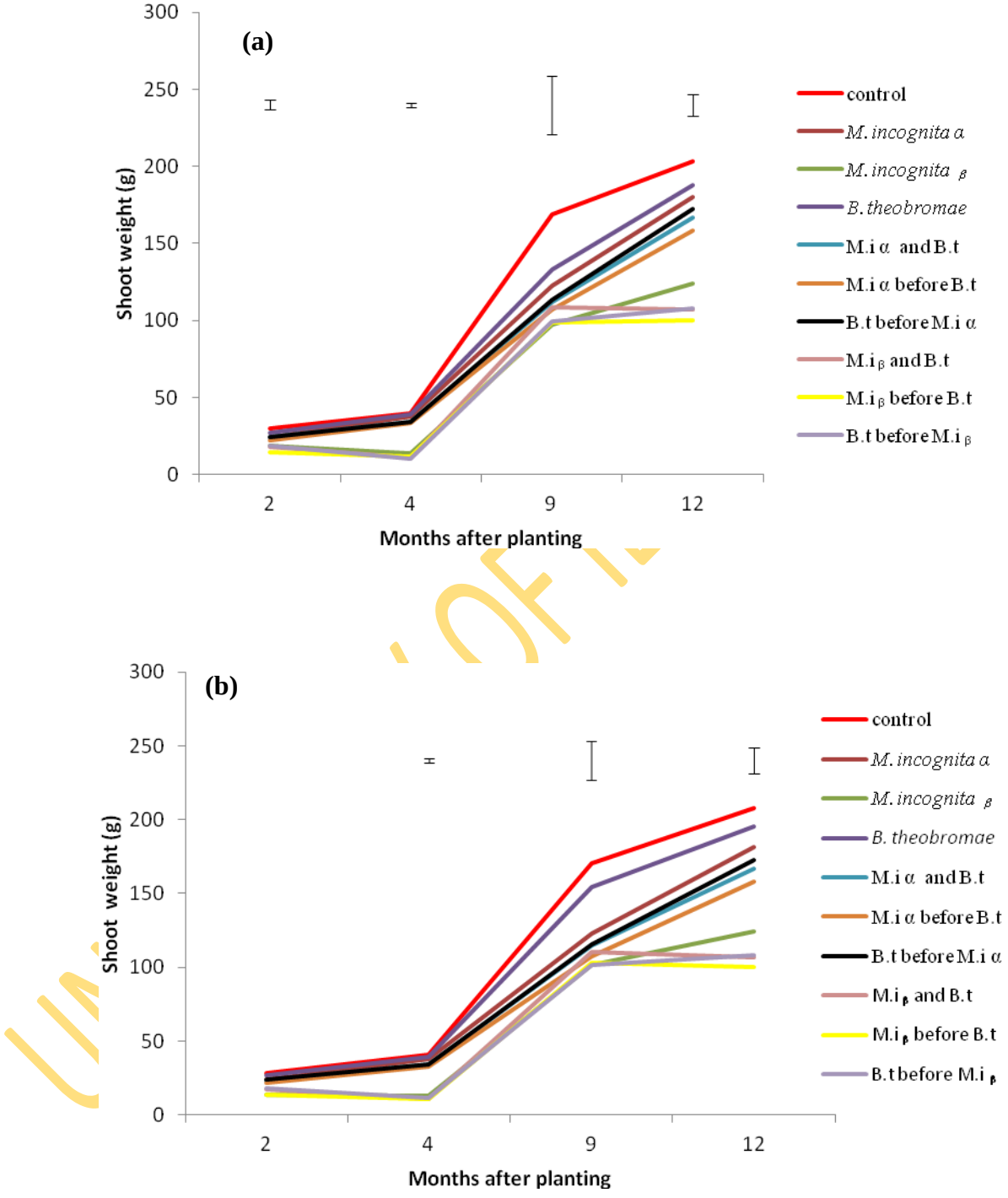


Fig 4.19: Effects of *M. incognita* and *B. theobromae* interaction on shoot weight of Ofege cassava cultivar. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i egg .i eggs.

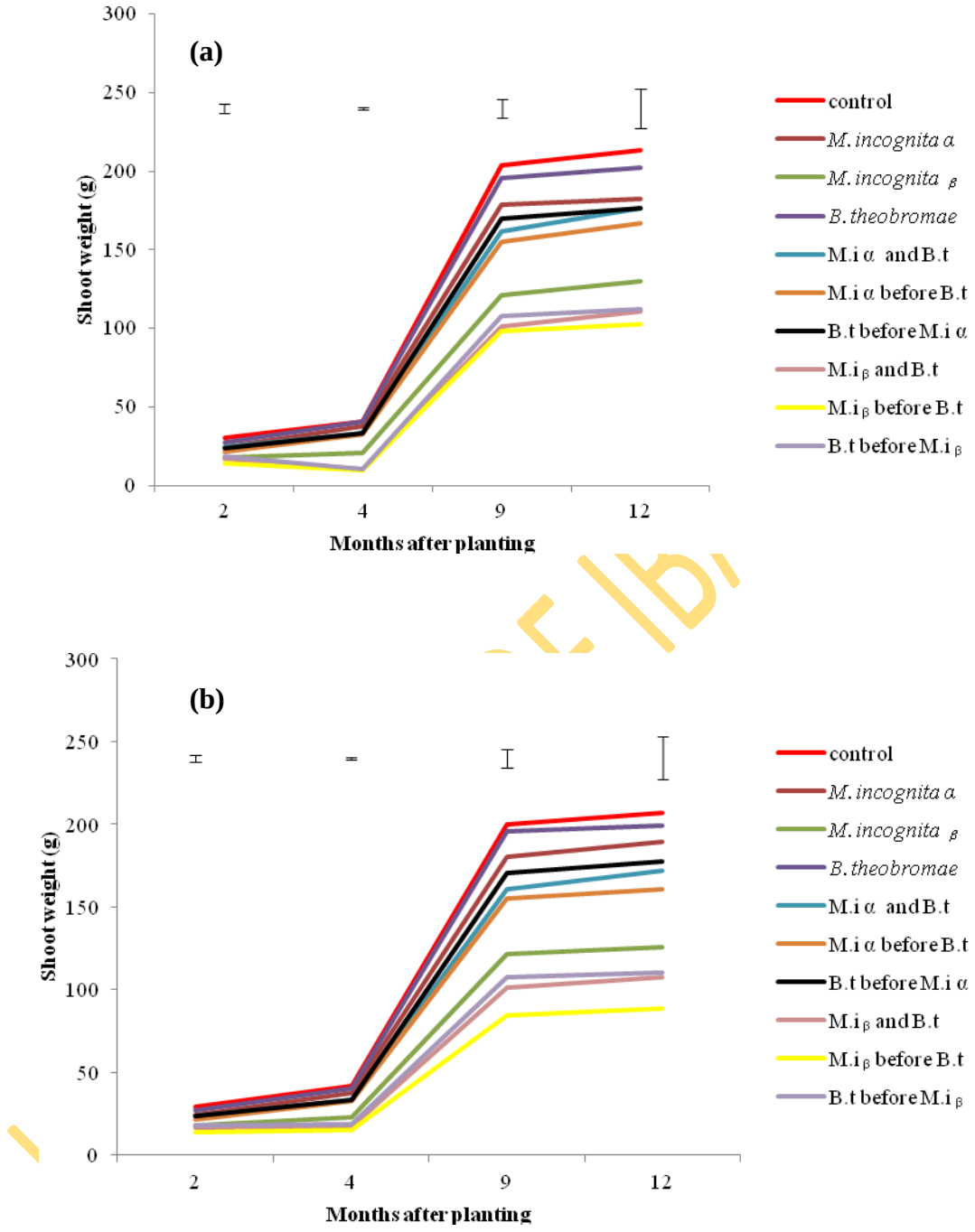


Fig 4.20: Effects of *M. incognita* and *B. theobromae* interaction on shoot weight of TME 1 cassava cultivar. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

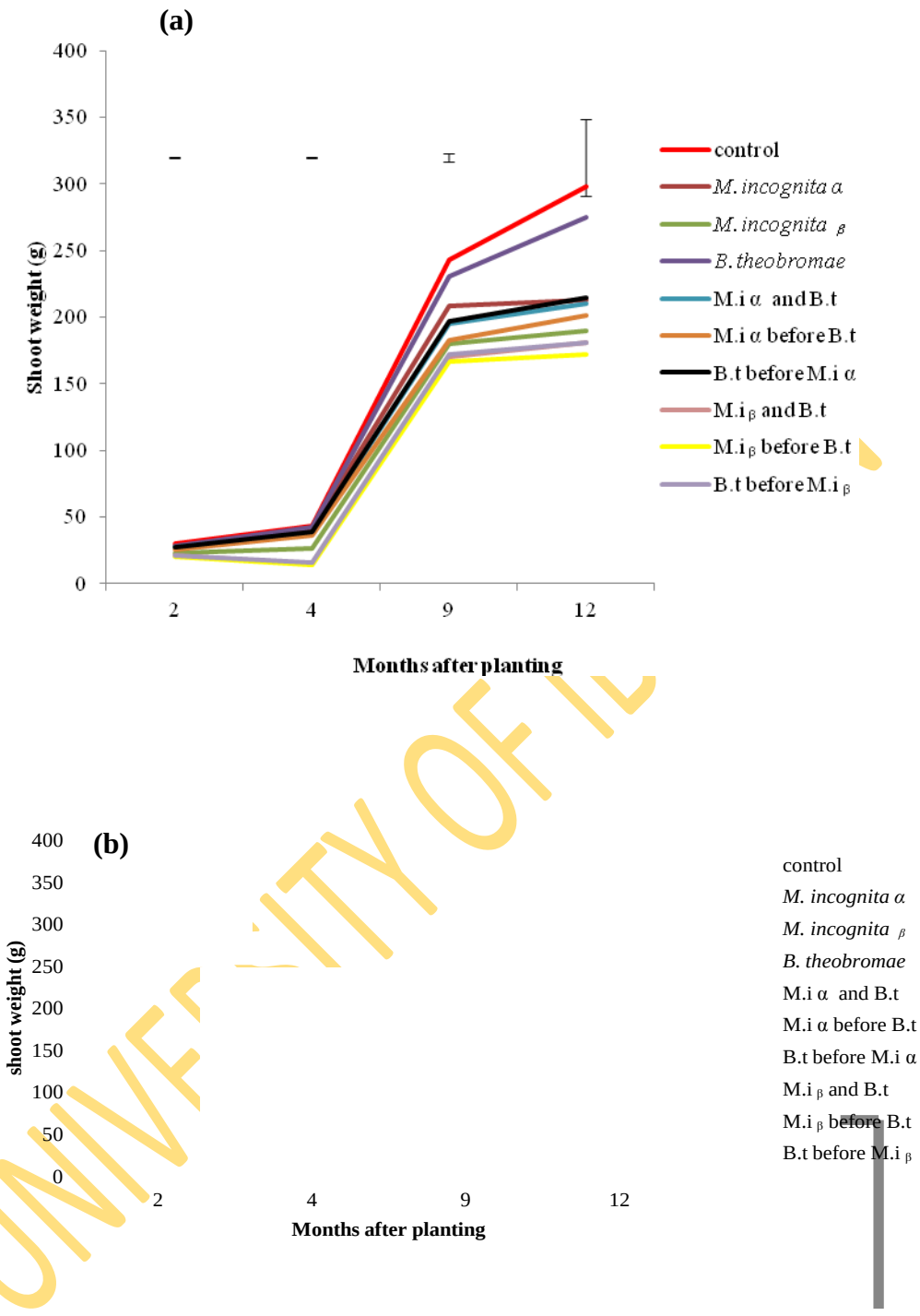
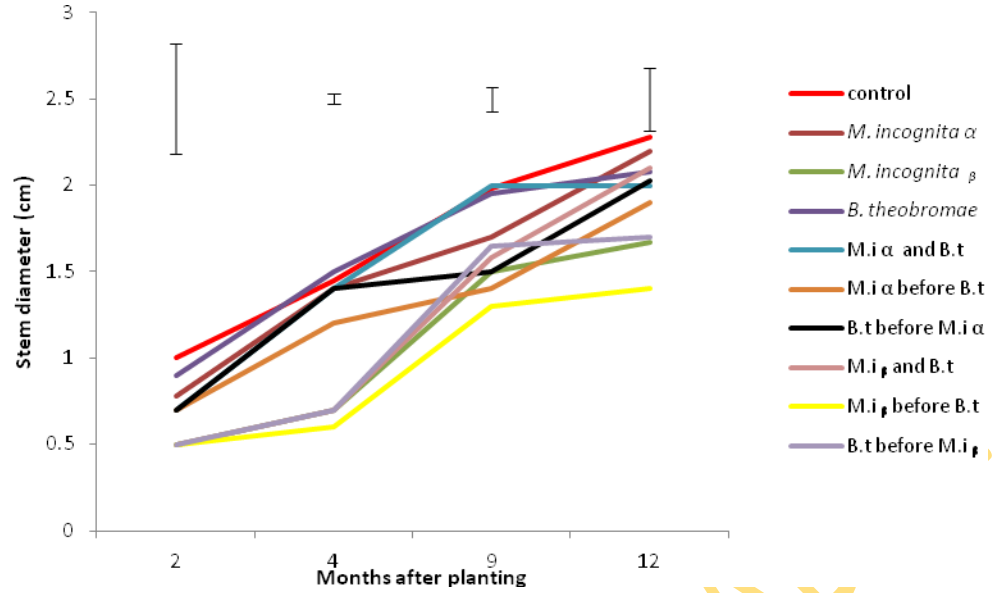


Fig 4:21 Effects of *M. incognita* and *B. theobromae* interaction on shoot weight of TMS 30572 cassava cultivar. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time. Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.



(b)

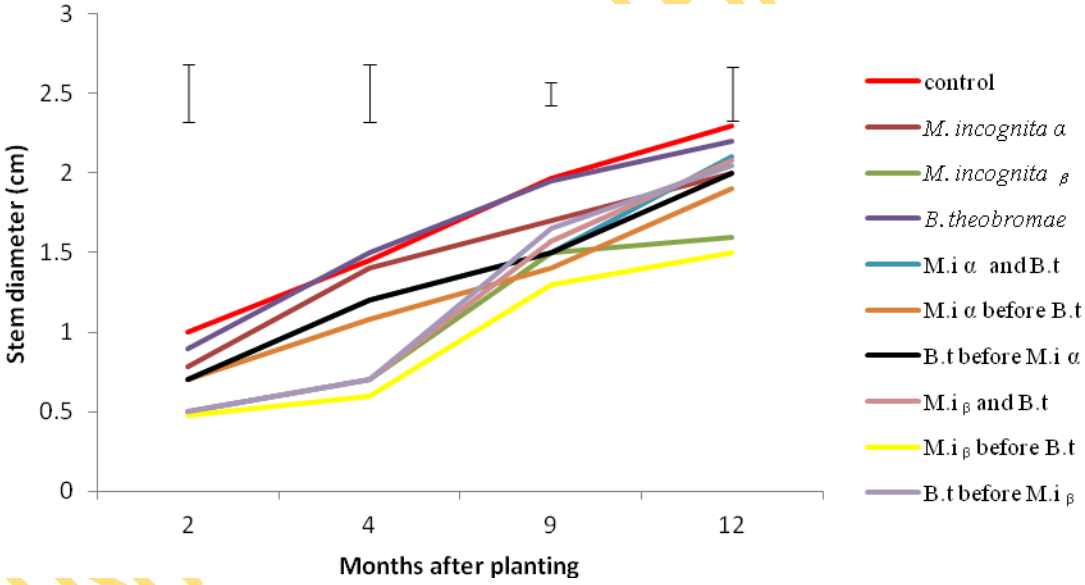


Fig 4.22: Effects of *M. incognita* and *B. theobromae* interaction on stem diameter of Ofege cassava cultivar. (a) = first trial, (b) = second trial.

LSD bar is for comparing treatment means at each specific sampling time. Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

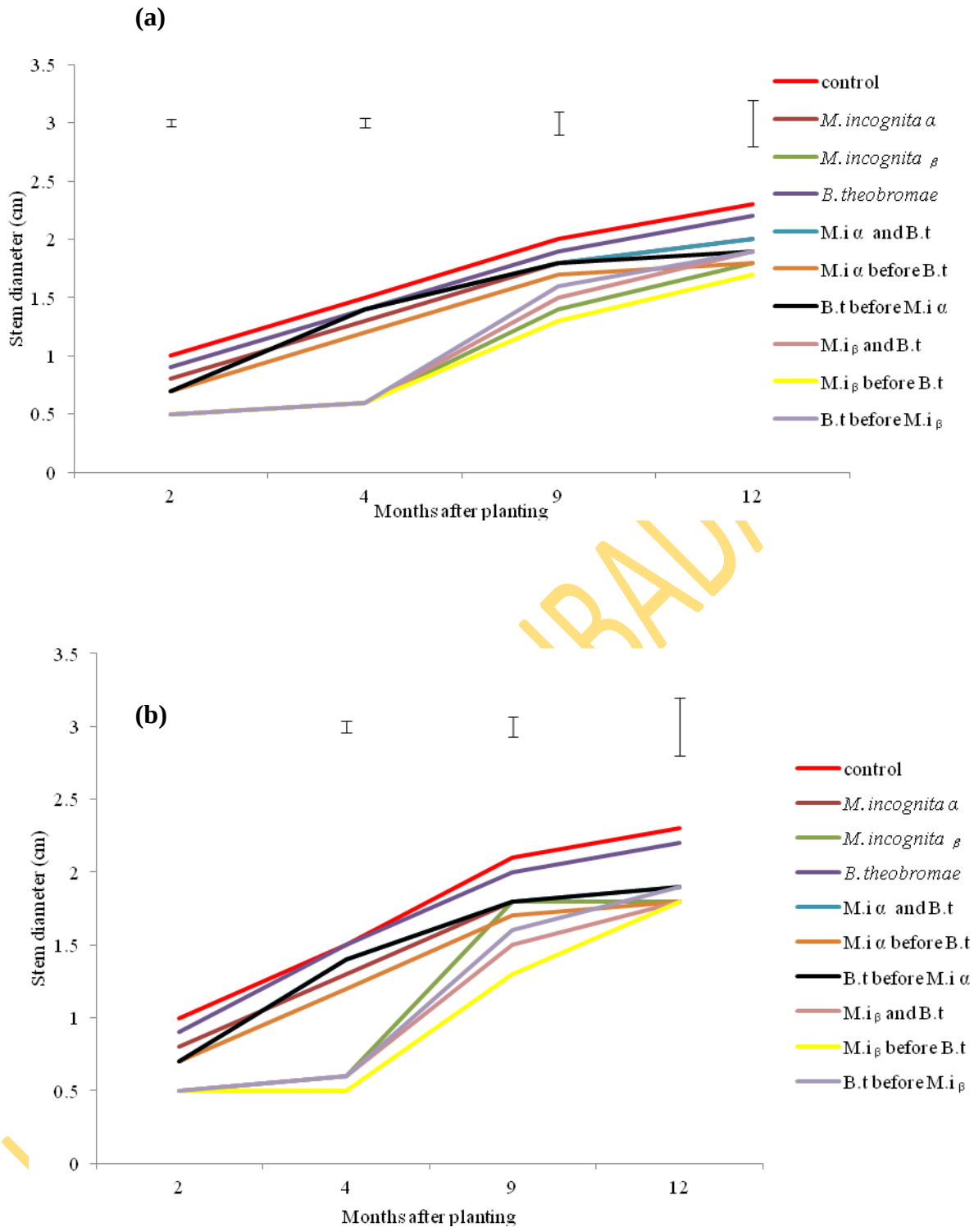


Fig 4.23: Effects of *M. incognita* and *B. theobromae* interaction on stem diameter of TME 1 cassava cultivar. (a) = first trial, (b) = second trial

LSD bar is for comparing treatment means at each specific sampling time. Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

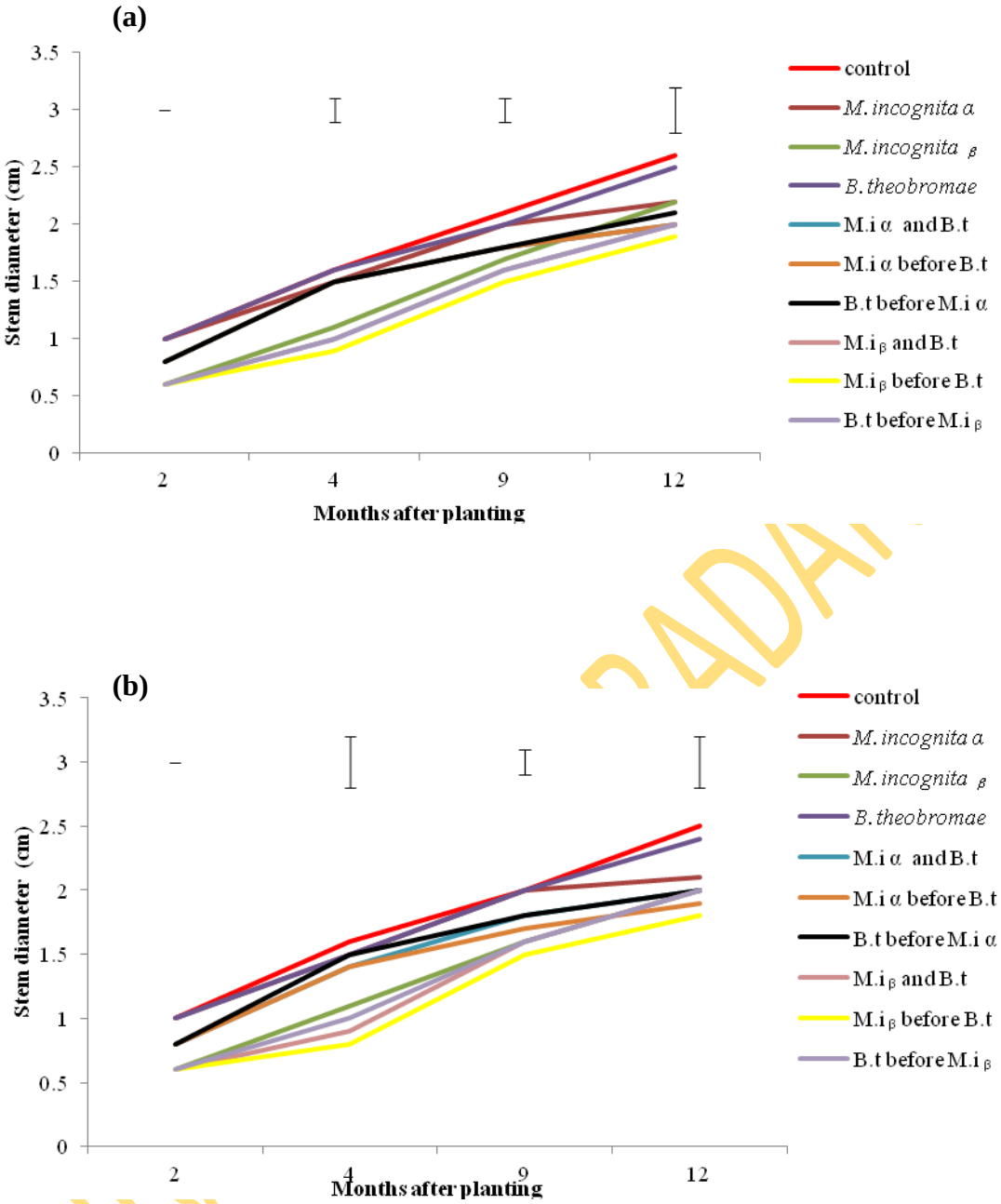


Fig 4.24: Effects of *M. incognita* and *B. theobromae* interaction on stem diameter of TMS 30572 cassava cultivar.

LSD bar is for comparing treatment means at each specific sampling time. Treatment: M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

one another (Figs. 4.22, 4.23 and 4.24). By the 9th month and 12th month after planting, the lowest values from the study was recorded from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode. This trend was recorded also in the second trial.

4.2.4: Effect of *M. incognita* and *B. theobromae* interaction on yield of three cassava cultivars

The cultivar Ofege had the highest mean number of tubers recorded from the uninoculated plants at 2, 4, 9, and 12 months after planting (2.3, 4.0, 5.0 and 5.8) (Table 4.5), though at 4 and 9 months after planting the values were the same with those of plants inoculated with *B. theobromae* alone (4.0 and 5.0) (Table 4.5). At 2 months after planting the number of tubers initiated from the uninoculated control (2.3) was significantly higher than all other treatments. The lowest mean number of tubers was recorded from plants inoculated with the combination of 10000 *M. incognita* eggs and *B. theobromae* regardless of the fungus coming 2 weeks before or after or simultaneously with the nematodes (0.0).(Table 4.5). At the 4th and 9th month after planting, there was no significant difference from the values recorded from the control (4.0, 5.0) and plants inoculated with *B. theobromae* alone (4.0, 5.0), and plants inoculated with 1000 *M. incognita* eggs + *B. theobromae* in combination with the fungus inoculated either 2 weeks before or 2 weeks after or simultaneous with the nematode (4.0, 5.0) all through the study and this was the trend in the two trials. The highest mean tuber weight was produced by uninoculated plants but was not statistically different from plants inoculated with *B. theobromae* alone at 2 MAP and 12 MAP (4.3 g and 128.6 g). The lowest mean tuber weight was recorded from plants inoculated with the combination of 10000 *M. incognita* eggs and *B. theobromae* with the fungus inoculated 2 weeks after the nematodes and this was the trend all through the period of the study, and also in both trials (90.2 g) (Table 4.5).

The mean number of rotted tubers also differed statistically ($P \leq 0.05$) from each other except at 2 months after inoculation when there was no rotted tuber observed (0) (Table 4.5) The least number of rotted tubers came from the uninoculated control, 1000 *M. incognita* eggs alone and 10000 *M. incognita* eggs alone and *B. theobromae* alone at 4 MAP (0.0) and the values were not different from each other. Also at 9 MAP the least number of rotted tubers came from the uninoculated control, 1000 *M. incognita* eggs alone and 10000 *M. incognita* eggs

alone (0.0) (Table 4.5). The highest mean number of rotted tubers were observed in plants inoculated with 10,000 *M. incognita* + *B. theobromae* with the fungus inoculated 2 weeks after the nematode inoculation at 9 and 12 months after planting (3.0 and 2.7) and were significantly different from other treatments except at 2 MAP when there was no tuber rot and at 4 MAP when it was not significantly different from treatments with combination of nematodes and fungus (1.0). This was the trend in the second trial. (Table 4.5)

In TME 1, the highest mean number of tubers was recorded from the uninoculated plants at 2, 4, 9, and 12 months after planting (3.3, 5.5, 6.3 and 8.3 respectively). At 2 months after planting the number of tubers harvested from the uninoculated control (3.3) was significantly different from all other treatments. At 4 MAP the number of tubers obtained from the uninoculated control (4.0) in the first trial was the same as the mean number of tubers produced by plants inoculated either singly with 1000 M. incognita alone or in combination with fungus. At 9 MAP the number of tubers produced by the uninoculated control in the first trial (5.0) was the same as treatment with 1000 eggs of *M. incognita* alone and treatment *B. theobromae* alone. But in the second trial the uninoculated control had the highest number of tubers (6.3). The lowest mean number of tubers was recorded from plants inoculated with the combination of 10000 *M. incognita* eggs and *B. theobromae* with the fungus coming 2 weeks after the nematodes (3.0) (Table 4.6).

The highest mean tuber weight was produced by uninoculated plants at 2, 4, 9 and 12 MAP (4.9 g, 10.0 g, 102.0g and 129.4 g). The lowest mean tuber weight at 4, 9 and 12 MAP were recorded from plants in soil inoculated with the combination of 10000 *M. incognita* eggs and *B. theobromae* with the fungus inoculated 2 weeks after the nematodes (2.1 g, 30.9 g and 54.9 g) and this was the trend all through the period of the study and also in both trials.

The mean number of rotted tubers also differed statistically ($P \leq 0.05$) from each other except at 2 MAP when there was no rotted tuber observed (0.0). The least number of rotted tubers came from the uninoculated control (0.0), 1000 *M. incognita* eggs alone (0.0) and 10000 *M. incognita* eggs alone (0.0) and *B. theobromae* alone (0.0) at 4 MAP and the values were not different from each other. Also at 9 MAP the least number of rotted tubers came from the uninoculated control (0.0), 1000 *M. incognita* eggs alone (0.0) and 10000 *M. incognita* eggs alone (0.0). The highest mean number of rotted tubers was observed in plants inoculated with 10000 *M. incognita* + *B. theobromae* with the fungus inoculated 2 weeks after the nematode inoculation (2.0, 2.5 and 3.3) and it was significantly different from other treatments

except at 2 MAP when there was no tuber rot. At 4 MAP, the first trial of the study showed no significant difference in the number of rotted tubers recorded for plants planted on soil inoculated with *M. incognita* + *B. theobromae* with the fungus inoculated 2 weeks after the nematode and all other treatments with nematode – fungus combination, but in the second trial it was significantly different from all other treatments. It was statistically higher than all other treatments (Tables 4.6).

In TMS 30572, the highest mean number of tubers was recorded from the uninoculated plants at 2, 4, 9, and 12 months after planting (2.0, 3.3, 7.8 and 8.0) though at 2 MAP it has the same value with plants treated with *B. theobromae* alone (2.0). At 4 MAP the number of tubers obtained from the uninoculated control was not significantly different from the mean number of tubers produced by plants inoculated with *B. theobromae* alone (3.3). At 9 MAP the lowest mean number of tubers was produced by plants inoculated with 10,000 *M. incognita* + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (5.0). The lowest mean number of tubers was recorded from plants inoculated with the combination of 10,000 *M. incognita* eggs and *B. theobromae* with the fungus coming 2 weeks after the nematodes all through the period of the experiment (0.0, 2.3, 5.0 and 4.3) (Table 4.7). The highest mean tuber weights were produced by uninoculated plants at 2, 4, 9, and 12 MAP (6.7 g, 13.5 g, 139.2 g and 188.6 g respectively). The lowest mean tuber weight was recorded from plants from soil inoculated with the combination of 10,000 *M. incognita* eggs and *B. theobromae* with the fungus inoculated 2 weeks after the nematodes (0.0 g , 5.0 g, 56.7 g and 68.1 g).

The mean number of rotted tubers also differed statistically ($P \leq 0.05$) from each other except at 2 MAP when there was no rotted tuber observed (0.0). At 4 MAP the highest number of rotted tubers was obtained from plants treated with simultaneous inoculation of 10000 *M. incognita* eggs + *B. theobromae* (1.0) and it was not significantly different from the mean number of rotted tubers obtained from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (0.8). Also at 9 MAP the highest mean number of rotted tubers came from plants inoculated with 10,000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (1.0). At 12 MAP, the highest mean number of rotted tubers was produced by plants inoculated with 10000 *M. incognita* eggs+*B. theobromae* (simultaneously) and 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (1.3). The values from both treatments were significantly different from other treatments (Table 4.7).

Table 4.5: Effects of *M. incognita* and *B. theobromae* interaction on yield parameters of cassava cultivar (OFEGE) in a pot experiment

	Number of Tubers				Tuber weight (g)				Number of Rotted Tubers			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial												
Control	2.3	4.0	5.0	5.8	4.3	9.7	99.8	128.6	0.0	0.0	0.0	0.0
M. incognita α	2.0	4.0	4.8	4.5	3.1	7.2	67.9	117.4	0.0	0.0	0.0	0.0
M. incognitai β	0.0	3.5	4.0	3.3	0.0	2.6	40.5	90.8	0.0	0.0	0.0	0.0
B. theobromae	2.0	4.0	5.0	5.5	4.1	9.6	74.8	124.6	0.0	0.0	0.3	0.3
M.i α and B.t	1.0	4.0	5.0	4.0	2.1	5.3	58.4	108.7	0.0	1.3	1.0	1.0
M.i α before B.t	1.0	4.0	5.0	4.3	1.9	4.6	51.8	102.8	0.0	1.3	1.7	1.5
B.t before M.iα	1.0	4.0	5.0	4.0	2.6	7.0	68.0	117.6	0.0	0.7	1.3	0.7
M.i β and B.t	0.0	2.3	4.0	3.3	0.0	2.5	40.0	121.0	0.0	1.0	2.5	1.7
M.i β before B.t	0.0	2.0	3.0	2.8	0.0	2.4	35.3	90.2	0.0	1.0	3.0	2.7
B.t before M.i β	0.0	2.2	4.0	3.5	0.0	2.6	39.8	94.1	0.0	1.0	2.0	1.3
LSD(P≤0.05)	0.2	0.4	0.2	0.9	0.4	0.1	0.8	15.1	0.0	0.5	0.5	0.7
Second trial												
Control	2.5	4.8	6.0	7.8	7.7	17.7	113.5	162.1	0.0	0.0	0.0	0.0
M. incognita α	2.3	4.5	5.3	5.0	6.1	14.5	77.0	144.8	0.0	0.0	0.0	0.0
M. incognita β	0.3	3.5	4.8	4.3	0.6	10.1	49.7	116.1	0.0	0.0	0.0	0.0
B. theobromae	2.3	4.8	5.3	5.8	7.4	16.7	83.6	132.2	0.0	0.0	0.3	1.0
M.i α and B.t	1.3	4.5	5.3	5.8	5.1	12.7	67.1	129.0	0.0	1.0	0.8	1.3
M.i α before B.t	1.3	4.3	5.3	5.3	5.4	11.8	60.8	1137.5	0.0	1.0	1.5	1.8
B.t before M.iα	1.3	4.5	5.3	6.0	5.8	14.4	76.8	137.5	0.0	0.8	1.0	1.3
M.i β and B.t	0.3	2.7	4.8	4.8	0.6	10.0	48.0	121.4	0.0	1.0	2.3	2.5
M.i β before B.t	0.3	3.0	4.5	4.0	0.5	9.9	44.4	103.3	0.0	1.0	2.5	2.5
B.t before M.i β	0.3	3.0	4.8	5.0	0.6	9.9	48.2	113.4	0.0	1.0	1.8	2.3
LSD(P≤0.05)	0.8	1.2	1.3	0.8	2.8	7.2	9.3	16.4	0.0	0.7	0.9	0.7

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, $5X10^5$ *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

Table 4.6: Effects of *M. incognita* and *B. theobromae* interaction on yield parameters of cassava cultivar TME 1 in a pot experiment

	Number of tubers				Weight of tubers (g)				Number of Rotted tubers			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial												
Control	2.8	4.0	5.0	5.5	4.9	10.0	102.0	129.4	0.0	0.0	0.0	0.0
M. incognita α	1.3	4.0	5.0	5.3	2.0	7.2	65.9	117.4	0.0	0.0	0.0	0.0
M. incognita β	0.0	3.8	4.0	3.3	0.0	2.4	36.8	88.2	0.0	0.0	0.0	0.0
B. theobromae	2.0	4.0	5.0	5.0	3.9	9.5	91.4	124.6	0.0	0.0	0.8	0.0
M.i α and B.t	1.0	4.0	4.0	4.0	1.4	5.2	56.3	106.9	0.0	1.3	1.3	1.0
M.i α before B.t	1.0	4.0	4.0	3.5	1.2	4.8	50.6	102.8	0.0	1.3	2.0	1.3
B.t before M.i α	1.0	4.0	4.0	3.8	1.4	6.1	64.7	106.4	0.0	1.0	1.5	1.0
M.i β and B.t	0.0	2.5	4.0	3.3	0.0	2.4	37.1	121.0	0.0	1.0	2.5	2.0
M.i β before B.t	0.0	2.3	3.0	2.5	0.0	2.1	30.9	54.9	0.0	1.3	3.0	2.5
B.t before M.i β	0.0	2.0	4.0	3.3	0.0	2.5	41.2	87.9	0.0	1.0	2.3	1.3
LSD(P≤0.05)	0.3	0.6	0.0	0.9	1.0	0.2	2.4	12.3	0.0	0.5	0.7	1.0
Second Trial												
Control	3.3	5.5	6.3	8.3	8.8	20.5	116.1	172.2	0.0	0.0	0.0	0.0
M. incognita α	1.3	4.8	5.5	6.3	5.6	14.7	75.5	150.1	0.0	0.0	0.0	0.0
M. incognita β	1.0	4.3	4.3	4.5	2.2	9.8	46.6	123.0	0.0	0.0	0.0	0.0
B. theobromae	2.3	4.3	5.5	7.0	7.2	18.5	100.7	157.5	0.0	0.0	0.8	1.3
M.i α and B.t	1.0	4.5	4.5	6.0	5.2	12.7	65.6	138.3	0.0	1.0	1.0	1.5
M.i α before B.t	1.0	4.3	4.0	4.5	5.0	12.3	60.7	135.0	0.0	1.0	1.3	1..5
B.t before M.i α	1.0	4.5	4.5	5.3	5.6	11.1	74.0	139.8	0.0	0.5	1.0	1.5
M.i β and B.t	0.3	3.3	4.5	4.5	1.1	9.9	47.0	108.6	0.0	1.3	2.0	2.8
M.i β before B.t	0.3	3.0	3.5	4.0	0.8	9.6	40.0	86.5	0.0	2.0	2.5	3.3
B.t before M.i β	0.3	2.5	4.5	5.8	1.1	10.0	50.7	105.3	0.0	1.0	1.8	2.8
LSD(P≤0. 05)	0.8	1.6	1.2	1.5	3.6	8.0	10.9	8.3	0.0	0.6	0.7	0.8

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

Table 4.7: Effects of *M. incognita* and *B. theobromae* interaction on yield parameters of cassava cultivar TMS 30572 in a pot experiment

	Number Of Tubers				Weight Of Tubers (g)				Number Of Rotted Tubers			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial												
Control	2.0	3.3	7.8	8.0	6.7	13.5	139.2	188.6	0.0	0.0	0.0	0.0
M. incognita α	1.0	3.0	6.0	5.5	3.7	10.6	80.8	117.4	0.0	0.0	0.0	0.0
M. incognita β	0.0	2.5	6.0	5.0	0.0	5.1	55.8	97.8	0.0	0.0	0.0	0.0
B. theobromae	2.0	3.3	6.0	5.5	6.0	11.5	97.6	124.6	0.0	0.0	0.0	0.3
M.i α and B.t	1.0	3.0	5.0	4.8	2.8	8.8	74.0	108.7	0.0	0.0	0.0	0.8
M.i α before B.t	1.0	3.0	6.0	5.3	2.7	8.6	69.5	104.2	0.0	0.0	0.0	0.8
B.t before M.i α	1.0	3.0	6.0	5.3	2.8	9.1	78.2	122.2	0.0	0.3	0.3	0.5
M.i β and B.t	0.0	2.3	6.0	5.3	0.0	5.2	59.1	116.9	0.0	1.0	0.8	1.3
M.i β before B.t	0.0	2.3	5.0	4.3	0.0	5.0	56.7	68.1	0.0	0.8	1.0	1.3
B.t before M.i β	0.0	2.5	6.0	5.3	0.0	5.5	60.9	103.3	0.0	0.3	0.3	1.0
LSD(P≤0.05)	0.0	0.6	0.2	1.4	0.6	0.7	2.5	23.7	0.0	0.4	0.4	0.6
Second Trial												
Control	2.5	5.3	9.0	11.0	6.7	22.3	163.2	224.5	0.0	0.0	0.0	0.0
M. incognita α	1.3	4.0	7.3	8.5	3.7	16.9	93.3	161.6	0.0	0.0	0.0	0.0
M. incognita β	0.5	3.8	6.3	7.3	0.0	11.4	68.5	134.3	0.0	0.0	0.0	0.0
B. theobromae	2.3	4.8	7.3	9.5	6.0	17.9	108.9	180.9	0.0	0.0	1.0	1.0
M.i α and B.t	1.3	3.8	6.3	7.0	2.8	15.2	82.1	144.3	0.0	0.0	0.8	1.3
M.i α before B.t	1.0	3.8	6.8	6.3	2.7	15.0	78.3	130.0	0.0	0.3	1.0	1.3
B.t before M.i α	1.3	3.8	6.8	7.3	2.8	15.9	88.9	142.7	0.0	0.0	0.8	1.3
M.i β and B.t	0.5	3.3	6.5	6.3	0.0	11.4	72.3	125.9	0.0	0.8	1.3	1.8
M.i β before B.t	0.5	3.0	5.8	5.8	0.0	11.3	67.3	102.7	0.0	0.8	1.3	2.0
B.t before M.i β	0.5	3.0	6.5	6.3	0.0	11.8	74.0	118.9	0.0	0.3	10	1.8
LSD(P≤0.05)	0.7	2.0	2.0	1.4	2.7	7.6	9.0	14.2	0.0	0.6	0.5	0.6

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

4.2.5: Effect of *M. incognita* and *B. theobromae* interaction on tuber rot severity and percentage tuber rot of three cassava cultivars

At 2 MAP there was no significant difference in the values obtained in rot severity from all the treatments in the cultivar Ofege (0.0), but at 4 MAP, 9 MAP and 12 MAP the treatments differed (P≤0.05) from one another. At 4 MAP, 9 MAP and 12 MAP the highest significant mean tuber rot severity was obtained from plants inoculated with 10000 *M. incognita* eggs prior *B. theobromae* (3.3, 4.5 and 4.3 respectively) (Table 4.8).

There was no significant difference in the percentage tuber rot at 2 MAP (0.0%) but the highest significant mean percentage tuber rot was obtained from plants inoculated with 10,000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode at 4 MAP, 9 MAP and 12 MAP (50%, 75% and 85.4% respectively). The lowest significant mean percentage tuber rot was observed in the uninoculated plants (0.0%) and plants inoculated with nematode eggs alone (0.0%). This trend was noticed in the second trial too (Table 4.8).

In TME 1, at 2 MAP there was no significant difference in the values obtained for tuber rot severity from all the treatments (0.0), but at 4 MAP, 9 MAP and 12 MAP the treatments differed (P≤0.05) from one another. At 4 MAP, 9 MAP and 12 MAP the highest significant mean rot severity was obtained from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (3.3, 4.8 and 4.8 respectively). (Table 4.9)

In the two trials, there was no significant difference in the percentage tuber rot at 2 MAP (0.0%). However at 4 MAP, in the first trial, the highest significant mean percentage tuber rot was obtained from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* (simultaneously) and 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (50.0% resptively). In the second trial the highest significant mean percentage tuber rot was obtained from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (68.3%). At 9 MAP and 12 MAP the lowest significant mean percentage tuber rot was observed in the uninoculated plants and plants inoculated with nematode eggs alone (0.%) while plants treated with 10,000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks

after the nematode had the highest significant mean percentage tuber rot (77.1% and 100% respectively). This trend was the same for the two trials (Table 4.9).

In 30572, the mean tuber rot severity recorded from all the treatments at 2 MAP were not significantly different from one another (0.0), but at 4 MAP, 9 MAP and 12 MAP the treatments differed ($P \leq 0.05$) from one another. At 4 MAP, 9 MAP and 12 MAP the highest significant mean tuber rot severity was obtained from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (2.5, 4.5 and 3.3) (Table 4.10)

In the two trials there was no significant difference in the percentage tuber rot at 2 MAP (0.0%). However at 4 MAP, the highest significant mean percentage tuber rot was obtained from plants inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (45.8%). At 9 MAP and 12 MAP the lowest significant mean percentage tuber rot was observed in the uninoculated plants and plants inoculated with nematode eggs alone (0.0%), while plants treated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode had the highest significant mean percentage tuber rot (30.0%, 26.1%). This trend was the same for the two trials (Table 4.10).

4.2.6: Effects of *M. incognita* and *Botryodiplodia theobromae* interaction on Gall Index and Nematode Reproductive Factor on three cassava cultivars

In Ofege, the least significant number of nematodes in the roots, soil and the total nematode recovered was recorded in the uninoculated control and plants inoculated with *B. theobromae alone* (0.0) (Table 4.11). Plants inoculated with 10,000 *M. incognita* eggs alone had the highest number of nematode in the roots at 2 MAP, 4 MAP, 9 MAP and 12 MAP (22565.0, 24509.8, 16808.0 and 8608.3 respectively) (Table 4.11). The treatment also had the highest significant number of nematode in the soil at 2 MAP, 4 MAP, 9 MAP and 12 MAP (342.5, 536.3, 1252.0 and 3444.3 respectively) (Table 4.11). The highest significant number of total nematode recovered were recorded from plants inoculated with 10000 *M. incognita* eggs alone at 2 MAP, 4 MAP, 9 MAP and 12 MAP (22907.5, 25046.1, 18060.0 and 126052.6) (Table 4.11).

Table 4.8: Effects of *M. incognita* and *B. theobromae* interaction on tuber rot severity and percentage tuber rot on cassava cultivar Ofege (Pot)

	Tuber rot severity*				Percentage tuber rot			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. theobromae	0.0	0.0	1.0	1.0	0.0	0.0	5.0	9.2
M.i α and B.t	0.0	1.0	1.5	1.5	0.0	31.3	20.0	19.6
M.i α before B.t	0.0	1.5	2.0	2.3	0.0	31.3	35.0	33.8
B.t before M.i α	0.0	1.0	1.5	2.0	0.0	18.8	25.0	17.5
M.i β and B.t	0.0	2.0	3.0	3.5	0.0	45.8	62.5	56.3
M.i β before B.t	0.0	3.3	4.5	4.3	0.0	50.0	75.0	85.4
B.t before M.i β	0.0	2.3	3.5	3.8	0.0	45.8	50.0	35.4
LSD (P≤0.05)	0.0	0.4	0.5	0.5	0.0	14.6	10.3	22.1
Second trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. theobromae	0.0	0.3	0.3	0.5	0.0	0.0	6.3	17.5
M.i α and B.t	0.0	0.8	1.5	1.5	0.0	0.0	13.4	21.7
M.i α before B.t	0.0	1.3	2.0	2.3	0.0	8.3	26.7	35.0
B.t before M.i α	0.0	1.0	1.5	1.5	0.0	0.0	18.4	20.9
M.i β and B.t	0.0	1.5	2.8	3.3	0.0	23.8	48.3	52.5
M.i β before B.t	0.0	2.3	3.0	3.5	0.0	29.2	52.5	64.2
B.t before M.i β	0.0	1.5	2.0	3.5	0.0	12.5	37.1	45.0
LSD (P≤0.05)	0.0	0.6	0.7	0.8	0.0	22.9	17.7	14.3

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, $5X10^5$ *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

*0= No rot, 1 = very mild rotting (Less than 5% of tuber rotted), 2 = Mild rotting (6-10% of tuber rotted), 3 = Moderate rotting (11-25% of tuber rotted), 4= Severe rotting (26-50% of tuber rotted) and 5 = Very severe rotting (more than 50% of tuber rotted)

Table 4.9: Effects of *M. incognita* and *B. theobromae* interaction on tuber rot severity and percentage tuber root on cassava cultivar TME 1 (Pot)

	Tuber rot severity*				Percentage tuber rot			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. theobromae	0.0	0.0	1.0	1.0	0.0	0.0	5.0	15.0
M.i α and B.t	0.0	1.0	2.3	2.3	0.0	31.3	25.8	31.3
M.i α before B.t	0.0	1.15	2.5	3.0	0.0	31.3	35.8	50.0
B.t before M.i α	0.0	1.0	1.8	2.3	0.0	18.8	25.3	37.5
M.i β and B.t	0.0	2.5	3.5	3.8	0.0	50.0	62.5	62.5
M.i β before B.t	0.0	3.3	4.8	4.8	0.0	50.0	77.1	100.0
B.t before M.i β	0.0	2.5	3.7	4.0	0.0	45.8	37.5	56.3
LSD (P≤0.05)	0.0	0.5	0.6	0.5	0.0	14.1	25.6	16.1
Second trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. theobromae	0.0	0.0	0.5	1.5	0.0	0.0	19.2	18.0
M.i α and B.t	0.0	1.8	1.3	1.8	0.0	22.5	22.5	25.0
M.i α before B.t	0.0	2.0	2.5	3.3	0.0	25.0	31.7	35.0
B.t before M.i α	0.0	1.0	1.3	2.5	0.0	11.3	22.5	30.9
M.i β and B.t	0.0	2.3	3.0	3.8	0.0	42.5	46.3	61.3
M.i β before B.t	0.0	3.0	4.0	5.0	0.0	68.3	78.8	85.4
B.t before M.i β	0.0	1.5	2.5	4.3	0.0	55.0	41.7	54.3
LSD (P≤0.05)	0.0	0.5	0.9	0.8	0.0	27.2	20.2	21.3

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, $5X10^5$ *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

*0= No rot, 1 = very mild rotting (Less than 5% of tuber rotted), 2 = Mild rotting (6-10% of tuber rotted), 3 = Moderate rotting (11-25% of tuber rotted), 4= Severe rotting (26-50% of tuber rotted) and 5 = Very severe rotting (more than 50% of tuber rotted)

Table 4.10: Effects of *M. incognita* and *B. theobromae* interaction on rot severity and Percentage tuber rot in cassava cultivar TMS 30572 (Pot)

	Tuber rot severity*				Percentage tuber rot			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. theobromae	0.0	0.0	0.5	0.5	0.0	0.0	16.7	5.0
M.i α and B.t	0.0	0.8	1.5	1.5	0.0	0.0	20.0	14.6
M.i α before B.t	0.0	1.3	2.0	2.3	0.0	8.3	20.8	14.4
B.t before M.i α	0.0	1.0	1.5	1.5	0.0	8.3	16.7	11.3
M.i β and B.t	0.0	1.5	2.8	3.3	0.0	33.3	25.0	25.4
M.i β before B.t	0.0	2.5	4.5	3.3	0.0	45.8	30.0	26.1
B.t before M.i β	0.0	1.5	2.8	3.3	0.0	12.5	20.8	19.2
LSD (P≤0.05)	0.0	0.6	0.6	0.6	0.0	17.8	13.1	14.6
Second trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita β	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B. theobromae	0.0	0.0	0.0	0.5	0.0	0.0	14.3	8.3
M.i α and B.t	0.0	1.0	2.5	1.5	0.0	0.0	11.9	18.4
M.i α before B.t	0.0	1.3	2.0	2.3	0.0	0.0	15.3	20.3
B.t before M.i α	0.0	1.0	1.5	1.5	0.0	0.1	11.1	17.6
M.i β and B.t	0.0	1.5	2.8	3.3	0.0	23.8	19.8	28.6
M.i β before B.t	0.0	2.5	4.0	3.5	0.0	29.2	22.8	35.0
B.t before M.i β	0.0	1.5	2.8	3.5	0.0	12.5	15.7	28.6
LSD (P≤0.05)	0.0	0.5	0.5	0.6	0.0	22.9	9.0	10.1

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

*0= No rot, 1 = very mild rotting (Less than 5% of tuber rotted), 2 = Mild rotting (6-10% of tuber rotted), 3 = Moderate rotting (11-25% of tuber rotted), 4= Severe rotting (26-50% of tuber rotted) and 5 = Very severe rotting (more than 50% of tuber rotted).

In TMS 30572, the least significant number of nematodes in the roots, soil and the total nematode recovered was recorded in the uninoculated control and plants inoculated with *B. theobromae alone* (0.0) (Table 4.12). Plants inoculated with 10,000 *M. incognita* eggs alone had the highest number of nematode in the roots at 2 MAP, 4 MAP, 9 MAP and 12 MAP (21998.0, 20755.5, 11690.5 and 9035.5 respectively) (Table 4.12). The highest significant number of total nematode recovered, were recorded from plants inoculated with 10000 *M. incognita* eggs alone at 2 MAP, 4 MAP, 9 MAP and 12 MAP (22270.0, 21121.5, 12343.5 and 9925.0) (Table 4.12).

In TME 1, the least significant number of nematodes in the roots, soil and the total nematode recovered was recorded in the uninoculated control and plants inoculated with *B. theobromae alone* (0.0) (Table 4.13). Plants inoculated with 10,000 *M. incognita* eggs alone had the highest number of nematode in the roots at 2 MAP, 4 MAP, 9 MAP and 12 MAP (30067.0, 32534.0, 31818.0 and 18541.3 respectively) (Table 4.13). The treatment also had the highest significant number of nematode in the soil at 2 MAP, 4 MAP, 9 MAP and 12 MAP (430.5, 576.3, 732.8 and 2193.8 respectively) (Table 4.13). The highest significant number of total nematode recovered was recorded from plants inoculated with 10000 *M. incognita* eggs alone at 2 MAP, 4 MAP, 9 MAP and 12 MAP (30497.5, 33110.3, 32550.8 and 20735.1 respectively)(Table 4.13).

The mean Reproductive Factor (R) on Ofege differed statistically ($P \leq 0.05$) from each other. The least significant reproductive factor came from soil of the control, soil inoculated with *B. theobromae* alone and soil inoculated with combination of 10000 *M. incognita* eggs + *B. theobromae* at 9 MAP in the first trial (0.0) (Table 4.14). At 12 MAP the least reproductive factor was obtained from uninoculated control soil, *B. theobromae* alone soil, soil inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus silmutaneously and soil inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (0.0).(Table 4.14).

The highest reproductive factor was obtained from plants from soil inoculated with 10000 *M. incognita* eggs alone at 2 MAP, 4 MAP, 9 MAP and 12 MAP (2.3, 2.5, 2.8 and 1.2 respectively) (Table 4.14)

The mean gall indices differed significantly with each treatment (Tables 4.14). The least gall index was obtained from uninoculated control plants and plants

inoculated with *B. theobromae* alone for both trials (0.0) and the values obtained were significantly lower than other treatments at 2 MAP and 4 MAP. Plants treated with 10,000 *M. incognita* eggs alone had the highest gall indices at 2 MAP, 4 MAP, 9 MAP and 12 MAP (3.2, 3.3, 4.5 and 3.3 respectively) and were significantly different from all other treatments (Table 4.14).

The mean Reproductive Factor (R) on TME 1 differed statistically (P≤0.05) from each other. The least significant reproductive factor came from soil of the control, soil inoculated with *B. theobromae* alone and soil inoculated with combination of 10000 *M. incognita* eggs + *B. theobromae* at 9 MAP in the first trial (0.0) (Table 4.15) At 12 MAP the least reproductive factor was obtained from uninoculated control soil, *B. theobromae* alone soil, soil inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus silmutaneously and soil inoculated with 10000 *M. incognita* eggs + *B. theobromae* with the fungus inoculated 2 weeks after the nematode (0.0).(Table 4.15).

The highest reproductive factor was obtained from plants from soil inoculated with 10000 *M. incognita* eggs alone at 2 MAP, 4 MAP, 9 MAP and 12 MAP (3.0, 3.3, 3.3 and 3.7 respectively) (Table 4.15)

The mean gall indices differed significantly with each treatment (Tables 4.12). The least gall index was obtained from uninoculated control plants and plants inoculated with *B. theobromae* alone for both trials (0.0) and the values obtained were significantly lower than other treatments at 2 MAP and 4 MAP, 9 MAP and 12 MAP. Plants treated with 10,000 *M. incognita* eggs alone had the highest gall indices at 2 MAP, 4 MAP, 9 MAP and 12 MAP (4.0, 2.5, 5.0 and 4.0 respectively) and were significantly different from all other treatments (Table 4.15).

The mean Reproductive Factor (R) on TMS 30572 differed statistically (P≤0.05) from each other. The least significant reproductive factor came from soil of the control, soil inoculated with *B. theobromae* alone and soil inoculated with combination of 10000 *M. incognita* eggs + *B. theobromae* at 9 MAP in the both trial (0.0) (Table 4.16). At 12 MAP the least reproductive factor was obtained from uninoculated control soil, *B. theobromae* alone soil (0.0) (Table 4.16).

The highest reproductive factor was obtained from plants from soil inoculated with 10000 *M. incognita* eggs alone at 2 MAP, 4 MAP, 9 MAP and 12 MAP (3.3, 3.0, 4.5 and 2.5 respectively) (Table 4.16).

The mean gall indices differed significantly with each treatment (Tables 4.16). The least gall index was obtained from uninoculated control plants and plants inoculated with *B. theobromae* alone for both trials (0.0) and the values obtained were significantly lower than other treatments at 2 MAP and 4 MAP, 9 MAP and 12 MAP (Table 4.16). Plants treated with 10,000 *M. incognita* eggs alone had the highest gall indices at 2 MAP, 4 MAP, 9 MAP and 12 MAP (2.2, 2.1, 1.2 and 1.6 respectively) and were significantly different from all other treatments (Table 4.16).

4.2.7: Effects of Main Treatments on growth, yield, galling index and reproductive factor of *M. incognita* on Cassava (across varieties) in microplots.

The highest significant plant height was obtained from the uninoculated control plants (275.9 cm) (Table 4.17). The least significant plant height was obtained from plants inoculated with 10,000 *M. incognita* eggs prior to inoculation of *B. theobromae* (207.1 cm) The highest significant mean shoot weight was obtained from the uninoculated control plants (7.8 kg) with the least significant mean shoot weight obtained from plants inoculated with 10,000 *M. incognita* eggs prior to *B. theobromae* inoculation (4.6 kg) (Table 4.17).

The uninoculated control plants had the highest significant number of tubers (8.7) but not significantly different from the number of tubers obtained from plants inoculated with *B. theobromae* alone (7.4) but significantly different from all other treatments, while the lowest significant number of tubers was recorded in plants inoculated with 10,000 *M. incognita* eggs prior to *B. theobromae* (5.4) (Table 4.17).

In the second trial, the uninoculated control plants had the highest significant number of tubers (8.8) but not significantly different from the number of tubers obtained from plants inoculated with *B. theobromae* alone (6.8) but significantly different from all other treatments, while the lowest significant number of tubers was recorded in plants inoculated with 10,000 *M. incognita* eggs prior to *B. theobromae* (5.4) Plants treated with 1000 eggs of *M. incognita* produced 6.4 tubers compared with 5.7 tubers recorded in plants treated with 10000 eggs of *M. incognita* (Table 4.18).

Table 4.11: Effect of *M. incognita* and *B. theobromae* interaction on nematode population in cassava cultivar OFEGE (Pot)

	Nematodes in root				Nematodes in soil (250ml soil)				Total nematodes			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First trial												
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1604.5	1952.3	2233	1861.8	79.50	141.8	118.8	162.7	1684.0	2094.0	2352.0	2024.5
M. incognita β	22565.0	24509.8	16808.0	8608.3	342.50	536.3	1252	3444.3	22907.5	25046.0	18060.0	12052.5
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1391.3	1001.8	766.0	396.3	34.0	56.8	69.3	235.5	1425.3	1058.5	835.0	631.8
M.i α before B.t	1646.5	898.3	563.0	186.8	67.0	71.5	37.5	25.0	1713.5	969.8	598.0	211.8
B.t before M.i α	1379.8	1027.3	636.0	123.0	33.50	73.8	80.3	60.3	1413.3	1101.0	716.0.0	486.3
M.i β and B.t	21825.5	12723.2	1169.0	701.8	312.5	426.3	86.8	101.8	22138.0	13149.5	1256.0	803.5
M.i β before B.t	21895.0	12490.3	401.0	123.0	278.0	367.0	35.0	30.0b	22173.0	12857.3	439.0	153.0
B.t before M.i β	21782.0	12800.5	1873.0	650.3	236.5	462.5	134.8	171.3	22018.5	13263.0	2008.0	821.5
LSD(P≤0.05)	627.3	946.5	3474.0	1607.1	17.2	42.5	516.7	1586.5	632.1	953.0	3847.7	602.7
Second trial												
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1665	1904.0	1894.0	1024.5	93.5	143.0	125.3	124	1161.0	1602.0	2040.0	1173.8
M. incognita β	22323.0	23741.3	14465.0	10251.8	335	546.3	1084.3	1211.5	18257.0	20813.0	17800.0	13264.5
B. theobromae	0.0	0.0	0.0	0.0b	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1489.0	926.0	598.0	403.8	40.5	57.3	86.3	86.8	734.0	879.0	1045.0	422.5
M.i α before B.t	1737.0	835.0	359.0	145.5	76.5	81.3	27.0	20.8	593.0	723.0	656.0	277.8
B.t before M.i α	1469.0	880.8	527.0	340.3	45.8	75.0	81.5	60.0	654.0	898.0	873.0	422.0
M.i β and B.t	16609.0	12735.8	830.0	699.3	821.0	451.3	84.5	101.8	14314.0	15683..0	4124.0	783.5
M.i β before B.t	16737.0	12240.3	262.0	116.3	776.5	367.0	23.3	27.3	9567.0	9994.0	2952.0	332..8
B.t before M.i β	16612.0	12833.5	1469.0	742.0	739.5	468.0	125.3	171.3	11245.0	11951.0	5024.0	1110.5
LSD(P≤0.05)	7953.2	484.53	3928.9	1028.1	791.9	60.34	383.9	381.2	3656.2	3219.6	3905.9	1160.4

LSD for comparing treatment means within the same column, M.i=*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

Table 4.12: Effect of *M. incognita* and *B. theobromae* interaction on nematode population in cassava cultivar TMS 30572 (Pot)

	Nematodes in root				Nematodes in soil (250 ml soil)				Total nematodes			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First trial												
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1444.0	1227.5	1069.0	834.8	36.0	57.0	214.5	201.3	1480.0	1284.5	1283.5	1036.0
M. incognita β	21998.0	20755.5	11690.5	9035.5	272.0	366.0	653.0	889.5	22270.0	21121.5	12343.5	9925.0
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1423.0	1055.5	481.5	222.5	31.0	45.5	68.0	65.5	1454.0	1101.0	549.5	288.0
M.i α before B.t	1429.0	1019.5	394.5	198.5	49.3	40.0	37.5	29.0	1478.0	1059.5	374.3	227.5
B.t before M.i α	1419.0	1065.0	466.88	368.3	35.0	54.5	71.8	121.3	1454.0	1120.3	1621.3	489.5
M.i β and B.t	14125.0	11186.5	1196.3	1026.5	287.5	350.5	139.0	135.5	14413.0	11537.0	1335.3	1162.0
M.i β before B.t	14294.0	10809.0	336.8	43.0	303.0	302.3	68.5	14.5	14597.0	11111.3	463.0	57.5
B.t before M.i β	14105.0	11133.3	1621.3	1226.8	281.0	403.0	185.8	303.8	14386.0	11536.3	1621.3	1530.5
LSD(P≤0.05)	5502.2	1971.6	1041.1	278.7	94.3	4.5	113.7	221.2	5573.4	1971.1	1122.2	307.3
Second trial												
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	968.0	1214.0	896.3	888.8	21.8	63.0	220.8	180.5	990.0	1277.0	1069.3	1069.3
M. incognita β	19538.0	20316.0	9020.5	9190.5	215.0	361.8	910.8	770.5	19753.0	20678.0	9938.0	9961.0
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1189.0	1101.0	263.5	233.5	25.5	41.0	110.0	76.0	1215.0	1142.0	309.5	309.5
M.i α before B.t	1200.0	1096.0	272.5	217.8	35.8	31.0	53.8	29.3	1236.0	1127.0	326.3	247.0
B.t before M.i α	1182.0	1132.0	368.3	368.3	21.8	53.0	121.3	121.3	1203.0	1185.0	489.5	489.5
M.i β and B.t	13733.0	11885.0	938.8	946.3	345.0	337.3	83.5	130.8	14078.0	12223.0	1077.0	1077.0
M.i β before B.t	13888.0	11353.0	222.8	247.5	373.0	253.3	26.3	60.8	14261.0	11607.	247.0	308.3
B.t before M.i β	13697.0	11512.0	1293.5	1360.5	329.0	330.3	259.5	283.8	14026.0	11842.0	1644.3	1644.3
LSD(P≤0.05)	4586.3	2447.1	218.3	267.9	102.6	66.0	137.9	213.7	4632.2	2462.4	291.7	292.9

LSD for comparing treatment means within the same column, M.i=*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

Table 4.13: Effect of *M. incognita* and *B. theobromae* interaction on nematode population in cassava cultivar TME 1 (Pot)

	Nematodes in root				Nematodes in soil (250ml soil)				Total nematodes			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First trial												
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	2219.0	2455.0	2547.3	921.8	79.5	158.5	190.8	91.3	2298.5	2613.5	2738.1	1013.1
M. incognita β	30067.0	32534.0	31818	18541.3	430.5	576.3	732.8	2193.8	30497.5	33110.3	32550.8	20735.1
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	2215.0	1305.0	610.8	441.8	70.5	102.0	94.0	120.0	2285.5	1407.0	704.8	561.8
M.i α before B.t	2212.0	4274.0	259.3	191.0	72.0	92.3	66.8	20.5	2284.5	4366.3	326.1	211.5
B.t before M.i α	2209.0	1326.0	619.5	484.5	64.5	108.3	87.5	77.3	2273.5	1434.3	707.0	561.8
M.i β and B.t	22442.0	15513.0	964.0	959.5	362.0	439.0	205.8	119.0	22804.0	15952.0	1169.8	1078.5
M.i β before B.t	22389.0	15126.0	310.8	170.8	362.0	401.0	108.8	17.3	22751.0	15527.0	419.6	188.1
B.t before M.i β	22370.0	15621.0	1133.5	1034.3	373.5	482.0	220	148.8	22743.5	5006.2	1353.5	1183.1
LSD(P≤0.05)	2306.1	4998.6	394.2	307.7	55.7	7.6	191.7	289.3	632.1	953.0	3847.7	602.7
Second trial												
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	2239.0	2449.0	1048.5	1048.5	91.8	173.5	176.0	168.5	2330.8	2622.5	3088.5	1216.5
M. incognita β	27583.0	32661.0	20551	19551.0	314.5	596.5	748.3	2458.8	27897.5	33257.5	21299.3	22009.8
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	2270.0	1436.0	462.3	462.3	56.5	105.3	157.8	132.3	2326.5	1541.3	620.1	594.6
M.i α before B.t	2281.0	4267.0	194.3	216.8	61.0	90.0	90.8	72.3	2342.0	4357	285.1	289.1
B.t before M.i α	225.0	1383.0	583.0	583.0	56.8	111.3	168.5	168.0	281.8	1494.3	751.5	751.0
M.i β and B.t	22392.0	15559.0	1009.0	1009.0	364.5	382.8	204.5	112.0	22756.5	15941.8	1213.5	1121.0
M.i β before B.t	22496.0	15199.0	186.3	200.8	430.5	278.5	113.8	19.8	22926.5	15477.5	300.1	220.6
B.t before M.i β	22252.0	15419.0	1030.0	583.0	389.5	450.8	264.3	224.5	22641.5	15869.8	1294.3	807.5
LSD(P≤0.05)	2663.5	4884.7	779.7	396.9	85.2	86.5	186.0	417.2	3656.2	3219.6	3905.9	1160.4

LSD for comparing treatment means within the same column, M.i=*Meloidogyne incognita*, 5X10^5 *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs.

Table 4.14: Effect of *M. incognita* and *B. theobromae* interaction on Nematode Reproductive Factor and galling index of cassava cultivar Ofege in a pot experiment

	Galling Index*				Reproductive Factor			
Treatment	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1.8	2.0	2.8	1.5	1.7	2.1	1.1	0.8
M. incognita β	3.2	3.3	4.5	3.3	2.3	2.5	2.8	1.2
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	2.0	1.8	2.0	1.5	1.4	1.1	0.8	0.6
M.i α before B.t	2.5	2.5	2.0	1.5	1.7	0.9	0.6	0.2
B.t before M.i α	1.3	2.0	2.0	1.0	1.4	1.1	0.7	0.5
M.i β and B.t	2.8	1.8	2.3	0.3	2.2	1.3	0.2	0.0
M.i β before B.t	3.0	2.0	2.8	2.0	2.2	1.3	0.0	0.0
B.t before M.i β	2.0	2.0	3.0	2.8	2.2	1.3	0.2	0.2
LSD(P≤0.05)	0.5	0.9	0.7	0.9	0.5	0.3	0.3	0.2
Second trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1.5	2.5	2.0	1.8	1.2	1.6	2.0	1.2
M. incognita β	2.8	3.5	3.8	3.5	1.8	2.1	1.8	1.3
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1.3	1.5	1.0	1.5	0.7	0.9	1.0	0.4
M.i α before B.t	1.5	1.8	1.0	1.5	0.6	0.7	0.7	0.3
B.t before M.i α	1.0	2.0	1.0	1.3	0.7	0.9	0.9	0.4
M.i β and B.t	2.0	1.5	1.3	1.0	1.4	1.6	0.4	0.1
M.i β before B.t	2.5	1.3	2.0	1.0	1.0	1.0	0.3	0.0
B.t before M.i β	1.3	2.0	1.5	1.0	1.1	1.2	0.5	0.1
LSD(P≤0.05)	0.8	0.6	0.6	0.7	0.5	0.5	0.7	0.2

LSD for comparing treatment means within the same column, M.i =*Meloidogyne incognita*, $5X10^5$ *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

*0 = no galls, 1 = 1-10% of the root system galled, 2= 11-20% of the root system galled, 3= 21- 70% of the root system galled, 4= 71-90% of the root system galled; and 5 = greater than 90% of the root system galled.

Table 4.15: Effect of *M. incognita* and *B. theobromae* interaction on Nematode Reproductive Factor and galling index of cassava cultivar TME 1 in a pot experiment

Treatment	Galling Index*				Reproductive Factor			
	2MAP	4MAP	9MAP	12MAP	2MAP	4MAP	9MAP	12MAP
First Trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1.3	2.1	3.0	2.0	2.3	2.6	2.7	2.0
M. incognita β	4.0	2.5	5.0	4.0	3.0	3.3	3.3	3.7
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	2.0	1.1	2.0	1.0	2.9	1.4	0.7	0.9
M.i α before B.t	2.0	0.9	2.3	1.3	2.3	1.3	0.3	0.3
B.t before M.i α	1.8	1.1	2.3	0.5	2.3	1.4	0.7	0.4
M.i β and B.t	3.0	1.3	2.5	1.3	2.3	1.6	0.1	0.2
M.i β before B.t	3.5	1.2	2.0	0.8	2.3	1.5	0.0	0.0
B.t before M.i β	2.8	1.3	3.0	1.0	2.3	1.6	0.1	0.0
LSD(P≤0.05)	0.5	0.3	0.5	0.8	0.2	0.4	0.1	0.4
Second trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1.3	2.3	2.3	3.0	2.3	2.6	1.2	2.7
M. incognita β	3.5	4.0	4.0	4.0	2.8	3.3	2.1	2.3
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1.8	1.5	1.8	1.8	2.3	1.5	0.6	0.3
M.i α before B.t	1.8	1.8	2.0	1.8	2.3	1.4	0.3	0.2
B.t before M.i α	1.5	1.5	2.0	1.0	2.3	1.5	0.7	0.3
M.i β and B.t	2.8	1.5	2.0	0.8	2.3	1.6	0.1	0.1
M.i β before B.t	3.3	1.5	2.3	1.0	2.3	1.5	0.0	0.0
B.t before M.i β	2.5	1.8	2.5	1.0	2.3	1.5	0.1	0.0
LSD(P≤0.05)	0.9	0.8	1.2	1.1	0.3	0.4	0.1	0.3

LSD for comparing treatment means within the same column, M.i=*Meloidogyne incognita*, $5X10^5$ *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

*0 = no galls, 1 = 1-10% of the root system galled, 2 = 11-20% of the root system galled, 3 = 21- 70% of the root system galled, 4 = 71-90% of the root system galled; and 5 = greater than 90% of the root system galled

Table 4.16: Effects of *M. incognita* and *B. theobromae* interaction on Nematode Reproductive Factor and galling index on cassava cultivar TMS 30572 in a pot experiment

	Galling Index*				Reproductive Factor			
Treatment	**2MAP**	**4MAP**	**9MAP**	**12MAP**	**2MAP**	**4MAP**	**9MAP**	**12MAP**
First Trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1.5	1.3	1.2	1.0	1.0	2.0	2.8	1.5
M. incognita β	2.2	2.1	1.2	1.6	3.3	3.0	4.5	2.5
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1.5	1.1	0.5	1.3	1.5	2.0	2.0	0.5
M.i α before B.t	1.5	1.0	0.4	0.4	2.0	1.0	2.0	1.3
B.t before M.i α	1.5	1.1	0.6	0.6	1.5	2.0	2.0	1.2
M.i β and B.t	1.4	1.2	0.1	0.2	2.3	2.0	2.3	1.3
M.i β before B.t	1.5	1.2	0.0	0.0	3.0	2.0	1.0	0.3
B.t before M.i β	1.4	1.2	0.1	0.1	2.0	2.0	3.0	1.3
LSD(P≤0.05)	0.7	0.6	0.2	0.4	0.5	0.0	0.7	0.6
Second trial								
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M. incognita α	1.0	2.0	2.0	1.5	1.0	2.0	2.8	1.5
M. incognita β	2.0	3.0	3.3	2.3	3.0	3.0	4.5	2.5
B. theobromae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
M.i α and B.t	1.2	2.0	1.5	1.5	1.5	2.0	2.0	0.5
M.i α before B.t	1.2	1.0	1.5	1.0	2.0	1.0	2.0	1.3
B.t before M.i α	1.2	1.8	1.5	1.5	1.5	2.0	2.0	13
M.i β and B.t	1.4	1.5	1.5	1.3	2.3	2.0	2.3	1.3
M.i β before B.t	1.4	1.3	2.0	1.0	3.0	2.0	0.7	0.5
B.t before M.i β	1.4	1.5	2.0	1.3	2.0	2.0	3.0	1.3
LSD(P≤0.05)	0.9	0.5	1.2	0.7	0.5	0.0	0.7	0.7

LSD for comparing treatment means within the same column, M.i=*Meloidogyne incognita*, $5X10^5$ *Botryodiplodia theobromae* (B.t) spores, α = 1,000 M.i eggs, β = 10,000 M.i eggs

*0 = no galls, 1 = 1-10% of the root system galled, 2 = 11-20% of the root system galled, 3 = 21-70% of the root system galled, 4 = 71-90% of the root system galled; and 5 = greater than 90% of the root system galled

The highest significant tuber weight was recorded in the uninoculated plants (7.8 kg) while the least significant tuber weight was recorded in plants inoculated with 10,000 *M. incognita* eggs prior to *B. theobromae* (4.0 kg). The highest mean number of rotted tubers (2.4) and percentage tuber rot (44.5%) were recorded in plants inoculated with 10,000 *M. incognita* eggs prior to *B. theobromae* while the uninoculated control plants did not record any rot at all (0.0) The severity of rot was more visible on plants inoculated with a combination of 10,000 *M. incognita* eggs and fungus than in plants inoculated with a combination of 1,000 *M. incognita* eggs and fungus (Tables 4.17 and 4.18).

The highest significant total nematode was recorded from plants inoculated with 10,000 *M. incognita* alone (17758.0) while the least significant total nematode was recorded in the uninoculated control (0.0) and plants inoculated with *B. theobromae* apores alone (0) and they were significantly different from all other treatments (Tables 4.17 and 4.18).

For both trials, the mean gall indices differed significantly among the treatments. Plants inoculated with 10,000 eggs of *M. incognita* eggs alone had the significantly highest gall index (2.3), followed by plants inoculated with 1,000 eggs of *M. incognita* alone (1.0) and plants inoculated with 10,000 *B. theobromae* spores prior to *M. incognita* inoculation after while the least significant gall index were recorded from uninoculated plants (0.0) and plants inoculated with *B. theobromae* alone (0.0). In treatments where nematode and fungus were in combination, the least gall index were recorded when the nematode was inoculated two weeks before the fungus inoculation. (Tables 4.17 and 4.18)

The plants inoculated with 10,000 *M. incognita* eggs alone had the highest significant reproductive factor (1.8) while the least reproductive factor was recorded from uninoculated plants (0.0) and plants inoculated with *B. theobromae* alone (0.0) and these values were not significantly different from values recorded from plants inoculated with 1,000 *M. incognita* eggs 2 weeks before fungus inoculation, and plants inoculated with 10,000 *M. incognita* eggs in combination with fungus before or after nematode or simultaneously (Tables 4.17). This followed the same trend in the second trial (Table 4.18)

Table 4.17: Effects of *B. theobromae* and *M. incognita* on growth, yield parameters and nematode reproduction on cassava (1st trial) (Field)

	TREATMENTS*										
Parameters	A	B	C	D	E	F	G	H	I	J	LSD (P≤0.05)
Mean Gall index**	0.0	1.0	2.3	0.0	0.5	0.4	0.7	0.8	0.3	1.0	0.4
Reproductive Factor	0.0	1.3	1.8	0.0	1.0	0.3	0.7	0.2	0.1	0.2	0.3
Plant Height (cm)	275.9	257.9	242.0	259.2	256.0	242.4	247.5	246.7	207.1	244.7	21.9
Stem diameter (cm)	4.5	4.0	4.2	4.1	3.9	3.9	3.9	4.1	3.8	4.0	0.5
Mean fresh shoot weight (kg)	7.8	6.3	5.4	7.0	5.5	5.9	6.0	6.0	4.6	6.0	2.4
Number of tubers	8.7	6.4	5.7	7.4	6.3	5.4	6.8	6.0	5.4	5.7	1.5
Tuber weight (kg)	7.8	5.5	5.3	6.5	4.6	5.3	6.7	4.9	4.0	5.3	1.9
No of Rotted tuber	0.0	0.0	0.0	0.6	0.5	0.7	0.3	1.0	2.4	1.1	0.8
Percentage tuber rot	0.0	0.0	0.0	8.1	6.9	12.6	3.3	24.7	44.5	18.7	14.1
Tuber rot severity***	0.0	0.0	0.0	1.4	1.5	1.5	1.2	2.3	2.7	1.9	0.6
Total nematode	0.0	1343.0	17758	0.0	987.0	289.0	661.0	1785.0	520.0	1597.0	2348.2

LSD for comparing treatment means within the same row

*Treatment Key: A= Control (no nematode, no fungus), B= 1,000 *M. incognita* eggs, C = 10,000 *M. incognita* eggs, D= $5X10^5$ *B. theobromae* spores, E= 1,000 *M. incognita*.eggs + $5X10^5$ *B. theobromae* spores + simultaneously, F= 1,000 *M. incognita* eggs + $5X10^5$ *B. theobromae* spores inoculated 2 weeks afte the nematode, G= $5X10^5$ *B. theobromae* spores + 1,000 *M. incognita* eggs inoculated 2 weeks after the fungus, H= 10,000 *M. incognita*.eggs $5X10^5$ *B. theobromae* spores + simultaneously, I= 10,000 *M. incognita* eggs + $5X10^5$ *B. theobromae* spores inoculated 2 weeks after the nematode, J= $5X10^5$ *B. theobromae* spores + 10,000 *M. incognita* eggs inoculated 2 weeks after the fungus

* *0 = no galls, 1 = 1-10% of the root system galled, 2= 11-20% of the root system galled, 3= 21- 70% of the root system galled, 4= 71-90% of the root system galled; and 5 = greater than 90% of the root system galled.

*** 0 = No rot, 1 = very mild rotting (Less than 5% of tuber rotted), 2 = Mild rotting (6-10% of tuber rotted), 3 = Moderate rotting (11-25% of tuber rotted), 4 = Severe rotting (26-50% of tuber rotted), 5 = Very severe rotting (more than 50% of tuber rotted).

Table 4.18: Effects of *B. theobromae* and *M. incognita* on growth, yield parameters and nematode reproduction on cassava (2nd trial) (Field)

Parameters	TREATMENTS*										
	A	B	C	D	E	F	G	H	I	J	LSD (P≤0.05)
Mean Gall index**	0.0	1.0	2.3	0.0	0.5	0.4	0.6	0.8	0.3	1.0	0.4
Reproductive Factor	0.0	1.4	1.9	0.0	1.1	0.3	0.7	0.2	0.1	0.2	0.3
Plant Height (cm)	284.8	257.9	242.0	252.1	256.0	242.4	247.5	246.7	212.9	240.9	23.1
Stem Diameter (cm)	4.6	4.1	4.0	4.2	3.9	3.9	3.9	4.1	3.8	3.8	0.6
Mean fresh shoot weight (kg)	8.3	6.3	5.4	7.0	5.5	5.9	6.0	5.9	4.6	6.2	6.2
Number of tubers	8.8	6.4	5.7	6.8	5.4	6.3	7.2	6.0	5.0	5.7	1.5
Tuber weight (kg)	7.2	4.1	4.0	5.4	3.6	4.4	5.24	3.76	2.0	3.4	1.9
Number of Rotted tuber	0.0	0.0	0.0	0.1	0.7	0.5	0.3	1.2	2.4	1.1	0.8
Percentage tuber rot	0.0	0.0	0.0	5.0	12.6	6.9	3.3	28.8	48.1	18.7	15.0
Rot severity***	0.0	0.0	0.0	1.4	1.4	1.4	1.1	2.3	2.9	2.0	0.6
Total nematode	0.0	1412.0	18928.0	0.0	1124.0	283.0	631.0	1736.0	541.0	1545.0	2273.8

LSD for comparing treatment means within the same row

*Treatment Key; A= Control (no nematode, no fungus), B= 1,000 *M. incognita* eggs, C = 10,000 *M. incognita* eggs, D= $5X10^5$ *B. theobromae* spores, E= 1,000 *M. incognita*.eggs + $5X10^5$ *B. theobromae* spores + simultaneously, F= 1,000 *M. incognita* eggs + $5X10^5$ *B. theobromae* spores inoculated 2 weeks after the nematode, G= $5X10^5$ *B. theobromae* spores + 1,000 *M. incognita* eggs inoculated 2 week after the fungus, H= 10,000 *M. incognita*.eggs $5X10^5$ *B. theobromae* spores + simultaneously, I= 10,000 *M. incognita* eggs + $5X10^5$ *B. theobromae* spores inoculated 2 weeks after the nematode, J= $5X10^5$ *B. theobromae* spores + 10,000 *M. incognita* eggs inoculated 2 weeks after the fungus.

** 0 = no galls, 1 = 1-10% of the root system galled, 2= 11-20% of the root system galled, 3= 21- 70% of the root system galled, 4= 71-90% of the root system galled; and 5 = greater than 90% of the root system galled.

*** 0 = No rot, 1 = very mild rotting (Less than 5% of tuber rotted), 2 = Mild rotting (6-10% of tuber rotted), 3 = Moderate rotting (11-25% of tuber rotted), 4 = Severe rotting (26-50% of tuber rotted), 5 = Very severe rotting (more than 50% of tuber rotted)

4.3.1: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on Height and Stem diameter of three cassava cultivars

The highest mean value was from plants inoculated with *G. mosseae* alone followed by values recorded from the uninoculated control plants throughout the study period. However, they were not significantly different from each other throughout the experiment.

At 2 MAP, the highest mean plant height from TMS 30572 was obtained from the plants inoculated with *G. mosseae* alone (43.7 cm) and followed by the uninoculated control plants (43.0 cm) but there was no significant difference between both. Plants inoculated with *G. mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs had significantly lower value (35.7 cm) than plants inoculated with 5,000 *M. incognita* with either of the two fungi alone (40.5 cm and 37.4 cm) (Fig. 4.25). There was no significant difference between plant height of plants inoculated with *G. mosseae* + 5000 *M. incognita* eggs (40.4 cm) and *P. lilacinus* + 5,000 *M. incognita* eggs (37.4 cm). At 12 MAP the highest mean plant height was recorded in plants inoculated with *G. mosseae* alone (289.2 cm) while the least mean plant height was recorded in plants inoculated with 5,000 *M. incognita* eggs alone (197.9 cm) (Fig. 4.25) It was significantly lower than values from all other treatments and this trend was recorded in the second trial (Fig. 4.25).

At 12 MAP, the highest mean plant height from TME 1 was obtained from the plants inoculated with *G. mosseae* alone (297.5 cm) and followed by plants inoculated with *G. mosseae* and *P. lilacinus* (295.1 cm), followed by the uninoculated control plants (294.7 cm) but there was no significant difference between the treatments (Fig. 4.26). Plants inoculated with *G. mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs had significantly lower value (272.1 cm) than plants inoculated with 5,000 *M. incognita* with either of the two fungi alone (280.7 cm and 284.4 cm) (Fig. 4.26). There was no significant difference between plant height of plants inoculated with *G. mosseae* + 5000 *M. incognita* eggs (280.7 cm) and *P. lilacinus* + 5,000 *M. incognita* eggs (284.4 cm). The least mean plant height was recorded in plants inoculated with 5,000 *M. incognita* eggs alone (228.1 cm) (Fig. 4.26). It was significantly lower than values from all other treatments and this trend was recorded in the second trial (Fig. 4.26).

In Ofege, the highest mean plant height at 12 MAP was obtained from the plants inoculated with *G. mosseae* alone (297.7 cm) and followed by plants inoculated with 5,000 *M. incognita* and carbofuran (297.5 cm), but there was no significant difference between the treatments (Fig. 4.27). Plants inoculated with *G. mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs had significantly lower value (271.9 cm) than plants inoculated with 5,000 *M. incognita* with either of the two fungi alone (281.6 cm and 288.1 cm) (Fig. 4.27). There was no significant difference between plant height of plants inoculated with *G. mosseae* + 5000 *M. incognita* eggs (281.6 cm) and *P. lilacinus* + 5,000 *M. incognita* eggs (288.1 cm). The least mean plant height was recorded in plants inoculated with 5,000 *M. incognita* eggs alone (228.5 cm) (Fig. 4.27). It was significantly lower than values from all other treatments and this trend was recorded in the second trial (Fig. 4.27).

The mean stem diameter recorded for various treatments differed statistically from each other. In TMS 30572 the highest mean stem diameter was produced by plants inoculated with *G. mosseae* alone, uninoculated control and a combination of *G. mosseae* and *P.lilacinus* (2.6 cm) at 12 MAP. They all have the same values and are not significantly different from one another (Fig 4.28). The lowest mean diameter was recorded from plants inoculated with a combination of *G. mosseae, P. lilacinus* and 5,000 *M. incognita* eggs (2.4 cm) (Fig. 4.28).

In TME 1 the highest mean stem diameter was produced by plants inoculated with *G. mosseae* alone and a combination of *G. mosseae* and *P.lilacinus* (2.6 cm) at 12 MAP. They both recorded the same values and are not significantly different from one another (Fig 4.29). The lowest mean diameter was recorded from plants inoculated with 5,000 *M. incognita eggs* alone (2.3 cm) (Fig. 4.29)

In Ofege the highest mean stem diameter was produced by plants inoculated with *G. mosseae* alone (2.7 cm) at 12 MAP followed by the uninoculated control (2.6cm), plants inoculated with a combination of *G. mosseae* and *P. lilacinus* (2.6cm) and plants inoculated with Carbofuran + 5,000 *M. incognita* eggs (2.6 cm) (Fig 4.30).

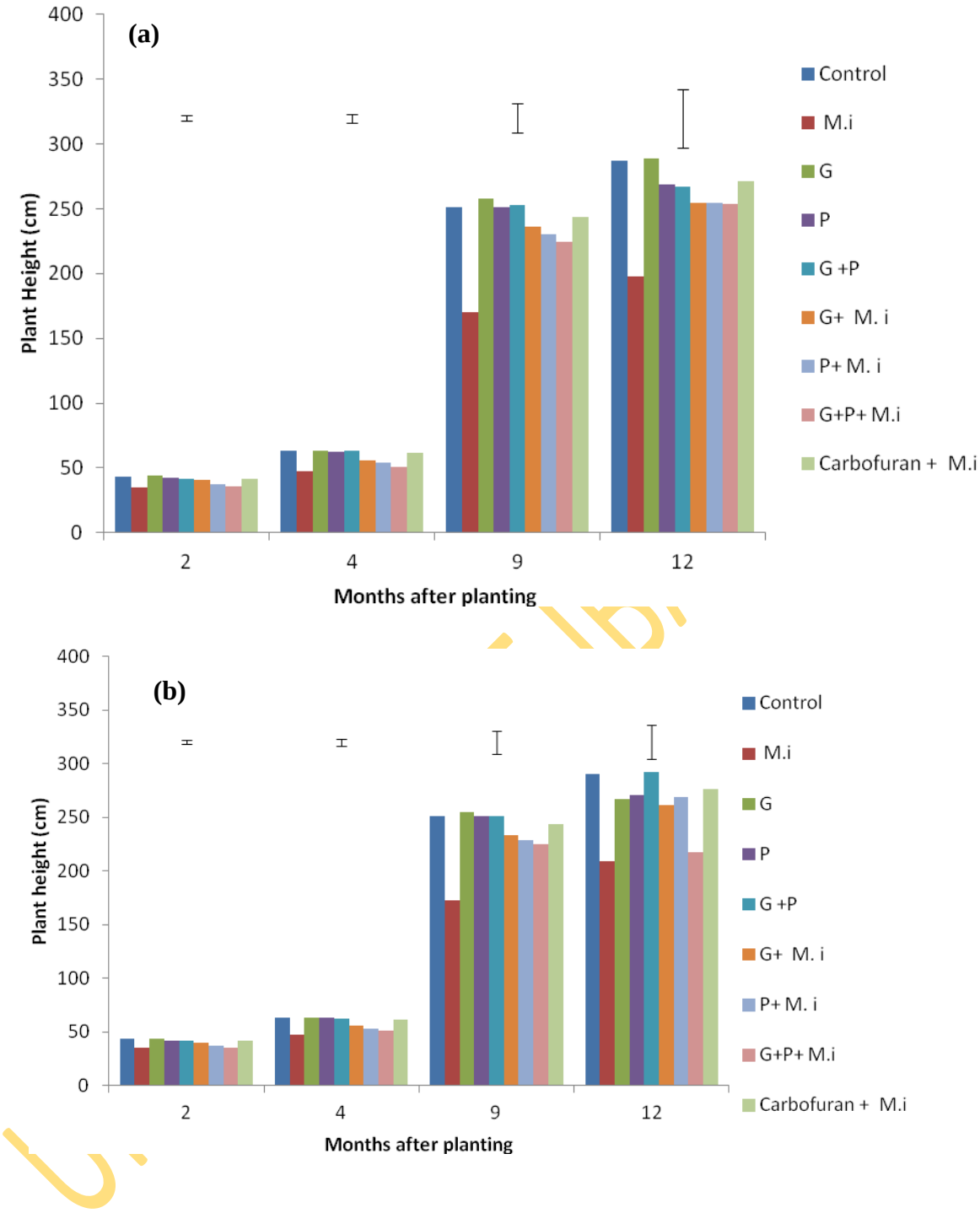


Figure 4.25: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on the plant heights of TMS 30572 cassava cultivar. (a) = first trial, (b) = second trial.

LSD bars are for comparing treatment means at each specific sampling time

Control = (no nematode, no fungus, no carbofuran), M.i = 5,000 *Meloidogyne incognita* eggs, G = 140 spores of *G. mossae*, P = 2x 10^6 ml $^{-1}$ spores of *P. lilacinus*, Carbofuran = Carbofuran 3 kg ai/ha

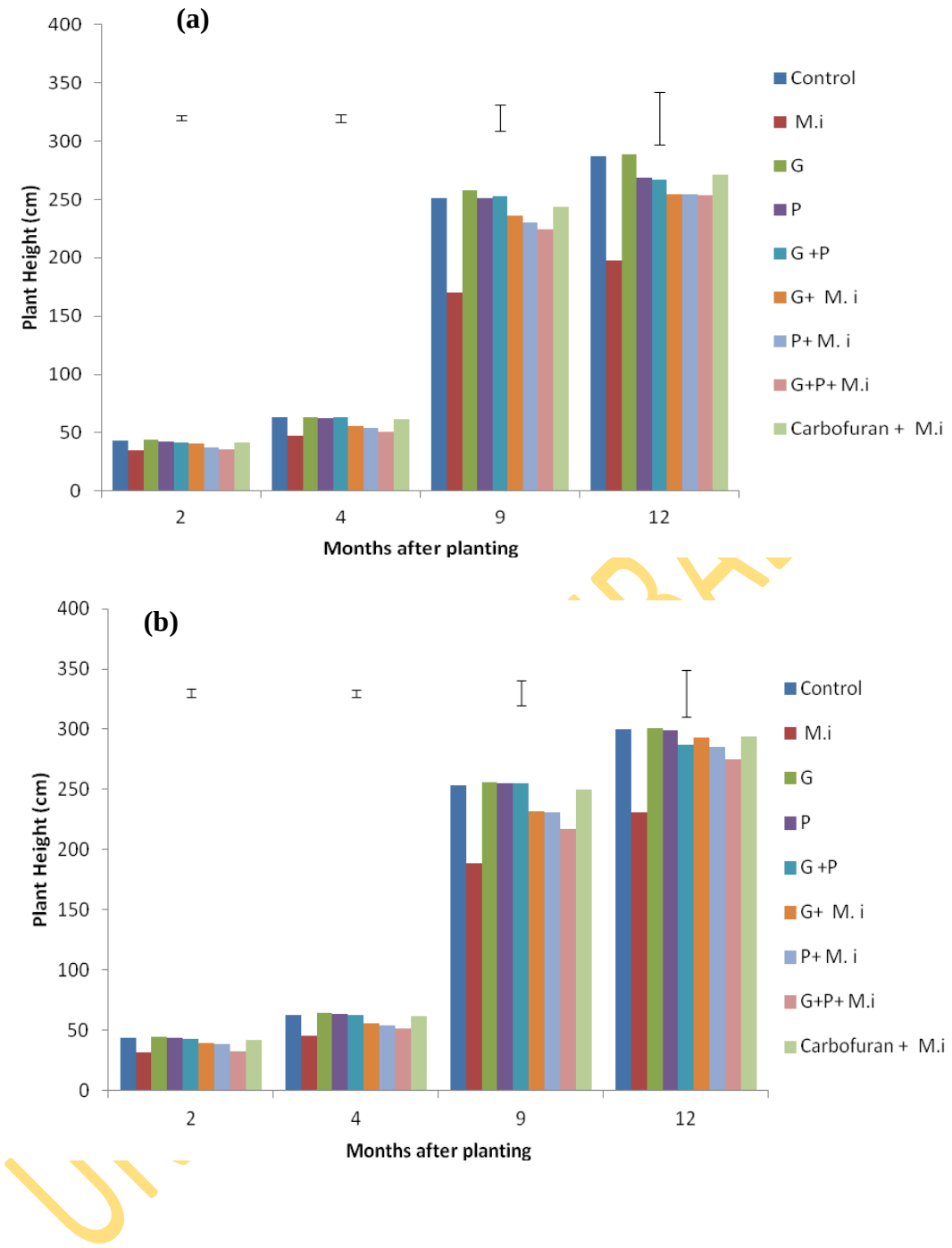


Figure 4.26: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on the plant heights of TME 1 cassava cultivar in a pot experiment.
(a) = First trial; (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

Control = (no nematode, no fungus, no carbofuran), M.i = 5,000 *Meloidogyne incognita* eggs, G = 140 spores of *G. mossae*, P = 2x 10^6 ml $^{-1}$ spores of *P. lilacinus*, Carbofuran = Carbofuran 3 kg ai/ha

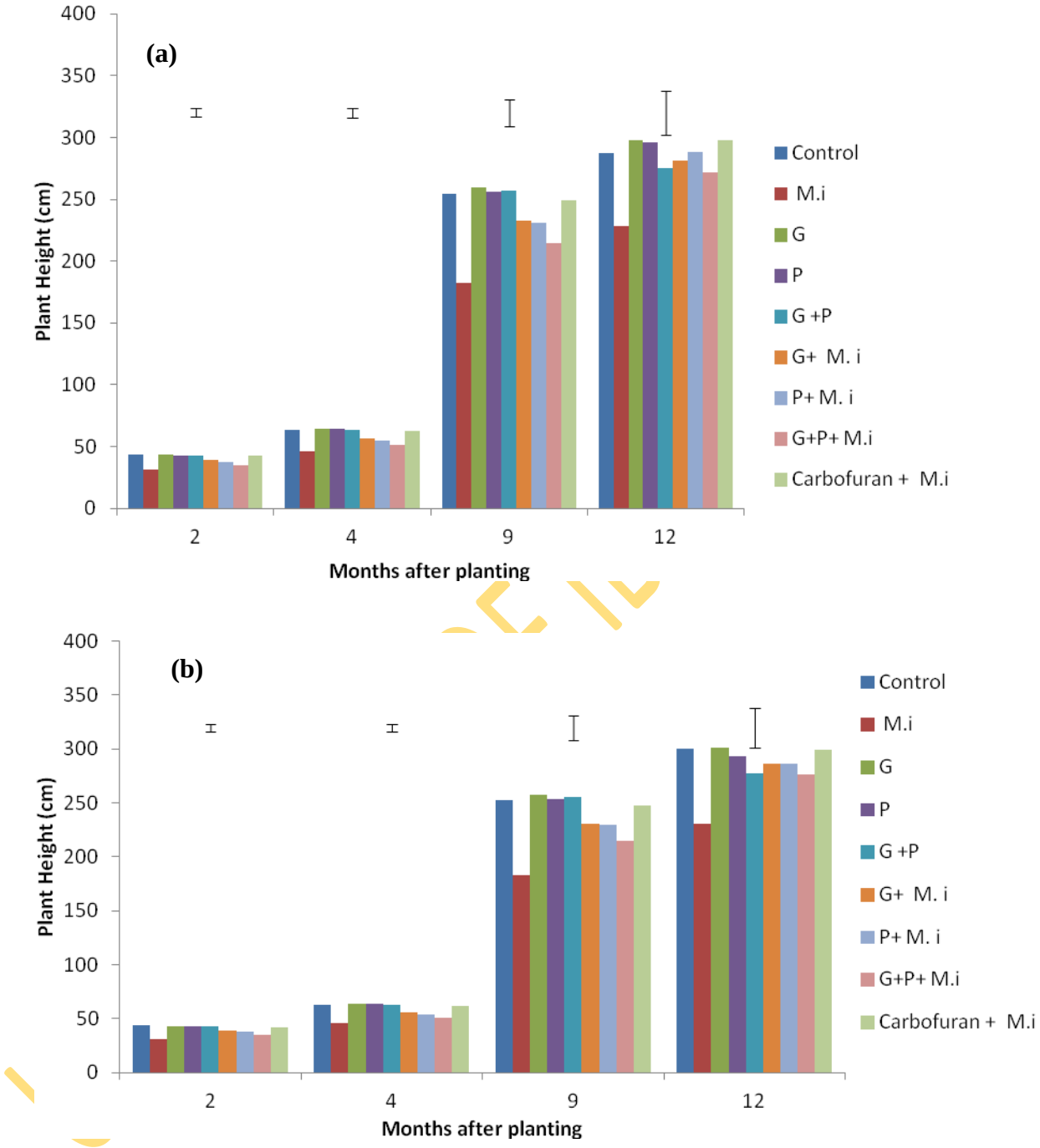


Figure 4.27: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on the plant heights of Ofege cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time.

Control = (no nematode, no fungus, no carbofuran), M.i = 5,000 *Meloidogyne incognita* eggs, G = 140 spores of *G. mossae*, P = 2x 10^6 ml $^{-1}$ spores of *P. lilacinus* , Carbofuran = Carbofuran 3 kg ai/ha

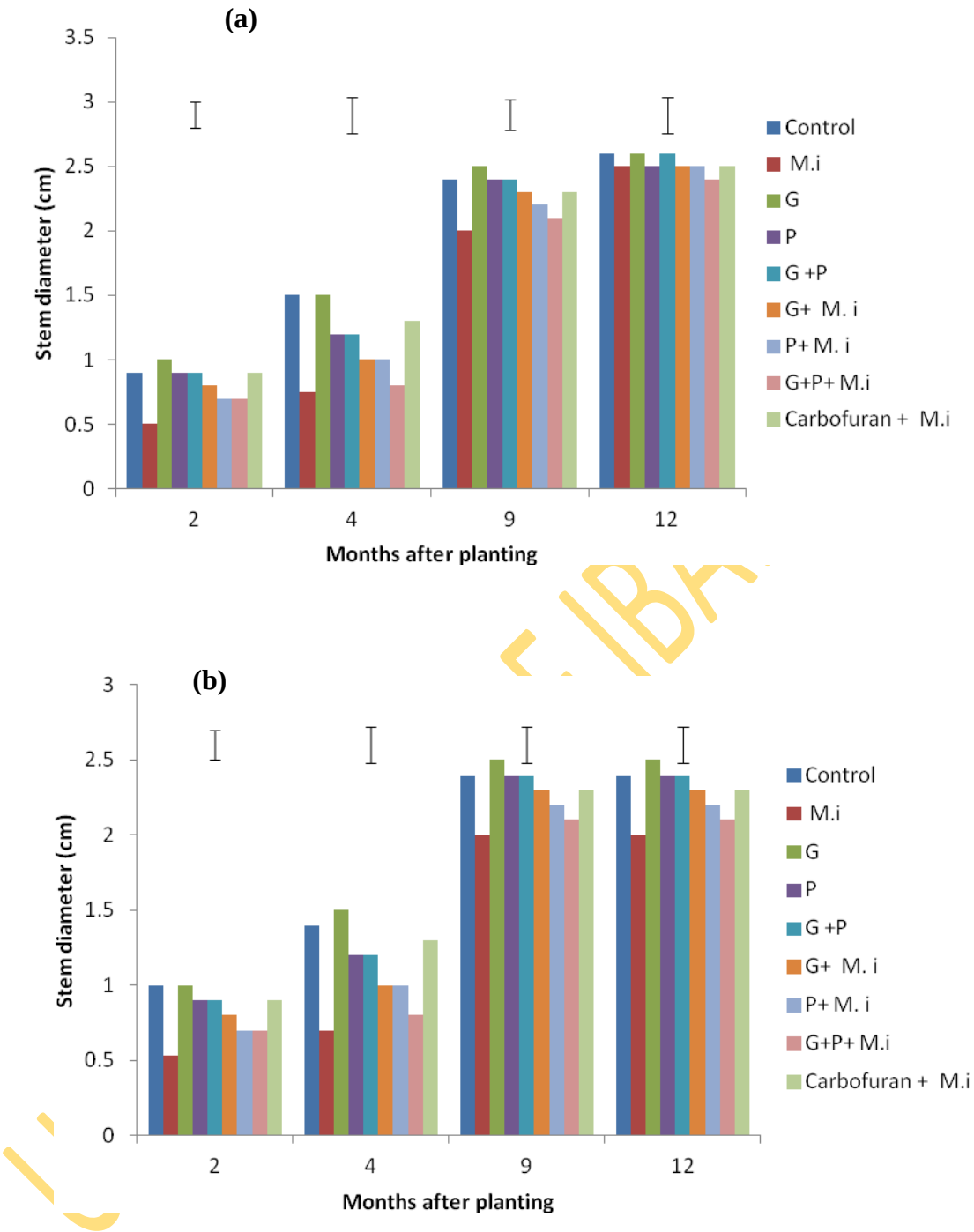


Figure 4.28: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on the stem diameter of TMS 30572 cassava cultivar in a pot experiment. (a) = first trial and (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time

Control = (no nematode, no fungus, no carbofuran), M.i = 5,000 *Meloidogyne incognita* eggs, G = 140 spores of *G. mossae*, P = 2x 10^6 ml $^{-1}$ spores of *P. lilacinus* , Carbofuran = Carbofuran 3 kg ai/ha

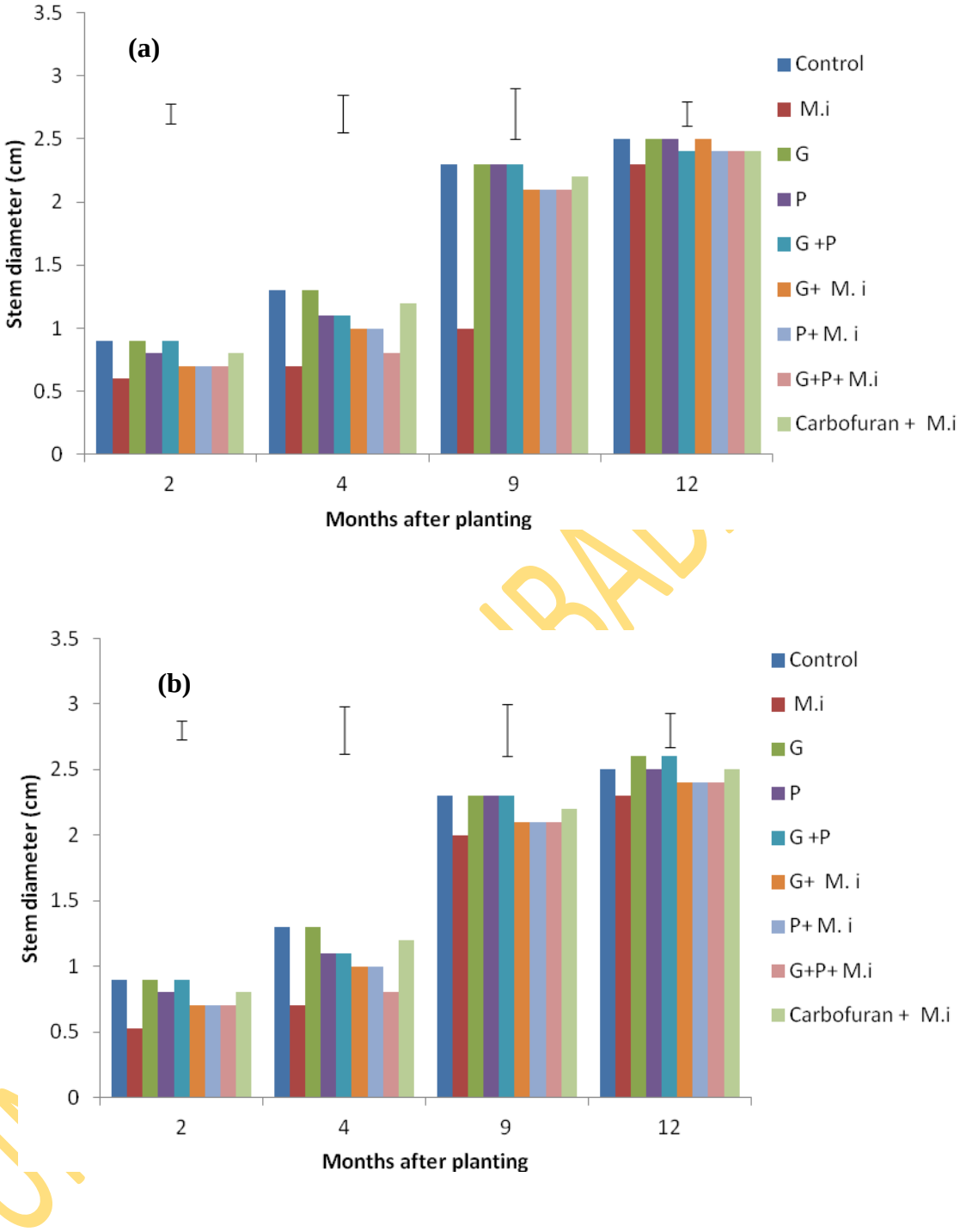


Figure 4.29: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on the stem diameter of TME 1 cassava cultivar in a pot experiment. (a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time

Control = (no nematode, no fungus, no carbofuran), M.i = 5,000 *Meloidogyne incognita* eggs, G = 140 spores of *G. mossae*, P = 2x 10^6 ml $^{-1}$ spores of *P. lilacinus*, Carbofuran = Carbofuran 3 kg ai/ha

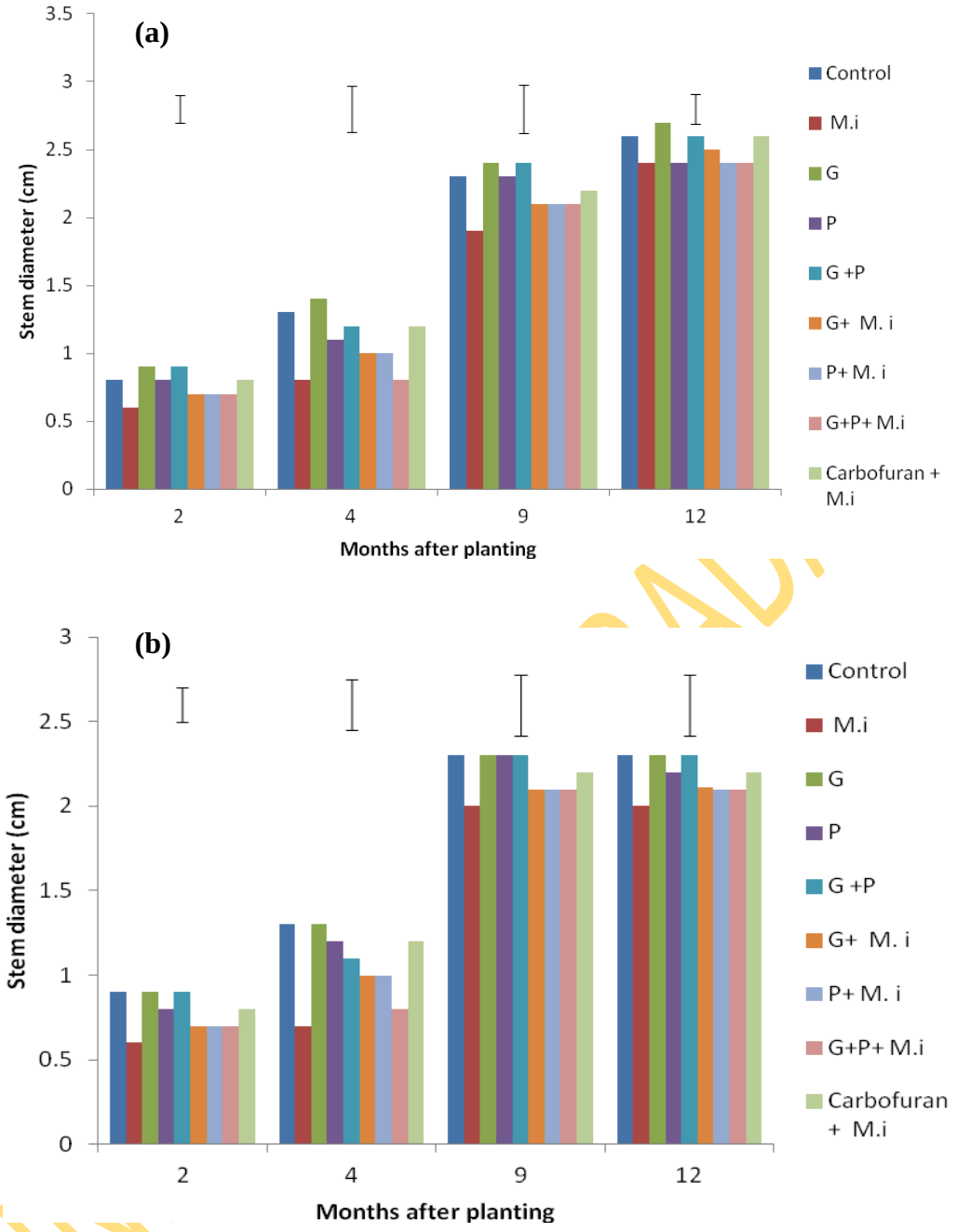


Figure 4.30: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on the stem diameter of Ofege cassava cultivar in a pot experiment.

(a) = first trial, (b) = second trial

LSD bars are for comparing treatment means at each specific sampling time

Control = (no nematode, no fungus, no carbofuran), M.i = 5,000 *Meloidogyne incognita* eggs, G = 140 spores of *G. mossae*, P = 2x 10^6 ml $^{-1}$ spores of *P. lilacinus*, Carbofuran = Carbofuran 3 kg ai/ha

4.3.2: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on fresh shoot weight of three cassava cultivars

Soil inoculated with *G. mosseae* + 5,000 *M. incognita* eggs and *P. lilacinus* + 5,000 *M. incognita* eggs influenced the fresh shoot weight of cassava than soil inoculated with 5000 *M. incognita* alone. In the first trial, TMS 30572, the mean fresh shoot weight was significantly higher (P≤0.5) in plants inoculated with *G. mosseae* alone (509.5 g), *P. lilacinus* alone (363.5 g), *G mosseae* + *P. lilacinus* in combination (368.9g), Carbofuran + 5,000 *M. incognita* eggs (372.3 g) and uninoculated soil (control) (394.9 g) than plants inoculated with 5000 *M. incognita* alone (159.4g), *G mosseae*+ 5,000 *M. incognita* eggs (242.1 g), *P. lilacinus* + 5,000 *M. incognita* eggs (292.6g) and *G. mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs (197.3 g) (Table 4.19).

In TME 1, the mean fresh shoot weight was significantly higher (P≤0.5) in plants inoculated with *G. mosseae* alone (221.5 g), *P. lilacinus* alone (195.1.5 g), *G mosseae* + *P. lilacinus* in combination (218.3 g), Carbofuran + 5,000 *M. incognita* eggs (205.7 g) and uninoculated soil (control) (213.2 g) than plants inoculated with 5000 *M. incognita* alone (121.5 g), *G mosseae*+ 5,000 *M. incognita* eggs (136.3 g), *P. lilacinus* + 5,000 *M. incognita* eggs (173.7 g) and *G. mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs (102.1 g) (Table 4.19).

In Ofege, the mean fresh shoot weight was significantly higher (P≤0.5) in plants inoculated with *G. mosseae* alone (238.2 g), *P. lilacinus* alone (317.5 g), *G mosseae* + *P. lilacinus* in combination (231.8 g), Carbofuran + 5,000 *M. incognita* eggs (222.1 g) and uninoculated soil (control) (206.3 g) than plants inoculated with 5000 *M. incognita* alone (99.38g), *G mosseae*+ 5,000 *M. incognita* eggs (192.4 g), *P. lilacinus* + 5,000 *M. incognita* eggs (208.9 g) and *G. mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs (165.2 g).The trend was the same in the second trial (Table 4.19). The highest mean value for the two trials in all the cassava cultivars used for the experiment was obtained from plants inoculated with *G. mosseae* alone while the lowest mean value in the two trials was obtained from plants inoculated with 5,000 *M. incognita* alone and it was significantly lower from all other treatments followed by plants inoculated with G. *mosseae* + *P. lilacinus* +5,000 *M. incognita* eggs (Table 4.19).

Table 4.19: Effects of *Glomus mosseae*. *Paecilomyces lilacinus* in combination or alone and carbofuran on shoot weight (g) of three cassava cultivars in a pot experiment.

Treatment*	TMS 30572		TME 1		OFEGE	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
Uninoculated soil (control)	394.9	466.0	213.2	296.2	206.3	324.2
5000 *M. incognita* eggs	159.4	257.3	121.5	215.6	99.38	194.6
G. mosseae alone	509.5	468.5	221.5	318.1	238.2	334.0
P. lilacinus alone	363.5	453.7	195.1	310.8	217.5	315.5
G . mosseae + *P. lilacinus*	368.9	433.6	218.3	317.4	231.8	316.3
G. mosseae + 5000 *M. incognita* eggs	242.1	334.7	136.3	232.7	192.4	287.6
P. lilacinus + 5000 *M. incognita* eggs	292.6	408.3	173.7	293.8	208.9	318.1
G. mosseae + *P. lilacinus* + 5000 *M. incognita* eggs	197.3	383.9	102.1	195.7	165.2	262.7
Carbofuran @ 3kg ai/ha + 5000 *M. incognita* eggs	372.3	406.2	205.7	299.9	222.1	318.1
LSD (P≤0.05)	165.6	51.6	41.6	36.7	49.1	39.7

LSD for comparing treatment means within the same column

*Treatment key: *P. lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus; G. mosseae* = 140 spores of *Glomus mosseae*

4.3.3: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on Galling Index and Nematode Reproduction on three cassava cultivars in pot

In TMS 30572, the least mean gall indices were obtained from uninoculated soil, *Glomus mosseae* alone, *P. lilacinus* alone and *Glomus mosseae* + *P. lilacinus* in combination (0.0) (Table 4.20). The values were not significantly lower than values from carbofuran treated soil in the first and second trial. The highest mean gall index was recorded from plants grown on soil inoculated with 5,000 *M. incognita* eggs alone (2.1)(Table 4.20). The least mean gall indices in TME1 were obtained from uninoculated soil, *Glomus mosseae* alone, *P. lilacinus* and *Glomus mosseae* + *P. lilacinus* in combination in the first and second trial and this trend was also recorded in Ofege (0.0). The highest mean gall index was recorded from plants grown on soil inoculated with 5,000 *M. incognita* eggs alone (3.6) (Table 4.20). This followed the same trend in the second trial (Table 4.20).

In Ofege, the least mean gall indices were obtained from uninoculated soil, *Glomus mosseae* alone, *P. lilacinus* and *Glomus mosseae* + *P. lilacinus* in combination in the first and second trial and this trend was also recorded in Ofege (0.0). The highest mean gall index was recorded from plants grown on soil inoculated with 5,000 *M. incognita* eggs alone (2.9) (Table 4.20). This followed the same trend in the second trial. In all the cultivars, the treatments with the combination of *G. mossaea*, *P.lilacinus* and *M. incognita* had higher gall index than when *G. mosseae* or *P. lilacinus* were inoculated singly (Table 4.20).

The mean nematode reproductive factor (RF) also differed statistically ($P \leq 0.05$) from one other in all the cultivars. In TMS 30572, the least reproductive factor came from uninoculated soil, *G. mosseae* alone, *P. lilacinus* alone and *G. mosseae* + *P. lilacinus* in combination for both trials (0.0) (Table 4.21). It was followed by soils treated with Carbofuran and 5,000 *M. incognita* eggs (0.1) and the values were not significantly different from the control. (Table 4.21) The trend was the same for the two trials (Table 4.21) Soils treated with the combination of *G. mosseae* + *P. lilacinus* + 5,000 eggs of *M. incognita* had reproductive factors higher than when the soil were inoculated with the fungi and 5000 *M. incognita* singly. The highest reproductive factor was observed in plants from soils inoculated with 5000

M. incognita eggs alone (2.1) and it was significantly higher than values from other treatments for both trials (Table 4.21)

In TME 1, the least reproductive factor were recorded from uninoculated soil, *G. mosseae* alone, *P. lilacinus* alone and *G. mosseae* + *P. lilacinus* in combination for both trials (0.0) (Table 4.21). It was followed by soils treated with Carbofuran and 5,000 *M. incognita* eggs (0.3) and the values were not significantly different from the control. (Table 4.21) The trend was the same for the two trials (Table 4.21) Soils treated with the combination of *G. mosseae* + *P. lilacinus* + 5,000 eggs of *M. incognita* had reproductive factors higher than when the soil were inoculated with the fungi and 5000 *M. incognita* singly. The highest reproductive factor was observed in plants from soils inoculated with 5000 *M. incognita* eggs alone (2.1) and it was significantly higher than values from other treatments for both trials (Table 4.21).

In Ofege, the least reproductive factor were recorded from uninoculated soil, *G. mosseae* alone, *P. lilacinus* alone and *G. mosseae* + *P. lilacinus* in combination for both trials (0.0) (Table 4.21). It was followed by soils treated with Carbofuran and 5,000 *M. incognita* eggs (0.0) and the values were not significantly different from the control. (Table 4.21) The trend was the same for the two trials (Table 4.21) Soils treated with the combination of *G. mosseae* + *P. lilacinus* + 5,000 eggs of *M. incognita* had reproductive factors higher than when the soil were inoculated with the fungi and 5000 *M. incognita* singly. The highest reproductive factor was observed in plants from soils inoculated with 5000 *M. incognita* eggs alone (2.2) and it was significantly higher than values from other treatments for both trials (Table 4.21).

4.3.4: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on the Yield of three cassava cultivars

The mean number of tubers recorded for various treatments were significantly different from each other ($P \leq 0.05$). In TMS 30572, the plants treated with *G. mosseae* alone produced the highest number of tubers (11.0) which did not differ significantly from the uninoculated control plants (10.0) (Table 4.22). The treatment *G. mosseae* + 5,000 *M. incognita* eggs had a higher number of tubers (9.0)

Table 4.20: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on root galling of three cassava cultivars in a pot experiment.

Treatment*	TMS 30572		TME 1		OFEGE	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
Uninoculated soil (control)	0.0	0.0	0.0	0.0	0.0	0.0
5000 *M. incognita* eggs	2.1	1.9	3.6	3.6	2.9	2.9
G. mosseae alone	0.0	0.0	0.0	0.0	0.0	0.0
P. lilacinus alone	0.0	0.0	0.0	0.0	0.0	0.0
G . mosseae + *P. lilacinus*	0.0	0.0	0.0	0.0	0.0	0.0
G. mosseae + 5000 *M. incognita* eggs	1.3	1.5	1.5	1.5	1.5	1.5
P. lilacinus + 5000 *M. incognita* eggs	1.3	1.3	1.3	1.3	1.5	1.8
G. mosseae + *P. lilacinus* + 5000 *M. incognita* eggs	1.7	1.8	2.2	2.1	1.8	1.8
Carbofuran @ 3kg ai/ha + 5000 *M. incognita* eggs	0.3	0.3	0.5	0.6	0.8	0.5
LSD (P≤0.05)	0.6	0.5	0.6	0.6	0.6	0.6

LSD for comparing treatment means within the same column

*Treatment key: *P. lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus; G. mosseae* = 140 spores of *Glomus mosseae*

* 0 = no galls, 1 = 1-10% of the root system galled, 2= 11-20% of the root system galled, 3= 21- 70% of the root system galled, 4= 71-90% of the root system galled; and 5 = greater than 90% of the root system

Table 4.21: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on nematode Reproductive Factor (RF) on three cassava cultivars in a pot experiment.

Treatment*	TMS 30572		TME 1		OFEGE	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
Uninoculated soil (control)	0.0	0.0	0.0	0.0	0.0	0.0
5000 *M. incognita* eggs	2.1	2.0	2.1	2.5	2.2	2.2
G. mosseae alone	0.0	0.0	0.0	0.0	0.0	0.0
P. lilacinus alone	0.0	0.0	0.0	0.0	0.0	0.0
G . mosseae + *P. lilacinus*	0.0	0.0	0.0	0.0	0.0	0.0
G. mosseae + 5000 *M. incognita* eggs	0.2	0.2	0.5	0.5	0.2	0.9
P. lilacinus + 5000 *M. incognita* eggs	0.2	0.2	0.2	0.2	0.2	0.2
G. mosseae + *P. lilacinus* + 5000 *M. incognita* eggs	1.2	0.5	1.4	1.3	1.2	0.9
Carbofuran @ 3kg ai/ha + 5000 *M. incognita* eggs	0.1	0.1	0.3	0.2	0.0	0.8
LSD (P≤0.05)	0.6	0.3	0.7	0.4	0.6	0.5

LSD for comparing treatment means within the same column

Treatment key: *G. mosseae* = 140 spores of *Glomus mosseae; P. lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*

*Reproductive Factor = Pf/Pi, where Pf = Final Nematode Population and Pi = Initial Nematode Population

than when it was inoculated in combination with *P. lilacinus* to control *M. incognita*.(7.0). The mean number of tubers were however not significantly different from each other. (Table 4.22). The lowest mean number of tubers was observed in plants inoculated with 5,000 *M. incognita* eggs alone (5.8) and was significantly lower (P≤0.05) than values from other treatments (Table 4.22). Increase in mean number of tubers was observed in treatments where the fungi were either inoculated singly or in combination along with 5,000 *M. incognita* eggs than in the treatment with 5,000 *M. incognita* alone (Table 4.22).

In TME 1, the plants treated with *G. mosseae* alone produced the highest number of tubers (7.5) which did not differ significantly from the uninoculated control plants (7.3) (Table 4.22). In the first trial, the treatment *G. mosseae* + 5,000 *M. incognita* eggs had the same number of tubers (6.0) as when *G. mosseae* is in combination with *P. lilacinus* to control *M. incognita* (6.0) but in the second trial plants with the treatment *G. mosseae* + 5,000 *M. incognita* eggs had a higher number of tubers (6.3) than when *G. mosseae* was inoculated in combination with *P. lilacinus* to control *M. incognita*.(5.6) (Table 4.22). The values were however not significantly different from each other (Table 4.22). The lowest mean number of tubers was observed in plants inoculated with 5000 *M. incognita* eggs alone (3.5) hand was significantly lower (P≤0.05) than the mean number of tubers from other treatments (Table 4.22).

In Ofege, the plants treated with *G. mosseae* and *P. lilacinus* produced the highest number of tubers (8.0) which did not differ significantly from the uninoculated control plants (7.3) (Table 4.22). The treatment *G. mosseae* + 5,000 *M. incognita* eggs had a higher number of tubers (6.8) than when it was inoculated in combination with *P. lilacinus* to control *M. incognita* (6.0). The mean number of tubers were however not significantly different from each other. (Table 4.22) The lowest mean number of tubers was observed in plants inoculated with 5,000 *M. incognita* eggs alone (5.0) and was significantly lower (P≤0.05) than values from other treatments (Table 4.22). Increase in mean number of tubers was observed in treatments where the fungi were either inoculated singly or in combination along with 5,000 *M. incognita* eggs than in the treatment with 5,000 *M. incognita* alone (Table 4.22).

Similarly, the mean tuber weights observed in various treatments were significantly different from each other (P≤0.05). In TMS 30572, the plants treated

with *G. mosseae* alone produced the highest mean tuber weight (296.9 g) which did not differ significantly from the uninoculated control plants (290.8 g) (Table 4.23). Plants inoculated with 5000 eggs of *M. incognita* alone recorded the lowest significant mean tuber weight (122.8 g). Mean tuber weight was significantly increased with application of *G. mosseae* or *P. lilacinus* either singly or in combination compared with the 5,000 *M. incognita* eggs inoculated plants (Table 4.23). Plants inoculated with the combination of *G. mosseae* and *P. lilacinus* in controlling 5,000 eggs of *M. incognita* recorded a lower mean tuber weight than when both fungi were applied in the control of *M. incognita* singly. The fungi significanltly reduced *M. incognita* (Table 4.23). This followed the same trend in the second trial.

In TME 1 the plants treated with *G. mosseae* alone produced the highest mean tuber weight (230.9 g) which did not differ significantly from the uninoculated control plants (224.5 g). Plants inoculated with 5000 eggs of *M. incognita* alone recorded the lowest significant mean tuber weight (88.1 g) (Table 4.23). Mean tuber weight was significantly increased with application of *G. mosseae* or *P. lilacinus* either singly or in combination compared with the 5,000 *M. incognita* eggs inoculated plants (Table 4.23). Plants inoculated with the combination of *G. mosseae* and *P. lilacinus* in controlling 5,000 eggs of *M. incognita* recorded a lower mean tuber weight than when both fungi were applied in the control of *M. incognita* singly. The fungi significanltly reduced *M. incognita* (Table 4.23). This followed the same trend in the second trial.

In Ofege the plants treated with *G. mosseae* alone produced the highest mean tuber weight (249.9 g) which did not differ significantly from the uninoculated control plants (237.4 g). Plants inoculated with 5000 eggs of *M. incognita* alone recorded the lowest significant mean tuber weight (93.6 g) (Table 4.23). Mean tuber weight was significantly increased with application of *G. mosseae* or *P. lilacinus* either singly or in combination compared with the 5,000 *M. incognita* eggs inoculated plants (Table 4.23). Plants inoculated with the combination of *G. mosseae* and *P. lilacinus* in controlling 5,000 eggs of *M. incognita* recorded a lower mean tuber weight than when both fungi were applied in the control of *M. incognita* singly. The fungi significanltly reduced *M. incognita* (Table 4.23).). This followed the same trend in the second trial.

4.3.5: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on the number of nematodes on three cassava cultivars

In TMS 30572, total nematode reproduction in plants inoculated with *M. incognita* alone (10455.0) was significantly higher than in plants inoculated with *G. mosseae* + 5,000 *M.* incognita eggs (888.0) , *P. lilacinus* + 5,000 *M. incognita* eggs (852.0) , *G. mosseae* + *P. lilacinus* + 5000 *M. incognita* eggs (6021.0) and carbofuran + 5,000 *M. incognita* eggs (441.0). This was significantly higher than mean nematode population in the uninoculated control (0.0), plants inoculated with *G. mosseae* alone (0.0), *P. lilacinus* alone and plants inoculated with the combination of *G. mosseae* and *P.lilacinus* alone used in the experiment (Table 4.24). Plants inoculated with G. *mosseae* +*P. lilacinus* + 5,000 *M. incognita* eggs had significantly higher number of nematodes (6021.0) than when the fungi were singly inoculated with 5,000 *M. incognita* (Table 4.24).

The uninoculated (control) plants, *G. mosseae* inoculated plants, *P. lilacinus* inoculated plants and *G. mosseae* + *P. lilacinus* inoculated plants that were not inoculated with 5,000 *M. incognita* eggs had no nematodes obtained from the treatments and had values that were not significantly different from plants inoculated with carbofuran+ 5,000 *M. incognita* eggs (441.0) (Table 4.24)

Numbers of *M. incognita* in roots were suppressed by *G. mosseae*, *P lilacinus* and both fungi in combination. The highest total nematode reduction was obtained from plants inoculated with Carbofuran + 5,000 *M. incognita eggs* (441.0) and the values were not significantly different from values obtained from plants inoculated with *G. mosseae* + 5,000 *M. incognita* (888.0) and *P. lilacinus* + 5,000 *M. incognita* (852.0). This followed the same trend in the two trials (Table 4.24).

In TME 1, total nematode reproduction in plants inoculated with *M. incognita* alone (12595.9) was significantly higher than in plants inoculated with *G. mosseae* + 5,000 *M.* incognita eggs (3002.1) , *P. lilacinus* + 5,000 *M. incognita* eggs

Table 4.22: Effect of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on number of tubers of three cassava cultivars in a pot experiment

Treatment*	TMS 30572		TME 1		OFEGE	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
Uninoculated soil (control)	10.0	10.3	7.3	7.3	7.3	8.0
5000 *M. incognita* eggs	5.8	6.3	3.5	3.9	5.0	4.8
G. mosseae alone	11.0	11.3	7.5	8.1	7.9	8.5
P. lilacinus alone	9.3	9.9	6.8	7.0	7.8	8.4
G . mosseae + *P. lilacinus*	9.8	9.9	6.8	7.6	8.0	8.5
G. mosseae + 5000 *M. incognita* eggs	9.0	8.5	6.0	6.3	6.8	7.0
P. lilacinus + 5000 *M. incognita* eggs	8.0	7.8	5.5	5.8	6.3	5.8
G. mosseae + *P. lilacinus* + 5000 *M. incognita* eggs	7.0	7.3	6.0	5.6	6.0	6.3
Carbofuran @ 3kg ai/ha + 5000 *M. incognita* eggs	9.0	9.4	6.8	6.5	7.3	7.8
LSD (P≤0.05)	1.2	1.2	1.1	1.0	1.0	1.3

LSD for comparing treatment means within the same column

*Treatment key: *G. mosseae* = 140 spores of *Glomus mosseae; P. lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*

Table 4.23: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on weight of fresh tubers (g) of three cassava Cultivars in a pot experiment.

Treatment*	TMS 30572		TME 1		OFEGE	
	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
Uninoculated soil (control)	290.8	387.0	224.5	325.7	237.4	335.7
5000 *M. incognita* eggs	122.8	219.0	88.1	179.3	93.6	189.7
G. mosseae alone	296.9	394.8	230.9	333.4	249.9	352.3
P. lilacinus alone	272.4	370.2	219.9	317.6	229.4	335.6
G . mosseae + *P. lilacinus*	282.2	377.7	224.5	325.7	234.4	335.6
G. mosseae + 5000 *M. incognita* eggs	236.9	332.5	186.7	300.7	216.1	315.6
P. lilacinus + 5000 *M. incognita* eggs	215.2	291.6	199.5	283.6	2202.7	299.9
G. mosseae + *P. lilacinus* + 5000 *M. incognita* eggs	148.6	246.5	105.9	206.3	164.7	260.5
Carbofuran @ 3kg ai/ha + 5000 *M. incognita* eggs	267.1	363.5	217.0	312.9	217.0	331.9
LSD (P≤0.05)	61.0	58.9	16.2	20.1	29.2	28.6

LSD for comparing treatment means within the same column

*Treatment key: *G. mosseae* = 140 spores of *Glomus mosseae; P. lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*

(1175.4), *G. mosseae* + *P. lilacinus* + 5000 *M. incognita* eggs (7186.3) and carbofuran + 5,000 *M. incognita* eggs (1374.8). This was significantly higher than mean nematode population in the uninoculated control (0.0), plants inoculated with *G. mosseae* alone (0.0), *P. lilacinus* alone and plants inoculated with the combination of *G. mosseae* and *P.lilacinus* alone used in the experiment (Table 4.24). Plants inoculated with G. *mosseae* + *P. lilacinus* + 5,000 *M. incognita* eggs had significantly higher number of nematodes (7186.3) than when the fungi were singly inoculated with 5,000 *M. incognita* (Table 4.24).

The uninoculated (control) plants, *G. mosseae* inoculated plants, *P. lilacinus* inoculated plants and *G. mosseae* + *P. lilacinus* inoculated plants that were not inoculated with 5,000 *M. incognita* eggs had no nematodes obtained from the treatments (Table 4.24).

Numbers of *M. incognita* on roots were suppressed by *G. mosseae, P lilacinus* and both fungi in combination. The highest total nematode reduction was obtained from plants inoculated with *P. lilacinus* + 5,000 *M. incognita* (1175.4) and the values were not significantly different from values obtained from plants inoculated with *G. mosseae* + 5,000 *M. incognita* (888.0) and *P. lilacinus* + 5,000 *M. incognita* (852.0). However, in the second trial, the highest total nematode reduction was obtained from plants inoculated with Carbofuran + 5,000 *M. incognita eggs* (1074.8) (Table 4.24).

In Ofege, total nematode reproduction in plants inoculated with *M. incognita* alone (11166.0) was significantly higher than in plants inoculated with *G. mosseae* + 5,000 *M.* incognita eggs (1055.0) , *P. lilacinus* + 5,000 *M. incognita* eggs (958.0) , *G. mosseae* + *P. lilacinus* + 5000 *M. incognita* eggs (6212.0) and carbofuran + 5,000 *M. incognita* eggs (778.0). This was significantly higher than mean nematode population in the uninoculated control (0.0), plants inoculated with *G. mosseae* alone (0.0), *P. lilacinus* alone and plants inoculated with the combination of *G. mosseae* and *P.lilacinus* alone used in the experiment (Table 4.24). Plants inoculated with G. *mosseae* +*P. lilacinus* + 5,000 *M. incognita* eggs had significantly higher number of nematodes (6212.0) than when the fungi were singly inoculated with 5,000 *M. incognita* (Table 4.24).

The uninoculated (control) plants, *G. mosseae* inoculated plants, *P. lilacinus* inoculated plants and *G. mosseae* + *P. lilacinus* inoculated plants that were not inoculated with 5,000 *M. incognita* eggs had no nematodes obtained from the

treatments and had values that were not significantly different from plants inoculated with carbofuran+ 5,000 *M. incognita* eggs (778.0) (Table 4.24)

Numbers of *M. incognita* on roots were suppressed by *G. mosseae, P lilacinus* and both fungi in combination. The highest total nematode reduction was obtained from plants inoculated with Carbofuran + 5,000 *M. incognita eggs* (778.0) and the values were not significantly different from values obtained from plants inoculated with *G. mosseae* + 5,000 *M. incognita* (1055.0) and *P. lilacinus* + 5,000 *M. incognita* (958.0). This followed the same trend in the two trials (Table 4.24).

4.3.6: Effects of *Glomus mosseae, Pacilomyces lilacinus* and Carbofuran on vegetative parameters of Cassava on the field

In Combined treatments of the bio-control fungal agents did not induce the greatest enhancement of the plant growth**.** The plants grown on carbofuran treated soil produced the highest mean plant height (306.3cm) and this did not differ significantly from the plant height of plants grown on *G. mosseae* treated soil and *P. lilacinus* treated soil. The least value came from the nematode-infested soil (210.5cm) (Table 4.25).

In the first trial, the highest mean stem diameter was recorded in plants grown on carbofuran treated soil (4.8cm) and there was no significant difference between this value and values recorded from soil treated singly with *P. lilacinus* (4.7cm) and *G. mossea* (4.2cm). The least value came from the nematode-infested soil (2.7cm) (Table 4.25). However, in the second trial the highest mean stem diameter was produced by plants grown on soil treated with *G. mosseae* (4.9cm) (Table 4.25).

Plants grown on carbofuran treated soil produced the highest significant shoot weight (5421.6g) in the first trial, while in the second trial the highest significant shoot weight was produced by plants grown on *G. mosseae* treated soil (5349.4g). The least significant shoot weight was recorded from plants grown on nematode infested soil (3654.8g). Combined treatments of the bio-control fungal agents did not induce the greatest enhancement of the plant shoot weight. (Table 4.25)

Table 4.24: Effects of *Glomus mosseae*, *Paecilomyces lilacinus* and Carbofuran on total nematode population in cassava cultivars in a pot experiment.

	Total Nematode Population (soil + roots)					
	TMS 30572		TME 1		OFEGE	
Treatment*	1st Trial	2nd Trial	1st Trial	2nd Trial	1st Trial	2nd Trial
Uninoculated soil (control)	0.0	0.0	0.0	0.0	0.0	0.0
5000 *M. incognita* eggs	10455.0	9993.8	12595.9	12694.2	11166.0	11348.4
G. mosseae alone	0.0	0.0	0.0	0.0	0.0	0.0
P. lilacinus alone	0.0	0.0	0.0	0.0	0.0	0.0
G . mosseae + *P. lilacinus*	0.0	0.0	0.0	0.0	0.0	0.0
G. mosseae + 5000 *M. incognita* eggs	888.0	1191.5	3002.1	2494.7	1055.0	589.0
P. lilacinus + 5000 *M. incognita* eggs	852.0	879.3	1175.4	1075.4	958.0	550.0
G. mosseae + *P. lilacinus* + 5000 *M. incognita* eggs	6021.0	2455.2	7186.3	6348.8	6212.0	4658.0
Carbofuran @ 3kg ai/ha + 5000 *M. incognita* eggs	441.0	448.9	1374.8	1074.8	778.0	499.0
LSD (P≤0.05)	3163.8	1681.1	2031.9	2013.1	2983.1	2521.1

LSD for comparing treatment means within the same column

*Treatment key: *G. mosseae* = 140 spores of *Glomus mosseae; P. lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*

4.3.7: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on yield of Cassava

The carbofuran treated soil produced plants with the highest mean number of fresh tubers (8.0). The least significant value came from the untreated soil (3.2) (Table 4.26). Plants grown on soil with the combination of the bio-control fungal agents produced lower number of tubers (5.2) compared with plants grown on soil treated with G. *mosseae* alone (6.8) and *P. lilacinus* alone (7.0) (Table 4.26).

In the second trial, the carbofuran treated soil produced plants with the highest mean number of fresh tubers (7.8). The least significant value came from the untreated soil (3.0) (Table 4.26). Plants grown on soil with the combination of the bio-control fungal agents produced lower number of tubers (5.4) compared with plants grown on soil treated with G. *mosseae* alone (7.2) and *P. lilacinus* alone (6.8) (Table 4.26).

The highest significant mean tuber weight was recorded from the carbofuran treated soil (7627.7 g) and was not significantly higher than plants grown on *G. mosseae* (7352.5 g) and *P. lilacinus* (7260.1 g) (Table 4.26). The least mean tuber weight came from plants grown on the untreated soil (2737.6 g) (Table 4.26). Plants grown on soil with the combination of the bio-control fungal agents produced lower weight of tubers (5608.2g) compared with plants grown on soil treated with G. *mosseae* alone (7352.5) and *P. lilacinus* alone (7260.1 g) (Table 4.26).

In the second trial, the highest significant mean tuber weight was recorded from the carbofuran treated soil (7531.3 g) and was not significantly higher than plants grown on *G. mosseae* (7318.1 g) and *P. lilacinus* (7494.3 g) treated soils. (Table 4.26). The least mean tuber weight came from plants grown on the untreated soil (2413.1 g) (Table 4.26). Plants grown on soil with the combination of the bio-control fungal agents produced lower weight of tubers (5336.9g) compared with plants grown on soil treated with G. *mosseae* alone (7318.1) and *P. lilacinus* alone (7494.3 g) (Table 4.26).

Table 4.25: Effects of *Glomus mosseae* and *Paecilomyces lilacinus* and carbofuran on shoot parameters of cassava cultivars in a field experiment.

Treatment*	Height (cm)		Stem diameter (cm)		Shoot weight (g)	
	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Carbofuran 3 kg ai/ha + N	306.3	309.3	4.8	4.4	5421.6	5265.4
Paecilomyces lilacinus + N	280.7	294.2	4.7	4.3	5248.2	5277.6
Glomus mosseae + N	288.7	194.2	4.2	4.9	5127.7	5349.4
G. mosseae + P. lilacinus+ N	259.6	267.1	3.3	3.4	4128.3	4189.3
Nematode infested soil	210.5	209.4	2.7	2.7	3654.8	3446.8
LSD (P<0.05)	21.8	22.4	0.6	0.6	329.1	324.0

LSD for comparing treatment means within the same column

*Treatment key: *Glomus mosseae* = 140 spores of *Glomus mosseae; Paecilomyces lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*; N = Nematode infested soil.

Table 4.26: Effectiveness of *Glomus mosseae* and *Paecilomyces lilacinus* and carbofuran on yield parameters of cassava in a field experiment.

Treatment*	Number of tubers		Weight of tubers (g)	
	1st trial	2nd trial	1st trial	2nd trial
Carbofuran 3 kg ai/ha + N	8.0	7.8	7627.7	7531.3
Paecilomyces lilacinus + N	7.0	6.8	7260.1	7494.3
Glomus mosseae + N	6.8	7.2	7352.5	7318.1
G. mosseae + *P. lilacinus* + N	5.2	5.4	5608.2	5336.9
Nematode-infested soil	3.2	3.0	2737.6	2413.1
LSD (P<0.05)	0.8	1.0	622.0	581.5

LSD for comparing treatment means within the same column

*Treatment key: *Glomus mosseae* = 140 spores of *Glomus mosseae; Paecilomyces lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*; N = Nematode infested soil.

4.3.8: Effects of *Glomus mosseae, Paecilomyces lilacinus* and Carbofuran on Galling Index and *Meloidogyne incognita* reproduction on Cassava in a field experiment.

The two bio-control fungal agents tested in this study decreased the negative effects of nematodes, leading to a decrease in root galling of cassava but the effect varied according to the treatment (Table 4.27). The lowest significant mean gall index came from the carbofuran-treated soil (0.6) and was followed by *G. mosseae* inoculated soil (1.4) and *P. lilacinus* inoculated soil (1.4) (Table 4.27). The values obtained from plants planted on soil inoculated singly with *G. mosseae* (1.4) and *P. lilacinus* (1.4) were not significantly different from each other but were significantly lower than plants from soil inoculated with a combination of both fungi.(2.7) The highest significant mean gall index was obtained from the plants grown on nematode-infested soil (4.2) (Table 4.27)

The least mean nematode population was recorded from plants raised in carbofuran treated soil (22.5) and this did not differ significantly from the values obtained from the plants treated with *G. mosseae* (649.3) and *P. lilacinus* (502.1) (Table 4.27). The plants from untreated soil produced the highest mean nematode population (3777.9) (Table 4.27). The values obtained from plants planted on soil inoculated singly with *G. mosseae* (649.3) and *P. lilacinus* (502.1) were not significantly different from each other but were significantly lower than plants from soil inoculated with a combination of both fungi.(2112.0) (Table 4.27).

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Table 4.27: Effect of Fungal Biocontrol Agents and carbofuran on galling index and *Meloidogyne incognita* population on cassava in a field experiment.

Treatment*	Galling Index		Soil nematode population (root+soil)	
	1st trial	2nd trial	1st trial	2nd trial
Carbofuran 3kg ai/ha + N	0.6	0.8	22.5	150.8
Paecilomyces lilacinus + N	1.4	1.7	502.1	391.6
Glomus mosseae + N	1.4	1.6	649.3	489.1
G. mosseae + *P. lilacinus* + N	2.7	2.7	2112.0	2110.8
Nematode-infested soil	4.2	4.2	3777.9	4227.9
LSD (P<0.05)	0.7	0.7	923.43	1531.0

LSD for comparing treatment means within the same column

*Treatment key: *Glomus mosseae* = 140 spores of *Glomus mosseae; Paecilomyces lilacinus* = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*, N =Nematode infested soil

Treatment key: G = 140 spores of *Glomus mosseae;* P = 2 x 10^6 spores ml^{-1} of *Paecilomyces lilacinus*

*0 = no galls, 1 = 1-10% of the root system galled; 2 = 11-20% of the root system galled; 3 = 21- 70% of the root system galled; 4 = 71-90% of the root system galled; and 5 = greater than 90% of the root system galled.

CHAPTER FIVE

DISCUSSION

The results of the pathogenicity investigations indicate that the pathogenic effect of root- knot nematode on cassava increased with increase in the initial nematode population density. The reduction of mean shoot and root weights by root-knot nematode observed in this work is similar to the findings of Gapasin (1980) who reported stunting and reduction of root and top weights of cassava as the *M. incognita* population levels increased. Reductions in root and top weights were different at the 1,000 and 10,000 inoculum levels over the uninoculated plants. In Philippines, losses of about 50% have been caused by root-knot nematode but could reach 100% with three continuous cropping in infested fields (Gapasin, 1984, 1986). Sasanelli *et al.* (1992) found out that cabbage plants attacked by *M. incognita* showed stunting and yellowing within two weeks of infestation. They also reported that top and root weight of the plants were greatly affected by the nematode. Some of the infected plants were dead 40 days after transplanting into infested soil.

Heterodera sacchari was also reported to cause significant decreases in the number of tillers, dry straw weight, dry pannicle weight and grain yield of rice with increasing inoculum level (Babatola, 1983). The fresh and dry top weights, height and number of nodes of sunflower were also severely affected by *M. incognita* race 1 (Sasanelli and Di Vito, 1992). Ononuju and Fawole (1999) found out that *M. incognita* race 2 led to poor performance of two banana cultivars (Fagamou and Paranta). There was reduction in pseudostem height, pseudostem girth, and number of developing suckers and emerging leaves of the two cultivars as nematode population increased. Okorocha and Ezeigbo (1992) reported that fresh and dry root weights of carrot were lower in *M.incognita*- infested soil than for control plants. The fresh and dry weights of roots (tuberized and lateral roots) decreased with increase in inoculum density. Di Vito *et al.* (2000) reported that the Venezuelan population of *M. exigua* affected the growth of coffee plants negatively. Reduction in top weight, height and internode length increased as nematode population

increased. Zahid *et al.* (2001) observed in their study that the dry shoot and root weights of white clover decreased significantly as *M. trifoliophila* inoculum level increased until a maximum value was reached above 10,000 eggs/ pot. Gergon *et al.* (2002) also observed reduction in plant height, leaf dry weight and root length with increase in the inoculum densities of *M. graminicola* in yellow granex bulb onion. They also reported that onion bulbs from the infested field were reduced by 16%, 32% and 35% in weight and 6%, 17%, and 18% in diameter when the percentage of galled roots was 10%, 50% and 100%, respectively. The growth of tomato and pepper was also reported to be curtailed by *M. incognita* which reduced the fresh weight of both crops (Mekete *et al.*, 2003). Walters and Barker (1993) reported that *Rotylenchulus reniformis* restricted storage root growth and increased the root necrosis on 'Beauregard' sweetpotato.

Reduction in top growth and subsequent yield reduction could be due to the distruption of the root system by the destructive, adaptive and neoplastic feeding behavior of *M. incognita* which interferes with the physiological processes involved in water, nutrient utilization and phytohormones originating in the root (primary factors), thereby creating a cascade effect of chlorophyll synthesis, photosynthesis and respiration in the shoot (secondary factors). The combination of these primary and secondary effects could subsequently lead to poor growth and productivity of infected cassava compared with uninfected plants in both pots and field trials. Also, there is a reduction in the photosynthetic rates of plants due to nematode infection and this contributed to reduction in growth rates (Loveys and Birds, 1973; Wallace 1974).

Generally, it is also observed in this study that galling indices increased with increase in inoculum density. This agrees with the findings Zahid *et al.* (2001) who reported that root-knot gall index increase exponentially with increase in initial population levels of *M. trifoliophila* on white clover. The root galling severity in tomato and pepper also increased with increase in inoculum level of *M. javanica* (Mekete *et al.*, 2003). Okorocha and Ezeigbo (1992) also reported a steady increase in galling index with increase in *M. incognita* population on carrot. Cassava TME 1 suffered most root damage compared with the remaining four cultivars namely TMS 326, TMS 30572, 492)1425 and Ofege. This confirms the high damage potential of *M .incognita* on cassava since gall index is a measure of root damage. The higher the initial nematode population, the higher the level of root damage of cassava. This is

evident in the field trial where yield of cassava was reduced from 52.5% to 61.3% with a corresponding increase in the population of nematodes. With continuous cropping on the same piece of land, there could be total crop failure if a susceptible cassava cultivar is grown in subsequent seasons. Even if a moderately resistant cassava cultivar is cultivated, there is high possibility of increased pressure of *M. incognita* on the cassava consequently causing a complete break down of the resistant factor and yield reduction.

The final population of *M. incognita* in this study increased with increasing inoculum level from 1,000 to 10,000 eggs per plant and then declined. Similar results were given by Sasanelli *et al.* (1992), Sasanelli and Di Vito (1992), Di Vito *et al.* (2000) and Zahid *et al.* (2001) who observed maximum reproductive rates at lower inoculum densities and this declined as the initial population density increased. The decline in nematode population at higher inoculum densities likely resulted from reduced food supply for nematodes because of poor plant growth and competition.

In this experiment, all the five cassava cultivars used were susceptible to root-knot nematode attack.. The plant height in all the cassava cultivars used in pot and field experiments was reduced at all inoculation densities compared with the control with a subsequent reduction in yield which ranged from 45% - 53% when the cultivars were inoculated with 10,000 nematode eggs.

Inoculated cassava cultivars suffered in terms of growth, root damage and yield compared with the uninoculated cassava plants. This confirms the high damage potential of *M. incognita* on cassava. The higher the initial nematode population, the higher the level of damage imposed on infected plants. Total nematode population recovered from soil and roots in the pot study increased with increasing inoculum levels from 1,000 to 10,000 eggs.

The high reproductive rate of *M. incognita* and degrees of root damage (galls or knots) exhibited by *M. incognita* on cassava indicates the suitability of cassava as a host for this nematode. It further demonstrates the pathogenic effect of *M. incognita* and indicates that severe damage could occur if the crop is grown in fields infested by the nematode. The ability of *M. incognita* to suppress cassava growth under controlled condition emphasizes the potential importance of the nematode. The information obtained in this study may prove useful in predicting the effect of different inoculum densities on the growth, development and yield of cassava. At an

inoculum density of 10,000 eggs per pot, cassava growth and development were reduced.

Furthermore, land tenure system and increased pressure on agricultural lands for industrial, infrastructural and urban development (Nwauzor and Ihediwa, 1992), make farmers to crop the same nematode-infested piece of land with the same type of crop year after year. This may result in the build up of nematode populations which can lead to total crop failure if a susceptible cassava cultivar is grown on the same land in the subsequent seasons. Besides, poor growth produces poor cassava stem cuttings for the next planting season.

Therefore, a nematode management programme is essential to reduce pre-plant nematode population in an infested soil as its high fecundity leads rapidly to economic threshold population level which may lead to damage and yield loss. Also considering the difficulty in controlling this nematode, awareness should be created among cassava farmers and other stakeholders about *M. incognita* and its potential threats to the sustainability of intensive cassava production particularly with the improved cultivars on infested soil, as it is evident that the Federal Government of Nigeria is interested in promoting the intensification of cassava production for food security. Therefore, it is important to incorporate several control options available for a viable nematode management. A suitable method of control should be formulated which incorporate two or more compatible measures (such as solarization, use of resistant crop cultivars, cultural method, biological method, chemical method etc.), with a view to being effective, environmentally safe and profit-oriented farmers.

The natural soil environment harbours a multitude of microorganisms. As many as 10^6 – 10^8 bacterial cells, 10^6- 10^7 actinomycete cells, 5.0 x10^4-10^6 fungal colony forming units (CFU), 10^5-10^6 protozoa were estimated to be present in a gram of field soil taken from the surface (Gottlieb, 1976), while Richards (1976) and Back *et al*., (2002) found 1.0 x10^7 nematodes in area of 1m^2 of fertile soil. Plant-parasitic nematodes are primarily regarded solely as plant pathogens, capable of producing disease (Powell, 1971, Prot, 1993 and Manzanilla-López *et al*., 2004). All root-parasitic nematodes cause mechanical injuries, either by simple micro-puncture or by rupturing or separating cells as they penetrate within or feed on root tissues. They may thereby either introduce a pathogen on or within their bodies or aid the

entry of a pathogen already present on the plant cell surface (Corbett and Hide, 1971; Manzanilla-López *et al*., 2004).

Synergistic interactions between nematodes and fungi have been recognized since 1892 when Atkinson (1892) reported that the infection of cotton by the root-knot nematode increased the severity of *Fusarium*. Certain nematodes like *Meloidogyne* predispose plants to attack of other pathogens or make plants susceptible to microorganisms that normally would not parasitize the plant. Such interactions are considered synergistic when the combined effects of two pathogens on the host plant results in more extensive damage than the sum of the effect when both are acting independently (Powell, 1979).

Most nematodes interact with other pathogenic organisms e.g. *Botryodiplodia theobromae* in disease complexes. Nematodes disrupt the normal translocation of water and nutrients where they form galls, breaking and deforming the vascular elements (Gapasin, 1980). These deformations and the physical demand caused by the nematodes to the roots also facilitate entry of secondary pathogens. The fungus-nematode interactions are numerous, and weakly parasitic fungal parasites can cause considerable damage once they gain entry into plant roots in the presence of feeding nematodes. (Giuseppe 1993).

Endoparasitic nematodes such as root-knot nematodes have long been known as primary pathogens for their ability to predipose plants to infection by secondary pathogens such as several species of *Fusarium, Phytophtora and Rhizoctonia*. However the presence of fungal infection can sometimes alter nematode growth and reproduction (Jorgenson 1970). Whitney (1974) attributed synergism between *Heterodera* and *Pythium* to an increase in the growth of the fungus when the nematode was present, leading as a final result to a more devastating fungal attack.

The results recorded in this study on the interaction between *M. incognita* and *B. theobromae* showed that the effect of the nematode in combination with the fungus worsened the suppression of growth and yield of plant and produced high tuber rot which is more pronounced than that of the fungus alone or the nematode alone. Inoculation of the nematode and fungus revealed a synergistic effect on growth retardation, yield of the plants and tuber rot. The resultant tuber rot was more in plants inoculated with nematode first followed by fungus inoculation (48.1%), than in simultaneous inoculation (28.8%), and fungus inoculation followed by nematode (18.7%).

The result obtained from the screenhouse and field microplot trials showed that *Meloidogyne incognita* and *B. theobromae* are capable of reducing the growth and yield of TME 1, TMS 30572 and Ofege cassava cultivars used. In the screen house experiment on the interaction study, it was clearly seen that both pathogens supppressed growth more than each pathogen did singly. This was also seen in the micro-plot when the experiment was repeated. This is in consonance with Noel and Edward (1989), who reported that apart from direct yield losses, *Meloidogyne* species interact with fungal pathogens in disease complexes which often damage roots more severely than either nematode or other pathogens**.** The study was also able to show that the cassava cultivar TMS 30572 was tolerant to the combination of nematode and fungal attack than the two other cultivars (Ofege and TME 1) used in the experiment. However, farmers should be advised not to plant this particular cultivar in nematode infested soil since it permits nematode reproduction. This could be more detrimental if susceptible cassava cultivars (TME 1) and early maturing cultivars like Ofege cultivar is planted after the TMS 30572 (which is a late-maturing cultivar).

The study also showed that all the cultivars had their highest percentage rot and very high tuber rot severity when the plants were inoculated with higher nematode population prior to fungus. This supports the work of Bergeson (1972) who demonstrated that severity of fungal-induced wilt diseases increased greatly when root-knot nematodes were inoculated 3-4 weeks prior to fungus in comparison to simultaneous inoculations of both pathogens. Mokbel *et al*. (2007) have also shown that rot severity is increased when root-knot nematodes interact with *Rhizoctonia solani*, *Macrophomina phaseolina* and *Fusarium solani* to increase the severity of root rot in sunflower.

M. incognita alone reduced number of tubers in cassava by 35%, B. *theobromae* reduced number of tubers by 22.7% while a higher inoulation of *M. incognita* in combination with *B. theobromae* reduced number of cassava tubers by 43%. The weight of the tubers produced by cassava was also reduced by *M. incognita* alone by 44% and *B. theobromae* alone reduced the weight of cassava tubers by 25% while a combination of the two pathogens reduced tuber weight by as high as 72%. The reduction of mean plant height, stem diameter, fresh shoot weight, number of tubers and weight of tubers caused by nematode and fungus interaction observed in this work are similar to the findings of Jaritz (1972); Sikora

(1977); Nordmeyer and Sikora (1983) who reported abnormal alteration of the roots as well as stunting and discoloration of subteraneum clover (*Trifolium subterraneum*). The presence of *Heterodera glycines* and *Fusarium solani* caused sudden death syndrome (SDS) in soybean. In a microplot experiment conducted by McLean and Lawrence (1993); Rupe *et al*. (1993) and Back *et al*. (2002) they reported the incidence of SDS symptoms in soybean plots containing both *H. glycines* and *F. solani* were 35% and 18% higher than in plots where the fungus was inoculated alone. Rupe (1989) observed root rot, crown necrosis, interveinal chlorosis, defoliation and abortion of pods in soybean plants. Xing and Westphal (2006) reported foliar symptoms (interveinal chlorosis and defoliation) of SDS-diseased soybean plants inoculated with both *F. solani* f.sp *glycines* alone in a field microplot test. In the microplot trials, reduction in number of tillers, number of panicles, top and root weights, total seed and 100 seed weights at the inoculation of nematode and fungus alone, combination of either nematode and fungus one week before or after and simultaneous inoculation were not different from one another but were different over the control. Total number of panicle was reduced by (50% - 64.9%), root weight (62.1%-75.6%), total seed weight (38.2%-65.1%) and weight of 100 seeds (12.5%- 25%).

It was also observed in this study that tuber rot was as high as 100% in TME 1 in the presence of the two pathogens (i.e. nematode inoculation first and followed by fungus one week later or vice-versa) unlike when the nematode or the fungus was inoculated alone (between 0 and 15%).This relationship can be summarized according to Back *et al*. (2002) as being positive when an association between nematode and pathogen results in plant damage exceeding the sum of individual damage by pest and pathogen.

The adverse effects caused by synthetic nematicides have led to strict legislations and banning of some of these toxic chemicals. Therefore search for environmentally friendly alternatives, such as the antagonistic microorganisms are inevitable. Although nematicides, crop rotation and fallow are effective in nematode management, it is important that bio-control option be considered in controlling the nematode problem of cassava because it is safe, cost-effective and long-lasting. Part of the objective of this study was to investigate the use of two fungi *Glomus mosseae* and *Paecilomyces lilacinus* for possible nematicidal properties and ability to improve growth and yield of cassava when applied under nematode pressure.

P. lilacinus and *G. mosseae* significantly reduced (P≤0.05) *M. incognita* density and improved root fresh weight, number of tubers, stem diameter, plant height and fresh shoot weight of cassava plants. These findings were in agreement with the results obtained in the research conducted by Roa *et al*. (1998). They reported that the final nematode population of *M. incognita* was significantly less in the treatment where *G. mosseae* and *P. lilacinus* were integrated. P*aecilomyces lilacinus* and *Verticilium chlamydosporium* (Stirling, 1991) may suppress nematode populations through predation and or parasitism. The rhizosphere microorganisms used for the control of plant parasitic nematodes have been reported to produce nematicidal compounds and could be exploited in the management of *M. incognita*.

Borowicz (2001) reported various studies where a decrease in reproduction of *M. incognita* occurred in the presence of arbuscular mycchoriza fungi, suggesting that this reduction was due to physiological changes produced by the fungus in the root system. The reduction of the number of eggs in the roots of sweet passion seedlings when *Scutellospora heterogama* was established indicated that the fungus enhanced the resistance to parasitism (Hussey and Roncadori, 1982). In soybean, resistance to *M. incognita* was promoted by *Glomus etunicatum* W.N. Becker and Gerd (Carling *et al*., 1989).

Plant resistance is governed by external or internal factors that can reduce the ability of the pathogen to infect or diminish the infection level (Agrios, 1988). The reduction in number of galls, egg masses and eggs observed on roots of the cassava plants could be due to competition between the root-knot nematode and the bio-control agents for infection sites, but other factors such as increase of lignin and phenols (Umesh *et al*., 1988) or nematicidal substances, such as phenylalanine and serine (Suresh *et al*., 1985) can be involved. Kellam and Schenck (1980) registered lower quantity of galls in mycorrhizal soybean plants than in the non-mycorrhizal. This could be a result of the reduction in the ability of the nematode to penetrate in the root or of the presence of the AMF affecting the formation of giant cells and further development of the nematodes. Increase in vigour also helped the mycorrhizal plants to endure the parasitism of the nematode. The nutritional benefit promoted by *G. etunicatum* on tomato plants contributed to the increase in the resistance to *M. javanica* (Cofcewicz *et al*., 2001). The establishment of mycorrizal association with *Scutellospora heterogama* prior to nematode contact was beneficial

for sweet passion fruit plants conferring conditions for improved plant growth in the presence of the pathogen. Mirghani and Elsheck (1996) had results that indicated that tomato growth could be improved by the addition of mycorrhiza, which could also be used as a control for root-knot nematodes. Shoot dry weights of plants inoculated with *Glomus* sp. before *M. incognita* were significantly higher compared to that of plants inoculated with *M. incognita* before or even with *Glomus* sp. They found that inoculation of tomato plants with *Glomus* sp before *M. incognita* significantly reduced nematode damage compared to inoculation with *M. incognita* before or with *Glomus* sp. These microorganisms could be alternatives for toxic nematicides that pollute the environment and not within the reach of the resource-poor farmers. These microorganisms used are natural inhabitants of soil and under natural conditions, will grow throughout the season.

Yield assessment as a result of the inoculation of these microorganisms in pot was significant, ($P \leq 0.05$). *Paecilomyces lilacinus* and *Glomus mosseae* are potential *M. incognita* biocontrol agents, if edaphic factors and their concentration in the rhizosphere of cassava are suitable. These microorganisms could be alternatives for replacing toxic nematicides that pollute the environment and not within the reach of the resource-poor farmers. They produce spores or propagules which parasitize or predate or antagonize plant parasitic nematodes. The microorganisms helped to reduce stress and as a result cassava production improved, due to enhanced root health. Also, the plant-parasitic nematodess may not be able to develop resistance against the microbial antagonists because they are not subject to the accelerated microbial degradation process in the soil. The microorganisms used in the biological control system are natural to the soil; it is also safe to workers when they handle them during application to the soil. Over-dosing cannot be possible, therefore making it unlikely for animals and plants on the farm to be harmed in any manner.

The arguments against chemical pesticides are that they not only kill the pest organism, but also many non-target species, including natural enemy species, which in turn, may increase the pest status of species that were previously unimportant or easy to control. Furthermore, chemical control is limited to the area within which the pesticide is applied, frequent application may be required, and this selects for pest resistance.

A comparison of the costs of both the development and the use of chemical and biological control indicates that, in both respects, natural enemies are more cost-

effective than pesticide. Cost–benefit analyses suggest that research on biological control is more cost-effective than on chemical control (30:1 and 5:1, respectively; Van Driesche & Bellows 1995). It is evident that many more chemical compounds have been tested than species of natural enemy (Bale *et al*., 2008), though there are still vast numbers of predator and parasitoid species that remain to be screened for their use in biological control. The success rate for finding a successful natural enemy is much higher than for a chemical compound, which is mainly attributable to the 'directed search' that is used for natural enemies compared with the more random approach for chemical compounds, although pesticide discovery and design has become far more rational over time. Development costs are much higher for chemical pesticides, largely as a result of the very stringent requirements for ecotoxicological studies as part of the registration process. (Bale *et al*, 2008). Interestingly, the developmental time of an effective product, be it a chemical pesticide or a natural enemy species, is the same. It is common for pests to develop resistance against chemical pesticides, whereas as resistance against natural enemies is unknown, at least to the extent that the control agent becomes ineffective. When compared on the criterion of specificity, even the most selective chemical is likely to kill many species of pests, whereas natural enemy species used in biological control are usually highly specific, killing only one or a few related species of prey. When selective natural enemies are used in biological control programmes, harmful side effects do not occur.

CHAPTER SIX

SUMMARY AND CONCLUSIONS

The results of the investigation on the pathogenicity of *Meloidogyne incognita* on cassava demonstrated the pathogenic effect of *M. incognita* on cassava. Top growth and yield were reduced. Root damage increased with increase in nematode inoculum density. TMS 30572 cassava cultivar which exhibited some level of tolerance could not withstand the pressure of increased *M. incognita* populations. This was confirmed in the yield quantity as the nematode population was increased from 1,000 eggs to 10,000 eggs of *M. incognita.* Therefore TMS 30572 could be improved upon through breeding programme for resistance to *M. incognita.* Furthermore, the investigation of the interaction between the root-knot nematode (*M. incognita)* and the fungus *B. theobromae* on cassava, showed that the presence of the two pathogens in various combinations synergistically inhibited the shoot and root growth, increased root damage and caused total yield loss. The resultant tuber rot was more in plants inoculated with nematode first followed by fungus inoculation, than in simultaneous inoculation, and fungus inoculation followed by nematode.

Therefore, there is the need to work out effective nematode control strategy in order to improve cassava 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 is the way out of this problem which involves the use of two or more compatible nematode control strategies which are cost -effective and ecologically-safe. Towards this end, the role of the use of fungal microorganisms e.g. *Paecilomyces lilacinus* and *Glomus mosseae*, is recommended for the management of *M. incognita*. They do not require special equipment, expertise or extra capital investment like chemicals. Above all, farmers and agricultural extension agents need to be enlightened about nematodes and their negative impacts on crops.

The two bio-control fungi *Glomus mosseae* and *Paecilomyces lilacinus* used for the control experiment were effective in reducing nematode reproduction and root damage compared with the control. However, carbofuran was superior to the fungi, either when used singly or in combination. Between the fungi, *G. mosseae* used alone to control *M. incognita gave* the higher tuber weight. The same observation was made in the number of tubers in cassava plants treated with *G mosseae* to control *M. incognita.*

From the foregoing, it is very clear that nematodes cause yield loss in cassava. 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. The study has also shown that *M. incognta* and *B. theobromae*, and the interaction of both organisms is a major constraint to cassava production both in screen house and field studies causing stunting, high percentage of tuber rot and, subsequently, poor yield. A high inoculum level of *M. incognita* in combination with *B. theobromeae* when the nematode is inoculated two weeks prior to the fungus inoculation revealed the greatest damage and tuber rot in cassava. This is because the nematode would have penetrated the roots thereby providing feeding sites for the fungus. *G. mosseae* and *P. lilacinus* were found to have biocontrol potentials in nematode control and in improving cassava growth and yield as a result of reduction in the negative impact of nematode attack.

As a result of the foregoing, an effective nematode control strategy needs to be worked out to improve the growth, yield and quality of cassava. The high cost of agrochemicals has put the items far above the reach of resource-poor African farmers apart from being environmentally-hazardous. The use of integrated nematode management is the way out of this problem which involves the use of two or more compatible nematode control strategies which is cost-effective and ecologically safe. While the development of *M. incognita*-resistant cassava cultivars, soil solarisation and other control measures are good recommendations for the management of *M. incognita* and *B. theobromae*, it is important to combine the use of bio-control agents which are natural and do not attack non-target pests. *Glomus mosseae* and *Paecilomyces lilacinus* have shown interesting prospect and are thus recommended to be used for the management of *M. incognita*. They are non-toxic and not harzardous. Above all, farmers need to be enlightened about nematodes and fungus interaction and their negative impacts on crops. These bio-control fungi represent a new tool for an integrated management program for palnt-parasitic nematodes. However more

researches are needed to fully evaluate the interactions between inoculum density and host-plant response to develop epidemiological close-response models for these fungi. Commercial production of these fungal bio-control agents for massive use by farmers is recommended to aid in the reduction of the effect of *M. incognita* in cassava production.

Similarly, cassava, sweet potato or yam should not be used in rotation schemes in order to reduce *M. incognita* population because this may probably lead to the build up of *B. theobromae* on the farm since the three crops are very good hosts to *B. theobromae* and other rot fungi. The interaction of the two organisms will lead to further damage on the crop.

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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

$$V_2 = \frac{3.5 \times 100}{0.5} = 700\text{ml}$$

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 2:

Potatoes Dextrose Agar (PDA)

Dissolve in 39g/L in distilled water bath or in a current of steam ; autoclave (15 min. at 121^{o}C)

PH : 5.6+ or –0.1 at 25^{o}C

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Proteose peptone no 3 20g ; Glycerol 15 ml

Magnesium sulphate 1.5g

Potassium phosphate K_2HPO_4 1.5g