CHAPTER ONE

1.0 INRODUCTION

Egusi melon is a well known vegetable crop in West Africa. It is commonly grown for its consumable seeds popularly called "*Egusi*" in Yoruba language (Adewusi *et al*., 2000; van der Vossen *et al*., 2004). The crop's family is cucurbitaceae (Schippers, 2000). Three genera of this family are referred to as melon and these are *Cucumis*, *Citrullus* and *Cucumeropsis.* Common examples of these genera in Nigeria are *Cucumis melo* L. (true melon), *Citrullus lanatus* Thunb. Matsum and Nakai (Watermelon) and *Citrullus lanatus* (Thumb) Mansf. (Brown-seeded *Egusi* melon) and *Cucumeropsis mannii* Naud. (syn. *C. edulis* (Hooker f.) cogn.). The species of the genus *Lagenaria* (gourd melons) used for *Egusi* melon is *Lagenaria abyssinica* (Ajuru and Okoli, 2013).

Egusi melon is a creeping, hairy, herbaceous annual crop (Agba *et al.,* 2009). It originated from Africa, and usually intercropped with yams, sorghum and cassava (Adewusi *et al.,* 2000; Agba, 2004). In Africa, Nigeria is the highest producer of melon with 581,344 tonnes over an area of 999,618 ha with an average yield of 581.6 kg/ha followed by Republic of Congo (62,039 tonnes) and Cameroun (53,064 tonnes) (FAO, 2017). The world total production has been put at about 962,301 tonnes, with Africa as the highest producer in the world, producing about 894,178 tonnes followed by Asia (63,039 tonnes) (FAO, 2017). It is drought tolerant and grown in commercial quantity in Southwest Nigeria, especially in some parts of Oyo, Ogun, Ondo, Osun and Ekiti states where its seeds serve as soup condiments (Kehinde, 2011).

Egusi melon seed is typically utilized in Nigeria as a thickening for sauces, melon ball snacks ("robo" in Yoruba) and fermented melon seed condiment ("ogiri" in Yoruba). There are variations in the quality and quantity of oil produce from different cultivar of

melon seeds (Adewusi *et al*., 2000) and the oil is commonly utilized for cooking, for and production of margarine (Ajibola *et al.*, 1990). The crop is also important in the farming system as it is used to suppress weeds, for mulching, restoring soil fertility and controlling soil erosion (Dupriez and Deleener, 1989 and Achigan -Dako *et al*., 2008).

In spite of the vast nutritional and medicinal importance of *Egusi* melon, its production and yield in Nigeria are very low due to excessive rainfall, low soil pH and increased incidence of fungi, bacteria and viruses (van der Vossen *et al*., 2004). Infact, this crop is named as neglected crops having received little attention compared with other crops (Ogbonna, 2013). Most diseases affecting *Egusi* melon are described in a way similar to other closely related crops such as watermelon (*Citrullus lanatus var. lanatus*) which has received more attention than other members of the family. The incidence and management of a variety of diseases have been reported on other members of the family cucurbitaceae, whereas, little information is available on *Egusi* melon. Some fungal diseases reported on the crop in Nigeria were Powdery mildew, Downy mildew, *Alternaria* leaf spot, Cercospora leaf spot, Anthracnose disease, leaf blight, vine blight and fruit rot (*Didymella bryoaniae)* and fruit and flower wet rot (*Choanephora cucurbitarum*) (Kehinde, 2008).

Among this, anthracnose and leaf blight, vine blight and fruit rot caused by *Colletotrichum lagenarium* and *Didymella bryoaniae* respectively were reported to be most prevalent in Southwestern Nigeria (Kehinde, 2011). Yield losses exceeding 30% have been reported to be caused by *D. bryoniae* due to wet weather conditions (Keinath, 2000).

The management of most fungal diseases of economic importance in *Egusi* melon such as leaf blight disease remains a challenge. Growers usually apply fungicide for the control of these diseases but these are expensive and become ineffective over time. Besides this, synthetic pesticides have adverse effects on the environment, pest and pathogen resurgence, residual effects of pesticides on the quality of agricultural produce and lethal effects on non-target organisms (Bonjar *et al*., 2006). Therefore, there is need for more eco-friendly management strategies for leaf blight disease of *Egusi* melon. Botanicals and biological control agents have been employed in disease control of many economically important crops like cowpea, maize and Irish potato among others. There

has not been extensive report on the control of fungal disease of *Egusi* melon. Some of these fungi *Phytophthora, Rhizoctonia, Sclerotinia, Sclerotium, Fusarium oxysporum* and *Verticillium* spp have been reported to be seed borne (Sudisha *et al*., 2004). However, the rate of seed transmission of pathogenic fungi which is very important in disease spread and management of the diseases has not been well reported.

Some plant extracts have been reported to produce effective anti-fungal effects on many agriculturally important crops. For instance, *Cymbopogon citratus* (lemon grass) and *Eugenia uniflora* (Surinam cherry) plant oils were fungitoxic on fungi associated with melon seeds in storage (Kehinde, 2008). Extracts of *Azadirachta indica* were also found to control the seed-borne fungi of cowpea (Ekpo, 1999). Owolade *et al.* (2000) reported the efficacy of *Ocimum gratissimmum* and *Vernonia amygdalina* in protecting maize from seed transmitted *Fusarium verticillioides.* However, fewer reports were available on the use of these biopesticides as a seed treatment in management of diseases on *Egusi* melon. Hence, there is need to investigate an effective extract with appropriate application rate as seed treatment to enhance production of disease-free *Egusi* melon plants. The main objective of this study is to investigate the incidence of leaf blight disease of *Egusi* melon varieties and its management using biopesticides in Southwestern Nigeria. The specific objectives are to:

1. determine the incidence, severity and pathogenicity of fungal isolates associated with leaf blight disease of *Egusi* melon in Southwestern Nigeria
2. evaluate the effect of leaf blight disease on the seed yield of *Egusi* melon
3. determine the rate of seed transmission of the causal organism and effective inoculation period of *Egusi* melon for blight disease development
4. evaluate the efficacy of some biological control agents and botanicals for *Egusi* melon seed treatment*s* against leaf blight disease causing organisms.
5. determine the bioactive metabolites responsible for fungitoxicity of some of the botanicals used.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, taxonomy and distribution of *Egusi* melon

Egusi melon has been reported to have derived its origin from Africa (Siemonsma and Piluerk, 1994: Sadiq *et al.*, 2013). It probably originated in eastern and north-eastern Africa where wild forms still occur. It reached the Mediterranean region some 2000 years ago and subsequently introduced into Asia (Siemonsma and Piluerk, 1994; Denton and Olufolaji, 2000). Its introduction into the Asian continent (South and South east) was possible because of its preference for tropical climate. The crops' family Cucurbitaceae is known for their high genetic diversity and extensive adaptation in tropical, subtropical, arid deserts and temperate regions (Abbah *et al*., 2014). It belongs to sub-family Cucurbitoideae, order Cucurbitales and tribe Benincaseae. It is predominantly grown in forest and savanna ecological zones of Nigeria. It is commonly referred to as "*egusi*" by Nigerians, "agushi" by Ghanians, while Congolese and Burundese call it "kokoliko" (SIF, 2019).

2.2 Production of *Egusi* melon

The world total production is about 962,301 tonnes, with Africa as the highest producer in the world, producing about 894,178 tonnes followed by Asia (63,039 tonness) (FAO, 2017). It is usually intercropped with arable crops like yams, maize and cassava in early and late cropping seasons (Adewusi *et al*., 2000 and Agba, 2004). An estimated range of 5,000 – 7,000 tonnes is traded from Nigeria to other West African countries annually (Van der Vossen *et al*., 2004).

2.3 Botany and morphology

Egusi melon is a trailing, herbaceous annual crop (Agba *et al*., 2009). The leaves are 10 - 15 cm long, glabrous or slightly scabrid and denticulated, pinnately dissected (deeply cleft) into five irregular lobes which are roughly serrated and alternately arranged (Kehinde, 2008). They have 3 - 7 divided lobes and very similar to Watermelon leaves **(**Schafferman *et al.,* 1998).

The stem is hairy, soft and ridge, later becoming woody at the base (Rice *et al*., 1987). Its stem spreads around and climbs over nearby object while the root system is fibrous (Kehinde, 2008).

Flowers are monoecious with female flowers slightly larger than male (Lomova and Terekhina, 2009). Female flowers possess globular and hairy inferior ovary. The first male flower opens 30 - 45 days after germination, while that of the female is at 40 - 55 day after germination. Flowering is at peak 50 - 80 days after germination. The flowers are usually pollinated by insect (mainly bees) in the morning. After 24 hours, there is an elongation of the pedicel and swelling of the ovary (van der Vossen *et al.,* 2004).

Fruit of *Egusi* melon is sphere-shaped and smooth, with a 5 to 10 cm diameter. It has dull skin colours, which are either green or green with white markings, depending on the variety, with the size range of 7 to 20 cm in diameter (Dupriez and Deleener, 1989). The mesocarp has a soft and cushion white pulp covering the seeds. A plant yields about 15 to 30 fruits **(**Schafferman *et al.,* 1998). The shape of the fruit does not determine the seed type. Thus any of the major seed types can be found in any shape of fruit (Denton and Olufolaji, 2000). Fruit weight varies considerably from very heavy (above 1500 g) to light (less than 300 g), and this is dominated by the white pulp containing 95% or more of water (Cobley, 1979). The green fruit stalk changes to brown at maturity and has bitter and inedible fruit pulp. The seed has varying weight, with 100 unshelled seeds weighing 10.9 to 16.10 g (Denton and Olufolaji, 2000).

2.4 *Egusi* melon species

Egusi melon species (Plate 2.1a-o) include white seeded melon (*Cucumeropsis manni*), gourd melon (*Lagenaria abyssinica*), Brown - seeded melon (*Citrullus lanatus* (Thumb) Mansf., etc. Their useful part is the seed, called '*Egusi*'(Burkill, 1985). All the species are found in Africa. However, (*Citrullus lanatus* (Thumb) Mansf., is the most commonly cultivated species in Nigeria (Denton and Olufolaji, 2000).

2.4.1 *Citrullus lanatus (*Thumb) Mansf.

The scientific name for this melon is variously given as *Citrullus lunatus* (Thumb.), *Citrullus vulgaris* Shrad or *Colocynthis citrullus* L. (Philip, 1977; Ogunremi, 1978). However, Lowe and Soladoye (1990) indicated that *Egusi* melon was formerly known as *Colocynthis citrullus* (Linn) O. Ktzer but is now confirmed to be *Citrullus lanatus* (Thumb) Mansf. *C. lanatus* (*Egusi*) is native to the West African region; although, it is the progenitor of the watermelon. It was grown mainly for its seeds in West Africa (Jackson *et al.*, 2013).

There are two common seeds of *Egusi* melon in Nigeria namely Bara and Serewe. They are differentiated by their seed edge. Those with prominent thick black or white colour seed edge are known as Bara while the one without pronounced seed edge are Serewe (van der Vossen *et al*., 2004). They can be regarded as cultivars of *Citrullus lanatus* (Thumb) Mansf.). Six different shapes of fruits are known in the local varieties but the most common are the round and oblong types. In the South western and Northern Nigeria, the most common type is known as "Bara" which is characterized by prominent thick, black or white edges (Oyolu, 1977; Fayemi, 1999). This variety has been found to have the widest distribution (Denton and Adeniran, 1990; Denton and Olufolaji, 2000).Out of the seed types, the characteristic thin seed coat (which makes it a bit difficult to shell) and the absence of seed edge has made the 'Serewe' seed type to have higher shelling percentage, thus providing more edible endosperm than Bara (Denton and Olufolaji, 2000).

2.4.2 *Lagenaria abyssinica* (Hook. F.) C. Jeffrey (Gourd melons)

This is a gourd-bearing plant in the squash family Cucurbitaceae. It derived its origin from tropical Africa and has six species (Morimoto *et al.*, 2004) namely *Lagenaria breviflora*,

2.1a *Lagenaria abyssinica* (Gourd melon / Bojuri) Plant

2.1b *Lagenaria abyssinica* (Gourd melon / Bojuri) fruits

2.1c *Lagenaria abyssinica* (Gourd melon / Bojuri) seeds

2.1d *Citrullus lanatus* (Bara) plant

2.1e *Citrullus lanatus* (Bara) fruits

2.1f *Citrullus lanatus* (Bara) seeds

2.1g *Citrullus lanatus* (Serewe) plants

2.1h *Citrullus lanatus* (Serewe) fruits

2.1i *Citrullus lanatus* (Serewe) seeds

Plate 2.1. The Plants, fruits and seeds of *Egusi* melon species

2.1j *Cucurbita moschata* (squash melon or pumpkin) Plants

2.1k *Cucurbita moschata* (squash melon or pumpkin) fruit

2.1i *Cucurbita moschata* (squash melon or pumpkin) Plants

2.1m *Cucumeropsis manni* (White seeded melon) Plants

2.1n *Cucumeropsis manni* (White seeded melon) fruit

2.1o *Cucumeropsis manni* (White seeded melon) seeds

Plate 2.1 contd: The plants, fruits and seeds of *Egusi* melon species

L. abyssinica, L. siceraria, L. rufa, L. sphaerica and *L. guineensis*. All six species are found in Africa. The best known species, the bottle gourd, (*L. siceraria*) has been domesticated and has spread beyond Africa while the other species are not cultivated (Morimoto *et al*., 2004). Several species of gourds may be used as vegetable. Often, it is used in making utensils (including musical instruments and containers) when harvested dried. *Lagenaria abyssinica* is a climber because of its climbing vine. The stem and branches are covered in hair-like spines. Its distribution ranges from Africa to Asia. The fruit is used to make bottle and instruments (The Plant list, 2014). *Lagenaria abyssinica* is dioecious, with separate male and female plant (Morimoto *et al.*, 2004).

2.4.3 *Cucumeropsis mannii*

Cucumeropsis mannii has consumable and oily seeds. In Southern part of Nigeria, its seeds are fried and chewed as delicacy. According to Ajuru and Okoli, (2013) *Cucumeropsis mannii* is an annual, creeping plant. The fruits are creamy green, spherically elongated, about 14 cm to 20 cm long. The flesh is white and not edible. The seeds are white, flat, smooth and about 1.8 cm by 0.9 cm wide (Ajuru and Okoli, 2013).

2.4.4 *Cucurbita moschata*

Cucurbita moschata has a creeping growth pattern. Its stem is light green and very hairy. The flower colour is yellow, with male and female flowers on different peduncle of same plant. The fruit is green round and rough in shape (Ajuru and Okoli, 2013). Young leaves are eaten as vegetable as well as the fruit. The dried fruit shell is sometimes used as a container (Okoli, 1984). *C. moschata* seeds are sometimes offered for sale as brown-seeded melon by traders (Ajuru and Okoli, 2013).

2.5 Utilization of *Egusi* melon

Egusi melon seed is used for several purposes in Nigeria: for sauces and '*egusi*' soup, snacks, seasoner (Denton and Olufolaji, 2000). Schippers (2002) acknowledged that the crop highly contributed to the income generation ability of peasant farmers in Africa. Seed yield ranged between 131 to 1005kg /ha in Nigeria (Ojo *et al*., 2002). It is commonly used in house hold cooking and industrial purposes (canned fish, margarine

and salad oil, medicine and soap) (Adewusi *et al.,* 2000). Other industrial usages are in the manufacturing of pomade, metal polish, lubricant, adhesive and candles (Osunde and Kwaya, 2012).The seed is rich in oil (53.1 %), protein and (69-78 % in the defatted meal) (Ogbonna and Obi, 2007; Ogbona, 2013).

The medicinal properties of *C. lanatus* subsp. *mucosospermus* had been reported by some farmers in Republic of Benin. Sliced young fruit of *Egusi* melon is used in healing stomach troubles (Achigan-Dako *et al*., 2008).

2.6 Cultivation

All year round, *Egusi* melon seeds are available. This is because it is dried after harvest, in this state, has long shelve-life. Planting is usually in April and harvesting in the months of July. Fields for planting are cleared and tilled before sowing. Melon seeds are sown in farms either on ridges, mounds or flat surfaces in a spacing of 1m x 1m. Germination takes place 4 to 7 days after sowing (Adewusi *et al*., 2000). Thinning is carried out three to four weeks after sowing. The plant suppresses weed because of its ability to cover the ground early (Abbah *et al.,* 2014). The total growing period is about 120 days (van der Vossen *et al.,* 2004).

2.7 Soil and Climatic Requirements

Egusi melon grows best in sandy loam soil with pH 6 to 7. Well aerated fertile soil is suitable for good and productive growth of melon while excessive rainfall or irrigation brings about diseases on the field (Schippers, 2002). It tolerates periods of low rainfall (at least 700 to 1000 mm) and temperature of 28–35ºC is required (Adewusi *et al*., 2000).

2.8 Harvesting, Processing and Storage of *Egusi* melon

Fruits of *Egusi* melon are harvested when the fruit stalk and ancillary tendrils are dried up. Fruits are broken with a strong wooden stick. Thereafter, they are gathered and covered to ferment for two weeks. Seeds are removed from the pulp, washed and sundried. Seeds harvested are marketed or stored in lofts, baskets, sacks and jars (Schippers, 2002). Storage of seed with seed coat prolonged seed viability beyond 36

months while seed without seed coat had viability reduced to 12 months (Adewusi *et al.*, 2000).

2.9 Constraints to production of *Egusi* melon

Despite the numerous importance of *Egusi* melon, its production in Nigeria is hindered by various biotic and abiotic factors.

2.9.1 Abiotic limiting factors

High disease infection especially leaf blight, with resulting poor yields is been enhanced by high rainfall and humidity. At lower soil pH values below 6 to 7, soil borne diseases for example damping-off and wilting could occur (Kehinde, 2011). A waterlogged soil makes *Egusi* melon susceptible to anthracnose and fruit rot diseases. Germination is poor under high temperature (van der Vossen *et al*., 2004).

2.9.2 Biotic limiting factors

The most important biotic constraints are diseases caused by viruses, bacteria, nematodes and fungi.

2.9.2.1 Bacterial diseases of *Egusi* melon

The major bacterial diseases that pose a serious threat to melon cultivation are angular leaf spot, bacterial fruit blotch and bacterial soft rot (Roberts and Kucharek, 2004)

2.9.2.2 Viral diseases of *Egusi* melon

Common virus diseases of *Egusi* melon are *Watermelon mosaic virus* (WMV-2), and *Papaya ringspot virus* (PRSV-W) which are all transmitted by silver leaf whitefly and aphids respectively (van der Vossen *et al.*, 2004).

2.9.2.3 Nematode diseases of *Egusi* melon

Root knot nematode (*Meloidogyne* spp.) is a notable disease of melon. This can be managed by soil solarization, regular weeding and grafting on resistant rootstocks

2.9.4 Fungal diseases of *Egusi* melon

The major fungal diseases of melon are Anthracnose, Vine wilt, Powdery mildew, Gummy stem blight, Wet rot of flower and fruit, *Alternaria* leaf spot, *Cercospora* leaf spot and Leaf blight (Keinath, 2008 and Kehinde, 2011).

2.9.4.1 Leaf blight disease

This disease is also referred to as leaf and stem blight disease (Kehinde, 2011). The causal organism was identified as *Didymella bryoniae.* This disease has been observed in Nigeria among the most important diseases of cucurbits. It is widely spread across Southwestern states of Nigeria. The symptoms begin on leaf as light brown, irregular spots with yellow borders which develop from the tip of the leaves and gradually progress backwards. Later, leaves turn yellow, then black and eventually die (Kehinde, 2013). This reduces the photosynthetic activities thereby affecting the patitioning of assimilates.

Yield losses resulting from leaf and stem blight disease can exceed 30% in early cropping season enhanced by wet weather conditions (Keinath, 2000).

2.10. Biological control methods

Biological control agents of plant diseases are often referred to as antagonists (Flint and Dreistadt, 1998). Disease suppression method employ differs depending on the microbial species (Bafti *et al.,* 2005 and Al-Mughrabi, 2010). Several researches have been carried out on *Bacillus subtilis* and *Trichoderma* species which have revealed fungicidal and bactericidal activities on several microorganisms and plant pathogens including *R. solani.* Bernard *et al.* (2011) reported that *Trichoderma* species produces antibiotics which help in reducing the growth of the plant pathogens. Many of the microorganisms are found naturally within the soil. Thousands of microorganisms have shown a high level of plant pathogen growth reduction in the laboratory, screenhouse and field (Bafti *et al.,* 2005; Henderson *et al.,* 2009).

2.11 Biopesticides

Biochemical pesticides are natural pesticides that employ naturally occurring substances instead of chemicals to control pest such as pheromones, plant hormones, plant-derived regulators and enzymes as the active ingredients (Chunxue *et al.,* 2010). They consist of biochemical substances that control pests, microbes, or plant-incorporated protectants that are pesticidal. They are less harmful to human and non-target organisms, making them more suitable for disease management. However, they are often used as preventive measures, because their speed of action may be slower compared with some synthetic chemical pesticides. Environmental Protection Agency registered biopesticides are the products approved for plant disease control (Chunxue *et al.,* 2010).

2.11.1 Microbial pesticides

Microbial pesticides has microorganism as the active ingredient such as beneficial bacteria, fungi, nematodes, viruses and their products. Examples include *Bacillus* spp. (*Bt* producing strains of *B. thuringiensis*), *Pseudomonas* spp., *Streptomyces* spp. and *Trichoderma* spp. (PMNI, 2013).

2.11.1. 1 *Trichoderma* species

Trichoderma are filamentous fungi the species of which were previously considered to be culture contaminants. *Trichoderma* is useful in industry and as biocontrol and a bane to other fungi. *Trichoderma* spp. is present in soils and other diverse habitats

Recently, appreciable success has been achieved by the use of fungal bioagent. But so far there is lack of feasible and effective formulation of bioagents to exploit it commercially. The production of different formations of *T. viride* Pers. that is effective, easy to apply and economically viable is important for improving the antimicrobial potentials of the bioagent. *Trichoderma* species commonly inhabit rhizosphere and assist in reducing the growth of many soil borne fungi that can cause plant disease (Chouaki *et al.,* 2002). Also, it increased plant growth response after application (Bravo *et al*., 2007). For instance, studies conducted by Ha (2010) indicated that *Trichoderma* spp. had the ability to suppress growth of fungal pathogens and enhance plant growth. The biocontrol

potential of *Trichoderma* spp. against phytopathogenic *Fusarium* was highly suggested by Calistru *et al*. (1997). This is associated with the strong saprotrophic and mycoparasitic behavior of *Trichoderma* spp. in colonizing the root epidermis and even a few cell layers below the epidermis (Harman *et al*., 2004). For instance, *Trichoderma* spp. were commonly use for effectively control of fungal pathogen in various fruit crops (Svetlana *et al*., 2010). Rice plants inoculated with *Trichoderma* spp. (T. *harzianum and T* viride) significantly reduce bakanae disease incidence and disease severity caused by *Fusarium fujikuroi* under greenhouse conditions (Ng *et al*., 2015).

Trichoderma viride, was effective on Rice *Curvularia lunata,* (Bhat *et al.* 2009), and *T. harzianum* on rice *Fusarium spp*., (Chakravarthy 2011). *Trichoderma koningii* was effective on rice disease by *Rhizoctonia solani*, (Gomathinayagam *et al.* 2010)

2.11.1.2 *Bacillus* species

Bacillus is a rod-shaped, gram-positive bacterium found naturally in soil, water, and grain dust (Chouaki *et al.,* 2002).There has been success in commercial preparations of *Bacillus* as microbial pesticides. It has been considered to be the most successful biological pest control products worldwide comprising 95% of microbial pesticides sold according to Bravo *et al*. (2007). Chouaki *et al.* (2002) confirmed crygene produced by *Bacillus* to be related to its toxicity.

2.12 Use of botanicals in controlling fungal diseases

The use of agrochemicals is becoming less favourable because of environmental pollution and detrimental consequences on a variety of non-target organisms (Bonjar *et al*., 2006). This has resulted in worldwide interest in the use of biocontrol methods because most of them are locally available, environmentally friendly, while development of resistance to diseases is rare (Okigbo and Ogbonnaya, 2006).

Fungicidal activity of botanicals on plants have been reported severally (Okigbo and Emoghene, 2004; Okigbo and Nneka, 2005). For instance, *Cymbopogon citratus* (lemon grass) and *Eugenia uniflora* (Surinam cherry) plant oils were fungitoxic on fungi associated with melon seeds in storage (Kehinde, 2008). Extracts of *Vernonia*

amygdalina Del. have been found to be effective on *Curvularia lunata* and *Fusarium semitectum* (Ekpo, 1991). Also, extracts of *Azadirachta indica* were found to control the seed-borne fungi of cowpea (Ekpo, 1999). It was found that spraying crude neem oil on lilac bushes when done before any outbreak, prevented outbreak of powdery mildew out for the rest of the season and also gave 100% control in hydrangeas better than Benlate (benomyl). Bean rust was also controlled by 90% when neem extract was applied before the plants were exposed to the fungus (Bernd, 1999). In another study by Madunagu and Ebena, (1994),extract of *Azadirachta indica, Carica papaya* L., *Costus afer* Ker-Gawl, *Mangifera indica* L. and *Ocimum gratissimum* were tested on *Aspergillus* spp., *Rhizopus stolonifer* and *Penicillium chrysogenum* and reports showed that *Costus afer* inhibited all the fungi isolated, *Azadirachta indica* inhibited all except *Rhizopus stolonifer. Carica papaya* had no effect on *Penicillium chrysogenum* while *Ocimum gratissimum* and *A. indica* inhibited *Penicillium sclerotigenum* (Madunagu and Ebena, 1994).

2.12.1 Review of plants used in this study

2.12.1.1 *Costus afer* Ker-Gawl

This plant is a member of the family Costaceae. It is found in countries such as Ghana, Cameroon and Nigeria (Edeoga and Okoli, 2000). The plant is popularly known as “ginger lily” or “bush cane”, “Okpete” in Igboland, “Kakizawa” in Hausa and “tete-egun” in Yoruba in Nigeria (Iwu, 1993).

Costus afer can grow up to 4 m. Its leaves are arranged spirally, simple and entire. Its white or yellow flowers are bisexual and zygomorphic **(**Omokhua, 2011). Its stem is used in treatment of cough, while the root could cure sleeping sickness and stomach ache. Phytochemical analysis of this plant showed the presence of alkaloids, saponins, flavonoids, tannins and phenols in aqueous stem extract (Ukpabi *et al*., 2012). Mothana *et al.* (2009) confirmed that chemical extracts of *Costus arabicus* roots have antimicrobial activity whereas, the essential oil, methanol, ethanol and aqueous extracts efficiently reduced bacteria growth. Also, Al-Kattan, (2013) observed the fatal influence of Indian Costus roots on *S. aureus* and *K. pneumonia* when, their growth is repeated at same concentration by inoculation. The SEM images taken by Al-Kattan, (2013) in his study demonstrate that *Costus* oil is effective in reducing the budding and damaged cell walls

of *Candida albicans*. Oil extract preparation of Indian *Costus* and Sea- Qust are a new antimicrobial treatment against opportunistic pathogens and can be employed for diabetic patients' foot infections to reduce the side effects caused by the antibiotics currently in use (Al-Kattan, 2013).

2.12.1.2 *Morinda lucida* Benth

Morinda lucida Benth belongs to *Rubiaceae* (Karou *et al.*, 2011). *Morinda lucida* is commonly referred to as Brimstone tree and locally called Oruwo by Yorubas, Nfia in Igbo (Nigeria) and Kon kroma in Twi (Ghana) (Nweze, 2011). It is a tree plant. Leaves are opposite, simple and entire (Zimudzi and Cardon, 2005). The wood of *M. lucida* produces yellow to red dyes used to dye textiles into scarlet red in Nigeria and Gabon. The roots are used as chewing sticks, while the trees are used for making charcoal, construction, furniture, poles and fuel wood.

Soladoye (2005) reported *Morinda lucida* as one of the medicinal plants in Nigeria. It is used in treating fever, malaria, ulcer, leprosy, hypertension, cerebral complication and gonorrhea (Kemabonta and Okogbue, 2000). The major constituents of *M. lucida* are anthraquinones, anthraquinols, tannins, flavonoids and saponosides according to Akinyemi *et al.* (2005) and Zimudzi and Cardon (2005). The crude ethanolic extract of the leaves is reported to contain alkaloids, cardenolides and saponins (Adomi and Umukoro, 2010). Ogundare and Onifade (2009) worked extensively on the efficacy of *M. lucida* leaf extract and reported its ability to suppress the growth of *Escherichia coli* in Petri plate and in the Screenhouse. The extract has showed an indication that it can treats enteropathogenic *E. coli* infection.

Morinda lucida extracts have the potential of reducing the growth of *Escherichia coli, Salmonella typhi, S. paratyphi,* and *S. typhorium* (Akinyemi *et al.,* 2005). Ogunsola and Aduramigba- Modupe (2013) reported that *Morinda lucida* and *Moringa oleifera* extracts inhibited *Botryodiplodia theobromae* at 1500 mg/ml concentration producing 16.67% and 16.27% inhibition respectively in post-harvest management of tuber rot of Irish potato (S*olanum tuberosum*).

2.12.1.3 *Azadirachta indica* Juss.

Azadirachta indica is a member of Meliaceae and order Sapindales (Dubey and Kashyap, 2016). It is a tree plant native to India and typically cultivated in tropics. Its seed is the source of neem oil. The common names for this plant are, Neem Tree (English), Dongonyaro (Hauss) (Anyaehie, 2009).

'Neem extracts' have the ability to control a large range of crop pests (Makein *et al.* 2007). Several insect-repellent products are extracted from the oil. Azidarachtin is the active principle in those products. Extract of neem leaves and bark were reported to have successfully controlled damping-off of soybean (Adeyeye and Olufolaji, 2004). Phytochemicals like saponins, tannins, glycosides, alkaloids, terpenes and flavonoids were observed leaves extracts of *Azadirachta indica* (neem) by Biu *et al.* (2009).

2.12.1.4 *Passiflora foetida* Linn.

Passiflora foetida also known as "Stinking passion" flower, belong to the family Passifloraceae and Order Malpighiales (Patil *et al.*, 2015). It is indigenous to tropical America which has now spread throughout America, Asia and the Pacific (Mohanasundari *et al*., 2007). This plant is widely grown as perennial climber. It is used as treatment for itching and coughs (Sathish *et al.*, 2011).

Pharmacological studies of passion flower revealed antimicrobial, sedative and hypotensive activity (Dhawan *et al*., 2003; Abascal and Yarnell, 2004). *Passiflora foetida* plant contains alkaloids, phenols, glycoside, flavonoids and cyanogenic compounds (Dhawan *et al*., 2004). Leaf extract of *Passiflora foetida* was significantly effective against human pathogenic bacteria such as *Pseudomonas putida* and *Streptococcus pyogenes* pathogens compared to fruits (Mohanasundari *et al*., 2007). No report of toxicity to plant pathogenic fungi available. *Passiflora foetida* extracts have potential tendency against anxiety, insomnia, sexual dysfunction, inflammation and cancer (Patil *et al.*, 2015).

2.12.1.5 *Phyllanthus amarus* Schum & Thonn

Phyllanthus amarus is of the family Phyllanthaceae and Order Malpighiales. It is used worldwide for medicinal purposes. It can grow up to 50 to 70 cm. It consists of pale

green flowers frequently blushed with red **(**FAFLOMO, 2013). Common names for *Phyllanthus* include Eyin Olobe in Yoruba and Ngwu in Ibo, Nigeria (Etta, 2008).

It is an essential medicinal plant of Indian Ayurvedic system of medicine used in the disorders of stomach, liver and kidney (Patel *et al*., 2011). Many compounds such as lignans, flavonoids, hydrolysable tannins, sterols and alkaloids were found present in *Phyllanthus amarus*. These compounds showed pharmacological activities including antiviral, antibacterial, and diurectic properties (Patel *et al*., 2011). *P. amarus* is most efficient in the control of leaf spot of maize caused by *C. lunata* (Akinbode, 2010). Extract of *Phyllanthus nururi* showed significant reduction in growth of *Pythium debaryanum*. *Phyllanthus amarus* leaf extracts insecticidal property on workers of *Macrotermes bellicosus in vitro* (Oyedokun *et al*., 2011)

2.12.1. 6 *Thevetia neriifolia* Juss.

Thevetia neriifolia belongs to the family Apocynaceae. The common names include yellow “oleander”, exile tree”, “exile oil tree”, “milk bush” (Irvine). In Nigeria (Yoruba) it is called “olómiòjò”. It is used for medicinal purpose such as laxatives. It is also used as ornamental, cultivated or partially tended. The whole plant consists of phytochemicals which include glycosides, saponins, steroids and fatty acids. The foliage is not grazed by livestock. The plant makes a useful enclosure hedge (Duarte and Debur, 2004). It stands clipping, endures drought and suppresses plants from its shade. The wood is used in Ghana sometimes to make axe-handles. The fruits and leaves have shown some insecticidal activity. The leaves contain several glycosides including neriantin and glycerides of oleic acid (Biu *et al.,* 2009). The plant produces latex substances (Duarte and Debur, 2004). Neriantin extracted from kernels, leaves and bark of the milk bush tree was toxic against insect pests (Biu *et al.,* 2009). No previous report on the antimicrobial activities of Thevetia neriifolia on plant pathogens.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 General methods

3.1.1 Location and Experimental Sites

This study was carried out in the laboratories, screenhouse and experimental field of the Nigerian Agricultural Quarantine Service (NAQS), Moor Plantation, Ibadan, and Pathology Research laboratory of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan, Nigeria.

3.1.2 Sources of materials

Three common *Egusi* melon varieties in Southwest Nigeria were used in this research work. These are Bara (NG/AO/11/085b), Serewe (NG/AA/SEP/09/148) and Bojuri (NG/TO/AUG/09/003) which were obtained from National Center for Genetic Resource and Biotechnology (NACGRAB), Moor Plantation. Six plants that are commonly found in Nigeria (*Costus afer, Morinda lucida, Azadirachta indica, Phyllanthus amarus, Passiflora foetida* and *Thevetia neriifolia*) were used as botanicals. *Morinda lucida, Azadirachta indica, Phyllanthus amarus, Passiflora foetida and Thevetia neriifolia* leaves, stems and roots were collected from the National Cereal and Research Institute (NCRI), Moor Plantation, Ibadan. *Costus afer was* collected from Southwestern University, Okun Owa, Ogun State. Also, four biological control agents (BCAs), *Trichoderma harzianum*, *Trichoderma pseudokoningii*, *Bacillus subtilis* and *Pseudomonas fluorescens*, used as biopesticides in this study were isolated from soil in *Egusi* growing farms in NCRI compound.

3.1.3 Sterilization of seeds

Egusi melon seeds were surface-sterilized by soaking in 10% sodium hypochlorite (NaOCl) for 1 minute and rinsed in five changes of sterile distilled water. Seeds were placed on sterile paper towel in a Laminar flow cabinet for 15 minutes to air-dry.

3.1.4 Sterilization of glassware and other materials

The glassware used were washed with detergent, rinsed with tap water and drained. Glass Petri dishes were placed in canisters while the pipettes were wrapped with aluminum foil. All glassware were sterilized in an oven at 160^{o}C for 2 hours while the inoculating needle was sterilized by flaming to red hot in spirit lamp. Liquid medium and the distilled water were sterilized in an autoclave at 121^{o}C for 15 minutes. Laminar flow hood (inoculating chamber) was sterilized by swabbing the work area with 70% ethanol and an ultra-violet light (253.7 nm) was put on for 30 minutes before use.

3.1.5 Preparation and sterilization of culture growth media

The isolated pathogens were cultured on Potato Dextrose Agar (PDA) which was routinely prepared. The PDA was prepared by dissolving 39 g of dehydrated PDA in 1 litre of sterile distilled water (SDW). Also, 28 g of Nutrient Agar (NA) was dissolved in 1 litre of SDW. The solution was dispensed into 500 mL conical flasks. The media were sterilized by autoclaving at 121^{o}C for 15 minutes and allowed to cool to 45^{o}C before being poured into sterile plates of 9 cm diameter. Lactic acid (1 mL in 1 L medium) was added shortly before pouring PDA into the Petri plates to suppress bacterial growth. Ten millilitres of PDA was dispensed into McCartney bottles and kept in a slanted position until the contents solidified, and then used for long term- storage of cultures.

3.1.6 Soil preparation in screenhouse

Top soil was collected at 0-15 cm depth from the former Practical Year Training Programme (PYTP) 7.4463^{o}N 3.9033^{o}E farm of Faculty of Agriculture and Forestry, University of Ibadan and sterilized at 170^{o}C for two hours, using Electrical Sterilizer in CPEB.

3.2 Field Sampling

3.2.1 Determination of the incidence, severity and distribution of leaf blight disease of *Egusi* melon in Southwestern Nigeria

Field sampling for leaf blight diseases of melon was conducted in Ogun, Oyo, Osun, Ekiti and Ondo states. In each state, five local government areas with three villages/towns per local government were purposely selected and sampled by the assistance of State Agricultural Development Project (ADP) extension agents. The selection of the surveyed locations in each state was done based on the information on high *Egusi* melon production available at the ADPs. Two farms per village were randomly sampled, with a total of six farms per local government areas (LGAs), 30 farms per state and 150 farms altogether. Coordinates of sampled location were recorded using Global Positioning System (GPS) (Figure 3.1). Sampling was conducted during the planting season of April – July (Kehinde, 2011) in the year 2016. Farms were rated as small, medium, or large if the size is < 0.3 ha, between 0.3 and 0.5 ha and > 0.5 ha, respectively (Kehinde, 2011). Melon plants were randomly selected by walking through the farm diagonally. Twenty, thirty and fifty plants were selected randomly for small, medium and large farms, respectively.

The distribution (%) of the *Egusi* varieties encountered on the farm was calculated.

$$\text{Distribution (\%)} = \frac{\text{No of farms where a particular variety was found}}{\text{Total no of farms visited in a local Government Area}} \times \frac{100}{1}$$

Disease incidence and severity on the fields was calculated using the formular of Cardoso *et al.* (2004); Rai and Mamatha (2005) and Islam *et al*. (2015)

$$\text{Incidence (\%)} = \frac{n}{N} \times 100$$

Where n is the number of plants showing symptoms on the field

N is the Total number of plant on the field

$$\text{Disease severity} = \frac{\text{Proportion of total damaged tissue}}{\text{Total surface area of the plant parts}} \times \frac{100}{1}$$

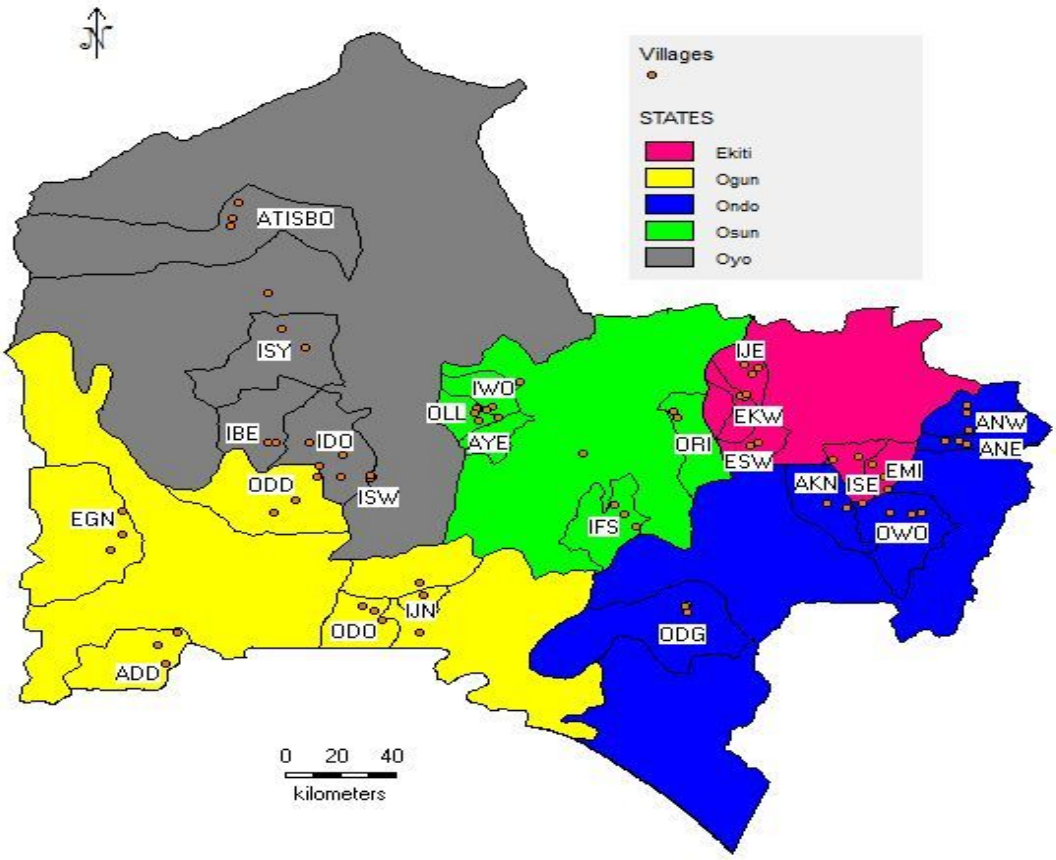


Figure 3.1. Map of locations visited in Southwestern Nigeria for the assessment of Leaf blight disease of Egusi melon.

ADO=Ado odo Ota; ANE=Akoko N/E; ANW= Akoko North West; AKN= Akure North; ATI= Atisbo; AYE= Ayedire; EGN= Egbado North; ESW= Ekiti S/W; EKW= Ekiti west; EMI= Emure/Ise orun; ISW= Ibadan S/W; IBE= Ibarapa East; IDO= Ido; IFS= Ife South; ISE= Ise; IJN= Ijebu North; IJE= Ijero; ISY= Iseyin; IWO= Iwo; ODD= Odeda; ODG= Odigbo; ODO= Odogbolu; OLL= Ola oluwa; ORI= Oriade; OWO=Owo

This was converted to a disease rating scale of 1- 5 using modified Kehinde, (2011) scale (Table 3.1). Typical disease symptoms of leaf blight disease were documented and infected leaf samples were taken to the laboratory for pathogen isolation and identification. Frequency of occurrence of the isolated fungi was calculated (Zakari *et al.,* 2015) as:

$$\text{Frequency of occurrence (\%)} = \frac{N}{T} \times 100$$

Where, N = No of time the organism occurred.

T = Total no of organisms isolated.

3.3 Isolation and identification of fungi associated with Leaf blight disease symptom on *Egusi* melon plants

Egusi melon plants showing leaf blight symptom were collected during field sampling. Diseased parts were washed with sterile distilled water and 5 mm^2 of the infected parts were cut at the boundary of the healthy and infected tissues. This was sterilized for two minutes in 10 % sodium hypochlorite (Klement *et al*., 1990), rinsed in five times with SDW and blotted dry between sterile paper towels before plating on PDA. These pieces were aseptically transferred with sterile scapel into sterile Petri dish containing 15 mL of PDA. The inoculated plates were incubated at 28 ± 2^oC for 3 to 7 days and examined for fungal growth. The fungal isolates from each sample were sub-cultured on PDA to obtain pure cultures for identification. The cultural features of the fungal isolates were carefully observed and recorded. Wet mounts of the isolate were prepared on a microscope slide and stained with lactophenol cotton blue. The prepared mounts were observed under compound microscope and detailed structural features of each isolate were recorded. The features of the organisms were compared with those described in a standard manual of fungi (Barnett and Hunter, 2003).

3.4 Pathogenicity of fungi associated with leaf blight disease of *Egusi* melon

Pathogenicity test was carried out in the screenhouse using 14 day-old melon seedlings. Fungal suspensions were prepared from the 7 days old culture plates of the isolated fungi. Pure cultures of the pathogen were sub-cultured.

Table 3.1 Disease severity rating scale fo leaf blight disease of egusi melon

Scale		Inference
1	(No symptom)	No symptom
1.1-2	(<10% damaged portion)	Mild symptom
2.1-3	(10-30% damaged portion)	Moderate symptom
3.1-4	(31-50% damaged portion)	Severe symptom
4.1-5	(>50% damaged portion)	Very severe symptom

Source: (Kehinde, 2008) modified

The mycelial mass of the fungus growth culture in the Petri dishes were scooped into a sterile conical flask, which contained 10 mL of SDW and a drop of Tween 20 detergent (for spore dispersal) was added (Williams, 2003; Kehinde, 2008). The spores were filtered off the mycelial and media fragment using a double layer of muslin sheet. Thereafter, 0.1 mL spore concentration was counted using heamocytometer under a compound microscope.

The surface of the haemocytometer was carefully cleaned before use. Care was taken to ensure that all surfaces are completely dried using non-linting tissue. Spore suspension of 10 microlitres was pipetted into the counting chambers. The suspension was thoroughly, but gently, and the chamber was slowly filled by avoiding injecting bubbles into the chambers. The spores were counted in each of the four $0.1mm^3$ corner squares labelled A through D (Figure 3.2).

The spores concentration were calculated using the equation:

$$\text{Spores/mL} = (\text{n}) \times 10^4$$

Where n equals the average cell count from the four corner squares, labelled A, B, C and D (Akinbode, 2013).

Suitable spore concentrations were determined by dispensing 0.1ml of the inoculum on a hemacytometer. Final spore count and expected or desired inoculum concentration was determined by the method of Berkane *et al*. (2002):

$C_1V_1 = C_2V_2$

Where:

C_1=initial inoculum concentration

V_1=initial volume of water used in streaking the culture plate

C_2=Final inoculum concentration desired

V_2=Final volume of water to be added to obtain desired concentration.

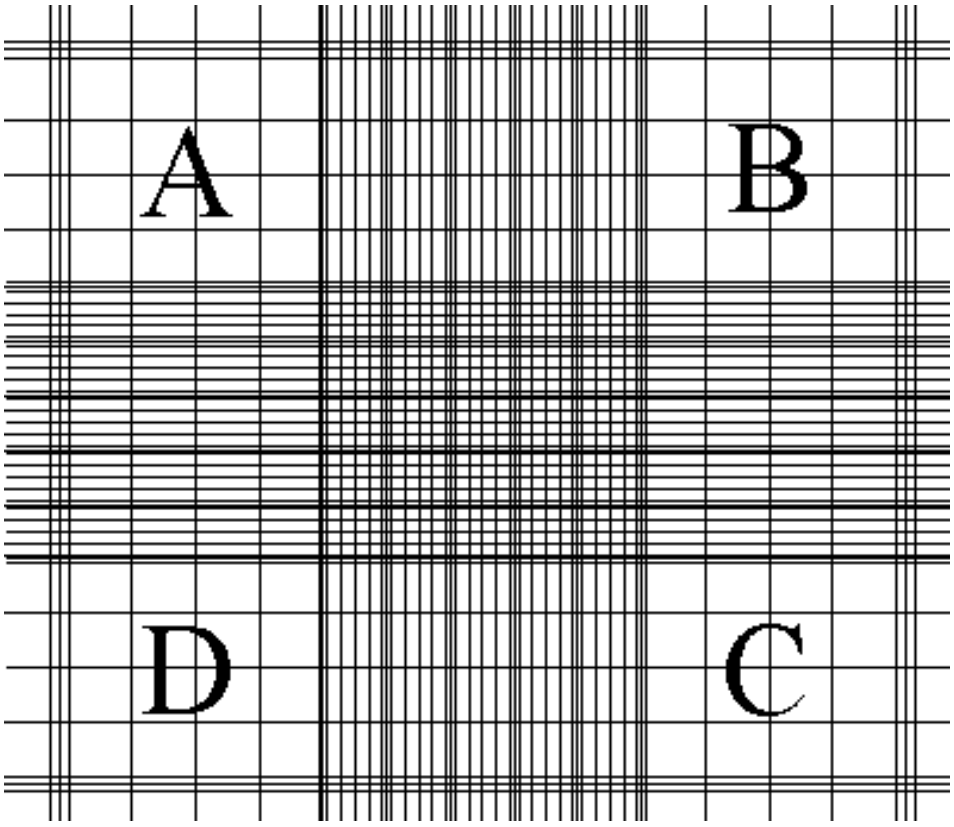


Figure 3.2. Diagrammatic representation of a Haemocytometer used in spore counting.

Source: Akinbode (2013)

The spore suspension of the fungi pathogens ($2.1x10^6$ spore/mL) were poured each into a hand sprayer for subsequent inoculation in the screenhouse.

All the isolates were used in the pathogenicity test. Each test was conducted in a 3 by 12 factorial experiment in a completely randomized design replicated three times. The factors were the three melon varieties (Serewe, Bara and Bojuri) and twelve fungal isolates. The seeds were planted in planting bags filled with 5kg of sterilized soil as previously described in Section 3.1.6. The spore suspension of $2.1x10^6$ spores /mL was sprayed on the leaves surface to run off using a 250 mL hand operated sprayer. Control plants were sprayed with distilled water. Two seedlings of *Egusi* melon of two weeks old per pot were inoculated and covered with a transparent polythene sheet for 48 hours after spraying to maintain high humidity required for disease initiation. Symptoms were observed for up to five weeks. During the test, plants were watered regularly. Re-isolation of the pathogen was done from the plant showing symptoms and identified using standard procedure to fulfill Koch's postulate

3.5 Field evaluation of the effect of leaf blight disease on seed and fruit yield of *Egusi* melon varieties

Field experiment was conducted on the research field of the National Cereals Research Institute (NCRI) Moor Plantation, Ibadan. Field with no cropping history of *Egusi* melon was used. Land preparation involved ploughing and harrowing. The experiment was in split-plot laid down in Randomised Complete Block Design (RCBD) with variety as the main plot and pathogens as sub-plot. The total field area was 1696 m^2. The field was divided into four blocks. Each block was split into 2 by 2 m alley for the inoculated and un-inoculated. Each block was 14 m x 4 m separated by 2 m alley (Figure 3.3). The block comprised of 3 plots on which each of the 3 melon varieties Bara (NG/AO/11/085b), Serewe (NG/AA/SEP/09/148) and Bojuri (NG/TO/AUG/09/003) were randomly planted per plot. Each plot, separated by 2 m alley, consisted of one row of melon plants. Test seeds were sown at 4 seed per hole at about 2cm depth and at a spacing of 1.5 m x 2 m. A week after emergence, seedlings were thinned to two per hole leaving 24 plants per plot

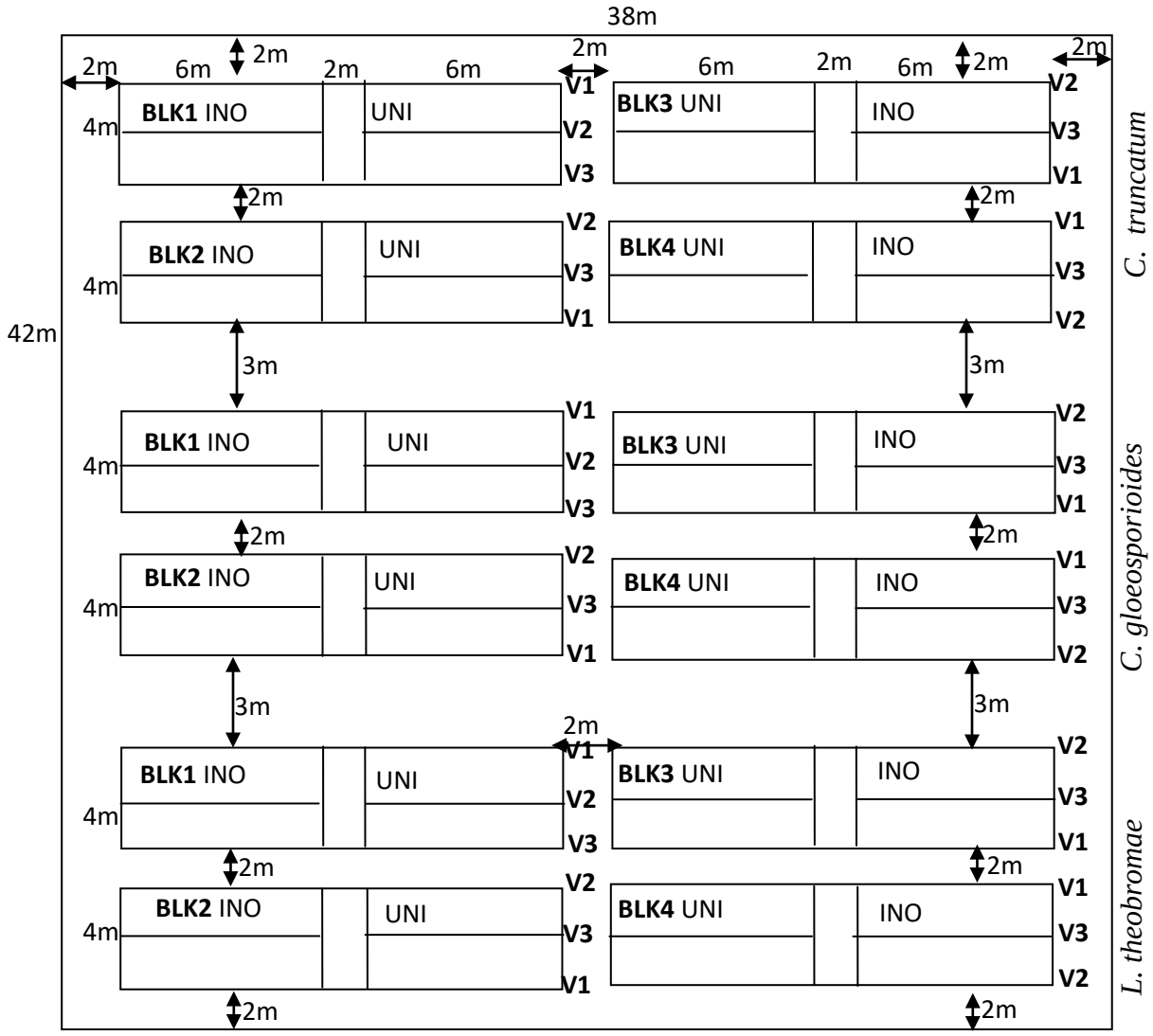


Figure 3.3. Layout for field experiment for the effect of Leaf blight disease on yield of *Egusi* melon; V1=Serewe, V2= Bojuri, V3= Bara variety, BLK= Blocks, UNI= Un-inoculated, and INO= Inoculated.

Test seedlings were artificially inoculated with *Colletotrichum truncatum, Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae* which were found pathogenic on *Egusi* melon in 2017 for two trials (April- June and August- October). Inoculation was carried out 14 days after planting by spraying the adaxial and abaxial surfaces of two youngest fully expanded leaves (4 leaf- stage) with fungal suspension ($2.1x10^6$ spores/ml) to run-off, using a hand-operated sprayer (Kehinde, 2008). Spore suspension was used immediately after preparation. Control plants were sprayed with SDW. Insect damage was controlled by spraying with Cypermethrin at 2 mL /litre. At maturity, all fruits were manually harvested and processed. Fruits harvested were heaped together per treatment, beaten with club or machete to break them and left to ferment for two weeks before washing and sun-drying. Dry seeds were stored with the seed coat in paper envelopes and refrigerated at 4^oC.

Data were collected on plant height, number of leaves, number of vine, number of fruits/plant, number of fruits/ha, weight of fruits/plant, seed weight per fruit, seed weight per plant and seed weight per hectare. Percentage yield reduction was calculated as below:

$$\text{Percentage yield reduction} = \frac{WC - WI}{WC} \times \frac{100}{1}$$

Where, WC = weight of seed per plant for control

WI = weight of seed per plant for inoculated plants

3.6 Seed-to-plant transmission of leaf blight disease in *Egusi* melon artificially inoculated with causal organism.

The laboratory and screenhouse experiments were conducted at Seed Health Testing Laboratory and screenhouse of the NAQS, Ibadan. A preliminary experiment was conducted to ascertain if the seed samples to be used in this experiment were contaminated with the causal organisms and the percentage germination of the seeds were determined by the standard Seed Health Testing procedures.

This preliminary experiment was conducted using blotter method described by ISTA (1993) by direct seed plating. Three layers of sterilized (at 121 ^oC in an autoclave) Whatman No 1 filter paper soaked in sterile distilled water were placed aseptically in

sterile 9 cm diameter Petri dishes using flamed forceps. Ten unshelled seeds of each melon varieties, surface sterilized with 10 % NaOCl, were placed equidistant from one another in each Petri dish, replicated thrice. The Petri dishes were incubated for seven days at 28^{o} C. Seeds were observed for fungal growth after 3 - 4 days. Percentage germination was calculated (Ng, *et al*., 2015) thus;

$$\text{Percentage germination} = \frac{\text{Number of seeds that germinated}}{\text{Total number of seeds planted}} \times \frac{100}{1}$$

Three *Egusi* melon varieties: Bara (NG/AO/11/085b), Serewe (NG/AA/SEP/09/148) and Bojuri (NG/TO/AUG/09/003) were evaluated for disease transmission. The experimental design was 3 x 4 factorial three varieties and three fungi; *Colletotrichum gloeosporioides, C. truncatum, Lasiodiplodia theobromae* and control (un-inoculated) in CRD with 3 replications. Thirty six pots were filled with 10 kg of sterilized soil as described in Section 3.1.6 each. Artificial inoculation was done by soaking the *Egusi* melon seed in fungal spore suspension. Spore suspension of $2.1x10^{6}$ spore /ml was prepared as in section 3.4. Seeds of each variety were soaked in spore suspension for 2 hours with gentle shaking on a shaker. Seeds for control experiments were soaked in sterile distilled water for 2 hours. The suspension was decanted and seeds were air-dried for 24 hours on sterile paper towel at 28 ± 2^{0}C (Burdman *et al*., 2005). Five seeds were planted randomly per pot. A week after planting, percentage emergence was determined and subsequently plants were thinned to two plants. Percentages of symptomatic seedlings and of seedling death were assessed 18 days after planting (DAP). Disease incidence and severity were calculated as in section 3.2.1. Disease severity was calculated at weekly intervals starting from the first appearance of disease symptoms until flowering and converted to disease rating scale of 1-5. Insect pests were controlled by the application of Cypermethrin at a rate of 2 mL per litre at 4 weeks after planting (WAP). At maturity, all fruits per replicate were harvested and processed.

Seeds harvested from infected plants in the screenhouse experiment were replanted in 5 kg sterilized soil in the screenhouse to evaluate seed-to-plant transmission of the causal organisms of leaf blight of *Egusi* melon. The experiment was laid out in

3 x 3 factorial in CRD comprising of three *Egusi*-melon varieties with three blight causing fungal pathogens. Seeds were sown at the rate of two seeds per pot, with a total of thirty plants per treatment. Data were calculated on percentage germination. Percentage seed transmission (Ogunsola, 2015) of leaf blight disease was calculated using the disease incidence value as shown below.

$$\text{Percentage seed transmission} = \frac{\text{Number of plant showing leaf blight symptom}}{\text{Total no of plant per treatment}} \times \frac{100}{1}$$

3.7 Determination of the effective inoculation period of *Egusi* melon for blight disease development

The experimental design was 3 x 3 x 3 factorial experiment in Completely CRD with 4 replications. Three organisms *(Colletotrichum truncatum, Colletotrichum gloeosporioides* and *Lasiodiplodia theobromae*), three varieties Bara (NG/AO/11/085b), Serewe (NG/AA/SEP/09/148) and Bojuri (NG/TO/AUG/09/003) and three periods of inoculation (Seed inoculation, 14 DAP, 28 DAP and un-inoculated). One hundred and eight pots were filled with 5 kg sterilized soil.

The spore suspension $2.1x10^6$ spores /mL was sprayed on the leaves surfaces to run-off using a 250 mL hand operated sprayer at 14 DAP and 28 DAP. Seed inoculation was done as in section 3.6. Inoculated *Egusi* melon seedlings were also covered with a polythene sheet for high humidity required for disease initiation. Then, the seedlings were kept in the screenhouse. The symptoms were observed for up to fruiting period. During the test, plants were watered regularly with hand sprayer. Control plants were sprayed with distilled water. Disease incidence and severity were calculated as in section 3.2.1. Disease severity was calculated at weekly intervals starting from the first appearance of disease symptoms until flowering and converted to disease rating scale 1-5.

3.8 Inhibitory effect of botanicals on the growth of leaf blight disease causing organism of *Egusi* melon *in vitro* and *in vivo*

3.8.1 Preparation of botanicals extract

Fresh leaves, stems and roots of *Costus afer, Morinda lucida, Azadirachta indica, Phyllanthus amarus, Passiflora foetida and Thevetia neriifolia* plants (Plate 3.1) were washed with distilled water and air-dried in the laboratory at 28 ± 2^0C for two days during hammerttan season. The dried plant parts were pulverized by blending for 2 - 4 minutes with a Warring Blender (Shaisho magic blender S-999). Crude water extract of the ground leaves were prepared by adding the varying weight of the powdered plant materials to 100 mL of SDW in a 500 mL beaker, stirred vigorously, allowing 24 hours for settling, and then filtered through four folds of sterile cheese cloth. Five extract concentrations were prepared by mixing 2.5 g, 5 g, 7.5 g, 10 g and 12.5 g of each plant sample in 100 mL of SDW to produce 2.5%, 5%, 7.5%, 10% and 12.5% (w/v) extract concentrations, respectively (Ojo and Olufolaji, 2011)

3.8.2 Determination of the inhibitory effects of botanical extracts on the mycelial growth and sporulation of leaf blight disease-causing organisms of *Egusi* melon

Effect of extracts on fungal growth *in vitro* was determined according to Nduagu *et al*. (2008). A 3.9 g of PDA was added to each crude water extract at different concentrations. The flasks containing the medium were autoclaved for 15 minutes (Onekutu *et al.,* 2001) and antibiotics streptomycin sulphate (100 mg/litre) was added. Media were allowed to cool to about 40^{o}C (Obagwu *et al*., 1997). Five millimeter diameter mycelial disc obtained from the colony edge of 7 days old culture of the test fungus was separately placed on the extract at the point of intersection of the two lines drawn at the bottom of the Petri dish. The experiment replicated three times. Control experiment was without plant extract on the PDA and inoculated with the test fungus. All plates were incubated at 28 ± 2^{o}C and fungal growth was measured on a daily basis for five days. The diameter of fungal growth was measured using meter rule along two directions on the perpendicular lines previously drawn on the plates.

Morinda lucida leaves

Morinda lucida stem

Morinda lucida root

Azadirachta indica leaves

Azadirachta indica stem

Azadirachta indica root

Plate 3.1. Plant materials used as seeds treatment against Leaf blight disease

Costus afer leaves

Costus afer stem

Costus afer rhizome

Passiflora foetida leaves

Thevetia neriiflora leaves

Phyllanthus amarus leaves

Plate 3.1 continued

Sporulation was determined 7 days after inoculation, by cutting out 5mm^2 agar disc of the mycelial mass of the test pathogens in Petri plates using cork borer. The inoculating loop was used to pick the punched mass into a 50 mL beaker and 3 mL of sterile distilled water was added. Stirring was done to dislodge the pathogen after which fitration was done using muslin cloth in other to have only the pathogen spores in the suspension. One hundred microlittre from each concentration was then placed, one after the other on haemocytometer slide, and viewed under a compound microscope at Magnification of X100.

The percentage reduction (M_r) or stimulation (Ms) of colony diameter and sporulation by each extract was calculated using the formula

$$Mr = \frac{M1 - M2}{M1} \times 100$$

Where M_r. = % reduction in colony diameter or sporulation;

M1 =Colony diameter or sporulation on the untreated medium (Control);

M2 = Colony diameter or sporulation on the treated medium

$$Ms = \frac{M2 - M1}{M2} \times 100$$

Where M_s = % stimulation in colony diameter or sporulation;

M2 = Colony diameter or sporulation on the treated medium;

M1 =Colony diameter or sporulation on the untreated medium (Enikuomehin *et al*., 2002; Nduagu *et al*., 2008).

Extracts were rated based on their inhibitory effects using modified Ejechi and Souzey (1999) scale.

-0% =stimulatory (ST)

0% = no effect (NE)

0.1 – 20% = slightly effective (SE)

20.1 - 40% = moderately effective (ME)

40.1 – 60% = effective (EE)

60.1% and above = highly effective (HE)

3.9 Isolation of Biological control organism

Test biological control organisms (*Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens and Bacillus subtilis*) used in this study were isolated from soil collected in *Egusi* melon farms (rhizosphere) of the NCRI compound in Ibadan and the soil collected were taken to the General Laboratory of the Nigeria Agricultural Quarantine Service, Moor Plantation, Ibadan.

3.9.1 Isolation of *Bacillus subtilis* and *Pseudomonas fluorescens*

One gram of soil was placed in McCartney bottle containing 10 mL of SDW. The soil was left in the water for 1 hr and shaken periodically at 20 minutes intervals to get the micro-organisms acclimatized to the ambient temperature. Serial dilution was carried out to obtain 10^8 cfu/mL for bacterial antagonists. Thereafter, 1 mL of each dilution was dispensed into separate 9 cm diameter sterile Petri dishes in triplicates (Dania *et al*., 2015). Nutrient Agar (NA) maintained at 45^{o}C was poured over the suspension. The plates were then swirled gently in both directions to mix the suspension and agar. The plates were incubated at 28±2^{o}C and examined for bacterial growth after 24 - 48 hours of incubation. In order to confirm the identification of *P. fluorescens* cultures, the bacterium was grown on King's B medium (king *et al*., 1954) which is selective for *Pseudomonas* and encourages it to form fluorescent pigments.

3.9.2. Isolation of *Trichoderma* spp.

One gramme of soil was placed in a McCartney bottle containing 10 ml of SDW. The soil was left in the water for 1hr and shaken periodically at 20 minutes intervals to get the micro-organisms acclimatized in the ambient temperature. Serial dilution was prepared to obtain 10^{-6} dilutions. Then, 1mL of each dilution was dispensed into separate 9cm diameter sterile Petri dishes in triplicates. Potato Dextrose Agar maintained at 45^{o}C was poured over the suspension. The plates were then swirled gently in both directions to mix the suspension and agar. Plates were incubated at 28^{o}C and examined for fungal growth after 48 hours of incubation. The fungal isolates from each

of the samples were sub-cultured on PDA to obtain pure cultures for identification. The cultural features of the fungal isolates were carefully observed and recorded. Wet mounts of the isolate were prepared on a microscope slide and stained with lactophenol cotton blue. The prepared mounts were observed under a compound microscope and detailed structural features of each isolate were recorded. The features of the organisms were compared with those described in a standard manual of fungi (Barnett and Hunter, 2003).

3.10 *In vitro* evaluation of biological control agents (BCAs) for the control of leaf blight disease- causing pathogens.

The dual culture method was used (Dania *et al.*, 2015). A freshly prepared PDA medium was inoculated with a fully grown culture of each BCAs with the aid of a 5 mm cork borer at four equidistant points, while the test pathogens were inoculated at the center of the plate in three replicates. The culture plates were incubated at 28±2^{o}C. Daily measurement of radial growth of the BCAs and pathogen were taken consecutively for 7 days. The pairing was done by introduction of the pathogen 24 hours after the BCAs. Where a zone of inhibition occurred, the length and the difference between mycelial growths was recorded, and expressed as a percentage of the growth away from the antagonist (Dania *et al.*, 2015).

3.11 Test for biotoxin production from BCAs

3.11.1 Evaluation of toxins from fully grown potential BCAs

Fully grown 7- day old culture of the BCA was scrapped off to remove surface mycelial growth and washed off twice with SDW. The BCA plate was inoculated with 7- day old culture of the test pathogens with the aid of a 5 mm cork borer and incubated at 28-30^{o}C in the incubator. A similar procedure was employed on 14 and 21 day-old cultures of the potential BCA. The PDA plates were inoculated with 7 day old test pathogens separately as control. Three replicates were maintained per treatment and observations were made from 3 day after inoculation till day 21.

3.11.2. Effect of heat on biotoxin production

Thirty-nine grammes PDA and 28g NA were prepared and poured in glass Petri dishes. These were inoculated with potential BCAs and incubated at 28-30^{o}C for 24 hours and 7 days for bacteria and fungi, respectively. At 24 hours and day 7 after inoculation, the potential BCAs were scooped into a conical flask containing 100 mL of fresh PDA. It was autoclaved at 121^{o}C for 15 minutes, allowed to cool and poured out at 20 mL per Petri plate. This was allowed to solidify and inoculated with the test pathogen. There were three replicates of the potential BCA and its growth observed was recorded until day 7 after inoculation (Dania, 2012).

3.12 Evaluation of botanicals and BCAs as seed treatment against leaf blight disease causing organism of *Egusi* melon

The *Egusi* melon variety with the highest disease severity in seed transmission experiment was used for the screenhouse trial to determine the effectiveness of plant extracts and BCAs as seed treatment against leaf blight disease of *Egusi* melon. *Egusi* melon seeds were sterilized in 10% sodium hypochlorite for 2 minutes and then rinsed in five changes of SDW. Fifty seeds were evaluated per treatment.

Seeds were soaked in fungal spore suspension of 2.1x10^{6} spores/mL for 2 hours with gentle shaking on a shaker and air-dried for 24 hour on sterile paper towel at 28-30^{o}C. Seeds for control experiments were soaked in SDW for 24 hour.

Cassava starch was used as an adhesive for botanicals and BCAs (Adekunle, 1998 and Akinbode, 2013). Twenty grammes of the cassava substrate was added to 100 mL of boiled distilled water and mixed thoroughly with glass rod.

For botanical treatment, the botanicals used were *Costus afer* root, *Phyllanthus amarus* leaf, *Passiflora foetida* leaf and *Costus afer* leaf. One hundred milliliter of SDW was added to 20 g cassava starch and boiled. A weight of 5 g, 10 g and 50 g of the botanicals powder were added to the starch in separate flasks. The slurry was mixed with 50 seed of *Egusi*, and left in the laminar flow for 12 hours to dry before planting.

Flasks containing boiled cassava starch were then inoculated with 1mL of *P. fluorescens* and *B. subtilis* at 10^{8}cfu/mL. The contents were shaken thoroughly to disperse the inocula evenly. The seeds were left for 10 minutes in the slurry before they

were removed and dry with paper towel in the laboratory before planting. Also, one gramme of mycelial mat of the two *Trichoderma* species were then added each to the boiled cassava starch in separate flasks. These were mixed with 50 seeds of *Egusi* melon seed, mixed, shaken and left to dry in the laminar flow for 12 hours before planting. There were two controls, the same 50 seeds were also treated with Mancozeb (80% WP) fungicide at a rate of 0.5 g/ 50 seeds and there were 50 seeds for untreated seed as negative control. Two seeds were planted randomly per pot in screen-house to seedling stage. Data were collected on germination, incidence and severity.

3.13 Qualitative and quantitative analysis of the botanicals

The phytochemical analysis of the plant extract with antifungal activity against the test pathogens was carried out in KAPPA Biotechnology laboratories using standard procedures to identify the constituents as described by Nduagu *et al*. (2008). The plant extracts used were those of *Costus afer* root, *Phyllanthus amarus* leaf, *Passiflora foetida* leaf, *Costus afer* leaf, *Azadirachta indica* leaf and *Thevetia neriifolia leaf*. The extracts were evaluated for the presence of tannins, saponins, flavonoids glycosides, steroids, terperoids, phenols and alkaloids (Padamaja, 1989; Okerulu and Ani, 2001; Hassan *et al*., 2004 and Makkar *et al*., 2007)

3.14 Data analyses

Data on percentage germinations, disease incidence and severity, colony diameter and sporulation were analyzed by descriptive statistics and ANOVA using SAS (2008) package. Means with significant differences were separated using Fishers Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT) at 5 % level of probability. Yield and growth parameters data were compared by t- test analysis using SAS (2008) package version 9.2 and means were separated using Fishers LSD at 5 % level of probability.

CHAPTER FOUR

4.0 RESULTS

4.1 Distribution of *Egusi* melon varieties in five states of Southwestern Nigeria.

Bara variety was cultivated in all the Local Government Areas (LGA) visited in the five states (Table 4.1). The distribution of *Egusi* melon varieties planted across the five states in year 2016 ranged between 16.7% and 100% (Bara), 16.7% and 66.7% (Serewe), and 16.7% and 100% (Bojuri). Four LGAs, Odeda (Ogun State), Ola Oluwa (Osun state), Ibarapa East and Ibadan Southwest (Oyo state) had the highest distribution (100%) of Bara variety. Serewe variety was not planted in Akure North (Ondo state), Ola Oluwa (Osun state), Ise Orun, Emure, Ekiti Southwest (Ekiti state) and Ibadan Southwest (Oyo state) LGAs while Bojuri was planted in all the LGAs except Odogbolu, Akoko N/W (Ondo state) Iwo, Olaoluwa (Osun state) and Ido, Ibarapa East and Ibadan Southwest (Oyo state) during the year (Table 4.1).

4.1.2 Incidence and severity of leaf blight disease of *Egusi* melon varieties in selected LGAs of five Southwestern States in Nigeria

From the studied locations, incidence of disease ranged between 0 and 95% while severity rating of 1 (no symptom) to 5 (highly severe) was observed among the surveyed location (Table 4.2). In Ogun State, 90% incidence and highly severe blight was observed on Bojuri variety in Odeda LGA which was significantly ($p = 0.05$) higher than those observed in all other farms. Significantly higher infection rate (70% incidence and severe severity) was also observed on Serewe at Ijebu North East of Ogun State. Although, high incidence and severity of blight were observed in all the LGAs of Ogun State. Bojuri variety was not planted at Odogbolu LGA.

Table 4.1. Distribution of three varieties of *Egusi* melon planted in selected areas surveyed in five states of Southwestern Nigeria in 2016

State	LGA	Distribution (%)		
		Bara	Serewe	Bojuri
Ogun	Odeda	100.0	16.7	16.7
	Ijebu North East	66.7	16.7	16.7
	Odogbolu	83.3	33.3	0.0
	Ado-odo Ota	66.7	66.7	66.7
	Yewa North	66.7	33.3	50.0
Ondo	Owo	66.7	16.7	66.7
	Odigbo	16.7	33.3	50.0
	Akure North	16.7	0.0	83.3
	Akoko North East	66.7	16.7	33.3
	Akoko North West	66.7	33.3	0.0
Osun	Ayedire	83.3	0.0	16.7
	Iwo	83.3	16.7	0.0
	Ola Oluwa	100.0	0.0	0.0
	Ife South	66.7	66.7	83.3
	Oriade	83.3	33.3	50.0
Ekiti	Ise-Orun	16.7	0.0	66.7
	Emure	50.0	0.0	50.0
	Ekiti South West	83.3	0.0	16.7
	Ekiti West	66.7	16.7	83.3
	Ijero	66.7	33.3	66.7
Oyo	Ido	83.3	16.7	0.0
	Ibarapa East	100.0	16.7	0.0
	Ibadan South West	100.0	0.0	0.0
	Atisbo	66.7	16.7	100.0
	Iseyin	33.3	66.7	100.0

Table 4.2. Incidence (%) and severity of leaf blight disease of three *Egusi* melon varieties in selected LGAs of five South Western States in Nigeria in 2016.

State	Variety	Local Government Area									
		Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity	Incidence	Severity
Ogun		**Odeda**		**Odogbolu**		**Ijebu North-east**		**Ado-odo Ota**		**Yewa North**	
	Bara	60.00b	3.75b	56.00a	4.00a	55.00a	3.25b	30.00a	4.00a	26.67a	3.25a
	Serewe	40.00c	1.50c	50.00a	3.50a	70.00a	5.00a	30.00a	4.00a	30.00a	3.00a
	Bojuri	90.00a	5.00a	-	-	40.00a	2.00c	32.50a	2.50a	55.00a	4.00a
Ekiti		**Emure**		**Ise-orun**		**Ekiti S/West**		**Ekiti West**		**Ijero**	
	Bara	63.33a	3.00a	70.00a	3.50a	65.00a	3.50a	23.75a	2.25a	47.50a	3.75a
	Serewe	40.00a	2.00a	-	-	-	-	6.67b	2.00a	40.00a	3.50a
	Bojuri	63.33a	3.33a	62.00a	3.20a	68.00a	3.80a	32.00a	2.80a	57.50a	4.00a
Ondo		**Owo**		**Odigbo**		**Akure North**		**Akoko N/East**		**Akoko N/West**	
	Bara	87.50a	4.50a	90.00a	4.00a	-	-	10.00a	1.50ab	27.50a	3.00a
	Serewe	70.00a	3.00a	80.00a	4.00a	-	-	20.00a	3.00a	30.00a	3.00a
	Bojuri	86.67a	4.33a	83.33a	4.00a	95.00	4.67	-	-	-	-
Osun		**Ayedire**		**Iwo**		**Ola Oluwa**		**Ife South**		**Ori-ade**	
	Bara	87.50a	4.50a	75.00a	4.20a	75.00a	4.17a	0.00b	1.00b	24.00a	3.40b
	Serewe	70.00a	4.00a	80.00a	4.00a	-	-	0.00b	1.00b	35.00a	3.50a
	Bojuri	-	-	-	-	-	-	34.00a	3.2a	23.33a	3.33c
Oyo		**Ibarapa East**		**Ibadan S/West**		**Ido**		**Atisbo**		**Iseyin**	
	Bara	77.00a	3.80a	50.00a	3.60a	35.00b	2.83b	35.00a	2.75c	60.00a	4.00a
	Serewe	60.00a	2.00a	56.67a	4.00a	70.00a	4.00a	40.00a	3.00a	60.00a	4.00a
	Bojuri	60.00a	2.00a	70.00a	4.00a	-	-	35.00a	2.83b	50.00a	3.83a

* - ; not found. Values with similar letter along the column within each state are not significantly different according to LSD (p = 0.05). Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe)

In Ekiti state, there was no difference in the disease incidence and severity of leaf blight among the three varieties across the LGAs except the incidence observed in Ekiti West. Highest incidence (70%) and severe blight (3.5) were observed on Bara at Ise-Orun LGA and moderate to severe blight (2.8 – 4.0) was generally observed on Bojuri variety in all the five LGAs in Ekiti state. Serewe was not planted at Ise - Orun and Ekiti South West LGA (Table 4.2).

High incidence (70 – 90%) and severe (3 – 4) blight infection were found on the three varieties in both Owo and Odigbo LGAs of Ondo state without significant difference ($p = 0.05$) in blight occurrence among the varieties (Table 4.2). Bara variety showed the highest incidence (90%) and severity (4.0) of blight infection at Odigbo while, least incidence (10%) and mild severity (1.5) was recorded at Akoko North East LGAs. Cultivation of Bojuri variety was not observed in Akoko North East and Akoko North West.

In Osun state, high incidence and severe blight disease symptom were observed on Bara and Serewe at Ayedire and Iwo LGAs, while cultivation of Bojuri by the farmers was not observed. Also, high blight incidence (75%) and very severe severity were recorded at Ola Oluwa on Bara which was the only variety planted in the LGA. However, farms planted with Bara and Serewe varieties at Ife south LGA were observed to be free of blight infection (Table 4.2).

High occurrence of blight disease symptoms were also observed in four out of the five LGAs in Oyo states. In Ibarapa East, high blight incidence (77%) and severe severity (3.8) was observed on Bara which was not different ($p = 0.05$) from that on other two varieties. Higher incidence and severe blight were observed in Ido LGA on Serewe (70%, 4) than Bara (35%, 2.8) variety respectively. Low incidence (35- 40%) and moderate (2.75 - 3.00) blight disease were recorded in Atisbo LGA. Variety Bojuri was not observed in Ido LGA in the period under study (Table 4.2).

Averagely, the incidence ranged from 45.7 to 61.8 while severity was between 3.3 and 3.6 (severe) in Osun and Ondo States (Table 4.3). Ondo State had the highest while the least was recorded in Osun State.

Table 4.3. Average Incidence and severity of leaf blight disease of Egusi melon in Southwestern Nigeria

State	Incidence (%)	Severity
Ogun	47.51	3.46
Ekiti	48.39	3.13
Ondo	61.81	3.55
Osun	45.73	3.3
Oyo	54.19	3.33

Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe).

4.2 Isolation and Identification of fungi on leaf blight infected *Egusi* melon

Symptoms of leaf blight were observed on *Egusi* melon farms in various LGAs in five States of the Southwestern Nigeria. Twelve fungal pathogens of ten genera were isolated from the *Egusi* plant samples collected from the farms in the five Southwestern states. The fungi were *Lasiodiplodia theobromae, Colletotrichum truncatum, C. gloeosporioides, Choanephora cucurbitarum, Curvularia lunata, Fusarium equiseti, F. verticillioides, F. solani, Sclerotium rolfsii, Corynespora cassiicola, Pestalotiopsis guepini* and *Rhizopus stolonifer*. (Plates 4.1: a - b).

The pathogenicity test showed that *C. gloeosporioides*, *L. theobromae* and *C. truncatum* caused the leaf blight disease on *Egusi* melon plants. *Choanephora cucurbitarum* caused wet rot of *Egusi* melon flowers. *Corynespora cassiicola* caused leaf spot disease, *F. verticillioides* and *Sclerotium rolfsii* caused vine wilt while *F. solani* caused dampimg-off of seedlings (Plate 4.2).

4.2.1 Frequency of occurrence of fungi isolated from leaf blight infected *Egusi* melon collected from farmer's field in five States of Southwestern Nigeria.

Choanephora cucurbitarum had the highest frequency of occurrence of 25.29% (Bara), 36.36% (Serewe) and 27.69% (Bojuri) (Table 4.4). This was followed by *L. theobromae* 18.39% (Bara), 24.24% (Serewe) and 35.38% (Bojuri). The least frequently encountered fungus across the three *Egusi* melon varieties was *R. stolonifer* with 1.15% incidence on Bara variety. Eight pathogens namely *L. theobromae, C. gloeosporioides, C. cucurbitarum, C. lunata, F. equiseti, F. verticillioides, F. solani* and *Pestalotia guepini* were all isolated from the three varieties of *Egusi* melon sampled. *Colletotrichum truncatum and C. cassiicola* were isolated from Bara and Bojuri but not observed on Serewe. Similarly, *S. rolfsii* was observed only on Bara and Serewe while, *R. stolonifer* was isolated only from Bara variety (Table 4.4).

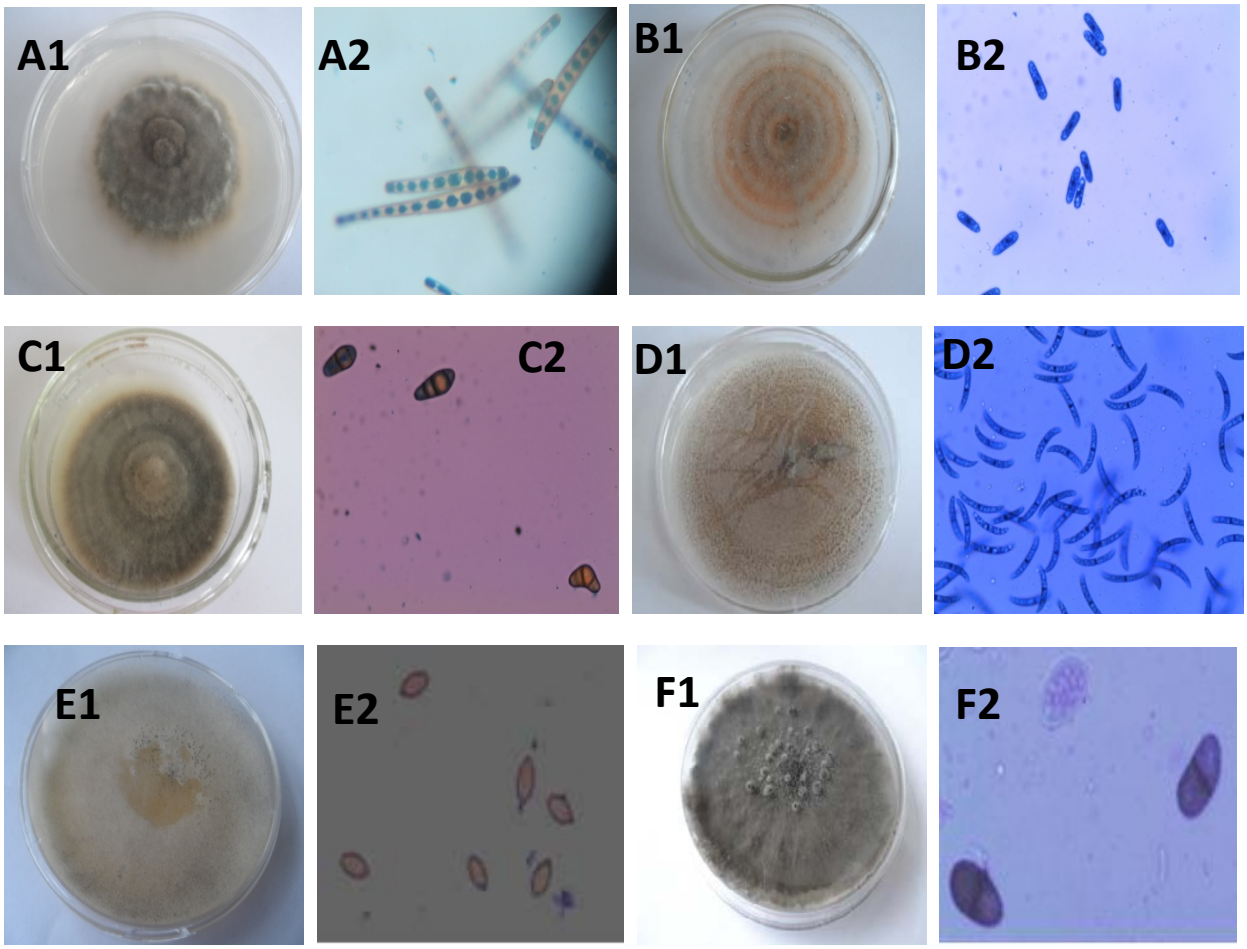


Plate 4.1a. Culture plates and photomicrographs of fungi isolated from leaf blight disease symptom of *egusi* melon in five states of South Western Nigeria: A1 and A2- culture plate and photomicrograph of *Corynespora cassiicola,* B1 and B2- *Colletotrichum gloeosporioides,* C1 and C2- *Curvularia lunata,* D1and D2- *Colletotrichum truncatum* E1 and E2- *Choanephora cucurbitarum,* and F1 and F2- *Lasiodiplodia theobromae*. (Mg = X40)

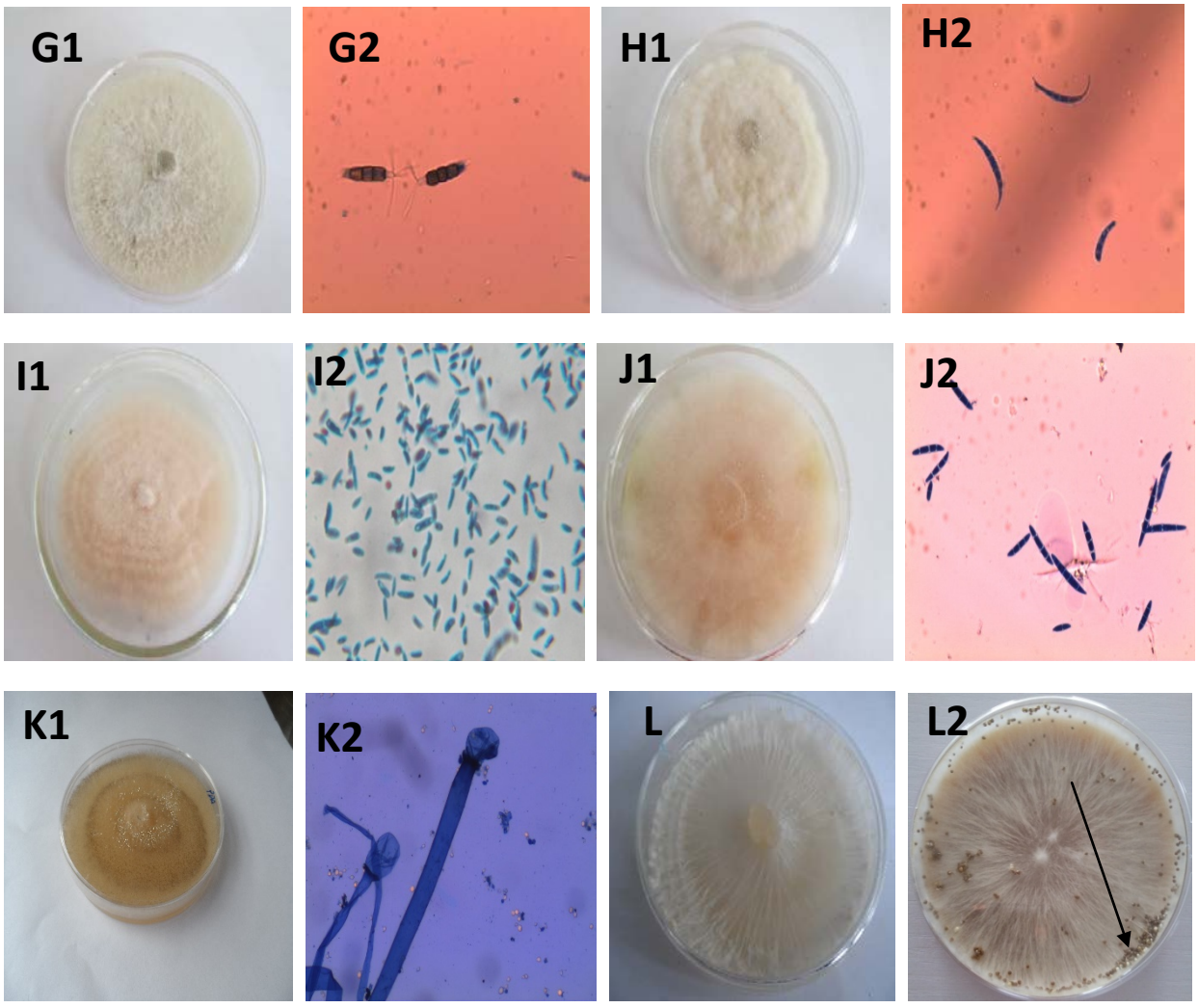


Plate 4.1b. Cultute plates and photomicrographs of fungi isolated from leaf blight disease symptoms of *egusi* melon in five states of Southwestern Nigeria: Culture plate and photomicrograph of *Pestalotiopsis* guepini G1 and G2- *Fusarium equiseti* H1 and H2- *F. solani* I1 and I2- *F. verticillioides* J1and J2- *Rhizopus nigricans* K1and K2- Culture plate of *Sclerotium rolfsii* (L1), and Sclerotia of *Sclerotium rolfsii* (L2). (Mg=X 40)

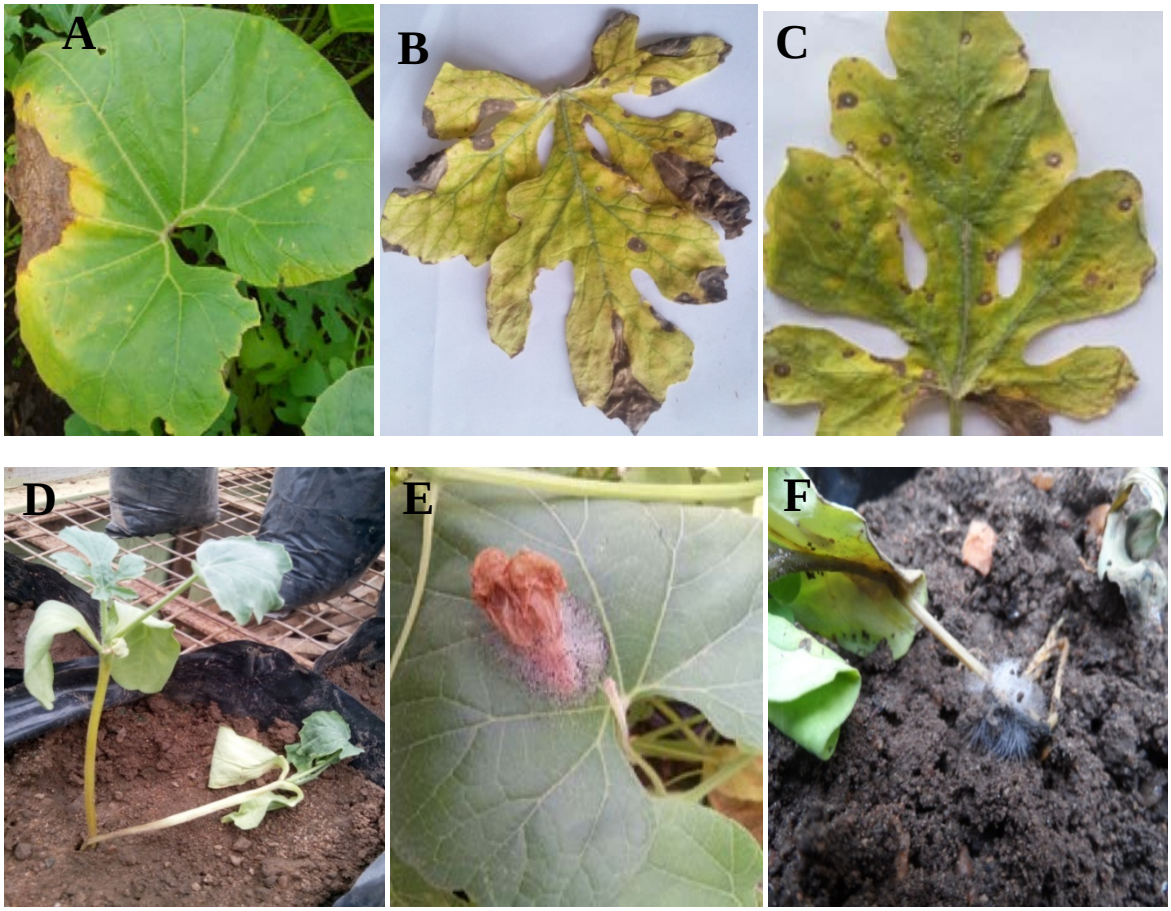


Plate 4.2. Disease symptoms on *Egusi* melon plants inoculated with isolated fungi. (A) Leaf blight caused by *Colletotrichum gloeosporioides* on Bojuri variety, (B) Leaf blight by *Colletotrichum truncatum* on Bara variety (C) Leaf spot disease by *Corynespora cassiicola* on Bara variety, (D) Damping-off caused by *Fusarium solani* on seedlings of Serewe variety, (E) Wet rot of flowers caused by *Choanephora cucurbitarum* on Bojuri variety (F) Vine wilt caused by *F. verticillioides* on serewe variety.

Table 4.4 Frequency of occurrence of fungi isolated from leaf blight infected *Egusi* melon varieties from farmers' fields in five states of Southwestern Nigeria

Fungi	Occurrence (%)		
	Bara	Serewe	Bojuri
Lasiodiplodia theobromae	18.4	24.2	35.4
Colletotrichum truncatum	4.6	0.0	3.1
C. gloeosporioides	8.1	9.1	9.2
Choanephora curcubitarum	25.3	36.4	27.7
Fusarium equiseti	6.9	3.0	4.6
F. verticillioides	10.3	9.1	3.1
Sclerotium rolfsii	2.3	3.0	0.0
Corynespora cassiicola	2.3	0.0	1.5
Pestalotia guepini	9.2	3.0	3.1
Curvularia lunata	8.1	9.1	10.8
Rhizopus stolonifer	1.2	0.0	0.0
Fusarium solani	3.5	3.0	1.5

4.3 Effects of Leaf blight Pathogens on the growth of *Egusi* melon on the field in 2017.

Table 4.5 shows the effect of *Colletotrichum truncatum* on growth of Egusi melon for the two trials in year 2017. There were significant (p = 0.05) differences in all the growth parameters of Bara variety inoculated with *C. truncatum* in both trials.

This fungus produced significant reduction in the vine length, number of leaves and number of vines in Bara variety. However, for Serewe and Bojuri varieties there was no significant difference between *C. truncatum* inoculated and un-inoculated plants in all the growth parameters in both trials.

The effect of *Colletotrichum gloeosporioides* on the growth of *Egusi* melon for the two trials in year 2017 is shown in Table 4.6. There was no significant (p = 0.05) difference between *C. gloeosporioides* inoculated and uninoculated plants in all the growth parameters under study among the three *Egusi* melon varieties in both trials.

Effect of *Lasiodiplodia theobromae* on the growth of *Egusi* melon for the two trials in year 2017 is presented in Table 4.7. *Lasiodiplodia theobromae* does not produce any significant effect on the growth of Egusi melon varieties

4.4 Effects of Leaf blight causal organisms on the seed yield of *Egusi* melon on the field in 2017

Colletotrichum truncatum produced significant (p = 0.05) differences in most of the seed yield parameters studied in the two trials only in Bara variety (Table 4.9). The fungus caused significant reduction in the fruit numbers per plant, weight of seed per fruit weight of seeds per plant and weight of seed per hectare in the first and second trials on Bara variety (Table 4.8). However, *C. truncatum* did not caused any significant yield loss on Serewe and Bojuri varieties in both trials. There was no difference between *C. gloeosporioides* inoculated and uninoculated plants in all the yield parameters under study among the three E*gusi* melon varieties in the two trials except in weight of seed per hectares in Bara varieties (1400kg to 795kg) in the second trials (Table 4.9). Also, *Lasiodiplodia theobromae* showed no significant (p = 0.05) the yield of Egusi melon plant in the two trials (Table 4.10)

Table 4.5 Effect of *Colletotrichum truncatum* on the growth parameters of *Egusi* melon artificially inoculated on the field in 2017

		Vine length		No of leaves		No of vines	
Varieties	TRT*	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Serewe	INO	53.89	70.50	85.40	69.5	17.96	7.80
	UNI	57.66	76.00	96.80	73.50	19.22	8.25
	LSD	ns	ns	ns	ns	ns	ns
Bojuri	INO	58.28	59.75	47.00	47.50	19.43	3.90
	UNI	70.28	61.00	56.00	48.75	23.43	4.20
	LSD	ns	ns	ns	ns	ns	ns
Bara	INO	25.21*	55.75*	96.60*	61.75*	8.40*	5.1*
	UNI	63.14*	78.75*	137.30*	74.00*	21.05*	8.1*
	LSD	21.71	16.55	33.25	13.84	7.23	2.61

*TRT: Treatment, INO: inoculated; UNI: Un-inoculated, LSD: ns: not significant; *, significant (p=0.05)

Table 4.6. Effect of *Colletotrichum gloeosporioides* on the growth parameters of *Egusi* melon artificially inoculated on the field in 2017

		Vine length		No of leaves		No of vines	
Varieties	TRT	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Serewe	INO	72.78	98.50	89.40	72.50	13.90	10.05
	UNI	68.70	99.25	102.10	77.75	15.80	10.05
	LSD	ns	ns	ns	ns	ns	ns
Bojuri	INO	37.85	78.75	47.20	46.00	7.40	4.80
	UNI	42.60	80.50	58.10	53.25	9.20	4.80
	LSD	ns	ns	ns	ns	ns	ns
Bara	INO	77.10	107.00	117.00	77.25	16.10	8.55
	UNI	73.80	98.50	112.10	68.25	12.00	6.90
	LSD	ns	ns	ns	ns	ns	ns

*TRT: Treatment, INO: inoculated; UNI: Un-inoculated, LSD: Least Significant Difference, ns: not significant; *, significant (p=0.05)

Table 4.7. Effect of *Lasiodiplodia theobromae* on the growth parameters of *Egusi* melon artificially inoculated on the field in 2017

		Vine length		No of leaves		No of vines	
Varieties	TRT	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Serewe	INO	37.50	18.90	41.50	36.90	3.75	6.40
	UNI	48.00	26.4	48.25	49.90	5.25	7.30
	LSD	ns	ns	ns	ns	ns	ns
Bojuri	INO	49.50	18.10	36.25	27.00	3.75	4.40
	UNI	51.50	20.60	39.00	31.3	3.00	5.30
	LSD	ns	ns	ns	ns	ns	ns
Bara	INO	44.5	38.40	50.00	62.40	4.95	9.30
	UNI	47.5	35.90	46.50	63.90	4.05	10.30
	LSD	ns	ns	ns	ns	ns	ns

*TRT: Treatment, INO: inoculated; UNI: Un-inoculated, LSD: Least Significant Difference, ns: not significant; *, significant (p=0.05)

Table 4.8. Effect of *Colletotrichum truncatum on* the yield parameters of *Egusi* melon artificially inoculated on the field in 2017

Variety	TRT	NFP		WFP (kg)		NFH		WSF (kg)		WSP(kg)		WSH(kg)	
		1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Serewe	INO	4.75	2.44	3.03	0.10	23750	14250	0.07	0.07	0.14	2.44	676.25	507.5.00
	UNI	4.80	3.8	3.28	0.14	24000	21000	0.07	0.06	0.15	3.80	745.00	700.00
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bojuri	INO	4.30	1.90	2.08	0.15	21500	7750	0.12	0.10	0.23	1.90	1161.30	742.50
	UNI	4.00	1.51	2.08	0.11	20000	7575	0.12	0.08	0.24	1.51	1468.80	550.00
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bara	INO	3.50*	1.69	2.26	0.15	17500	13000	0.07	0.06*	0.13*	0.08*	437.50*	395.00*
	UNI	4.50*	3.07	2.68	0.18	22500	20000	0.08	0.17*	0.19*	0.17*	671.25*	865.00*
	LSD	0.8	ns	ns	ns	ns	ns	ns	0.24	0.05	0.07	231.7	314.32

*Trt: Treatment, INO: inoculated; UNI: Un-inoculated, LSD: Least Significant Difference, NFP: No of fruit/plant, WFP: Weight of fruit/plant, WSF: Weight of seed /fruit, WSP: Weight of seed/Plant, WSH: Weight of seed/hectare, NFH: No of fruit/hectare, ns, not significant; *, significant (p=0.05)

Table 4.9. Effect of *Colletotrichum gloeosporioides* on the yield parameters of *Egusi* melon artificially inoculated on the field in 2017

Variety	TRT	NFP		WFP (kg)		NFH		WSF (kg)		WSP (kg)		WSH(kg)	
		1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Serewe	INO	3.55	2.55	1.98	2.15	17750	12750	0.11	0.10	0.10	1.69	512.50	727.50
	UNI	3.95	3.40	1.91	2.88	19750	17000	0.15	0.08	0.13	3.08	637.50	915.00
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bojuri	INO	4.00	1.60	2.01	1.51	20000	8000	0.11	0.09	0.26	0.09	1285.00	470.00
	UNI	4.40	2.00	2.29	2.12	22000	10000	0.16	0.09	0.22	0.15	1095.00	765.00
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bara	INO	4.00	3.40	2.42	2.82	20000	17000	0.07	0.09	0.10	0.16	511.25	795.00*
	UNI	4.45	5.45	2.54	4.26	22250	27250	0.08	0.13	0.13	0.28	606.25	1400.00*
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	560.36

Trt: Treatment, INO: inoculated; UNI: Un-inoculated, LSD: Least Significant Difference, NFP: No of fruit/plant, WFP: Weight of fruit/plant, WSF: Weight of seed /fruit, WSP: Weight of seed/Plant, WSH: Weight of seed/hectare, NFH: No of fruit/hectare, ns, not significant; *, significant (p=0.05)

Table 4.10. Effect of *Lasiodiplodia theobromae* on the yield parameters of *Egusi* melon artificially inoculated on the field in 2017

VAR	TRT	NFP		WFP (kg)		NFH		WSF (kg)		WSP(kg)		WSH(kg)	
		1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial	1st trial	2nd trial
Serewe	INO	2.80	3.7	1.99	3.33	14000	18500	0.06	0.07	0.06	0.15ns	305.00	725.00
	UNI	3.05	5.00	2.25	4.32	15250	25000	0.07	0.08	0.08	0.24ns	421.25	1195.00
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bojuri	INO	2.85	1.85	2.14	1.26	14250	9250	0.11	0.06	0.18	0.15	875.00	772.50
	UNI	3.25	1.70	2.21	1.87	16250	8500	0.12	0.07	0.21	0.12	1053.80	592.50
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Bara	INO	3.55	3.25	2.26	2.66	17750	16250	0.06	0.06	0.10	0.18	498.75	920.00
	UNI	3.85	3.85	2.55	2.89	19250	19250	0.08	0.06	0.12	0.22	587.50	1082.5
	LSD	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

*Trt: Treatment, INO: inoculated; UNI: Un-inoculated, LSD: Least Significant Difference, NFP: No of fruit/plant, WFP: Weight of fruit/plant, WSF: Weight of seed /fruit, WSP: Weight of seed/Plant, WSH: Weight of seed/hectare, NFH: No of fruit/hectare, ns, not significant; *, significant (p=0.05)

The percentage yield reduction caused by the causal pathogens on *Egusi* melon varieties artificially inoculated on the field in two tials in 2017 is shown in Table 4.11. The three blight-causing pathogens caused reduction in the yield of Bara and Serewe varieties in the two trials. The highest yield reduction of 53.6% caused by *C. truncatum* was observed on Bara varieties followed by 52.9% caused by *C. gloeosporioides* in same varieties. Meanwhile, there was no consistency in the yield reduction caused by the three pathogens on Bojuri variety in both trials.

4.5 Seed-to-plant transmission of leaf blight disease in *Egusi* melon artificially inoculated with causal organisms

Seeds inoculated with leaf blight causal pathogens showed lower percentage germination than uninoculated control (Table 4.12). Germination of the inoculated seeds at 7 days after planting (DAP) ranged between 66.7 – 80.0%, 73.3 – 86.7% and 51.9 – 66.7 for seed inoculated with *C. truncatum, L. theobromae* and *C. gloeosporioides*, respectively. Highest percentage seed germination (86.7%) was observed on Serewe and Bojuri varieties in *L. theobromae* inoculated seeds while the lowest (51.9%) was on Serewe seed inoculated with *C. gloeosporioides.* None of the three fungal inocula showed blight symptom on the *Egusi* melon seedlings at 18 DAP. However, at 42 DAP, all the pathogens caused blight symptom. Highest blight incidence of 100% was observed on Bara and Serewe seeds inoculated with *C. truncatum* while, least blight symptom (33.3%) was observed from *C. gloeosporioides* inoculated seeds of Serewe variety.

Table 4.13 shows the incidence and severity of leaf blight disease on *Egusi* melon seedlings raised from seeds inoculated with fungal pathogens. The three inoculated fungi produced infection symptom in all the *Egusi* melon varieties. Incidence and severity of blight disease progressed from week four to seven.

There was no significant difference ($p = 0.5$) in the incidence and severity of the three *Egusi* melon varieties inoculated with the three fungi pathogens at 4WAP except for Bara where each of the fungus produced similar incidence of blight that was higher than in uninoculated plants (Table 4.13). For Serewe variety, Only *L. theobromae* produced significantly higher incidence (66.67%) and mild severity at 5 WAP and this high incidence and severity increased progressively from 5 WAP to 7 WAP.

Table 4.11. Yield reduction (%) caused by leaf blight disease on *Egusi* melon varieties artificially inoculated on the field in two trials in 2017

Variety	Trial	Fungus		
		C. truncatum	*C. gloeosporioides*	*L. theobromae*
Serewe	1st	6.67	23.1	25.0
	2nd	35.8	45.1	37.5
Bojuri	1st	4.2	-18.2	14.29
	2nd	-25.8	40.0	-25.0
Bara	1st	31.58	37.5	16.67
	2nd	53.6	52.94	18.18

Table 4.12. Effects of blight causal pathogens on the increased progressively from 5 WAP to 7 WAP **growth of *Egusi* melon plants**

Variety	Fungi	G (%)	SD (%)	SP (%)*		
		7 DAP	18 DAP	18 DAP	28 DAP	42 DAP
Bara	*C. truncatum*	66.7	0.0	0.0	16.7	100.0
	C. gloeosporioides	60.0	0.0	0.0	16.7	83.3
	L.theobromae	73.3	0.0	0.0	16.7	66.7
	Control	93.3	0.0	0.0	0.0	16.7
Serewe	*C. truncatum*	73.3	0.0	0.0	16.7	100.0
	C. gloeosporioides	51.9	0.0	0.0	0.0	33.3
	L.theobromae	86.7	0.0	0.0	16.7	83.3
	Control	93.3	0.0	0.0	0.0	16.7
Bojuri	*C. truncatum*	80.0	0.0	0.0	0.0	66.7
	C. gloeosporioides	66.7	0.0	0.0	0.0	83.3
	L.theobromae	86.7	0.0	0.0	0.0	83.3
	Control	93.3	0.0	0.0	0.0	0.0

DAP: Days After Planting, G: Seed Germination, SD: Seedling death, SP: Symptommatic plant

Table 4.13. Incidence and severity of leaf blight disease on Egusi melon varieties raised from seed inoculated with thee fungal pathogens at varying periods

Variety	ORG	Incidence (%)				Severity			
		4WAP	5WAP	6WAP	7WAP	4WAP	5WAP	6WAP	7WAP
Serewe	CT	16.67	100.00	100.00	100.00	1.33	1.17	2.50	3.17
	CG	0.00	33.33	33.33	83.33	1.17	1.17	1.33	1.83
	LT	16.67	83.33	83.33	100.00	1.67	1.67	2.50	2.50
	CTRL	0.00	16.67	16.67	16.67	1.00	1.00	1.17	1.17
	LSD	38.43	47.07	47.07	38.43	0.46	0.46	0.81	0.54
Bojuri	CT	0.00	66.67	66.67	83.33	1.33	1.33	1.83	1.67
	CG	0.00	83.33	83.33	66.67	1.17	1.17	1.83	1.67
	LT	0.00	83.33	83.33	33.33	1.33	1.33	2.33	1.33
	CTRL	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00
	LSD	0.00	47.07	47.07	66.57	0.5	0.5	0.81	0.54
Bara	CT	16.67	100.00	100.00	100.00	1.67	1.67	2.00	2.67
	CG	16.67	83.33	83.33	100.00	1.50	1.50	2.5	3.33
	LT	16.67	66.67	66.67	66.67	1.83	1.83	1.83	1.83
	CTRL	0.00	16.67	16.67	16.67	1.00	1.00	1.17	1.17
	LSD	4.07	66.57	66.57	60.77	0.52	0.52	0.72	0.67

WAP: Week after planting. CT: *Colletotrichum truncatum*, CG: *Colletotrichum gloeosporioides*, LT: *Lasiodiplodia theobromae*, ORG: Organisms, CTRL: Control. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Means along thesame column within each variety are separated by Lsd (P= 0.05).

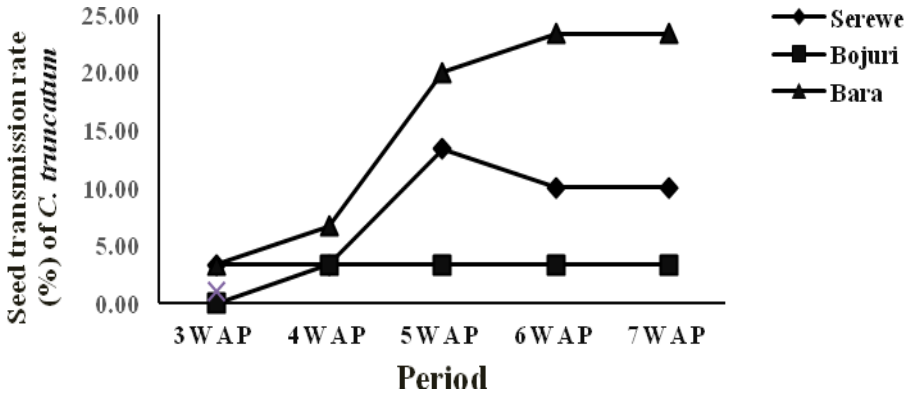
. *Colletotrichum truncatum* also induced higher blight incidence and severity from 6 WAP (100% and 2.50) to 7 WAP (100% and 3.17). Hence, *C. truncatum* produced highest severe blight symptom among the three pathogens on serewe variety. There was no significant effect of the fungal isolates on Bojuri variety at 4 and 5 WAP. At 6 WAP (Table 4.13), the three pathogens produced similarly higher blight incidence and severity than the control plants and at 7 WAP only two pathogens (*C. truncatum* and *C. gloeosporioides*) showed significantly higher disease incidence and severity than uninoculated control. Highest incidence (100%) of blight was observed on seedlings of Bara seed inoculated with *C. truncatum* and *C. gloeosporioides* at 6 to 7 WAP. *C. truncatum and L. theobromae* also caused 100% disease incidence on Serewe variety. The least incidence (33.33%) and severity (1.33) of blight disease was observed on Bojuri variety inoculated with *L. theobromae* (Table 4.13).

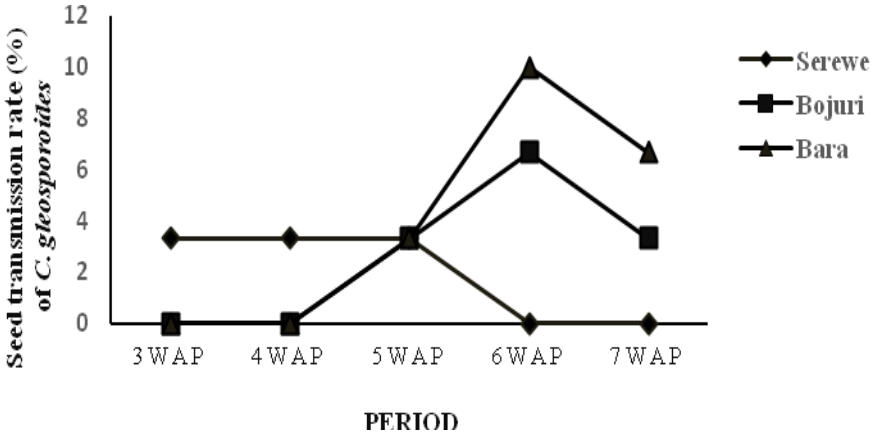
4.6 Seed transmission of leaf blight disease on *Egusi* melon inoculated with leaf blight fungal pathogen

Figure 4.1 shows the results of rate of seed transmission of leaf blight disease on *Egusi* melon. Seed transmission rates varied with pathogen as well as variety. Six weeks after planting, the highest percentage seed transmission (23.33%) was observed on *C. truncatum* in Bara variety whereas C. *gloeosporioides* was not transmitted in Serewe seeds. *C. truncatum* transmission rate was lowest (3.33%) in Bojuri, C. *gloeosporioides* transmission rate was highest in Bara (10%) while *Lasiodiplodia theobromae* transmission was highest (16.67%) in Bara and lowest (3.33%) in Bojuri variety. The transmission rates were either stable or decreased after week six (Figure 4.1).

4.7 Effective inoculation period of *Egusi* melon for leaf blight disease development

Table 4.14 shows the incidence (%) and severity of *Egusi* melon inoculated with fungal pathogens under varying inoculation periods at 6 WAP. Effective inoculation period of the three blight causing pathogens varied with *Egusi* melon varieties.





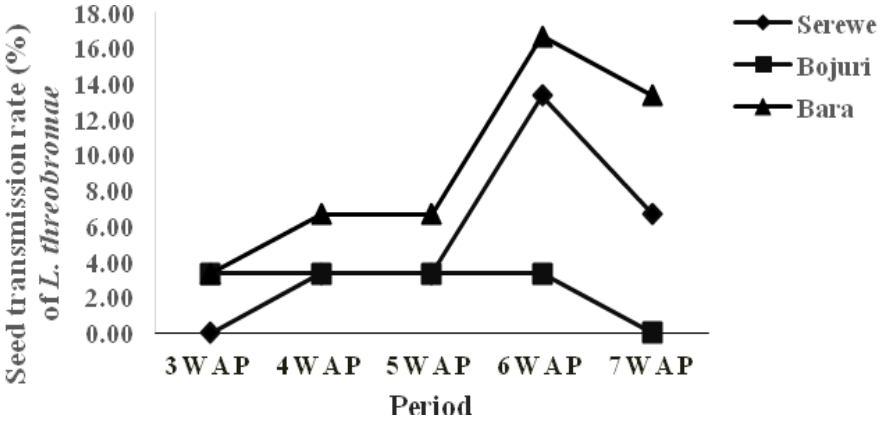


Figure 4.1. Rate of transmission of leaf blight disease on *Egusi* melon seeds harvested from plants inoculated with causal pathogens

Table 4.14. Incidence and severity of *Egusi* melon inoculated with fungal pathogens at varying periods of growth.

		Fungus					
		C. truncatum		*C. gloeosporioides*		*L. theobromae*	
Variety	Inoculation period	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity
Bara	Seed inoculation	100.0	2.5	100.0	3.2	66.7	2.2
	14 DAP	66.7	2.5	66.7	2.7	33.3	1.7
	28 DAP	66.7	2.2	100.0	2.9	33.3	2.7
	LSD	27.4	0.0	27.4	0.3	33.3	0.4
Serewe	Seed inoculation	100.0	3.4	100.0	2.7	33.3	1.9
	14 DAP	100.0	2.3	66.7	2.5	66.7	2.7
	28 DAP	66.7	1.7	66.7	2.3	66.7	1.7
	LSD	27.4	1.2	27.4	0.7	33.3	0.8
Bojuri	Seed inoculation	66.7	1.7	50.0	2.3	100.0	2.0
	14 DAP	33.3	1.3	33.3	1.7	0.0	0.0
	28 DAP	33.3	1.3	33.3	1.7	33.3	1.2
	LSD	33.3	0.37	15.1	0.6	33.3	0.7

DAP = Days after planting, WAP = Weeks after planting. Means along thesame column within each fungal pathogen are separated by LSD (p = 0.05). Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe)

There were differences (p = 0.05) in the period of inoculation among the three pathogens tested. Highest incidence and severity of blight disease was observed on the three melon varieties inoculated with *C. truncatum* before planting. Similarly higher incidence (100%) was observed only in Serewe inoculated 14 DAP but with moderate severity (2.3) than seed inoculated plants (3.4). Severity of blight disease on the three melon varieties inoculated with *C. truncatum* at 28 DAP were significantly lower than that of the seed inoculated plants (Table 4.14).

Seed inoculation of *C. gloeosporioides* caused the highest incidence and moderate severity of 100% and 3.2, 100% and 2.7 and 50 % and 2.3 on Bara, Serewe and Bojuri respectively. There was no difference in the incidence of blight observed from seed inoculated and 28 DAP on Bara but significantly different from 14 DAP. Inoculation of *C. gloeosporioides* at both 14 DAP and 28 DAP produced similar blight incidence and severity among the varieties except in Bara (Table 4.14).

Effects of *L. theobromae* inoculation also varied with inoculation period as well as variety. Incidence (%) and severity of *L. theobromae* inoculated through seeds were significantly (p = 0.05) higher in Bara (66.7% and 2.2) and Bojuri (100% and 2.0) varieties than seedlings inoculated at 14 DAP and 28 DAP. However, inoculation of the pathogen on seedling at 14 DAP produced a significantly higher disease incidence and severity (66.7% and 2.7) than seed inoculation before planting in Serewe (Table 4.14).

4.8. *In vitro* and *in vivo* evaluation of biological control agents for leaf blight control

4.8.1 Effect of *Trichoderma* spp. on *Colletotrichum truncatum*

Mycelial growth inhibition of *C. truncatum* inoculated 24 hours after *T. harzianum harzianum* and *T. pseudokoningii* was 41.50% on the 3rd day (Figure 4.2). The inhibition increased to 55.06% with *T. harzianum* and 68.91% by *T. pseudokoningii* on the 4th day respectively. Further mycelial growth was inhibited beyond this dimension at 5 days after inoculation. There was no significant difference (P < 0.05) between *T. harzianum* and *T. pseudokoningii* in their inhibitory effects on the test pathogen at day 7.

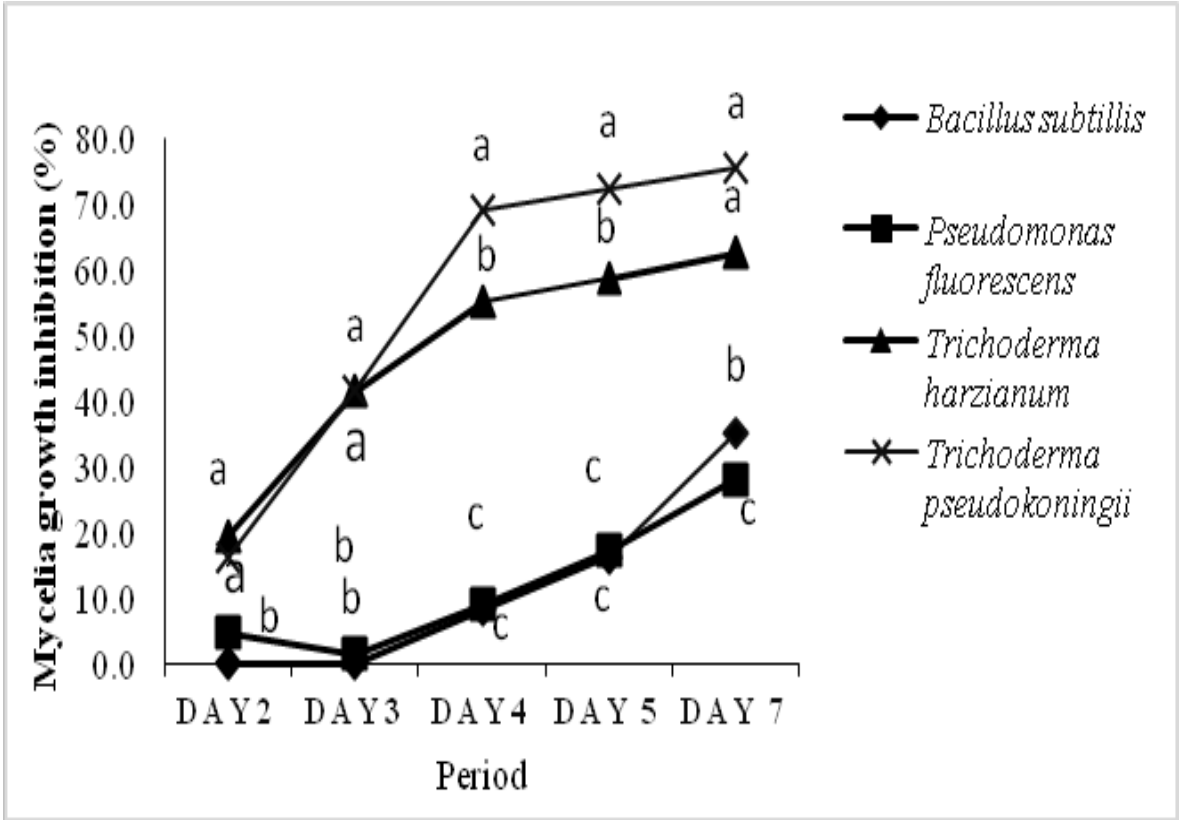


Figure 4.2. Effect of Biological control agents on the mycelial growth of *Colletotrichum truncatum* in the laboratory.

All bioagent induced reduction in mycelial growth of *C. truncatum* except *Bacillus subtillis* in first 3 days. *Trichoderma* spp performed better than other bioagents at day seven (Plate 4.3a).

4.8.2 Effect of *Trichoderma* spp. on *Colletotrichum gloeosporioides*

At 3 days after inoculation (3DAI), the mycelial growth inhibition of *C. gloeosporioides* inoculated 24 hours after *T. harzianum* and *T. pseudokoningii* was 53.63% and 62.15% respectively (Figure 4.3). The inhibition increased to 63.74% with *T. harzianum* and 79.21% with *T. pseudokoningii* on the fourth day. *Trichoderma pseudokoningii* induced a better growth inhibition of the test pathogen (83.43%) than *T. harzianum* (70.73%) but were not significantly different from each other (Figure 4.3), (Plate 4.3b). In all cases, no zone of inhibition was observed between *C. gloeosporioides* and the biocontrol agents.

4.8.3 Effect of *Trichoderma* spp. on *Lasiodiplodia theobromae*

The mycelial growth inhibition of *L. theobromae* inoculated 24 hours after *T. harzianum* and *T. pseudokoningii* was 83.67% and 86.67%, respectively on the 3rd day (Figure 4.4). There was no significant difference ($P < 0.05$) in the effect of the *T. harzianum* (84.56%) and *T. pseudokoningii* (91.33%) on the mycelial growth of *L. theobromae.* Species of *Trichoderma* was significantly superior to other bioagents in reducing the mycelial growth of *C. gloeosporioides.* Apart from the fast growth of *T. harzianum and T. pseudokoningii*, a greenish yellow secretion was observed on the media with *T. pseudokoningii* and around the edge of the test pathogen (Plate 4.3c).

4.8.4 Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on *C. gloeosporioides*

The mycelial growth inhibition on *C. gloeosporioides* was 26.79% and 29.10% when paired with *B. subtilis* and *P. fluorescens* respectively at 4 DAI (Figure 4.3)

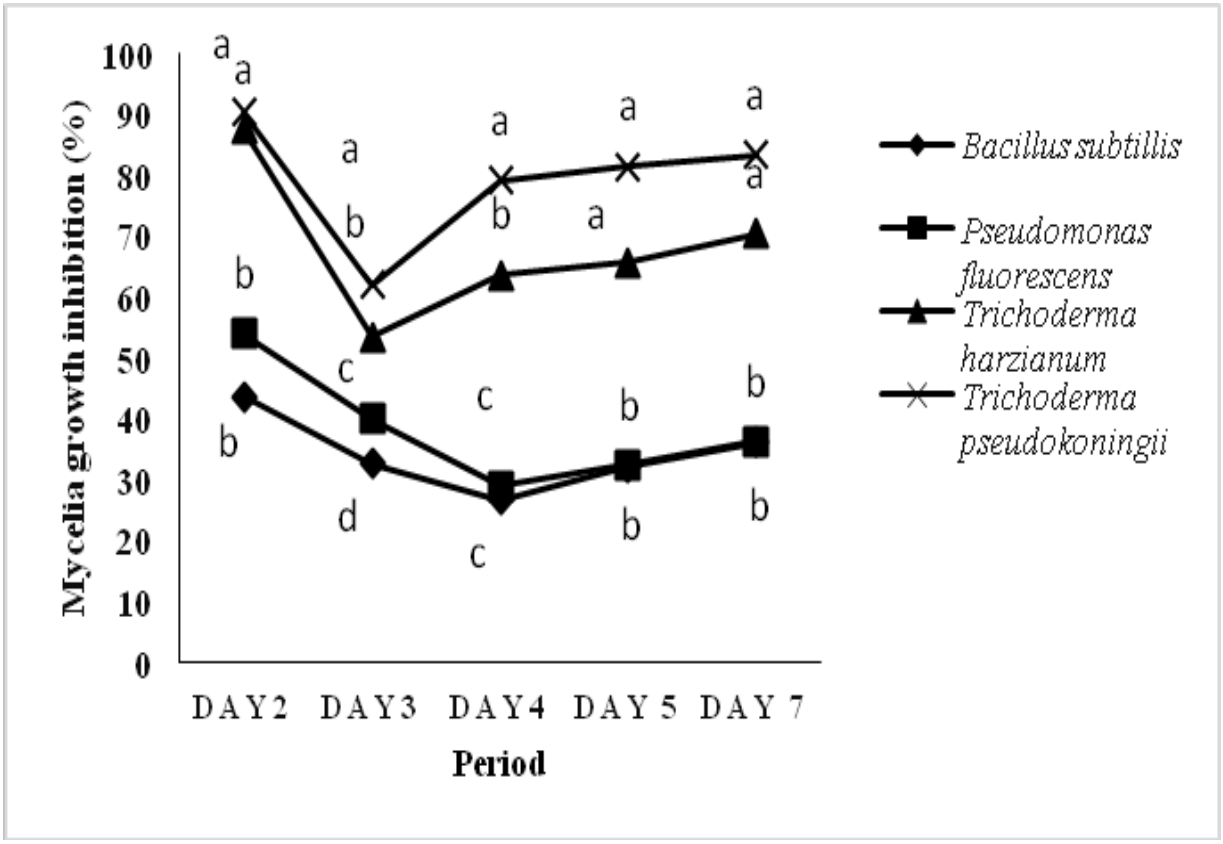


Figure 4.3. Effect of Biological control agents on the mycelial growth of *Colletotrichum gloeosporioides* in the laboratory.

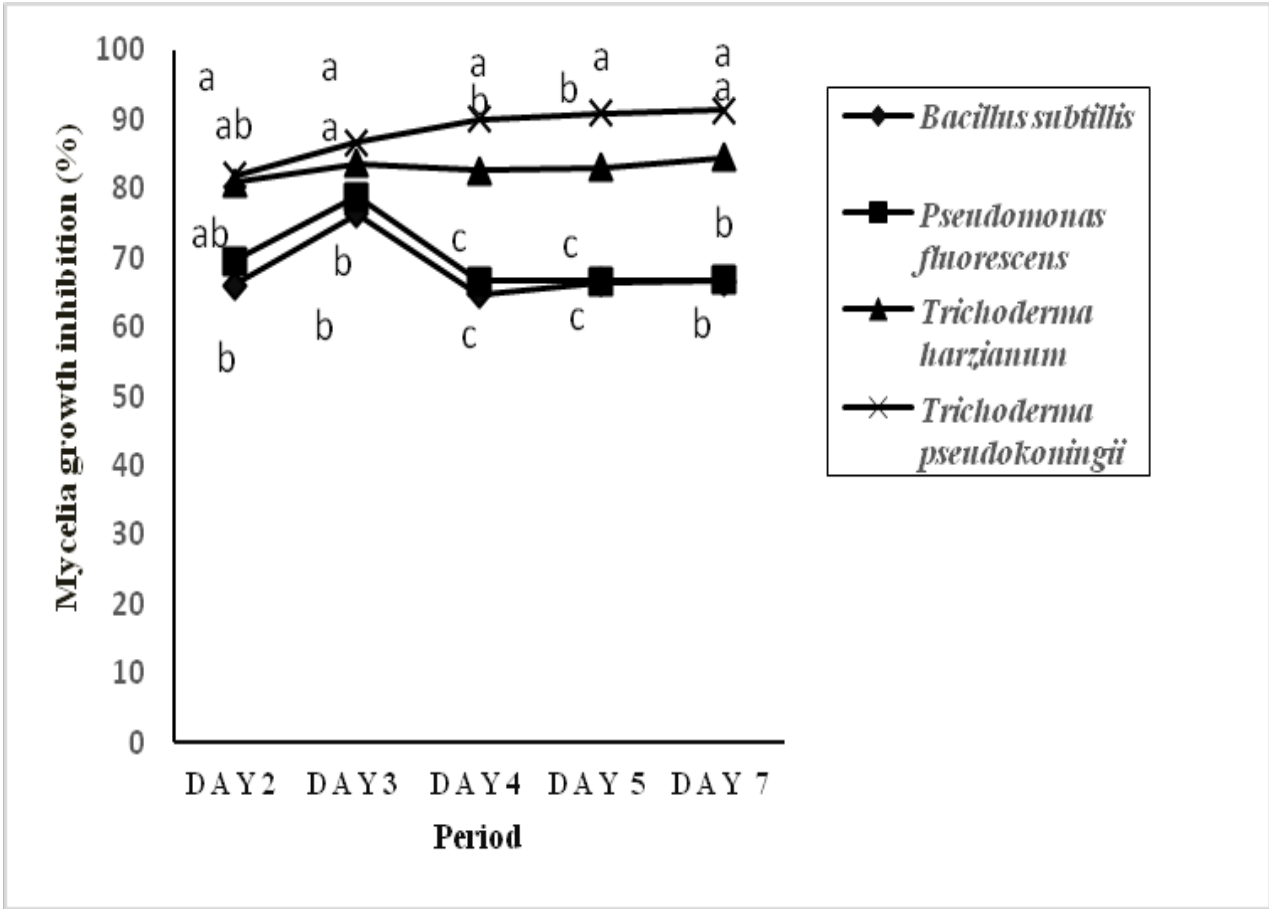


Figure 4.4. Effect of Biological control agents on the mycelial growth of *Lasiodiplodia theobromae* in the laboratory

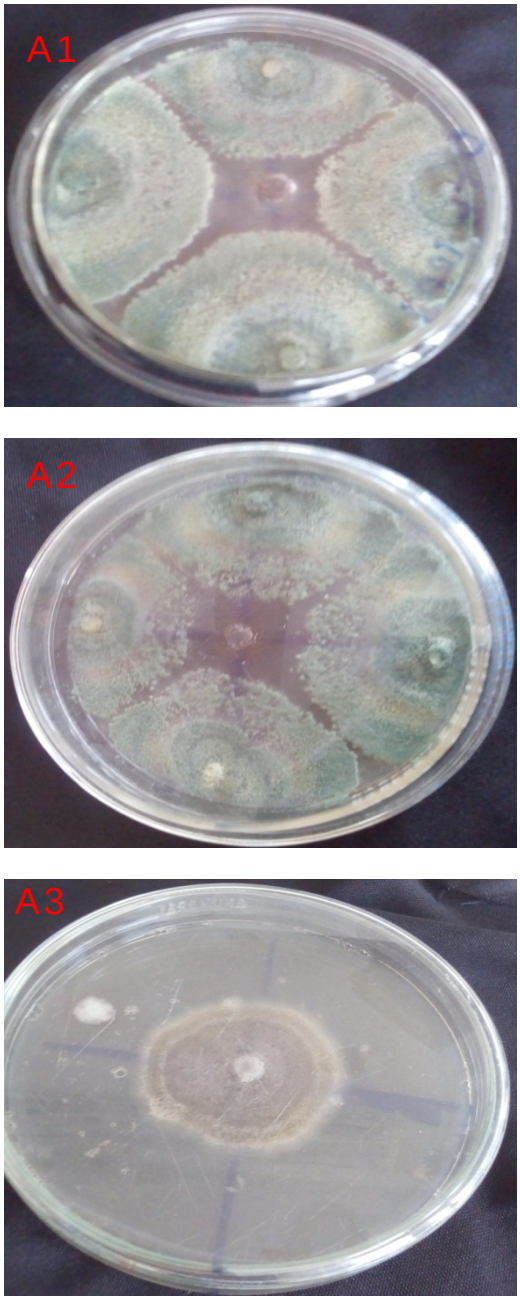


Plate 4.3a. Effect of *Trichoderma* spp. on mycelial growth of *C. truncatum* paired 24 hours before the test pathogen at 5 days after inoculation

Where: A1= *C. truncatum* at the centre paired with *T. harzianum* at the periphery
A2 = *C. truncatum* at the centre paired with *T. pseudokoningii* at the periphery
A3= Control plate of *Colletotrichum truncatum* at the centre.

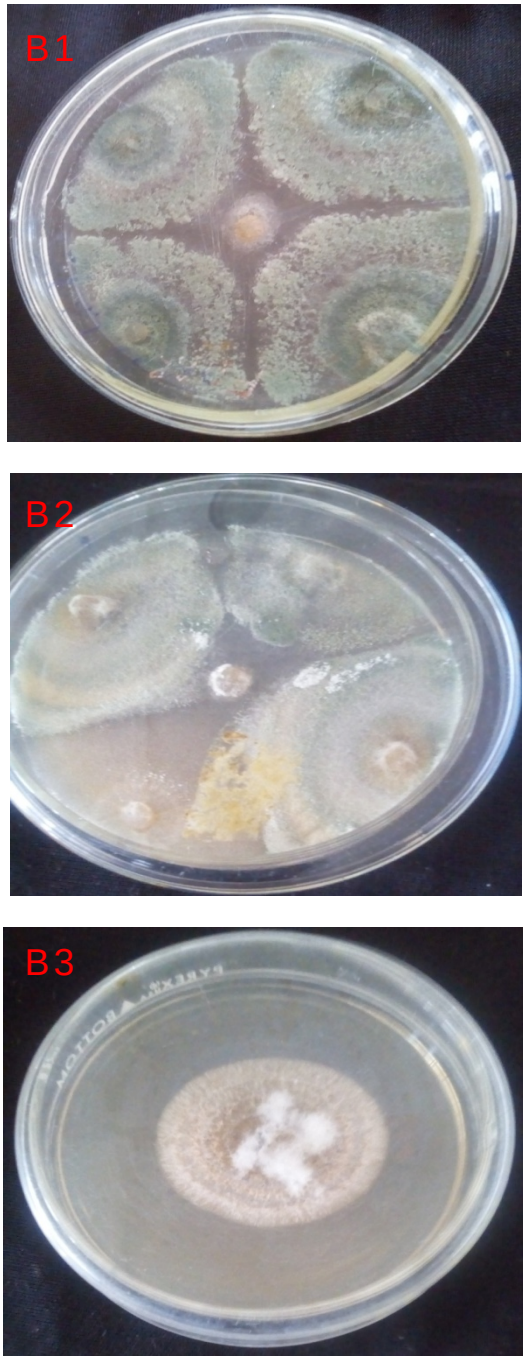


Plate 4.3b. Effect of *Trichoderma* spp. on mycelial growth of *C. gloeosporioides* paired 24 hours before the test pathogen at 5 days after inoculation
Where: B1= *C. gloeosporioides* at the centre paired with *T. harzianum* at the periphery
B2 = *C. gloeosporioides* at the centre paired with *T. pseudokoningii* at the periphery
B3= Control plate of *Colletotrichum gloeosporioides* at the centre.

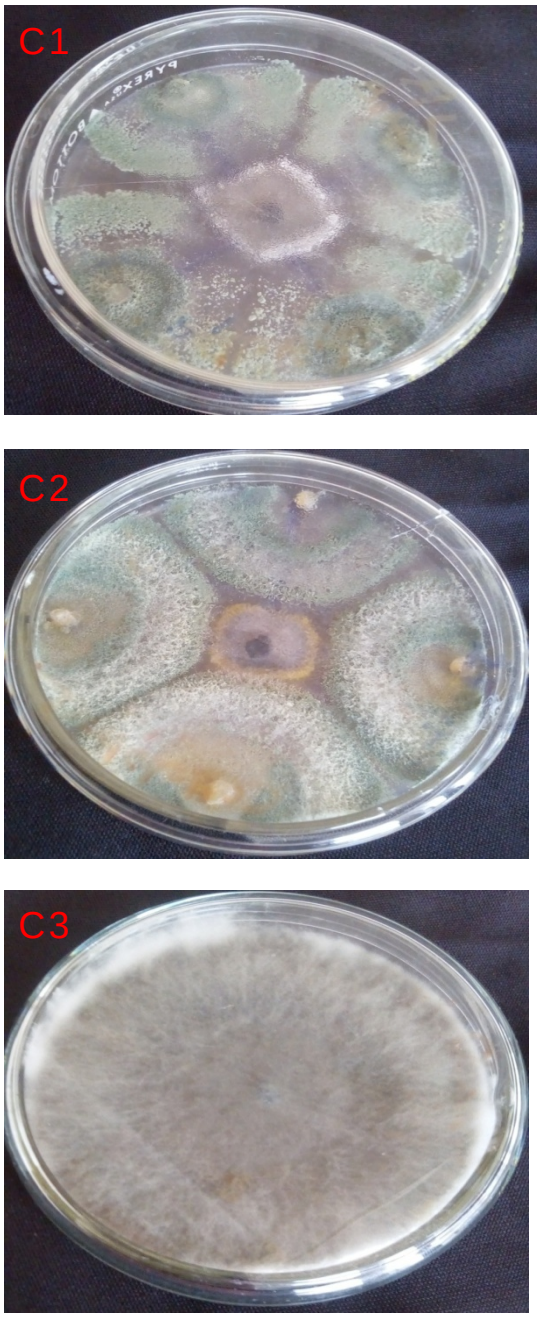


Plate 4.3c. Effect of *Trichoderma* spp. on mycelial growth of *Lasiodiplodia theobromae* paired 24 hours before the test pathogen at 5 days after inoculation

Where: C1= *L. theobromae* at the centre paired with *T. harzianum* at the periphery
C2 = *L. theobromae* at the centre paired with *T. pseudokoningii* at the periphery
C3= Control plate of *Lasiodiplodia theobromae* at the centre.

However, both BCAs restricted growth of *C. gloeosporioides* beyond day 4 with an inhibition zone of 0.8 cm and 1.0 cm maintained by *B. subtilis* and *P. fluorescens* respectively. There was no significant difference ($P < 0.05$) between *B. subtilis* (36.17%) and *P. fluorescens* (36.38%) in their inhibitory effects on the test pathogen (Plate 4.3d).

4.8.5 Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on *Colletotrichum truncatum*

There was no inhibitory effect on the mycelial growth of *C. truncatum* when paired with *B. subtilis* at 3 DAI. The growth inhibition increased to 8.24% on the 4th day (Figure 4.2). A well-defined zone of inhibition measuring 1.5 cm and 1.8 cm was established between the test pathogen and *B. subtilis* and *P. fluorescens* respectively (Plate 4.3e). However, both BCAs restricted growth of *C. gloeosporioides* beyond day 4. There was no difference ($P < 0.05$) between *B. subtilis* (16.21%) and *P. fluorescens* (17.24) in their inhibitory effects on the test pathogen. They inhibited the pathogen from a distance (Figure 4.2).

4.8.6 Effect of *Bacillus subtilis* and *Pseudomonas fluorescens* on *L. theobromae*

Lasiodiplodia theobromae grew appreciably when inoculated 24 hours after *B. subtilis* and *P. fluorescens* (Figure 4.4). It had mycelial growth inhibition of 32.81% and 40.06% when inoculated with *B. subtilis* and *P. fluorescens* at 3 DAI respectively. This inhibitory effect reduced to 26.79% with *Bacillus subtilis* and 29.10% with *P. fluorescens* on the 4th day (Figure 4.4). A clear zone of inhibition measuring 1.20 cm was clearly established by *P. fluorescens* and *B. theobromae.* However, the zone of inhibition (0.7 cm) between *L. theobromae* and *B. subtilis* was not as distinct as that of *P. fluorescens*. In both cases, the inhibitory effects of the bioagents prevented further growth of the test pathogen beyond the zone of inhibition on the fourth day (Plate 4.3f).

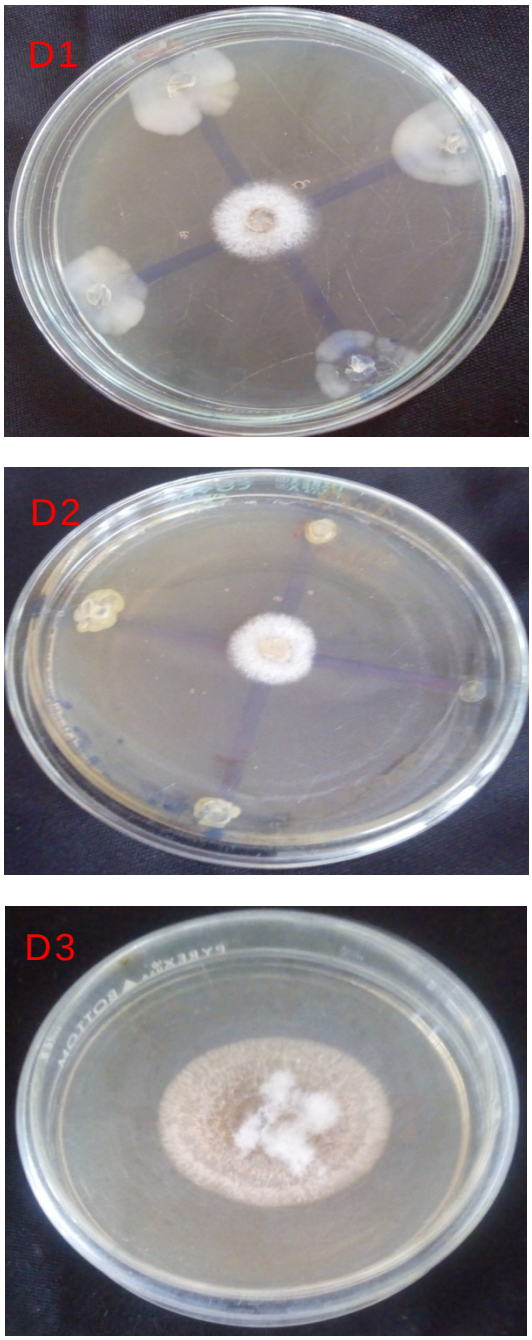


Plate 4.3d. Effect of *B. subtillis* and *P. fluorescens* on mycelial growth of *C. gloeosporioides* paired 24 hours before the test pathogen at 5 days after inoculation

Where: D1= *C. gloeosporioides* at the centre paired with *Bacillus subtilis* at the periphery
D2 = *C. gloeosporioides* at the centre paired with *P. fluorescens* at the periphery
D3= Control plate of *C. gloeosporioides* at the centre

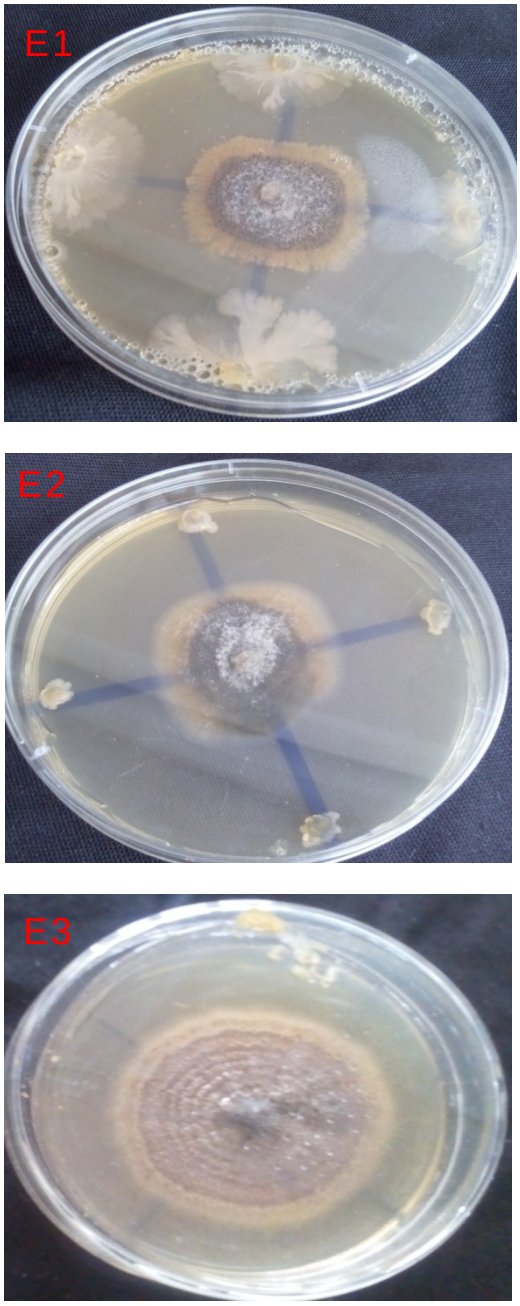


Plate 4.3e. Effect of *B. subtillis* and *P. fluorescens* on mycelial growth of *C. truncatum* paired 24 hours before the test pathogen at 5 days after inoculation

Where: E1= *C. truncatum* at the centre paired with *Bacillus subtilis* at the periphery
E2 = *C. truncatum* at the centre paired with *Pseudomonas fluorescens* at the periphery
E3= Control plate *C. truncatum* at the centre

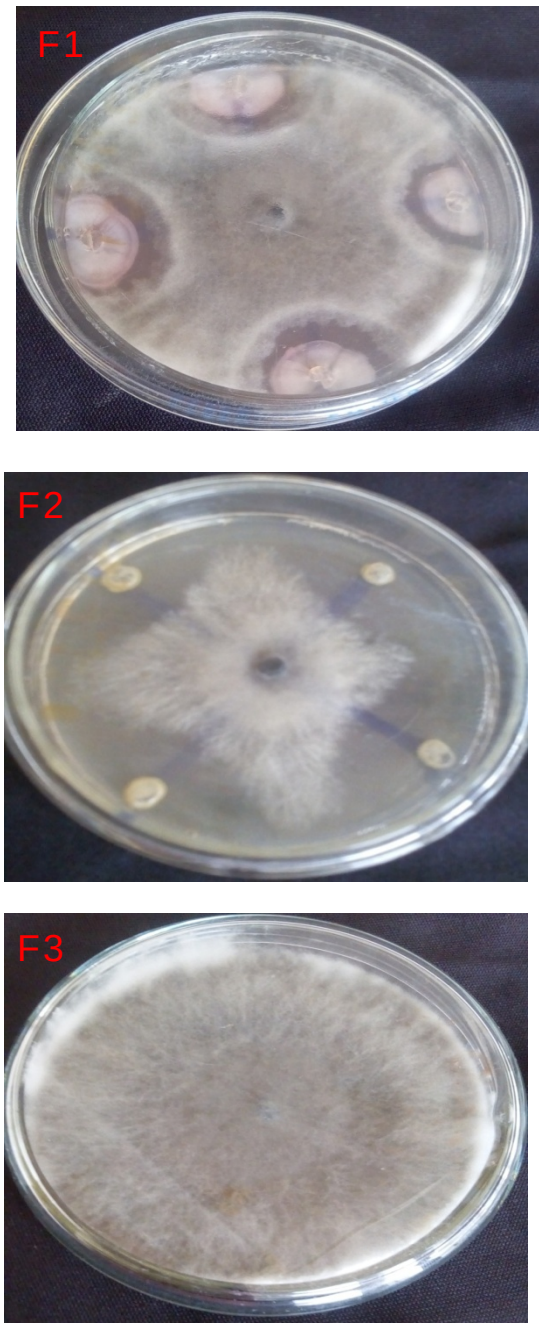


Plate 4.3f. Effect of *B. subtillis* and *P. fluorescens* on mycelial growth of *Lasiodiplodia theobromae* paired 24 hours before the test pathogen at 5 days after inoculation: Where: F1= *L. theobromae* at the centre paired with *Bacillus subtilis* at the periphery F2 = *L. theobromae* at the centre paired with *Pseudomonas fluorescens* at the periphery F3= Control plate *Lasiodiplodia theobromae* at the centre.

4.8.7 *In vivo* evaluation of BCAs as seed treatment for leaf blight control

The effect of the BCAs as seed treatment were tested on the disease incidence and severity, germination percentage and growth of Bara variety of *Egusi* melon which had the highest disease incidence and severity in the previous studies

4.8.8 Effect of BCAs as seed treatment on the disease incidence and severity of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP

There were significant differences (P = 0.05) in the disease incidence and severity of plants inoculated with the three fungal pathogens when treated with the BCAs and Mancozeb compared with the un-inoculated plants at 6 WAP (Table 4.15). *Trichoderma pseudokoningii* effectively prevented incidence of blight on plants treated with *C. truncatum*, while other biocontrol agents also controlled the disease in the same rate as the fungicide used as control. *P. fluorescens* was however not effective only on plant inoculated with *L. theobromae* producing the highest incidence of blight but the severity was mild (Table 4.15). Disease incidence and severity was significantly higher in all the control plants without any biocontrol treatment.

4.8.9 Effect of Biological control agents as seed treatment on the germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogen at 6WAP.

Table 4.16 shows the effect of BCAs as a seed treatment on germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens. The percentage germination of the inoculated seed treated with BCAs and Mancozeb were significantly (P = 0.05) different from the un-treated control plants except for those inoculated with *C. truncatum*. All BCAs protected and enhanced germination of seeds inoculated with *C. gloeosporioides* and *L. theobromae*. Germination of seeds with *C. truncatum* and treated with BCAs was comparable to those of untreated seeds. There was no significant (P = 0.05) difference in the vine length, number of leaf and number of vine of plant inoculated with C. truncatum and treated with BCAs, Mancozeb and the untreated control plants. For plants inoculated with *C. gloeosporioides*, there was no significant difference in the

Table 4.15. Effect of BCAs as seed treatment on the incidence and severity of *Egusi* melon seeds inoculated with leaf blight pathogens at 6 WAP

	C. truncatum		*C. gloeosporioides*		*L. theobromae*	
BCA	Incidence (%)	Severity	Incidence (%)	Severity	Incidence (%)	Severity
Trichoderma harzianum	16.67b	1.17b	33.33b	1.33b	50.00bc	1.67b
Trichoderma pseudokoningii	0.00b	1.00b	16.67b	1.17b	16.67c	1.17b
Bacillus subtillis	50.00b	1.67b	33.33b	1.33b	16.67c	1.17b
Pseudomonas fluorescens	33.33b	1.33b	16.67b	1.17b	83.33ab	1.83b
Mancozeb	16.67b	1.17b	0.00b	1.00b	16.67c	1.17b
Control	100.00a	3.83a	100.00a	3.83a	100.00a	4.00a

WAP= Weeks after planting. Values with similar letter along the column are not significantly different according to DMRT (p = 0.05). Severity scale 1= No symptom; 2 = <10% damaged portion (mildly severe); 3 = 10-30% damaged portion (moderately severe); 4 = 31-50% damaged portion (severe) and 5 = >50% damaged portion (highly severe).

Table 4.16. Effect of BCAs as seed treatment on the germination and growth of *Egusi* melon seeds inoculated with leaf blight fungal pathogens at 6WAP

	Colletotrichum truncatum				*Colletotrichum gloeosporioides*				*Lasiodiplodia theobromae*			
BCA	% G	VL (cm)	NL	NV	% G	VL (cm)	NL	NV	% G	VL (cm)	NL	NV
TH	100.00a	81.65a	45.00a	1.17a	100.00a	80.85ab	50.00a	1.00a	100.00a	89.15a	50.00ab	1.17a
TPK	100.00a	68.35ab	46.65a	1.33a	100.00a	58.35b	48.35a	1.33a	100.00a	81.65a	44.15b	1.17a
B	100.00a	71.65ab	50.85a	1.00a	100.00a	61.65b	51.65a	1.00a	100.00a	68.35a	47.50ab	1.00a
PF	100.00a	68.35ab	50.00a	1.00a	100.00a	64.15b	51.65a	1.17a	100.00a	89.15a	50.00ab	1.00a
Mancozeb	100.00a	87.00a	53.00a	1.33a	100.00a	98.35a	54.15a	1.17a	100.00a	94.15a	48.35ab	1.17a
Untreated	83.33a	54.15a	46.65a	1.00a	77.8b	65.85b	51.65a	1.17a	77.8b	85.00a	54.15a	1.00a

BCAs = Biological control agents, TH= *Trichoderma harzianum*, TPK= *Trichoderma pseudokoningii*, B= *Bacillus subtillis*, PF= *Pseudomonas fluorescens*, WAP= Weeks after planting, % G= % Germination, VL= vine length, NL= no of leaves and NV= no of vines. Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

effect of all the Biocontrol agents, Mancozeb and the untreated control in the number of leaves and number of vine produced by the plants. However, *T. harzianum* gave the highest vine length among the BCAs which was not significantly different from that recorded on the plants treated with Mancozeb. Similar result was observed on plants inoculated with *L. theobromae* except the number of leaves of plants treated with *T. pseudokoningii* which was significantly different from the un-treated control.

4.9 Heat stability of biological control agents (BCAs)

4.9.1 Effect of heat on biotoxin production

The effect of heat on the toxin produced by Biocontrol agents (*B. subtilis, P. fluorescens, Trichoderma harzianum* and *Trichoderma pseudokoningii* culture) on the growth of *C. truncatum* is shown in Fig 4.5. There was no growth of the biocontrol agents in all replicate plates, suggesting that the spores had been killed at high temperature during autoclaving at 121^{o}C for 15 mins. *C. truncatum*, however, grew on inoculation on the biocontrol plates. There were differences in the effects of the BCAs on mycelial growth by all the pathogens (P= 0.05). Radial growth of *C. truncatum* on *B. subtilis* was 0.93cm, *P. fluorescens* (1.80 cm), *Trichoderma harzianum* (1.20 cm), *T. pseudokoningii* (1.07 cm) and 2.16 cm for control plates. C*olletotrichum truncatum* had the least radial growth of 1.63cm on *B. subtilis agar* plate followed by *T. harzianum* (2.63cm). *Pseudomonas fluorescens* had the highest radial growth (2.90cm) among the BCAs.

There was a substantial reduction in the radial growth of *Colletotrichum gloeosporioides* on the BCAs agar plates (Fig 4.6) at 3, 5 and 7 DAI compared with the control. The lowest mycelial growth of the test pathogen was recorded on *Trichoderma pseudokoningii* agar plate at 5 DAI (2.27cm) and 7 DAI (3.70cm) followed by *B. subtilis* with 1.63cm 2.53cm and 3.60 respectively. Culture plates of *B. subtilis, P. fluorescens,*

Trichoderma harzianum and *Trichoderma pseudokoningii* significantly gave radial growth of 8.50cm, 8.50cm, 7.03cm and 8.33cm for *L. theobromae* at 3 DAI respectively while there was no further reduction of the mycelial growth 7 DAI (Fig 4.7). The lowestradial growth was recorded from *T. harzianum* culture plate which was significantly different from other BCAs and control.

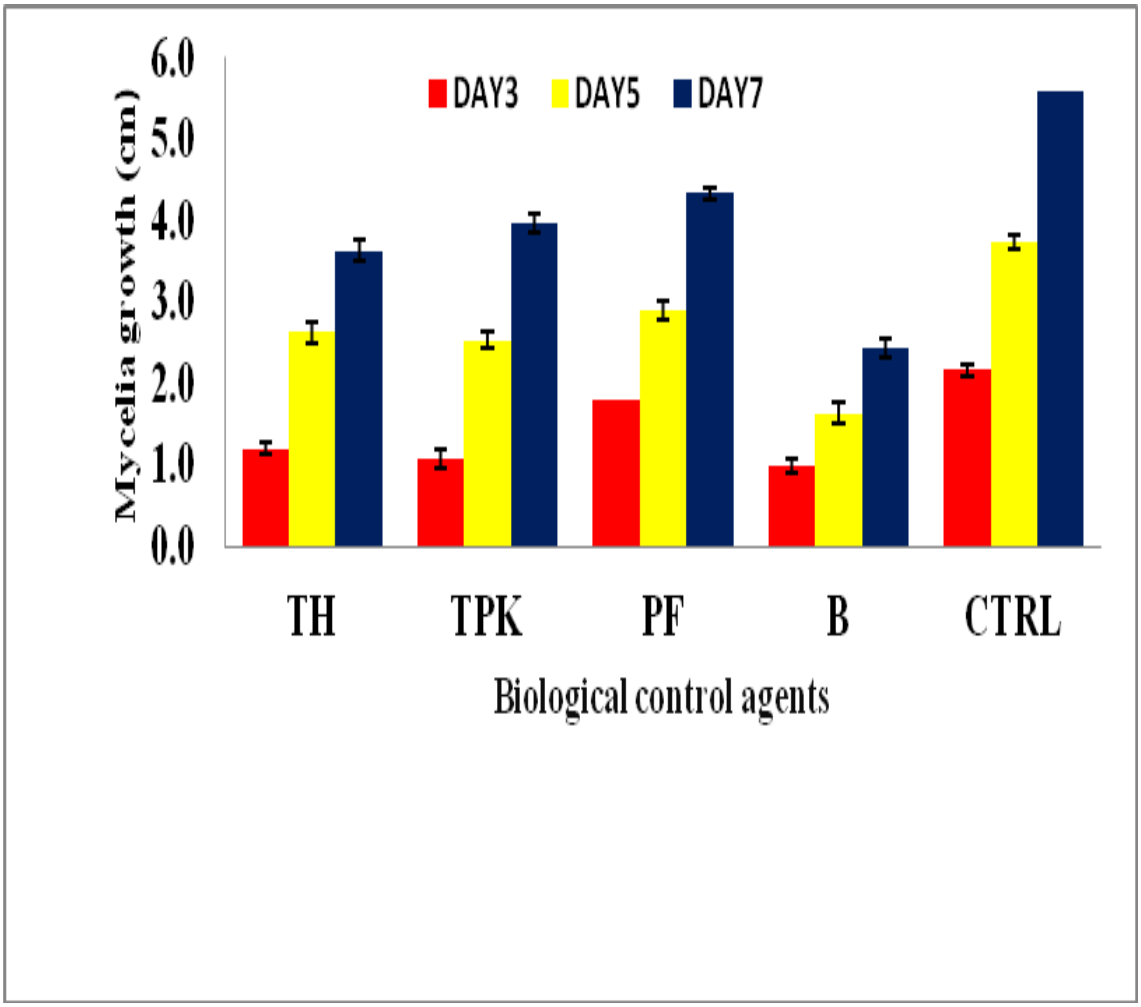


Fig 4.5. Heat stability level of BCA after autoclaving and the inhibitory effect of its biotoxin on the mycelial growth of *C. truncatum* at 3, 5 and 7 DAI

Where: TH= *Trichoderma harzianum*

TPK =*Trichoderma pseudokoningii*

PF = *Pseudomonas fluorescens*

BS = *Bacillus subtillis*

CTRL= Control (PDA)

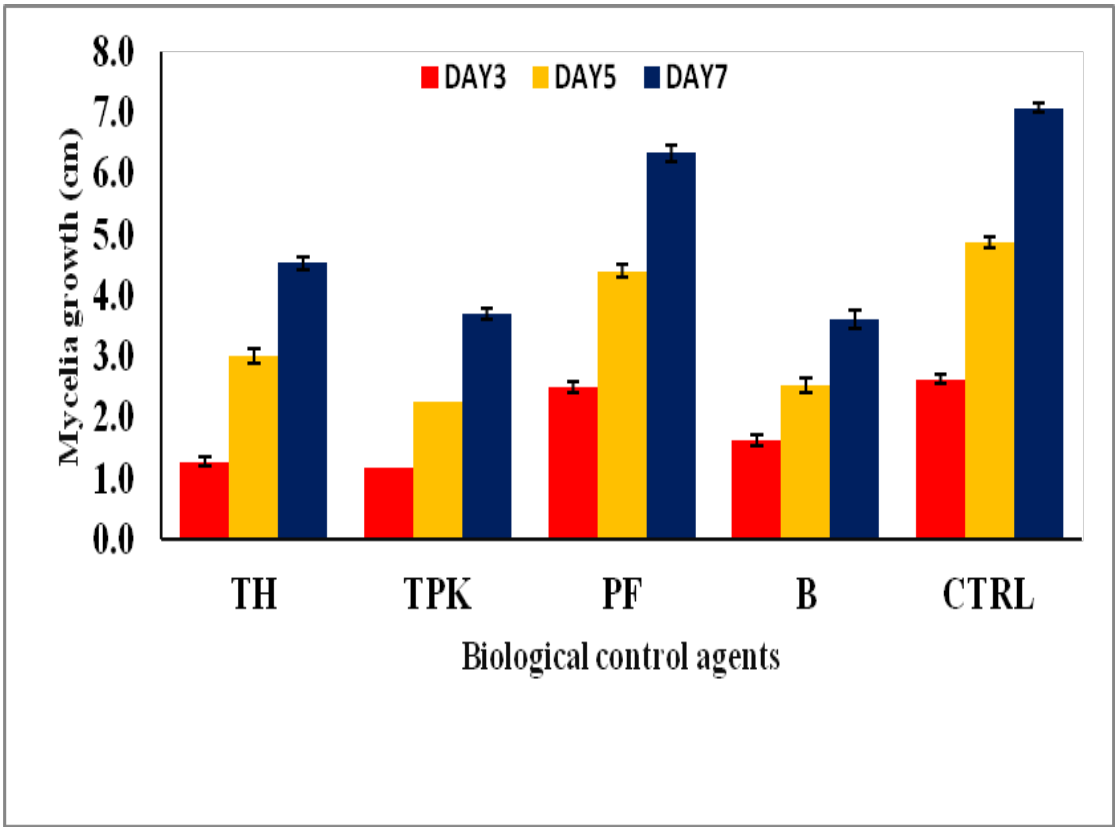


Fig 4.6. Heat stability level of biological control agents (BCAs) after autoclaving and the inhibitory effect of its biotoxin on the mycelial growth of *C. gloeosporioides* at 3, 5 and 7 DAI

Where : TH= *Trichoderma harzianum*

TPK =*Trichoderma pseudokoningii*

PF = *Pseudomonas fluorescens*

BS= *Bacillus subtillis*

CTRL= Control (PDA)

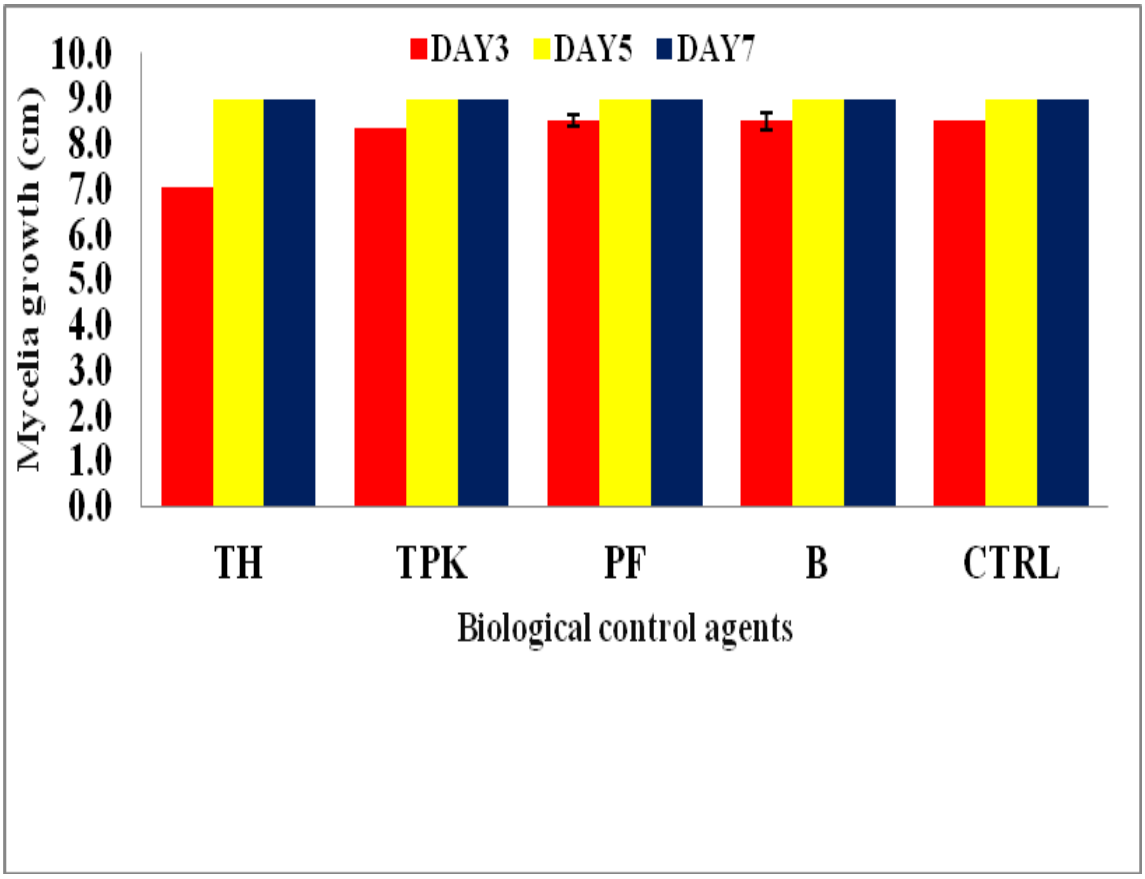


Fig 4.7. Heat stability level of biological control agents (BCAs) after autoclaving and the inhibitory effect of its biotoxin on the mycelial growth of *L. theobromae* at 3, 5 and 7 DAI

Where : TH= *Trichoderma harzianum*

TPK =*Trichoderma pseudokoningii*

PF = *Pseudomonas fluorescens*

BS= *Bacillus subtillis*

CTRL= Control (PDA)

4.10 *In vitro* evaluation of botanical extracts for leaf blight disease control

4.10.1 Effects of different concentrations of *Phyllanthus amarus, Thevetia neriifolia* and *Passiflora foetida* plant extracts on the colony diameter and no of spores of *C. truncatum*

Most of the botanicals produced significant inhibitory effects on the growth of the causal organisms of blight disease of *Egusi* melon (Table 4.17). There were significant (P=0.05) differences in the colony diameter and no of spores of *C. truncatum* on PDA amended with *P. amarus T. neriifolia* and *P. foetida* 5 and 7 days after inoculation (Table 4.17). Extract of *P. amarus* slightly reduced the colony diameter of of *C. truncatum* at all concentrations and effectively reduced the no of spores at higher concentrations of 7.5 - 12.5% w/v. Sporulation of *C. truncatum* was enhanced when treated with *P. amarus* at 5.0% w/v.

Thevetia neriifolia leaf extract was slightly effective in reducing the colony diameter of *C. truncatum* at lower concentrations of 5%, 7.5% and 10% w/v but stimulatory at 12.5% concentration (Table 4.17). The sporulation was highly reduced at 12.5% concentration. The extract of *T. neriifolia* at 7.5% w/v concentration stimulated the no of spores of *C. truncatum* by 25.67% while the other concentrations were slightly effective. Colony diameter of *C. truncatum* on PDA impregnated with *P. foetida* was moderately reduced at 5%, 7.5% and 12.5% w/v concentrations and slightly effective at 10% concentration. The extract moderately reduced the sporulation at 10% and 12.5% w/v concentrations but stimulated the growth at 5% and 7.5% w/v concentrations (Table 4.17).

Table 4.17. Effects of different concentrations of *Phyllantus amarus, Thevetia neriifolia* and *Passiflora foetida* leaf extracts on the colony diameter and sporulation of *Colletotrichum truncatum* 5 and 7 days after inoculation respectively

*Concentration (%) (w/v)	*P. amarus*		*T. neriifolia*		*P. foetida*	
	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)
12.5	5.50 (0.5)SE	79.42(41.5)EE	5.63 (1.6)ST	39.75 (70.8)HE	4.17 (24.6)ME	83.00 (39.0)ME
10.0	5.33 (3.6)SE	87.75(35.52)ME	4.47 (19.2)SE	120.50 (11.5)SE	4.40 (20.4)ME	97.17 (28.6)ME
7.5	5.06 (8.5)SE	104.81(23.0)ME	4.87 (11.9)SE	183.08 (25.7)ST	3.83 (30.7)ME	143.36 (5.1)ST
5.0	5.10 (7.8)SE	145.17(6.3)ST	4.40 (20.4)ME	120.50 (11.5)SE	3.87 (30.0)ME	140.64 (3.2)ST
Control	5.53	136.08	5.53	136.08	5.53	136.08
LSD	0.29	49.83	0.20	49.87	1.87	43.54

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). NE= Not effective; SE= Slightly effective; ME= Moderately effective; EE = Effective; HE= Highly effective; ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P = 0.05).

4.10.2 Effects of different concentrations of *Phyllanthus amarus, Thevetia neriifolia* and *Passiflora foetida* leaf on the colony diameter and no of spores of *Colletotrichum gloeosporioides* at 5 and 7 days after inoculation.

There was no significant (P=0.05) difference in the colony diameter and sporulation of *C. gloeosporioides* on PDA amended with *P. amarus T. neriifolia and P. foetida* leaves at 5 and 7 days after inoculation (Table 4.18). *P. amarus* extract moderately reduced the colony diameter of *C. gloeosporioides* at all concentrations used and effectively reduced the no of spores at 5% and 10% concentrations by 42.51% and 47.33% reduction respectively.

The highest concentration of 12.5% (w/v) produced the lowest percentage reduction of no of spores (Table 4.18). Colony diameter and no of spore of *C. gloeosporioides* on PDA impregnated with *Thevetia neriifolia* leaf extract were significantly lower than control but there was no significant difference among the concentrations used. *Thevetia neriifolia* leaf slightly reduced the colony diameter of *C. gloeosporioides* at all concentrations evaluated and highly effective in reducing no of spores at 5% w/v (72.9%), 7.5% w/v (77.28%) and 12.5% w/v (94.6%).

Passiflora foetida leaf extract moderately reduced the colony diameter of *C. gloeosporioides* at all concentrations (Table 4.18). There were significant differences in the colony diameter of PDA amended with *P. foetida* at all concentrations and the control. The highest reduction of sporulation was obtained at 5% level of concentration by 95.98% which was not significantly different in effectiveness from 96.38% and 95.11% obtained at 7.5% and 10% concentrations respectively.

4.10.3 Effect of *Phyllanthus amarus, Thevetia neriifolia* and *Passiflora foetida* leaves on the colony diameter of *Lasiodiplodia theobromae* at 2 and 5 days after inoculation.

The effect of different concentrations of *P. amarus, T. neriifolia* and *P. foetida* leaves on colony diameter of *L. theobromae* at 2 days after inoculation is shown in Table 4.19. *Phyllantus amarus* leaf moderately reduced the colony diameter of

Table 4.18. Effects of varied concentrations of *Phyllanthus amarus, Thevetia neriifolia* and *Passiflora foetida* leaf extracts on the colony diameter and sporulation of *Colletotrichum gloeosporioides* 5 and 7 days after inoculation respectively.

*Concentration	*P. amarus*		*T. neriifolia*		*P. foetida*	
(%) w/v	CD (Cm)	NS ($x10^4$)	CD (Cm)	NS ($x10^4$)	CD (Cm)	NS ($x10^4$)
12.5	5.13 (25.7)ME	95.58 (9.6)SE	7.00 (6.7)SE	5.58 (94.6)HE	5.37 (28.4)ME	46.83 (55.7)EE
10.0	5.23 (24.2)ME	55.33 (47.6)EE	6.07 (19.1)SE	42.25 (59.9)EE	5.53 (26.3)ME	5.17 (95.1)HE
7.5	5.23 (24.2)ME	75.41 (28.6)ME	6.00 (20.0)SE	23.92 (77.3)HE	5.00 (33.3)ME	3.83 (96.4)HE
5.0	4.90 (29.0)ME	60.75 (42.5)EE	6.17 (17.7)SE	28.50 (72.9)HE	5.27 (29.7)ME	4.25 (95.9)HE
Control	6.90	105.67	7.50	105.67	7.50	105.67
LSD	0.40	83.91	1.30	30.99	2.04	28.07

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). SE= Slightly effective; ME= Moderately effective; EE = Effective; HE= Highly effective; ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P=0.05).

Table 4.19. Effect of *Phyllanthus amarus, Thevetia neriifolia* and *Passiflora foetida* leaf on the colony diameter of *Lasiodiplodia theobromae*

Concentrations (%)	*P. amarus*		*T. neriifolia*		*P. foetida*	
w/v	2DAI	5DAI	2DAI	5DAI	2DAI	5DAI
12.5	5.90 (34.44)ME	9.00 (0)NE	7.13 (20.78)ME	9.00 (0)NE	5.87 (34.78)ME	9.00 (0)NE
10.0	6.60 (26.67)ME	9.00 (0)NE	6.07 (24.78)ME	9.00 (0)NE	7.03 (21.89)ME	9.00 (0)NE
7.5	6.07 (32.56)ME	9.00 (0)NE	6.77 (24.78)ME	9.00 (0)NE	5.33 (40.78)EE	9.00 (0)NE
5.0	6.07 (32.56)ME	9.00 (0)NE	5.93 (34.11)ME	9.00 (0)NE	5.87 (34.78)ME	9.00 (0)NE
Control	9.00	9.00	9.00	9.00	9.00	9.00
LSD	0.50	0.00	0.53	0.00	3.40	0.00

DAI= Days after inoculation; NE= Not effective; ME= Moderately effective; EE = Effective. Values in parenthesis represent % reduction or stimulation of colony diameter of the test pathogen. Means in the same column are separated with LSD (P=0.05).

L. theobromae at all concentrations used (2DAI), but not effective at 5DAI. The highest colony diameter of 6.60cm was obtained on PDA treated with *P. amarus* at 10% w/v concentration. *Thevetia neriifolia* leaf extract moderately reduced the colony diameter of *C. gloeosporioides* at all concentrations, 2DAI but not effective at 5DAI (Table 4.19). The colony diameter of *L. theobromae* across all concentration showed significant differences among 5.93cm, 6.77cm 6.07cm and 7.13cm at 5%, 7.5%, 10% and 12.5% on PDA treated with *T. neriifolia* leaf extract respectively.

There was no difference in colony diameter of 5.87, 5.33, 7.03 and 5.87 obtained on PDA treated with *P. foetida* at 5%, 7.5%, 10% and 12.5% concentrations respectively. *P. foetida* was however effective at 7.5% concentration with 40.78 % reduction and moderately effective with 34.78%, 21.89% and 34.78% reduction at 5%, 10% and 12.5% respectively (Table 4.19).

4.10.4 Effect of different concentrations of root bark, stem bark and leaf extract of *Azadirachta indica* on the colony diameter (cm) and sporulation of *Colletotrichum truncatum* 5 and 7 days after inoculation respectively.

The effect of different concentrations of root bark, stem bark and leaf extract of *A. indica* on the colony diameter and no of spores of *C. truncatum* are presented in Table 4.20. Root bark extract of *A. indica* produced the lowest colony diameter of 4.67cm at 10% concentration which was not significantly different from 4.47cm obtained at 5% concentration. The extract slightly reduced the colony diameter of *C. truncatum* at all concentrations tested. Also, there was no (P=0.05) difference in the reduction of no of spores at all concentrations used. The botanical was highly effective (74.04%) in reducing the no of spores produced by *C. truncatum* at 7.5% concentration while 57.20%, 49.52% and 43.53% reduction obtained at 5%, 10% and 12.5% level of concentrations respectively were also rated effective (Table 4.20).

Table 4.20. Effects of varied concentrations of root bark, stem bark and leaf extracts of *Azadirachta indica* on the colony diameter (cm) and sporulation of *Colletotrichum truncatum* 5 and 7 days after inoculation respectively.

*Concentration	*A. indica* root		*A. indica* stem		*A. indica* leaf	
(%) w/v	CD (cm)	NS (x104)	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)
12.5	5.07 (8.3)SE	33.75 (43.5)EE	4.73 (14.5)SE	36.67 (38.6)ME	5.40 (2.4)SE	23.75 (60.3)HE
10.0	4.67 (15.6)SE	30.17 (49.5)EE	4.50 (18.6)SE	38.00 (36.4)ME	5.43 (1.8)SE	12.08 (79.8)HE
7.5	4.83 (12.7)SE	15.5(74.1)HE	4.43 (19.9)SE	29.08 (51.4)EE	5.77 (4.2)ST	22.50 (62.4)HE
5.0	4.47 (19.2)SE	25.58 (57.2)EE	4.37 (20.9)ME	23.50 (60.7)HE	5.50 (0.5)NE	26.5 (55.7)EE
Control	5.53	59.77	5.53	59.77	5.53	59.77
LSD	0.24	25.14	0.18	23.65	0.21	23.65

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). NE= Not effective; SE= Slightly effective; ME= Moderately effective; EE = Effective; HE= Highly effective; ST= Stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P=0.05).

The stem extract of *A. indica* reduced the colony diameter of *C. truncatum* when compared with control. *C. truncatum* had the lowest colony diameter (4.37cm) at the lowest extract concentration (5%) which was not significantly (P=0.05) different from 4.43cm and 4.50cm obtained at 7.5% and 10% w/v concentration respectively. The extract slightly reduced the colony diameter at 12.5%, 10% and 7.5% w/v concentrations while at 5% concentration, colony diameters were moderately reduced by 20.98%. The percentage sporulation reduction of *C. truncatum* on PDA amended with *A. indica* stem increases from highest (12.5%) level of extract concentration to the lowest (5%). The no of spores was moderately reduced at 12.5% and 10% w/v concentration while it was highly reduced at 5%.The colony diameter of *C. truncatum* on the leaf extract of *A. indica* ranged from 5.43cm -5.53cm. The extract was not effective against the mycelial growth at 5% concentration (0.54%) and even stimulated the growth with 4.16% at 7.5% concentration. It was also highly effective in reducing the no of spores of *C. truncatum* at all concentrations used except at 5% which was effective (55.66%) (Table 4.20).

4.10.5 Effect of different concentrations of root bark, stem bark and leaf extract of *Azadirachta indica* on the colony diameter and no of spores of *Colletotrichum gloeosporioides* 5 and 7days after inoculation respectively.

The effect of different concentrations of root bark, stem bark and leaf extract of *Azadirachta indica* on the colony diameter and no of spores of *C. gloeosporioides* 5 and 7 days after inoculation is presented in Table 4.21. Colony diameter of *C. gloeosporioides* on PDA amended with root bark extract of *A. indica* ranged from 5.33cm - 7.50cm while the percentage reduction also ranged from 18.27% - 28.12%. There was no significant (P=0.05) differences in the colony diameters obtained at 5%, 7.5%, 10% and 12.5% w/v concentrations tested. It slightly reduced the growth at higher concentrations while moderate reduction (28.93%) of the colony diameter was observed at 5% concentration. Although, the extract reduced the colony diameter at all concentrations, it was only able to reduce the no of spores at 12.5% concentration while the sporulation was stimulated at 10%, 7.5% and 5% w/v concentrations (Table 4.21).

Table 4.21. Effect of different concentrations of root bark, stem bark and leaf extractsof *Azadirachta indica* on the colony diameter and sporulation of *Colletotrichum gloeosporioides* 5 and 7 days after inoculation respectively.

Concentrations (%) w/v	*A indica* Root		*A.indica* Stem		*A.indica* leaf	
	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)
12.5	6.13 (18.27)SE	3.42 (55.41)EE	6.17 (17.73)SE	1.16 (84.88)HE	6.70 (10.67)SE	1.67 (78.23)HE
10.0	6.03 (19.60)SE	10.00 (23.30)ST	5.47 (27.07)ME	1.25 (83.70)HE	6.97 (7.07)SE	1.67 (78.23)HE
7.5	6.07 (19.07)SE	13.92 (44.90)ST	5.50 (26.67)ME	4.08 (46.81)HE	6.93 (7.60)SE	4.77 (37.81)ME
5.0	5.33 (28.93)ME	10.67 (28.12)ST	5.57 (27.73)ME	2.17 (71.71)HE	7.03 (6.27)SE	3.00 (60.89)HE
Control	7.50	7.67	7.50	7.67	7.50	7.67
LSD	1.28	5.31	1.28	3.38	1.27	6.34

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). NE= Not effective; SE= Slightly effective; ME= Moderately effective; EE = Effective; HE= Highly effective; ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P= 0.05).

There was no significant difference in the colony diameter (cm) and no of spores of *C. gloeosporioides* on *A. indica* stem extract (Table 4.21). This extract slightly reduced the colony diameter by 17.73% at 12% concentration and moderately reduced (27.07%, 26.67% and 27.73%) it at 10%, 7.5% and 5% levels of concentration. The effects of the extract on the no of spore were highly effective at all concentrations.

There was no difference (P=0.05) in the colony diameter and no of spores of the test pathogens treated with *A. indica* leaf at all extract concentrations used Table 4.21). The colony diameters were slightly reduced, while the no of spores were highly effectively reduced at all concentrations.

4.10.6 Effect of different concentrations of root bark, stem bark and leaf extract of *Azadirachta indica* on the colony diameter of *Lasiodiplodia theobromae* at 2 and 5 days after inoculation.

The effect of root bark, stem bark and leaf extract of *Azadirachta indica* on the colony diameter of *L. theobromae* is shown in Table 4.22. There were significant (P=0.05) differences in the colony diameter of *L. theobromae* treated with root bark and stem extracts of *A. indica* at varying concentrations. Colony diameter of the test pathogen ranged from 5.50cm - 9.00cm. The extract was not effective at 12.5% concentration on the percentage colony reduction, slightly effective (18.56%, 3.33%) at 10% and 7.5% and moderately reduced the colony diameter (38.89%) at the lowest (5%) concentration. Its stem extract was both effective and significantly reduced the colony diameter of the pathogen at 7.5% concentration and only moderately effective at other levels of concentrations. However, for *A. indica* leaf extract there was no significant (P=0.05) difference in the colony diameter of *L. theobromae* produced at all extract concentrations and the control. The extract was not effective at 5% and 10% but slightly effective (1.89% and 1.89%) at 7.5% and 12.5% concentration (Table 4.22).

Table 4.22. Effect of root bark, stem bark and leaf extracts of *Azadirachta indica* on the colony diameter of *Lasiodiplodia theobromae* two and five days after inoculation

Concentrations (%)	*A. indica* Root		*A. indica* Stem		*A. indica* leaves	
w/v	2DAI (cm)	5DAI (cm)	2DAI (cm)	5DAI (cm)	2DAI (cm)	5DAI (cm)
12.5	9.00 (0.00)NE	9.00 (0)NE	6.23 (30.78)ME	9.00 (0)NE	8.83 (1.89)SE	9.00 (0)NE
10.0	7.33 (18.56)SE	9.00 (0)NE	7.13 (20.78)ME	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE
7.5	8.70 (3.33)SE	9.00 (0)NE	5.17 (42.56)EE	9.00 (0)NE	8.83 (1.89)SE	9.00 (0)NE
5.0	5.50 (38.89)ME	9.00 (0)NE	7.13 (20.78)ME	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE
Control	9.00	9.00	9.00	9.00	9.00	9.00
LSD	0.42	0.00	0.47	0.00	0.30	0.00

DAI= Days after inoculation; NE= Not effective; SE= Slightly effective; ME= Moderately effective; EE = Effective. Values in parenthesis represent % reduction or stimulation of colony diameter of the test pathogen. Means in the same column are separated with LSD (P=0.05).

4.10.7 Effect of different concentrations of rhizome, stem and leaf extract of *Costus afer* on the colony diameter and sporulation of *Colletotrichum truncatum* 5 and 7 days after inoculation respectively.

Effect of different concentrations of rhizome, stem and leaf extract of *Costus afer* on the colony diameter and no of spores of *C. truncatum* inoculation is shown in Table 4.23. For rhizome extract, the colony diameters ranged from 3.07cm – 5.53 cm while, the percentage reduction ranged from 23.51% - 44.48%. It was effective against the test pathogen at 5% and 10% and moderately effective at 7.5% and 12.5% concentrations. However, this extract highly reduced the no of spores with 90.93%, 70.87%, 65.02% and 84.94% at 5%, 7.5%, 10% and 12.5% w/v concentrations, respectively. The highest reduction in no of spores was observed at 5% w/v (Table 4.23).

Costus afer stem extract at higher concentration (12.5% and 10%) had the highest colony diameters of 4.00 cm and 3.67cm which were slightly effective and not different from each other (Table 4.23). However, at 5% and 7.5%, the extract effectively reduced the colony diameter each by 43.40%. The stem extract stimulated production of spores of the test pathogen at all concentration used.

The lowest colony diameter (2.77cm) of *C. truncatum* leaf extract was observed at 5% concentration which was significantly (P=0.05) different from colony diameter at 10% (3.57cm) and 12.5% (3.60cm). It effectively reduced the colony diameter (40.17%) at 5% concentration, while it was moderately reduced at 7.5%, 10% and 12.5% w/v concentrations (Table 4.23).

4.10.8 Effect of rhizome, stem and leaf extract of *Costus afer* on the colony diameter and no of spores of *Colletotrichum gloeosporioides* 5 and 7 days after inoculation respectively.

There was no significant (P=0.05) differences in the colony diameter and sporulation of *C. gloeosporioides* on PDA amended with *Costus afer* rhizome (Table 4.24). *C. afer* rhizome extract moderately reduced the colony diameter of *C. gloeosporioides* at 5% and 10% concentrations with 26.67% and 27.60% reduction respectively. There was a slight reduction in the colony diameter at 7.5% and 12.5% concentrations, while no of spores were stimulated at all concentrations. The stem extract

Table 4.23. Effect of different concentrations of rhizome, stem and leaf extracts of *Costus afer* on the colony diameter and no of spores of *Colletotrichum truncatum* 5 and 7 days after inoculation respectively,

Concentration	*C.afer* rhizome		*C.afer* stem		*C. afer* leaf	
(%) w/v	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)
12.5	4.23 (23.5)ME	9.00 (84.9)HE	4.00 (13.6)SE	143.83 (24.3)ST	3.60 (22.3)ME	220.67 (50.7)ST
10.0	3.07 (44.5)EE	20.91 (65.0)HE	3.67 (20.7)SE	166.75 (34.7)ST	3.57 (22.9)ME	216.50 (49.7)ST
7.5	3.83 (30.7)ME	17.41 (70.8)HE	2.63 (43.4)EE	175.39 (37.9)ST	3.00 (35.2)ME	172.58 (36.9)ST
5.0	3.07 (44.5)EE	5.42 (90.9)HE	2.63 (43.4)EE	125.17 (13.1)ST	2.77 (40.2)EE	147.08 (26.0)ST
Control	5.53	59.77	4.63	108.83	4.63	108.83
LSD	0.14	20.48	0.59	61.88	0.60	62.72

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). SE= Slightly effective; ME= Moderately effective; EE = Effective; HE= Highly effective ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (<0.001

Table 4.24. Effect of varied concentrations of rhizome, stem and leaf extracts of *Costus afer* on the colony diameter and sporulation of *Colletotrichum gloeosporioides* 5 and 7 days after inoculation respectively

Concentration	*C. afer* rhizome		*C. afer* stem		*C. afer* leaves	
(%) w/v	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)
12.5	6.43 (14.27)SE	11.67 (34.3)ST	5.83 (6.42)SE	9.83 (61.04)ST	5.30 (29.33)ME	17.53 (78.15)ST
10.0	5.43 (27.60)ME	34.58 (77.8)ST	5.53 (11.24)SE	11.17 (65.71)ST	5.11 (31.87)ME	69.00 (94.45)ST
7.5	6.23 (16.93)SE	21.25 (63.9)ST	5.17 (17.01)SE	8.50 (54.94)ST	5.45 (27.33)ME	22.67 (83.11)ST
5.0	5.50 (26.67)ME	17.00 (54.9)ST	5.33 (14.45)SE	5.97 (35.85)ST	5.50 (26.67)ME	75.25 (94.91)ST
Control	7.50	7.67	6.23	3.83	7.50	3.83
LSD	1.28	21.68	0.17	8.63	0.13	50.57

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). NE= Not effective; SE= Slightly effective; ME= Moderately effective; ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P=0.05)

slightly reduced the colony diameter and stimulated the no of spores of the test pathogen at all concentrations. Also, *C. afer* leaf moderately reduced the colony diameter while no of spores produced were stimulated at all concentrations used (Table 4.24). The least colony diameter was observed at 10% (5.43cm) concentration which was significantly (P=0.05) different from that recorded in other concentrations.

4.10.9 Effect of rhizome, stem and leaf extract of *Costus afer* on the colony diameter of *Lasiodiplodia theobromae*

Effect of rhizome, stem and leaf extract of *Costus afer* on the colony diameter of *L. theobromae* is shown in Table 4.25. Rhizome extract effectively reduced the colony diameter of *L. theobromae* at all concentrations. The least colony diameter (4.13 cm) observed at 10% level of concentration was not significantly (P=0.05) different from 4.57 cm and 4.70 cm obtained at 7.5 % and 12.5 % concentrations. There was no significant (P=0.05) difference in the colony diameter of PDA treated with *C. afer* stem and leaf at all concentrations used. *Costus afer* stem and root extracts were not effective in reducing the colony diameter of *L. theobromae* (Table 4.25).

4.10.10 Effects of different concentration of root, stem and leaf extract of *Morinda lucida* on the colony diameter and no of spores of *Colletotrichum truncatum* 5 and 7 days after inoculation.

Effects of root, stem and leaf extract of *Morinda lucida* on the colony diameter and no of spores of *C. truncatum* is presented in Table 4.26. Colony diameter of *C. truncatum* on PDA treated with *M. lucida* root ranged from 3.23 cm- 4.50 cm and significantly (P=0.05) increases with increase in concentration.

The extract moderately reduced the colony diameter of the test pathgen at 7.5% and 5% w/v concentrations but was slightly effective (16.41% and 2.81%) at 10% and 12.5% concentrations. The least colony diameter was observed at 5% concentration while no of spores was stimulated at all concentrations. *Morinda lucida* stem extract moderately reduced the colony diameter and has stimulatory effect on spore production of the test pathogen (Table 4.26). The leaf extract was slightly effective

Table 4.25. Effect of rhizome, stem and leaf extracts of *Costus afer* on the colony diameter of *Lasiodiplodia theobromae* two and five days after inoculation

Concentration (%)	Roots		Stems		Leaves	
w/v	2DAI (cm)	5DAI (cm)	2DAI (cm)	5DAI (cm)	2DAI (cm)	5DAI (cm)
12.5	4.70 (47.8)EE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE
10.0	4.13 (54.1)EE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE
7.5	4.57 (49.2)EE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE
5.0	5.30 (41.1)EE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE	9.00 (0)NE
Control	9.00	9.00	9.00	9.00	9.00	9.00
LSD	0.60	0.00	0.00	0.00	0.00	0.00

DAI= Days after inoculation; NE= Not effective; EE = Effective. Values in parenthesis represent % reduction or stimulation of colony diameter of the test pathogen. Means in the same column are separated with LSD (P=0.05).

Table 4.26. Effects of different concentrations of root bark, stem bark and leaf extracts of *Morinda lucida* on the colony diameter and sporulation of *Colletotrichum truncatum* five and seven days after inoculation, respectively

Concentration	*M. lucida* root		*M. lucida* stem		*M. lucida* leaf	
(%) w/v	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)	CD (cm)	NS ($x10^4$)
12.5	4.50 (2.8)SE	178.00 (38.9)ST	3.53 (23.8)ME	118.33 (8.0)ST	4.43 (4.3)SE	113.08 (3.8)ST
10.0	3.87 (16.4)SE	139.00 (21.7)ST	3.23 (30.2)ME	144.08 (24.5)ST	4.33 (6.5)SE	94.00 (13.6)SE
7.5	3.53 (23.8)ME	158.50 (31.3)ST	3.33 (28.1)ME	159.33 (31.7)ST	3.73 (19.4)SE	149.17 (27.0)ST
5.0	3.23 (30.2)ME	179.91 (39.5)ST	2.90 (37.4)ME	152.00 (28.2)ST	3.43 (25.9)ME	171.75 (36.6)ST
Control	4.63	108.83	4.63	108.83	4.63	108.83
LSD	0.15	87.96	0.95	57.37	0.28	40.74

*CD = Colony diameter (cm) and NS = no of spores ($x10^4$). NE= Not effective; SE= Slightly effective; ME= Moderately effective; ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P=0.05

at 12.5%, 10% and 7.5% w/v concentrations and moderate at 5% w/v. Number of spore was only slightly reduced at 10%w/v and stimulated at other concentrations (Table 4.26).

4.10.11 Effects of root, stem and leaf extract of *Morinda lucida* on the colony diameters and no of spores of *Colletotrichum gloeosporioides* 5 and 7 days after inoculation respectively.

There was no significant difference in the colony diameter of *C. gloeosporioides* on PDA treated with *M. lucida* root (6.33cm and 6.20cm) at 12.5% and 10% w/vconcentrations compared

with the control (6.23cm) (Table 4.27). The extract slightly reduced the colony diameter of the test pathogen at 7.5% and 5% concentrations with 2.57% and 1.61% respectively and highly reduced the spore production of the test pathogen at all concentrations except at 12.5% w/v concentration. Effect of *M. lucida* stem was slightly effective (14.45%, 7.38%, 11.72% and 8.51%) on colony diameter and highly effective (67.38%, 78.33%, 78.33% and 69.45%) on the spore reduction of *C. gloeosporioides* at 5%, 7.5%, 10% and 12.5% respectively. There were significant differences in the concentration of *M. lucida* leaf extract used. The extract slightly reduced the colony diameter and stimulates spore formation of the test pathogen at all concentrations (Table 4.27).

4.10.12 Effect of root, stem and leaf extract of *Morinda lucida* on the colony diameter of *Lasiodiplodia theobromae*.

There was no significant difference in the colony diameter of *L. theobromae* on PDA treated with *M. lucida* root, stem and leaf at all concentrations used. The extract was not effective in controlling the growth and sporulation of *L. thebromae* (Table 4.28).

4.11 *In vivo* evaluation of botanical extracts as seed treatment of leaf blight disease

4.11.1 Effect of *Costus afer* rhizomes extract as seed treatment on disease incidence and severity of *Egusi* melon seed inoculated with leaf blight fungal pathogen.

The effect of *Costus afer* rhizomes extract as seed treatment on the disease incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum truncatum* are shown in Table 4.29

Table 4.27. Effects of varied concentrations of root bark, stem bark and leaf extracts of *Morinda lucida* on the colony diameter (cm) and sporulation (x10^4) of *Colletotrichum gloeosporioides* five and seven days after inoculation respectively

Concentration (%)	*M. lucida* Root		*M. lucida* stem		*M. lucida* leaf	
w/v	CD (cm)	NS (x10^4)	CD (cm)	NS (x10^4)	CD (cm)	NS (x10^4)
12.5	6.33 (1.6)ST	1.25 (67.4)HE	5.70 (8.5)SE	1.17 (69.5)HE	5.70 (8.5)SE	4.50 (14.9)ST
10.0	6.20 (0.5)NE	1.58 (58.8)EE	5.50 (11.7)SE	0.83 (78.3)HE	5.80 (6.9)SE	11.00 (65.2)ST
7.5	6.07 (2.6)SE	2.25 (41.3)EE	5.77 (7.4)SE	0.83 (78.3)HE	5.50 (11.7)SE	4.83 (20.7)ST
5.0	6.13 (1.6)SE	1.25 (67.4)HE	5.33 (14.5)SE	1.25 (67.4)HE	5.73 (8.0)SE	11.33 (66.2)ST
Control	6.23	3.83	6.23	3.83	6.23	3.83
LSD	0.13	2.23	0.18	2.36	0.23	12.81

*CD = Colony diameter (cm) and NS = no of spores (x10^4). NE= Not effective; SE= Slightly effective; EE= Effective; HE = Highly effective; ST= stimulatory. Values in parenthesis represent % reduction or stimulation of colony diameter and sporulation of the test pathogen. Means in the same column are separated with LSD (P=0.05).

Table 4.28. Effect of root bark, stem bark and leaf extracts of *Morinda lucida* on the colony diameter (cm) of *Lasiodiplodia theobromae* 2 and 5 days after inoculation

Concentrations (%)	*M. lucida* root		*M. lucida* stem		*M. lucida* leaf	
w/v	2DAI	5DAI	2DAI	5DAI	2DAI	5DAI
12.5	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE
10.0	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE
7.5	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE
5.0	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE	9.00 (0.00)NE	9.00 (0)NE
Control	9.00	9.00	9.00	9.00	9.00	9.00
LSD	0.00	0.00	0.00	0.00	0.00	0.00

DAI= Days after inoculation; NE= Not effective; EE = Effective. Values in parenthesis represent % reduction or stimulation of colony diameter of the test pathogen. Means in the same column are separated with LSD (P=0.05).

Table 4.29. Effect of *Costus afer* rhizome extract as seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum truncatum*

	Incidence (%)			Severity		
Treatments	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
CAR 5%	16.65b	16.67a	66.67abc	1.17b	1.17a	1.67b
CAR 10%	16.65b	33.33a	83.33ab	1.17b	1.33a	2.00b
CAR 50%	0.00b	33.33a	33.33bc	1.00b	1.33a	1.50b
Mancozeb	0.00b	0.00a	16.67c	1.00b	1.00a	1.17b
Control	66.67a	50.00a	100.00a	1.67a	1.17a	3.83a

WAP: Weeks after planting, CAR: *Costus afer* rhizome. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

At all Concentrations, extract of *Costus afer* rhizome protected seedlings from leaf blight caused by seed-infected *C. truncatum.* The fungitoxic potentials of the extract was comparable to the effect of the synthetic fungicide, mancozeb. The concentrations of the extract were not significantly different from each other in respect of their potential to reduce incidence of *C. truncatum* induced leaf blight. Incidence of leaf bight increased progressively from 4 to 6 WAP. Range of incidence among extract treated plants was 0.0-83.3% while range in control (unprotected) seedling is 50.0-100.0% (Table 4.29).

Effects of *C. afer* rhizomes extract as a seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *C. gloeosporioides* are shown in Table 4.30. The disease incidence began from 4WAP and increases across the week. There was no significant difference in the severity of the disease on plant treated with *C. afer* rhizomes at 5% concentration and the untreated control plant. *C. afer* rhizome protected seedling from leaf blight caused by seed-infected *C. gloeosporioides* at all concentrations. Incidence of leaf blight progressed from week 4 to 6WAp. Range of incidence among extract treated plants was 0.0-50%, while range in unprotected control seedlings is 66.67-100.0%. The fungitoxicity potential of the extract was comparable to the effect of the synthetic fungicide, mancozeb. There were no significant differences in the potential of the extract to reduce leaf blight incidence caused by *C. gloeosporioides* at different concentrations (Table 4.30).

Table 4.31 shows the effects of *C. afer* rhizomes extract as a seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *L. theobromae*. There were significant (P = 0.05) differences in the effect of *C. afer* rhizomes extract as seed treatment between plants inoculated with *L. theobromae* through seed and the untreated control plants across the weeks. The leaf blight incidence and severity increased across the weeks. There was no incidence of leaf blight disease on the plant treated with *C. afer* rhizomes at 5% and Mancozeb which shows significantly different effect from incidence (50%) and severity (1.67) recorded on untreated control plants at 4WAP.

Table 4.30. Effect of *Costus afer* rhizome extract as seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum gloeosporioides*

	Incidence (%)			Severity		
Treatments	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
CAR 5%	16.67b	33.33ab	50.00b	2.00a	1.33ab	1.67b
CAR 10%	0.00b	0.00b	20.00bc	1.00b	1.00b	1.20b
CAR 50%	0.00b	16.67ab	16.67bc	1.00b	1.17ab	1.17b
Mancozeb	0.00b	16.67ab	0.00c	1.00b	1.17ab	1.00b
Control	100.00a	66.67a	100.00a	2.00a	1.67a	3.83a

WAP: Weeks after planting, CAR: *Costus afer* rhizome Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

Table 4.31. Effect of *Costus afer* rhizome extract as seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *Lasiodiplodia theobromae*

	Incidence (%)			Severity		
Treatments	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
CAR 5%	0.00b	25.00b	25.25b	1.00b	1.25b	1.75b
CAR 10%	16.67ab	0.00b	50.00ab	1.17ab	1.00b	1.50b
CAR 50%	16.67ab	16.67b	33.33b	1.17ab	1.17b	1.33b
Mancozeb	0.00b	0.00b	16.67b	1.00b	1.00b	1.17b
Control	50.00a	83.33a	100.00a	1.67a	2.33a	4.00a

WAP: Weeks after planting, CAR: *Costus afer* rhizome Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

However, there was no significant (P = 0.05) difference in the incidence and severity of disease observed on the plant treated with *C. afer* rhizomes extract at all concentration and Mancozeb at 5WAP and 6WAP but were different from the untreated control plant except incidence on plants treated with *C. afer* rhizomes extract at 10% concentration. The fungitoxic potentials of the extract was comparable to the effect of the synthetic fungicide, mancozeb. (Table 4.31).

4.11.2 Effect of *Phyllanthus amarus* leaf extract as a seed treatment on the disease incidence and severity of *Egusi* melon seed inoculated with leaf blight fungal pathogens.

The effect of *Phyllanthus amarus* leaf extract as a seed treatment on the incidence and severity of blight on *Egusi* melon seed inoculated with *C. truncatum* are shown in Table 4.32. The incidence and severity increased across the week. There was no incidence of leaf blight on plants treated with *P. amarus* leaf extract at 50% and Mancozeb at 4WAP and 5WAP. However, incidence of leaf blight on plant treated with *P. amarus* leaf extract at 10% concentration was not significantly different from that of the untreated control plants. At all Concentrations, extract of *Phyllanthus amarus* leaf protected seedlings from leaf blight caused by seed-infected *C. truncatum.*

Table 4.33 shows the effect of *P. amarus* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *C. gloeosporioides*. At 4WAP, incidence (33.33%) and severity (1.33) of plant treated with *P. amarus* at 50% concentration were significantly (P = 0.05) different from 100.0% and 2.0 recorded on the untreated control plants respectively. Meanwhile, there was no significant difference in blight incidence and severity across the treatments at 5WAP. At 6WAP, the incidence and severity on untreated control plant were significantly (P = 0.05) different from the plant treated with *P. amarus* at all concentrations and Mancozeb.

The effect of *P. amarus* leaf extract as seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *L. theobromae* are presented in Table 4.34. Blight incidence increased across the week. There was no incidence of leaf blight on plant treated with *P. amarus* at 10% concentration and Mancozeb at 4WAP to 5WAP, which was significantly (P = 0.05) than incidence and severity observed on untreated

Table 4.32. Effect of *Phyllanthus amarus* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum truncatum*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	0.00b	33.33a	33.33b	1.00b	1.33a	1.33b
PAL 10%	16.67b	16.67a	50.00ab	1.17b	1.17a	1.50b
PAL 50%	0.00b	0.00a	16.67b	1.00b	1.00a	1.17b
Mancozeb	0.00b	0.00a	16.67b	1.00b	1.00a	1.17b
Untreated	66.67a	50.00a	100.00a	1.67a	1.17a	3.83a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

Table 4.33. Effect of *Phyllanthus amarus* leaf extract as a seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum gloeosporioides*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	50.00abc	50.00a	50.00b	1.50abc	1.67a	1.83b
PAL 10%	66.67ab	16.67a	16.67b	1.67ab	1.17a	1.33b
PAL 50%	33.33bc	33.33a	50.00b	1.33bc	1.33a	1.67b
Mancozeb	0.00c	16.67a	0.00b	1.00c	1.17a	1.00b
Untreated	100.00a	66.67a	100.00a	2.00a	1.67a	3.83a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05)

Table 4.34. Effect of *Phyllanthus amarus* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Lasiodiplodia theobromae*

	Incidence (%)			Severity		
Treatments	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	16.67ab	16.67b	33.33b	1.17ab	1.17b	1.33b
PAL 10%	0.00b	0.00b	16.67b	1.00b	1.00b	1.17b
PAL 50%	16.67ab	0.00b	16.67b	1.17ab	1.00b	1.67b
Mancozeb	0.00b	0.00b	16.67b	1.00b	1.00b	1.17b
Untreated	50.00a	83.33a	100.00a	1.67a	2.33a	4.00a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

control plant at 4WAP (50% and 1.67) and 5WAP (83.33% and 2.33) respectively. At 6WAP, the incidence and severity of the untreated control plant were significantly different from the plant treated with *P. amarus* leaf extract at all concentrations and Mancozeb (Table 4.34).

4.11.3 Effect of *Costus afer* leaf extract as seed treatment on the incidence and severity of *Egusi* melon seed inoculated with leaf blight fungal pathogens.

The effect of *Costus afer* leaf extract as a seed treatment on the disease incidence and severity of *Egusi* melon seed inoculated with *C. truncatum* are shown in Table 4.35. At all Concentrations, extract of *Costus afer* leaf protected seedlings from leaf blight caused by seed-infected *C. truncatum.* The fungitoxic potentials of the extract was comparable to the effect of the synthetic fungicide, mancozeb. The concentrations of the extract were not significantly different from each other in respect of their potential to reduce incidence of *C. truncatum* induced leaf blight. Incidence of leaf bight increased progressively from 4 to 6 WAP except at 50% concentration. Range of incidence among extract treated plants was 0.0-33.3% while range in control (unprotected) seedling is 50.0-100.0% (Table 4.35). The severity of leaf blight on seedlings treated with extract was mild, while it was severe on unprotected control.

Table 4.36 presents the effect of *C. afer* leaf extract as a seed treatment on the blight incidence and severity of *Egusi* melon seed infected with *C. gloeosporioides*. All through the weeks, there was no significant (P = 0.05) difference in the fungitoxicity potential of the extract to reduce incidence and severity of *C. gloeosporioides* at different concentrations. This effect was comparable to the effect of Mancozeb. However, the incidence (0.00%) and severity (1.00) of plant treated with *C. afer* leaf at 10% concentration were different from the untreated control plant (66.67% and 1.67) at 5WAP.

The effect of *C. afer* leaf extract as a seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *L. theobromae* are shown in Table 4.37. There was no incidence of leaf blight disease on plant treated with *C. afer* leaf at 10% concentration across the weeks. The fungitoxicity potentials of the extract was comparable to the effect of the synthetic fungicide, mancozeb except at 6WAP. The concentrations of the extract

Table 4.35. Effect of *Costus afer* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum truncatum*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	16.67ab	16.67b	33.33b	1.17ab	1.17b	1.33b
PAL 10%	0.00b	0.00b	16.67b	1.00b	1.00b	1.17b
PAL 50%	16.67ab	0.00b	16.67b	1.17ab	1.00b	1.67b
Mancozeb	0.00b	0.00b	16.67b	1.00b	1.00b	1.17b
Untreated	50.00a	83.33a	100.00a	1.67a	2.33a	4.00a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

Table 4.36. Effect of *Costus afer* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum gloeosporioides*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	0.00b	16.67ab	16.67b	1.00b	1.17b	1.17b
PAL 10%	20.00b	0.00b	0.20b	1.20b	1.00b	1.40b
PAL 50%	33.33b	16.67ab	33.33b	1.33b	1.17ab	1.33b
Mancozeb	0.00b	16.67ab	0.00a	1.00b	1.17ab	1.00b
Untreated	100.00a	66.67a	100.00a	2.00a	1.67a	3.83a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05)

Table 4.37. Effect of *Costus afer* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Lasiodiplodia theobromae*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	50.00a	50.00ab	100.00a	1.50a	1.50ab	2.00b
PAL 10%	0.00b	0.00b	0.00b	1.00a	1.00b	1.00b
PAL 50%	66.67a	33.33ab	50.00ab	1.67a	1.33b	1.83b
Mancozeb	0.00b	0.00b	16.67c	1.00a	1.00b	1.67b
Untreated	50.00a	83.33a	100.00a	1.67a	2.33a	4.00a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

were not significantly different from each other in respect of their potential to reduce incidence of *L. theobromae* induced leaf blight. Range of incidence among extract treated plants was 0.0-100.0% while range in control (unprotected) seedling is 50.0-100.0% (Table 4.37). The severity of leaf blight on seedlings treated with extract was mild, while it was mild to severe on unprotected control. *Costus afer* leaf at 10% concentration was significantly better in reducing incidence of *Lasiodiplodia theobromae* induced leaf blight.The fungitoxicity of the extract was comparable to the effect of mancozeb (Table 4.37).

4.11.4 Effect of *Passiflora foetida* leaf extract as seed treatment on the disease incidence and severity of *Egusi* melon seed inoculated with leaf blight fungal pathogen.

The effect of *Passiflora foetida* leaf extract as a seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *C. truncatum* are shown in Table 4.38. There was no significant difference in the incidence and severity of the plant treated with *P. foetida* at 5%, 10% and 50% concentrations and Mancozeb at the varying weeks but they were different from the untreated control plant except the severity at 5WAP. The fungitoxicity of the extract was comparable to the effect of mancozeb. Range of incidence among extract treated plants was 0.0-33.3% while range in control (unprotected) seedling is 50.0-100.0% (Table 4.38). The severity of leaf blight on seedlings treated with extract was mild, while it was mild to severe on unprotected control.

The effects of *P. foetida* leaf extract as seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *C. gloeosporioides* are presented in Table 4.39. The concentrations of the extract were not significantly different from each other in respect of their potential to reduce incidence of *C. truncatum* induced leaf blight. However, all the treatment gave significantly (P = 0.05) low incidence and severity of leaf blight compared with the untreated control plant except at 50% concentration. The fungitoxicity of the extract was comparable to the effect of mancozeb.

Table 4.40 shows the effect of *P. foetida* leaf extract as a seed treatment on the incidence and severity of *Egusi* melon seed inoculated with *L. theobromae***.**

Table 4.38. Effect of *Passiflora foetida* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum truncatum*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	0.00b	16.67ab	16.67b	1.00b	1.17a	1.17b
PAL 10%	33.33ab	0.00b	0.00b	1.33ab	1.00a	1.00b
PAL 50%	0.00b	0.00b	33.33b	1.00b	1.00a	1.33b
Mancozeb	0.00b	0.00b	16.67b	1.00b	1.00a	1.17b
Untreated	66.67a	50.00a	100.00a	1.67a	1.17a	3.83a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05)

Table 4.39. Effect of *Passiflora foetida* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Colletotrichum gloeosporioides*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	0.00b	0.00b	0.00b	1.00b	1.00b	1.00b
PAL 10%	0.00b	0.00b	20.00b	1.00b	1.00b	1.20b
PAL 50%	0.00b	40.00ab	40.00b	1.00b	1.40ab	1.40b
Mancozeb	0.00b	16.67ab	0.00b	1.00b	1.17ab	1.00b
Untreated	100.00a	66.67a	100.00a	2.00a	1.67a	3.83a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05)

Table 4.40. Effect of *Passiflora foetida* leaf extract as seed treatment on the blight incidence and severity of *Egusi* melon seed inoculated with *Lasiodiplodia theobromae*

	Incidence (%)			Severity		
Treatments (w/v)	4WAP	5WAP	6WAP	4WAP	5WAP	6WAP
PAL 5%	66.67a	33.33ab	33.33b	1.67a	1.33b	1.67b
PAL 10%	16.67ab	16.67b	66.67ab	1.17ab	1.17b	1.83b
PAL 50%	16.67ab	50.00ab	50.00ab	1.17ab	1.50b	1.67b
Mancozeb	0.00b	0.00b	16.67b	1.00b	1.00b	1.17b
Untreated	50.00a	83.33a	100.00a	1.67a	2.33a	4.00a

WAP: Weeks after planting PAL: *Phyllanthus amarus* leaf. Severity scale 1.0= No symptom; 1.1-2.0 = <10% damaged portion (mildly severe); 2.1-3.0 = 10-30% damaged portion (moderately severe); 3.1- 4.0 = 31-50% damaged portion (severe) and 4.1- 5.0 = >50% damaged portion (highly severe). Values with similar letter along the column are not significantly different according to DMRT (p = 0.05

At 4WAP, there was no significant (P = 0.05) difference in the fungitoxicity potential of *P. foetida* leaf extract as seed treatment on *L. theobromae* infected seedling at 5%, 10%, 50% concentration compared with untreated control plant. The fungitoxicity potential of the extract was comparable to the effect of the synthetic fungicide, mancozeb.

4.12 Effect of botanical extracts as seed treatment on seeds germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP.

The percentage germination of the inoculated seeds treated with *C. afer* rhizome extracts and mancozeb was not significantly (P = 0.05) better than the untreated control plant for the three pathogen tested (Table 4.41). However, the vine length of the plants inoculated with *C. truncatum* and *L. theobromae* and treated with this extract at 5%, 10% and 50% were significantly longer than the untreated control plant. Vine length of plants inoculated with *L. theobromae* and treated with *C. afer* rhizomes at 10% concentrations was not different from the untreated control plant.

The number of leaves produced from plant inoculated with *C. truncatum* and treated with *C. afer* rhizomes at 5%, 10% and 50% concentrations (16.00, 18.25 and 15.60) were similar to that of the untreated control plant (16.00) but were significantly lower than those plants treated with Mancozeb (26.10) (Table 4.41). In contrast, the vine length produced by plant inoculated with *L. theobromae* and treated with *C. afer* rhizomes extract at the three concentrations was not significantly shorter than that of the plants treated with Mancozeb. Meanwhile, there was no significant (P = 0.05) difference in the vine length, number of leaves and no of vines of the plant inoculated with *C. gloeosporioides*. Also, there was no significant difference in number of vines produced by plants inoculated with *C. truncatum, C. gloeosporioides* and *L. theobromae* and treated with *C. afer rhizomes* at 6WAP.

The percentage germination and number of vines produced by the plants inoculated with *C. truncatum, C. gloeosporioides* and *L. theobromae* and treated with *P. amarus* at 5%, 10%, 50% was not significantly lower than plants treated with synthetic fungicide, mancozeb (Table 4.42). The vine length produced by plant inoculated

Table 4.41. Effect of *Costus afer* rhizome extract as a seed treatment on seeds germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6 WAP

	Colletotrichum truncatum				*Colletotrichum gloeosporioides*				*Lasiodiplodia theobromae*			
Treatment	% G	VL	NL	NV	% G	VL	NL	NV	% G	VL	NL	NV
CAR5	77.78a	75.00ab	16.00b	1.17a	77.78a	17.00a	72.00a	1.25a	50.00a	97.65a	18.38ab	1.25a
CAR10	100.00a	95.40a	18.25b	1.67a	88.89a	15.38a	72.00a	1.00a	66.67a	94.20ab	21.00a	1.67a
CAR50	77.78a	72.36ab	15.60b	1.20a	100.00a	15.00a	60.30a	1.50a	77.78a	102.00a	19.00ab	1.33a
Mancozeb	100.00a	65.25b	26.10a	1.67a	100.00a	16.25a	73.75a	1.83a	100.00a	102.63a	22.05a	2.33a
Untreated	83.33a	40.63c	16.00b	1.60a	77.78a	15.50a	49.38a	1.83a	77.78a	63.75b	16.25bc	1.50a

WAP= Weeks after planting, CAR: *Costus afer* rhizome, % G= % Germination, VL= vine length, NL= no of leaves and NV= no of vines. Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

Table 4.42. Effect of *Phyllanthus amarus* leaf as seed treatment on seeds germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP

	Colletotrichum truncatum				*Colletotrichum gloeosporioides*				*Lasiodiplodia theobromae*			
Treatment	% G	VL	NL	NV	% G	VL	NL	NV	% G	VL	NL	NV
PA5%	100.00a	80.70a	18.00a	1.50a	100.00a	37.800ab	25.29a	1.60a	66.67a	69.12a	15.90ab	1.40a
PA10%	77.78a	53.40ab	16.50b	1.33a	88.89a	40.20a	23.69ab	2.00a	66.67a	95.40a	18.75a	1.75a
PA50%	88.89a	66.30ab	15.50b	1.50a	77.78a	34.00ab	18.00bc	1.50a	100.00a	69.60a	16.00ab	1.50a
Mancozeb	100.00a	65.25ab	26.10a	1.60a	100.00a	34.50ab	18.43bc	1.83a	100.00a	70.63a	16.00ab	2.33a
Untreated	83.33a	40.63b	16.25b	1.67a	77.78a	31.00b	12.35c	1.83a	77.80a	63.75a	14.50b	1.50a

WAP= Weeks after planting, CAR: *Costus afer* rhizome, % G= % Germination, VL= vine length, NL= no of leaves and NV= no of vines. Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

with *C. truncatum, C. gloeosporioides* and *L. theobromae* and treated with *P. amarus* leaf extract at all concentrations was not significantly shorter than plant treated with Mancozeb. However, *Egusi* melon plants inoculated with *C. truncatum* and *C. gloeosporioides* and treated with *P. amarus* leaf extract at 5% (80.70 cm) and 10% (40.20 cm) concentrations were significantly (P = 0.05) longer than untreated control plants (40.63 cm and 31.00 cm) at 5% level of concentration (Table 4.42).

The number of leaves produced by the plant inoculated with *C. truncatum* and treated with *P. amarus* at 5% concentration (18.00) was similar to that of plants treated with mancozeb (26.10), but higher than that of untreated control plant (16.25). This indicates the effectiveness of *P. amarus* at 5% concentration on blight disease. Plants inoculated with *C. gloeosporioides* and treated with *P. amarus* at the same concentration significantly gave the highest number of leaves of 25.29 which though not significantly different from 23.69 recorded at 10% concentration, was higher than those of other treatments. The number of leaves produeced at 10% concentration was significantly higher than that of untreated plants (Table 4.42).

Table 4.43 shows the effect of *Costus* afer leaf extracts as seed treatment on seeds germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP. There were significant (P = 0.05) differences in the percentage germination of the plant inoculated with the leaf blight fungal pathogens except *C. truncatum.* The percentage was highest on plant treated at 50% (88.89% and 100.00%) concentration and lowest at 10% (77.78%) and 5% (11.11%) for *C. gloeosporioides* and *L. theobromae* respectively. There was no difference in the number of vines of the plant inoculated with the three pathogens at all concentrations and the untreated control plants. Vine length were significantly (P = 0.05) longer in plants inoculated with *C. truncatum* and treated with *C. afer* leaf at 10% (86.40cm) and 50% (75.15 cm) concentrations than in untreated control plants (40.63 cm). Number of leaves was not different at varying levels of concentrations from the untreated control plants but were lower significantly (P = 0.05) from the plants treated with Mancozeb. *Costus afer* leaf was effective as seed treatment by producing longer vine length. *C. afer* leaf at 50% concentration significantly gave the highest vine length of 95.40cm

Table 4.43. Effect of *Costus afer* leaf as seed treatment on seed germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP

	Colletotrichum truncatum				*Colletotrichum gloeosporioides*				*Lasiodiplodia theobromae*			
Treatment	% G	VL	NL	NV	% G	VL	NL	NV	% G	VL	NL	NV
CAL 5%	77.78a	58.80ab	14.00b	1.17a	88.89a	71.70b	15.00a	1.00a	11.11c	43.80b	12.67b	1.00a
CAL 10%	66.67a	86.40a	15.38b	1.25a	77.78a	78.84ab	16.50a	1.60a	55.55b	51.00ab	12.50b	1.00a
CAL 50%	66.67a	75.15a	16.13b	1.00a	88.89a	95.40a	16.50a	1.17a	100.00a	81.36a	15.60ab	1.60a
Mancozeb	100.00a	65.25ab	26.10a	1.60a	100.00a	73.75ab	16.25a	1.83a	100.00a	70.63ab	16.25a	1.50a
Untreated	83.33a	40.63b	16.25b	1.67a	77.80b	49.38c	15.50a	1.83a	77.80ab	63.75ab	14.50ab	2.33a

WAP= Weeks after planting, CAL: *Costus afer* leaf, % G= % Germination, VL= vine length, NL= no of leaves and NV= no of vines. Values with similar letter along the column are not different by DMRT (p = 0.05).

which was significantly longer than those at 5% concentration (71.70 cm) and the untreated control plants (49.38 cm). This extract treatment on plant inoculated with *C. gloeosporioides* had no significantly (P = 0.05) different in the number of leaf produced.

For plant inoculated with *L. theobromae,* the highest vine length (81.36 cm) and number of leaves (15.60) were observed at 50% concentration which was not significantly (P = 0.05) different from the untreated control plant, while the least vine length (43.80 cm) was observed at 5% concentration (Table 4.43).

Table 4.44 shows the effect of *Passiflora foetida* leaf extracts as seed treatment on germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP. There was no significant difference in the number of vines of the plant inoculated with *C. truncatum, C. gloeosporioides* and *L. theobromae* and treated with *P. foetida* at varying concentrations and the controls. The vine length of 40.63 cm recorded on the plant inoculated with *C. truncatum* but not treated was significantly (P = 0.05) lower than that observed from the treated plant at varying concentrations. Meanwhile, only number of leaves of plants inoculated with *C. truncatum* and treated with Mancozeb was significantly higher. Germination of 44.44% observed on plant inoculated with *C. gloeosporioides* and treated with *P. foetida* leaf extracts at 5% concentration was significantly (P = 0.05) lower than 55.56% observed at 50% concentration. The least vine length (37.80 cm) was recorded at 10% concentration which was not significantly different from the untreated control plant (49.38 cm) but lower than the vine length of plants treated with 50% *P. foetida* (73.80 cm) and Mancozeb (73.75 cm). Likewise, the 11.40 number of leaf at 10% concentration of *P. foetida* was not significantly (P = 0.05) different from that of the untreated control plant (11.20) but different from plant treated with Mancozeb. For *Egusi* melon plants inoculated with *L. theobromae,* there is no significant difference in the germination percentage of the plant treated with *P. foetida* leaf extracts at 5%, 10% and 50% compared with the untreated control plant. The vine length and number of leaves of plants treated at 5% (90.60 cm and 17.75) concentration were significantly (P = 0.05) different from the untreated control plant (63.75 cm and 14.50) (Table 4.44).

Table 4.44. Effect of *Passiflora foetida* leaf as a seed treatment on germination and growth of *Egusi* melon plant inoculated with leaf blight fungal pathogens at 6WAP

	Colletotrichum truncatum				*Colletotrichum gloeosporioides*				*Lasiodiplodia theobromae*			
Treatment	% G	VL	NL	NV	% G	VL	NL	NV	% G	VL	NL	NV
PFL5%	100.00a	84.60a	15.50b	1.17a	44.44c	50.70ab	14.50ab	1.00a	55.56b	90.60a	17.75a	1.33a
PFL10%	88.89a	82.44a	14.70b	1.40a	88.89a	37.80b	11.40b	1.60a	77.78ab	61.20b	12.60c	1.20a
PFL50%	77.78a	78.00a	17.25b	1.67a	55.56bc	73.80a	14.40ab	1.20a	77.78ab	73.50ab	14.75bc	1.50a
Mancozeb	100.00a	65.25a	26.10a	1.60a	100.00a	73.75a	16.25a	1.83a	100.00a	70.63ab	16.25ab	2.33a
Untreated	83.33a	40.63b	16.25b	1.67a	77.80ab	49.38ab	11.20b	1.83a	77.80ab	63.75b	14.50bc	1.50a

WAP= Weeks after planting, PFL: *Passiflora foetida* leaf, % G= % Germination, VL= vine length, NL= no of leaves and NV= no of vines. Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

4.13 Phytochemical composition of some botanicals assessed for antifungal activities against leaf blight pathogens

The phytochemicals present in the botanicals used against leaf blight pathogens are alkaloids, flavonoids, glycosides, tannins, steroids, phenols and saponins. The quantities of these phytochemicals are presented in Table 4.45. There were significant differences (p= 0.05) in the concentrations of phytochemicals across the botanicals screened.

4.13.1. Total alkaloids in the botanicals used against leaf blight pathogens

Phyllanthus amarus leaf significantly had the highest total alkaloids (658.00 mg/100g) amongst all the botanicals screened followed by *Azadirachta indica* stem (596.67mg/100g) (Table 4.45). There was no significant difference in the total alkaloids obtained from *Passiflora foetida* leaf (526.67 mg/100g) and *Thevetia neriifolia* leaf (513.33 mg/100g) while the least was recorded from *Costus afer* leaf (286.67 mg/100g).

4.13.2. Total flavonoids in the botanicals used against leaf blight pathogens

The highest quantity of flavonoids was observed in *Azadirachta indica* stem (541.67 mg/100g) which was significantly higher when compared with the flavonoids in the other botanical used. *C. afer* root and *T. neriifolia* leaf had 142.33 mg/100g and 128.33 mg/100g respectively which were not significantly different from each other but significantly higher than total flavonoids observed in *Costus afer* leaf (86.67 mg/100g) (Table 4.45)

4.13.3. Total saponins in the botanicals used against leaf blight pathogens

Costus afer root and *Azadirachta indica* stem had 316.67 mg/100g and 311.67 mg/100g of the total saponins which were not significantly different from each other (Table 4.45). The total saponins in both *C.afer* leaf (251.67 mg/100g) and *T. neriifolia* leaf (257.67 mg/100g) were significantly

Table 4.45. Phytochemical composition of some botanicals assessed for antifungal activities against leaf blight pathogens

	Phytochemical components mg/100ml						
Extract	Alkanoids	Flavonoids	Saponins	Tannins	Glycosides	Steroiodes	Phenols
Phyllantus amarus leaf	658.00a	226.33c	223.33c	723.30b	45.00a	116.67d	54.60b
Passiflora foetida leaf	526.67c	321.67b	176.67d	1548.30a	28.33bc	258.33b	48.37d
Costus afer leaf	286.67f	86.67f	251.67b	48.30d	36.67ab	90.00e	45.33e
Costus afer root	326.67e	142.33e	316.67a	451.30c	15.00d	273.33b	39.17g
Azadirachta indica leaf	458.33d	275.00c	148.33e	653.30bc	18.33d	213.33c	64.50a
Azadirachta indica stem	596.67b	541.67a	311.67a	480.00c	23.33cd	273.33b	51.33c
Thevetia neriifolia leaf	513.33c	128.33e	251.67b	183.30d	38.33a	313.33a	43.40f

Each value is a mean of three replicates. Values with similar letter along the column are not significantly different according to DMRT (p = 0.05).

higher than those found in the *P. amarus* leaf (223.33 mg/100g) and *P. foetida* leaf (176.67 mg/100g) while the lowest was recorded in leaf of *A. indica* (148.33 mg/100g) (Table 4.45).

4.13.4. Total glycosides in the botanicals used against leaf blight pathogens

The highest quantity of glycosides was found in *P. amarus* leaf (45.00mg/100g), but this was not significantly higher than alkaloids found in *T. neriifolia* leaf (38.33 mg/100g) and *C. afer* leaf (36.67 mg/100g) (Table 4.45). The least quantity of alkaloids was found in *C. afer* root (15.00 mg/100g) which was not significantly different when compared with the alkaloids content in the *A. indica* leaf (18.33 mg/100g) and stem (23.33 mg/100g).

4.13.5. Total steroids in the botanicals used against leaf blight pathogens

Thevetia neriifolia leaf significantly had the highest steroids content of 313.33 mg/100g) compared with other botanicals. *C. afer* root and *A. indica* stem had similar steroids content of 273.33 mg/100g which was not significantly different from 258.33 mg/100g recorded in *P. foetida* leaf. The least was recorded in *C. afer* leaf (Table 4.45).

4.13.6 Total phenols in the botanicals used against leaf blight pathogens

There were significant differences in the phenol content in all the botanical used. The highest quantity was found in *A. indica* leaf (64.50 mg/100g) while the least was recorded from *T. neriifolia* leaf (Table 4.45).

4.13.7 Total tannins in the botanicals used against leaf blight pathogens

Passiflora foetida leaf significantly had the highest tannins content of 1548.30 mg/100g) compared with other botanicals (Table 4.45). The least quantity of tannins was found in *C. afer* leaf (48.30mg/100g) which was not significantly different when compared with the tannins content in the *T. neriifolia* leaf (183.30 mg/100g).

CHAPTER FIVE

5.0 DISCUSSION

Sampling *Egusi* melon farms for blight infections revealed that Bara was the most cultivated variety in all the LGAs of the five states, followed by Bojuri and Serewe. This widest distribution of Bara among other varieties in Southwestern Nigeria has earlier been reported (Fayemi, 1999; Denton and Olufolaji, 2000).

Blight disease symptom observed in all the five Southwestern states on the three *Egusi* melon varieties confirms the presence of the disease on the crop in Nigeria as previously reported (Chiejina, 2006; Kehinde, 2008). Incidence of leaf blight observed in this study was close the range of 82 - 100% earlier reported by Kehinde, (2008). Higher incidence and severity of leaf blight disease on Bara and Bojuri varieties is conceivable due to their wider distribution among Southwestern states, arising from their preference by the farmers than Serewe variety.

Twelve fungal pathogens from ten genera isolated and identified from the infected samples namely: *Lasiodiplodia theobromae, Colletotrichum truncatum, C. gloeosporioides, Choanephora cucurbitarum, Curvularia lunata, Fusarium equiseti, F. verticillioides, F. solani, Sclerotium rolfsii, Corynespora cassiicola, Pestalotiopsis guepini* and *Rhizopus stolonifer* have some similarity with previous report. Although there is limited information on pathogenic fungi of *Egusi* melon, some of the isolated fungi have been reported from melon in Southeastern Nigeria (Chiejina, 2006). Other studies on stored *Egusi* melon seeds of Serewe variety in Nigeria showed incidence of *Aspergillus. niger, A. flavus, A. fumigatus, A. granulosis, F. solani, Botrydiplodia theobromae* and *Penicillum citrinum* (Bankole and Joda, 2004). Similarly, Kehinde, (2013) reported isolation of *Erysiphe cucurbitarum* (Powdery mildew), *Peronospora cucurbitarum*, (Downy mildew) *Alternaria cucumerina*, (*Alternaria* leaf spot) *Cercospora citrullina* (*Cercospora* leaf spot), *Colletotrichum lagenarium* (Anthracnose), *Didymella bryoaniae* (leaf and vine blight, fruit rot) and *C. cucurbitarum* (Wet rot of flower) on *Egusi* melon in Nigeria. However, the most frequently occurring fungi

pathogen of *Egusi* melon was *C. cucurbitarum* followed by *L. theobromae* and *C. lunata* in this study. The occurrence and distribution of these pathogens were not equal in all the states. This corresponds to the report that there is natural uneven occurrence and distribution of plant pathogenic fungi, which is determined by the host plant, soil, climate, cropping patterns, cropping history (Baimey *et al*., 2009).

In this study, *C. gloeosporioides*, *L. theobromae*, and *C. truncatum* were observed to be causal pathogens of leaf blight on *Egusi* melon. This is contrary to the report of Kehinde (2013) that *Didymella bryoaniae* is the causal pathogen of leaf blight of *Egusi* melon. The characteristics gummy stem blight symptom or exudation of gummy material and water soaked leaf lesions of the Egusi melon plants infected by *D. bryoaniae* were not encountered in the study area. This difference in observation might be due to the possibility of *D. bryoaniae* having a better alternative host other than *Egusi* melon or environmental factors capable of affecting fungi pathogenicity (Topit and Sovali, 2010).

The three pathogens have been reported to have caused various diseases in many crops (Kumar and Singh, 2009; Pedroso *et al*., 2011). *Lasiodiplodia theobromae* has been reported to have a very wide host range, causing diseases such as, blight, dieback, root rot and post-harvest rot in different plants in the tropical regions (Urbez-Torres *et al*., 2008; Kumar and Singh, 2009). It was reported as one of the major field pathogens in South East Nigeria (Bankole, 1998). *Colletotrichum* spp. infects various fruit crops in tropical and subtropical regions, causing serious damage and significant yield losses in all growth phases of crops (Suparman *et al.,* 2018). Apart from causing anthracnose, these pathogens also incites other diseases such as dieback, leaf spot, flower rot, fruit rot, stem end rot, and root rot (Bruce *et al.,* 2013; Nascimento *et al.,* 2010). *Colletotrichum gloeosporioides* causes 10-80 % loss in garden egg (Pedroso *et al*., 2011). In the humid forest of Southwestern Nigeria, *Colletotrichum truncatum* caused brown blotch of cowpea (Adebitan, 1984).

The observed occurrence of other diseases of *Egusi* melon apart from leaf blight on the collected samples corresponds with previous reports. *Choanephora cucurbitarum* causing wet rot of *Egusi* melon flowers, *C. cassiicola* as a causal pathogen of leaf spot, *F. verticillioides* and *Sclerotium rolfsii* causing vine wilt disease and *F. solani* causing damping off (foot rot) of seedlings are in conformity with the findings of Kehinde (2011).

Similar diseases by these pathogens on other crops have been reported in many countries. Wet rot (soft rot) caused by *C. cucurbitarum* was earlier reported on Okra (*Abelmoschus esculentus* (L.) Moench) in Egypt (Hussein and Ziedan, 2013) and Korea (Park *et al*., 2014) as well as on Cowpea (*Vigna unguiculata*) causing lamb's tail pod rot (Singh and Allen, 1980).

The reduction in the yield of Bara and serewe varieties with the highest recorded from *C. truncatum* on Bara variety conformed with report of Manandhar and Hartman (1999) that *C. truncatum* reduced seed germination, quality and yield of soybean. About 50% and 100% yield loss have been reported in Thailand and India, respectively due to anthracnose caused by *C. truncatum* on Soybean (Manandhar and Hartman, 1999). *Colletotrichum truncatum* inoculated Egusi melon (Bara) had significantly reduced growth in two field trials. However, *C. gloeosporioides* and *L. theobromae* inoculated plants produced blight symptom but without significant reduction in plant growth parameters on three varieties. This could be due to late development of blight symptoms (4 to 6 WAP) generally observed on *Egusi* melon plants and could be ability of the plant over time to tolerate the pathogens which agrees with Odubanwo *et al.* (2013). Mossler and Nesheim (2005) in their work reported that yield loss is directly correlated with disease severity and the lengths of time plant have been infected.

Seed transmission studies help to confirm the seed-to-plant transmission and to further confirm pathogenicity which helps in designing a suitable management strategy (Sajeesh *et al*., 2014). This study showed that the causal agents of leaf blight are seed transmitted with the rates ranging from 0 to 23.33%. The rate of seed transmission varied with pathogen as well as crop variety with maximum rates of 23.33% of *Colletotrichum truncatum*, 10.00% of C. *gloeosporioides* and 16.67% of *L. theobromae* transmission observed in Bara variety. This variation in seed transmission rate could be as a result of genotypic differences among the varieties (Chiejina, 2006) or virulence of the pathogen. Bankole *et al*. (2004) reported similar observations of fungal infection on melon seeds caused reduced seed germination, decreased nutritive value and mycotoxin production. Seed infection caused by *C. truncatum* may inflict considerable damage to seeds after harvest as reported by Begum *et al.* (2008). This implies that farmers should apply appropriate seed treatment to check the spread of blight disease of *Egusi* melon while the

regulatory authorities should apply adequate phytosanitary measures to prevent introduction and spread of blight disease through seed movement within and across nations. Begum *et al.* (2008) reported that *C. truncatum* remained in seed as latent infection without any visible symptoms and affect soybean seed as planting materials by reducing germination and vigour and caused pre and post emergence damping-off of seedlings.

The effective inoculation period for the three blight causing fungi for disease development varied with variety. However, seed inoculation for Bara and seedling inoculation (at 28 DAP) for Serewe were most effective. Time of first blight symptom appearance ranged between 4 and 6 WAP and this varies with fungi and *Egusi* melon variety, with symptom appearing earlier in Bara and Serewe than Bojuri. This agrees with the findings of Kehinde (2008) that highest incidence of blight disease occurred around week four to seven in *Egusi* melon varieties. Severity of blight disease in all the varieties increased with increase in incidence similar to the report of Than *et al*. (2008) that disease incidence caused by *Colletotrichum* species increased exponentially with increase in disease severity.

This study showed that four bioagents: *Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens* and *Bacillus subtilis* could be used in the management of *Egusi* melon leaf blight disease, with *T. pseudokoningii* and *T. harzianum* being more effective than the others. Earlier reports have noted the potential of *Trichoderma* spp. as biological control agents for the protection of various seed and soil borne diseases of crops (Adekunle *et al*., 2001; Peluola, 2005; Akinbode, 2013). The two *Trichoderma* species possessed the ability to grow aggressively to colonize and occupy the Petri plate within five days. Similar action of *Trichoderma* species had been observed by Akinbode, (2013). Prescott *et al*. (2002) stated that a good antagonist should have ability to compete well for space and nutrients. The action of the *Trichoderma* species against *C. truncatum, C. gloeosporioides* and *L. theobromae* proved the antagonist to be a stronger competitor for food and space. The fading colour of the *C. truncatum* and *C. gloeosporioides* from pinkish white to whitish black when the two *Trichoderma* species grew over its mycelial mass could indicate gradual death. Yellowish pigment found around the edge of the mycelial mass of *L. theobromae* paired with *T. pseudokoningii*

might be due to the yellowish-green metabolite produced by the antagonist. The growth of the two *Trichoderma* species on some part of the mycelial mass of the pathogens suggested hyperparasitism (Akinbode, 2013), a form of minor morphological disturbances and hyphal distortion of one fungus by another. The hyperparasitism action of the antagonist against the blight pathogens correspond with the reports of Adekunle *et al.* (2001); Peluola (2005) and Akinbode (2013).

Bacillus subtilis produces antibiotics such as bacillomycin, surfactin (Tsuge *et al.,* 1995), bacitracin, subtenolin, and subtilin (Manjula *et al.,* 2005). These antibiotics could be accountable for the inhibition observed in this study. *Lasiodiplodia theobromae* grew well when paired with *B. subtilis* with a thin clear zone of inhibition. This could have resulted from the fast growth ability of the test pathogen. It could also be, as suggested by Onyeka (1997) that *B. subtilis* produces antibiotics in response to the presence of selected organisms.

The lower incidence and severity of leaf blight disease and high percentage germination observed on seed inoculated with the causal pathogens and treated with biological control agents might be as a result of rapid multiplication of biological control agents in the soil and its establishment in the roots of seedlings, preventing pathogen establishment in the rhizosphere as reported by Akinbode (2013).

The significant reduction in the growth of the three blight causing fungi (*C. truncatum, C. gloeosporioides and L. theobromae*) on authoclaved *T. harzianum, T. pseudokoningii, P. fluorescens* and *B. subtilis* suggests that the effective toxins earlier produced by the biocontrol agents were heat-resistant and thermostable. This report agrees with that of Taylor *et al.* (2005) that toxins produced by *B. cereus* is heat stable, though their potency was halved. Effect of heat treatment on a biotoxin only inactivates its concentration by about half (Dania, 2012). Peluola (2005) also reported that high heat treatment of *T. pseudokoningii* and *P. fluorescens* at 121^{o}C for 15 mins had a limited effect on the potency of the pathogens. This indicated that heat does not affect the potency of the toxins produced by these pathogens.

Management of blight disease of *Egusi* melon using botanicals showed that botanicals from different parts of plants vary in their inhibitory effects on the growth and sporulation of *C. truncatum, C. gloeosporioides and L. theobromae. Phyllanthus amarus*

leaf, *Costus afer* rhizome and *P. foetida* leaf reduced both the colony diameters and the number of spores of the *Colletotrichum* species. Extract from some of the plants reduced the mycelial growth but stimulated sporulation of the fungus while other reduce sporulation but stimulate mycelial growth. This showed that there is need for frequent application of the extract in other to effectively control the pathogen. *Morinda lucida* root, stem and leaf extract effectively inhibited the mycelial growth of *C. truncatum* and stimulated sporulation which is in agreement with Nduagu *et al*. (2008) and Adeniyi and Joseph (2015). This shows that botanicals could have chemical components with variable effects on pathogens. Kurucheve *et al.* (1997) and Nduagu *et al.* (2008) reported that botanicals that stimulated mycelial growth of the test pathogens could be added to Potato Dextrose Agar to prepare a selective media for isolating and culturing fungi. Likewise, those that stimulated the sporulation could also be used as a medium for easy identification of those fungi that usually take longer period before sporulating.

Water extracts of *Azadirachta indica* root bark, stem bark and leaf at 5%, 7.5% and 10% concentrations effectively inhibited mycelial growth of *L. theobromae* and slightly inhibited that of *C. truncatum* and *C. gloeosporioides in vitro*. Effective antimicrobial properties of botanicals (*Piper guineense* and *Xylopia aethiopica*) on *L. theobromae* in watermelon have been reported (Abiala *et al*., 2015). In another study, Nduagu *et al*. (2008) controlled *Colletotrichum capsici* using leaf extract of *A. indica* and *Vernonia amygdalina*. Mycelial growth of *Phytophthora capsici* was significantly reduced by *Azadirachta indica in vitro* (Sahu *et al*., 2012). Extract of *Costus afer* rhizome, *P. amarus* leaf, *T. neriifolia* leaf, *P. foetida* leaf and *A. indica* stem inhibited *L. theobromae* whereas *M. lucida* was not effective at all concentrations against the pathogen. This agrees with the report of Ogunsola and Aduramigba-Modupe (2014) that *Morinda lucida* and *Moringa oleifera* could not inhibit the mycelial growth of *L. theobromae* and *Fusarium verticillioides in vitro*. Opara and Nwokocha (2015) however reported that *C. afer* stem extract reduced the growth of *Aspergillus* spp, and *Botryodiplodia* spp except *Rhizopus stolonifer.*

Costus afer leaf, stem and rhizome (5% and 10%) and *Morinda lucida* stem (5% - 12.5%) significantly reduced mycelial growth of *C. gloeosporioides* while *M. lucida* leaf stimulated its sporulation. This sugests the presence of antifungal compounds in different

extracts which agrees with the report by many researchers on different pathogens (Bahraminejad *et al*., 2011; Muthukumar, 2010; Uchegbu *et al*., 2016).

Phyllanthus amarus leaf, *C. afer* leaf and stem and *P. foetida* leaf extract effectively reduced the mycelial growth of *C. truncatum*, while its sporulation was stimulated by *Morinda lucida* root, stem and leaf, *C. afer* stem and leaf extracts. These differences in the antimicrobial activities of the botanicals used may be as a result of variations in the inhibitory effect of plant extracts which may be due to qualitative and quantitative differences in antifungal principles (Kurucheve *et al.*, 1997). The reduced efficiency of some botanicals such as ineffectiveness of *M. lucida* on *L. theobromae* and *C. truncatum*, that of *C. afer* stem and leaf on *L. theobromae* and the observed stimulatory effects of some extracts on the blight fungi is probably due to the tolerance of some fungi to the antimicrobial phenolics in plant extracts. It is also observed that some fungi produce phenol oxidases, which help in the degradation of phenols (Sharma *et al*., 2016). Most of the botanicals used in this study generally performed better at lower extract concentrations of 5% and 7.5% *in vitro*. These could be that the quantity of water added at higher concentrations was not enough to extract the active ingredient. It could also be that the extracts contain some additives which at higher concentrations might stimulate the growth of the fungi though effective at lower concentrations in reducing the fungal growth.

In vivo study confirms the effectiveness of the botanicals in controlling leaf blight disease on *Egusi* melon plants when used as a pre-planting seed treatment. Highest protection of leaf blight was obtained when *Egusi* melon seeds were treated with *C. afer* leaf extract (10%w/v/50seeds) before planting. Seed dressing with *C. afer* rhizome, *Phyllanthus amarus* leaf, *P. foetida* leaf extract and *C. afer* leaf protected the plant from leaf blight infection and were as effective as macozeb (chemical fungicide) which resulted in higher plant growth. *In vivo* inhibition of *Fusarium oxysporum* and *Botryodiploidia theobromae* in yam have been reported in Nigeria (Uchegbu *et al*., 2016)

The wide scope of antimicrobial activity of the plant species correspond with the presence of saponins, alkaloids and tannins (Ndukwe *et al.*, 2005). Antifungal activities of the botanicals used in this study could probably be attributed to the combined actions of the phytochemicals: flavonoids, saponins, tannins, glycosides, steroids and phenols

observed. This agrees with the findings of Muthukumar (2010) who attributed antifungal activity of some botanicals as a result of the presence of saponins in their content. These entire chemical constituents were found in all the botanical evaluated but with variations in quantity of each of the chemical constituents and in different parts of the botanicals. Significant amounts of saponins, alkaloids, flavonoids and tannins found in *P. amarus* leaf, *Costus afer* rhizome, *P. foetida* leaf, and *Costus afer* leaf might have contributed to its antimicrobial effectiveness compared to other botanicals in reducing the leaf blight infection on melon seedlings. This might suggest that saponins, alkaloids, flavonoids and tannins contributed more to the management of fungi on melon than the other phytochemicals in the amendments.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSIONS

This study was conducted to investigate the incidence and management of leaf blight disease of *Egusi* melon varieties in Southwestern Nigeria. The objectives were to determine the incidence, severity and pathogenicity of fungal isolates associated with leaf blight disease of *Egusi* melon in Southwestern Nigeria, and evaluates the effect of blight on the seed yield of *Egusi* melon. Also, to determine the rate of seed transmission of the causal organism and effective inoculation period of *Egusi* melon for blight disease development, evaluate the efficacy of some biological control agents and botanicals for *Egusi* melon seed treatments against blight disease causing organisms and determine the bioactive metabolites responsible for fungitoxicity of some of the botanicals used.

Leaf blight infected samples were randomly collected from 150 farms in five Southwestern states. Disease incidence and severity were measured using % infection and 1 (no symptom) to 5 (highly severe) scale, respectively. Causal pathogens were identified by pathogenicity test. Effect of the causal pathogens on the growth and yield of melon was evaluated on three varieties in two field trials. Seed-to-plant transmission of leaf blight disease was studied by artificially inoculating *Egusi* with causal organisms. Six botanicals and four biological control agents were evaluated in the management of leaf blight disease under laboratory and screenhouse conditions as seed treatment. Bioactive metabolites responsible for fungitoxicity of the botanicals used were also determined.

The conclusions and recommendation were as follows:

- This research confirms the presence of leaf blight disease on the three *Egusi* melon varieties on the farms visited in five Southwestern states.
- In Southwestern Nigeria, leaf blight disease of *Egusi* melon is most prevalent in Ondo state and least in Osun state

- *Colletotrichum truncatum, C. gloeosporioides* and *Lasiodiplodia theobromae* were the causal organisms of leaf blight disease of *Egusi* melon in Southwestern Nigeria.
- The three blight-causing pathogens reduced the yield of *Egusi* melon varieties with the highest reduction (53.6%) by *Colletotrichum truncatum* on Bara variety.
- Incidence of leaf blight symptom begins at 4 weeks and get to the peak at 6 weeks after planting.
- The study also confirms that the leaf blight causing fungi can be transmitted from infected *Egusi* melon seeds to plants at the rate of 0 to 23%
- Also, the effective inoculation periods for disease development of the three blight causing fungi *(Colletotrichum truncatum, C. gloeosporioides* and *Lasiodiplodia theobromae*) on the three *Egusi* melon varieties are seed inoculation and 14 days after planting.
- The most effective biocontrol agent against leaf blight disease is *Trichoderma pseudokoningii* at (1g/50seeds).
- *Passiflora foetida* leaves and *Costus afer* leaves each at 10%w/v/50seeds applied as seed treatments proved to be promising in the management of leaf blight disease of *Egusi* melon.
- Flavonoids, alkaloids, saponins, tannins, glycosides, steroids and phenols were the anti-microbial factors in the botanicals
- Further studies are required on field application of the botanicals and biological control agents.
- Farmers are advised to apply appropriate seed treatment before planting to check the occurrence and spread of blight disease of *Egusi* melon and plant healthy or resistant seed varieties
- Exploitation of naturally available biochemicals would be an effective disease management method
- Hence, these bioagents and botanicals, after further studies on application, are recommended as cheaper and ecofriendly biofungicides.

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Appendix

Field Survey of *Egusi* melon in South Western Nigeria 2015			
Sheet No		**Age (wks)**	
Field No (GPS)		**Variety**	
Village/Town		**Intercrops**	
LGA		**Crops in Neighbouring field**	
State		**Farmers name**	
Latitude		**Percentage infection**	
Longitude		**Average severity**	
Elevation/Altitude		**Severity range**	
Field size			
Plant No	**symptoms**	**severity score**	**insects**
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
		Scoring scale	
	Leaf and stem		
1	No visible symptoms		
2	<10% damage portion		
3	10 - 30% damage portion		
4	31 - 50% damage portion		
5	>50% damage portion		
Summary:			