

A SEMINAR PRESENTATION

ON THE TOPIC:

**EVALUATION OF PLANT EXTRACTS IN THE CONTROL OF LEAF SPOT DISEASE
OF COWPEA (*Vigna unguiculata*)**

BY:

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Cowpea (*Vigna unguiculata* L. Walp) is the most indigenous grain legume in Nigeria and it is popularly known as "beans" in the local markets with different varietal names attached (Timko *et al.*, 2014). At present, more than 70% of the world cowpea production is concentrated in three countries -Nigeria, Brazil and Niger (Agbogidi and Egho, 2012). It is a rich source of plant protein, containing greater than 25% of protein (Timko *et al.*, 2014). However, there are many species of *Vigna unguiculata* (L) Walp. Cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world, where it is a major source of dietary protein that nutritionally complements staple low-protein cereal (rice) and tuber crops (such as yam), and is a valuable and dependable commodity that produces income for farmers and traders (Singh, 2002; Langyintuo *et al.*, 2003). Cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al.*, 2002; Tarawali *et al.*, 2002; Sanginga *et al.*, 2003).

Cowpea grain production estimates by Singh *et al.* (2002) are slightly higher than FAO estimates, with worldwide production of 4.5 million (mt) on 12 to 14 million ha. About 70% of this production occurs in the drier Savanna and Sahelian zones of West and Central Africa, where the crop is usually grown as either a sole crop or intercropped with other crops such; cassava, millet, wheat, etc. (Langyintuo *et al.*, 2003). Other important production areas include lower elevation areas of eastern and southern Africa and in South America (particularly in northeastern Brazil and in Peru), parts of India, and the southeastern and southwestern regions of North America (Tarawali *et al.*, 2002). Nigeria is the largest producer and consumer of cowpea grain, with about 5 million ha and over 2 million mt production annually, followed by Niger (650,000 mt) and Brazil (490,000 mt) (Singh *et al.*, 2002). As a result of this shift in production and the adoption of new varieties and improved production systems, worldwide cowpea production has gone from an annual average of about 1.2 million mt during the decade of the 1970s to ca. 3.6 million mt per annum (during the five-year period spanning 1998 to 2003) according to the FAOSTAT (2016). Rapidly growing populations with high per-capita cowpea consumption in the West and Central African regions have fueled demand for cowpea grain during this period, and the trend is expected to continue. Consequently, cowpea production is crippled with myriads of challenges, but significantly diseases such as; leaf spot disease (*Cercospora canescens*) is also cumbersome to manage. To mitigate these challenge of disease infestation, strategies such as the use of fungicides, cultural practices and integrated disease management have been employed. Recently, the use of botanicals or plant extract to control plant diseases have attracted interest by researchers (Suleiman and Emua, 2009; Seint and Masaru, 2011) for further exploration, which is aim this work is geared.

1.2 Justification of the Study

Cowpea are susceptible to a wide range of pests and pathogens that attack the crop at all stages. In Nigeria, fungi constitute the major limiting factor to the production of cowpea. Losses caused by fungi attack vary from 20 to almost 30% (William, 1975). Since the end of the Second World War, there has been a great boom in the use of fungicides throughout the world. After the great justified alarm in the early 60s about the dangerous consequences to man and environment in the area of phytotoxicity (Williams, 1975), there is an urgent need for alternative method of plant disease control. This scenario necessitates the search for and the development of ecologically sustainable fungi control method which are effective against the target species but create minimal adversity for non-target species. Historical successes have been recorded in the use of azadirachtin (from neem) and similar alkaloids, flavonoids, terpenoids from aloe, ginger and bitter kola as biopesticides and fungicides. Cold-water extracts of *A. indica* (neem), Siam weed and bush cane leaf extract at various concentrations possess fungicidal activity against the mycelial growth and sclerotial germination of fungus as reported by Wokocha and Okereke (2005). Due to identifiable problems (e.g. chemical residues, biodegradation, phytotoxicity, pollution, etc.) associated with chemical control strategies, alternative control methods are being attempted. The aim of this research is to provide useful information on cheaper, affordable, natural and environmentally friendly pesticide in the control of Cercospora leaf spot disease (*Cercospora canescens*) of *Vigna unguiculata*.

1.3 Objective of the Study

The major objectives of this study are;

1. To determine the effect of neem leaf extract in the control of Cercospora leaf spot disease of cowpea (*Vigna unguiculata*)
2. To determine the effect of Siam weed extract in the control of Cercospora leaf spot disease of cowpea (*Vigna unguiculata*)
3. To determine the effect of bush cane leaf extract in the control of Cercospora leaf spot disease of cowpea (*Vigna unguiculata*)

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and Distribution of Cowpea (*Vigna unguiculata*)

Cowpea (*Vigna unguiculata* L. Walp.) is a member of the Phaseoleae tribe of the Leguminosae family. Members of the Phaseoleae include many of the economically important warm season grain and oilseed legumes, such as soybean (*Glycine max*), common bean (*Phaseolus vulgaris*), and mungbean (*Vigna radiata*) (Singh *et al.*, 2002). The name “cowpea” probably originated from the fact that the plant was an important source of hay for cows in the southeastern United States and in other parts of the world. Some important local names for cowpea around the world include “niebe,” “wake,” and “ewa” in much of West Africa and “caupi” in Brazil. In the United States, other names used to describe cowpeas include “southern peas,” “blackeyed peas,” “field peas,” “pinkeyes,” and “crowders.” These names reflect traditional seed and market classes that developed over time in the southern United States (Timko *et al.*, 2014). Cowpea most certainly evolved in Africa, as wild cowpeas only exist in Africa and Madagascar. Interestingly, while West Africa appears to be the major center of diversity of cultivated forms of cowpea and was probably domesticated by farmers in this region, the center of diversity of wild *Vigna* species is southeastern Africa (Ba *et al.*, 2004). Some evidence that domestication occurred in northeastern Africa, based on studies of amplified fragment length polymorphism (AFLP) analysis, has also been presented (Coulibaly *et al.*, 2002). The wild cowpea *Vigna unguiculata* ssp., *unguiculata* var. *spontanea* is the likely progenitor of cultivated cowpea (Pasquet, 1999). It is likely that the crop was first introduced to India during the Neolithic period, and therefore India appears to be a secondary center of genetic diversity. “Yardlong beans,” a unique cultivar group (*Sesqui pedialis*) of cowpea that produces very long pods widely consumed in Asia as a fresh green or “snap” bean, apparently evolved in Asia and is rare in African landrace germplasm. Cowpea has been cultivated in southern Europe at least since the 8th century BC and perhaps since prehistoric times (Tosti and Negri, 2002). Cowpea was introduced to the West Indies in the 16th century by the Spanish and was taken to the USA about 1700. Presumably it was introduced into South America at about the same time.

2.2 Botany and Cultivars of cowpea

Cowpea is an annual herb with a strong principal root and many spreading lateral roots in surface soil. The root system having large nodules is more extensive than those of soybean (Tosti and Negri, 2002). *Bradyrhizobium* spp are the specific symbiotic nodular bacteria. Growth forms vary and may be erect, trailing, climbing or bushy, usually indeterminate under favourable conditions. Leaves are alternate and trifoliate usually dark green. The first pair of them is simple and opposite. Stems are striate, smooth or slightly hairy, sometimes tinged with purple. (Aveling, 1999). Flowers are self-pollinating and may be white, dirty yellow, pink, pale blue or purple in colour. They are arranged in raceme or intermediate inflorescences in alternate pairs. Flowers open in the early day and close at approximately midday, after blooming they wilt and collapse (Carsky *et al.*, 2002). Pollinating insect activities are beneficial in increasing the number of pod set, the number of seeds per pod or both; Fruits are pods that vary in size, shape, colour and texture. They may be erect,

crescent shaped or coiled (Aveling, 1999). Usually yellow when ripe, but may also be brown or purple in colour. There are usually 8-20 seeds per pod. Seeds vary considerably in size, shape and colour. They are relatively large, 2-12 mm long and weigh 5-30 g/100 seeds. Seed shape could be reniform or globular (Carsky *et al.*, 2002). The testa - the coat covering the grain - may be smooth or wrinkled; white, green, red, brown, black, speckled, blotched, eyed (the hilum - central line - is white surrounded by a dark ring) or mottled in colour. (Aveling, 1999).

Cultivated cowpeas have been divided into five cultivar groups based mainly on pod and seed characteristics as shown in Table 2.1 (Pasquet 1999). Cultivar group *Unguiculata* is the largest and includes most medium- and large-seeded African grain and forage-type cowpeas. Cultivar group *Melanophthalmus* includes “blackeye pea”-type cowpea with large, somewhat elongated seeds with wrinkled seed coats and fragile pods (Pasquet 1998). Members of cultivar group *Biflora* (also known as “*catjang*”) are common in India and characterized by their relatively small smooth seeds borne in short pods that are held erect until maturity. Cultivar group *Textilis* is a rather rare form of cowpea with very long peduncles that were used in Africa as a source of fiber. Cultivar group *Sesquipedialis* (known as “yardlong bean,” “long bean,” “Asparagus bean,” or “snake bean”) is widely grown in Asia for production of its very long (40 to 100 cm) green pods that are used as “snap” beans (Aveling, 1999).

Table 2.1: The five cultivar groups of cultivated cowpea

Cultivar group	Selected feature
Unguiculata	Includes most African grain and forage types. More than 16 ovules/pod.
Melanophthalmus	Blackeye pea types. Less than 17 ovules/pod. Grown mostly in the Americas.
Biflora (Catiang)	Smooth seed in short erect pods. Common in India. Less than 17 ovules/pod.
Sesquipedalis	Asparagus or yard-long beans. Very long pods consumed fresh, especially in the People’s Republic of China.
Textilis	Rare form with very long peduncles once used for fibre in Africa.

Source: Pasquet, 1999

2.3 Classification of Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp.) belongs to the family *Fabaceae* (*Leguminosae* is also used as the family name with *Papilionoideae* as the subfamily), genus *Vigna*, and section *Catjang*.

Kingdom	<i>Plantae</i>
Order	<i>Fabales</i>
Family	<i>Fabaceae</i>
Genus	<i>Vigna</i>
Species	<u><i>unguiculata</i></u>

Botanical varieties 1. *Vigna unguiculata unguiculata* var. *unguiculata*

2. *Vigna unguiculata unguiculata* var. *spontanea*

Sources: *Pasquet, 1999; Timko et al., 2014*

2.4 Economic Importance of Cowpea

Cowpea can be used at all stages of growth as a vegetable crop, and the leaves contain significant nutritional value (Ahenkora *et al.*, 1998). The tender green leaves are an important food source in Africa and are prepared as a pot herb, like spinach. Immature green pods are used in the same way as snap beans, often being mixed with cooked dry cowpeas or with other foods. Nearly mature “fresh-shelled” cowpea grains are boiled as a fresh vegetable or may be canned or frozen. Cowpea plays a critical role in the lives of millions of people in Africa and other parts of the developing world, where it is a major source of dietary protein that nutritionally complements staple low-protein cereal and tuber crops, and is a valuable and dependable commodity that produces income for farmers and traders (Singh, 2002; Langyintuo *et al.* 2003). Cowpea is a valuable component of farming systems in many areas because of its ability to restore soil fertility for succeeding cereal crops grown in rotation with it (Carsky *et al.*, 2002; Tarawali *et al.*, 2002; Sanginga *et al.*, 2003). Early maturing cowpea varieties can provide the first food from the current harvest sooner than any other crop (in as few as 55 days after planting), thereby shortening the “hungry period” that often occurs just prior to harvest of the current season’s crop in farming communities in the developing world. Dry grain for human consumption is the most important product of the cowpea plant, but fresh or dried leaves (in many parts of Asia and Africa) (Nielsen *et al.*, 1997; Ahenkora *et al.*, 1998), fresh peas (the southeastern USA and Senegal), and fresh green pods (humid regions of Asia and in the Caribbean) may be the most important in some local situations. Cowpea hay plays a particularly critical role in feeding animals during the dry season in many parts of West Africa (Singh and Tarawali 1997; Tarawali *et al.*, 2002). Besides being low in fat and high in fiber, the protein in grain legumes like cowpea has been shown to reduce low-density lipoproteins that are implicated in heart disease (Phillips *et al.* 2003). In addition, because grain legume starch is

digested more slowly than starch from cereals and tubers, their consumption produces fewer abrupt changes in blood glucose levels following consumption (Phillips *et al.*, 2003). Innovative and appealing processed-food products using dry cowpea grain, such as cowpea-fortified baked goods, extruded snack foods, and weaning foods, have been developed (Phillips *et al.*, 2003). Protein isolates from cowpea grains have good functional properties, including, solubility emulsifying and foaming activities (Rangel *et al.*, 2004), and could be a substitute for soy protein isolates for persons (especially infants) with soy protein allergies (Timko *et al.*, 2014).

2.5 Proximate and Nutritional Composition of Cowpea

The nutritional content of cowpea grain is important because it is eaten in quantity by millions of people who otherwise have diets lacking in protein, minerals, and vitamins. The nutritional profile of cowpea grain is similar to that of other pulses, with a relatively low fat content and a total protein content that is two to four times greater than cereal and tuber crops. Like other pulses, the protein in cowpea grain is rich in the amino acids lysine and tryptophan, compared to cereal grains. However, it is deficient in methionine and cystine when compared to animal proteins. In a study of 100 cowpea breeding lines in the IITA collection, seed protein content ranged from 23 to 32% of seed weight. Similarly, protein content of 12 West African and US cultivars ranged from 22 to 29%, with most accessions having protein content values between 22 and 24% (Hall *et al.*, 2003). These results suggest that sufficient genetic variation exists to develop new cowpea cultivars with protein content of at least 30%. Cowpea grain is also a rich source of minerals and vitamins (Hall *et al.*, 2003) and it has one of the highest levels of any food of folic acid, a crucial B vitamin that helps prevent spinal tube defects in unborn children. Fat contents of 100 advanced breeding lines from IITA showed a range in fat contents from 1.4 to 2.7%, while fiber content is about 6%.

Table 2.2. Nutrient composition of cowpea (%)

Nutrient	Seeds	Hay	Leaves
Carbohydrate (%)	56	66	8
Protein (%)	22	24	4.7
Water (%)	11	18	85
Crude fibre (%)	5.9-7.3	9.6	2
Ash (%)	3.4	3.9	23.3
Fat (%)	1.3-1.5	11.3	0.3
Phosphorous (%)	0.146	2.6	0.063
Calcium (%)	0.104	0.076	0.256
Iron (%)	0.005	0.005	0.005

Source: Quass, 1995; OECD. 2015

2.6 Global Cowpea Production Statistics

Cowpea is one of the most important legume crop globally. The United Nations Food and Agricultural Organization (FAO, 2016) estimates that nearly 4 million metric tons (mt) of dry cowpea grain is produced annually on about 10 million ha worldwide. Cowpea grain production estimates by Singh *et al.*, (2002) are slightly higher than FAO estimates, with worldwide production of 4.5 million (mt) on 12 to 14 million ha. About 70% of this production occurs in the drier Savanna and Sahelian zones of West and Central Africa, where the crop is usually grown as an intercrop with pearl millet (*Pennisetum glaucum* (L.) R.Br.) or sorghum (*Sorghum bicolor* (L.) Moench) and, less frequently, as a sole crop or intercropped with maize (*Zea mays* L.), cassava (*Manihot esculenta* Crantz), or cotton (*Gossypium* sp.) (Langyintuo *et al.*, 2003). Other important production areas include lower elevation areas of eastern and southern Africa and in South America (particularly in northeastern Brazil and in Peru), parts of India, and the southeastern and southwestern regions of North America. Nigeria is the largest producer and consumer of cowpea grain, with about 5 million ha and over 2 million mt production annually, followed by Niger (650,000 mt) and Brazil (490,000 mt) (Singh *et al.*, 2002). Estimates of cowpea grain production in Latin America and East and southern Africa, regions of the world that produce significant quantities of common beans (*Phaseolus vulgaris* (L.)), may be underestimates because cowpea grain is not always distinguished from common bean grain during collection of production statistics. Trades in dry cowpea grain and cowpea hay are important to the economy of West Africa in particular, with substantial quantities of cowpea grain being traded at the local and regional level (Singh 2002; Langyintuo *et al.* 2003). The large urban centers of coastal West Africa are huge markets for cowpea produced further inland where climates are drier and favorable to production of high-quality grain. The United States produces about 80,000 mt, in several southern states (Alabama, Arkansas, Georgia, Louisiana, Missouri, Tennessee) and in Texas and California (Fery, 2002). A long-term drought in the Sahelian zone of West Africa has caused many farmers in this part of Africa to shift more of their production to cowpea because of its drought tolerance (Duivenbooden *et al.*, 2002). As a result of this shift in production and the adoption of new varieties and improved production systems, worldwide cowpea production has gone from an annual average of about 1.2 million mt during the decade of the 1970s to ca. 3.6 million mt per annum (during the five-year period spanning 1998 to 2003) according to the FAOSTAT (2016). Rapidly growing populations with high per-capita cowpea consumption in the West and Central African regions have fueled demand for cowpea grain during this period, and the trend is expected to continue.

2.9 Agronomic Characteristics and Ecology of Cowpea

Cowpea is an herbaceous warm-season annual having leaves are generally darker green, shinier, and less pubescent (Duivenbooden *et al.*, 2002). Plant growth habit can be erect, semierect, prostrate (trailing), or climbing depending mostly on genotype, although photoperiod and growing conditions can also affect plant stature. Most cowpea accessions have indeterminate stem and branch apices. Early flowering cowpea genotypes can produce a crop of dry grain in 60 days, while longer season genotypes may require more than 150 days to mature depending on photoperiod (Tarawali *et al.*, 2002). Cowpea has considerable adaptation to high temperatures and

drought compared to other crop species (Hall *et al.* 2002; Hall 2004). As much as 1000 kg ha⁻¹ of dry grain has been produced in a Sahelian environment with only 181 mm of rainfall and high evaporative demand (Hall, 2004). Presently available cultivars of other crop species cannot produce significant quantities of grain under these conditions. The crop is more tolerant of low fertility, due to its high rates of nitrogen fixation, effective symbiosis with mycorrhizae and ability to better tolerate soils over a wide range of pH when compared to other popular grain legumes (Fery 1990; Tarawali *et al.*, 2002).

2.10 Constraints of Cowpea

The production statistics of cowpea would be increase, but there are some setbacks due to challenges faced by producers. According to Timko *et al.*, 2014 and Tarawali *et al.*, 2002, small holders and medium scale farmers are facing five factors that constitute the mayor constraint on cropping, storage and consumption of cowpea. These constraints include the following:

1. **Abiotic:** erratic rainfall, high soil temperatures, low soil fertility and degraded fragile soils
2. **Biotic:** insect pests, parasitic weed, diseases induced by fungi, viruses and nematodes
3. **Socio-economic:** farmer capacity to produce inputs is limited and input delivery systems function poorly. Seed of improved varieties (e.g. Melakh and Mouride) is not widely available. The difficulty is linked to the high value of cowpea green pods for family consumption and sale. Farmers are reluctant to leave any improved variety mature for seed.
4. **Socio-cultural:** low acceptability of cowpea new formulation as well as low adoption of some improved post-harvest technologies. Change in taste and urbanization, which has favoured the importation of food and the neglect of indigenous food crops.
5. **Political:** negative or neglected position of the developing countries governments to resolve the problems associated with the development of post-harvest systems.

2.10.1 Pest of Cowpea

Pest Species Most cowpea farmers in sub-Saharan Africa are confronted with low yields, caused by insect pests and diseases. Over the past few years, however, this picture has been gradually changing due to the establishment of a regional pest management project. Cowpeas are susceptible to a wide range of pests and pathogens that attack the crop at all stages of growth. These include insects, bacteria, viruses, fungi and weed. Some 40 species of fungi are cowpea pathogens (Hall *et al.* 2002). Insects are the main pests during the growing season are the aphids, the main storage pests are the bruchids. Both of these pests can severely reduce the yield of cowpea or the stored grain (Quinn, 1999). The primary insect pest causing losses to stored cowpeas in West Africa is the cowpea weevil, *Callosobruchus maculatus*. Infestation begins in the field at low levels. After the crop is placed in storage, the insect population continues to grow until there is an obvious, severe infestation. Another bruchid pest of cowpea is *Bruchidus atrolineatus*. These insect causes losses primarily around harvest time, and does not reproduce in storage. (Ntoukam, *et al.*, 2000). Some common pests of cowpea include; Pod borer (*Maruca vitrata* and *Heliotis ssp*), Thrips (*Megalurothrip sjostedti*), Cowpea weevil (*Callosobruchus maculatus*), Another bruchid (*Bruchidus atrolineatus*), among others.

2.10.2 Diseases of Cowpea

Cowpea are susceptible to a wide range of pathogens that attack the crop at all stages causing disease. The pathogenic agents of this diseases include; viruses, bacteria, nematodes and fungi (Olotuah and Akerele, 2011). The common diseases reported are fusarium wilt, bacterial canker, southern stem blight, cowpea mosaic virus (and several other less prominent viruses), cercospora leaf spot, rust and powdery mildew. Table 2.3 is a summary of pathogens and the diseases the cause.

Table 2.3: Common Diseases of Cowpea

Fugal and Bacterial
<i>Septoria vignae</i> ; <i>S. vignicola</i>
Scab (<i>Elsinoë phaseoli</i>)
Brown blotch (<i>Colletotrichum capsici</i> and <i>C. truncatum</i>)
Cercospora leaf spot (<i>Cercospora canescens</i>)
Fusarium wilt (<i>Fusarium spp.</i>)
Rusts – <i>Uromyces appendiculatus</i> ; <i>Phakopsora pachyrhizi</i>
Anthracnose (<i>Colletotrichum destructivum</i>)
Powdery mildew (<i>Erysiphe polygoni</i>)
Ashy stem blight (<i>Macrophomina phaseolina</i>)
Ascochyta blight (<i>Ascochyta phaseolorum</i>)
Pythium stem rot (<i>Pythium aphanidermatum</i>)
Sclerotium stem rot (<i>Sclerotium rolfsii</i>)
Bacterial blight (<i>Xanthomonas campestris</i>)
Bacterial pustule (<i>Xanthomonas axonopodis</i>)
Viruses
Cowpea aphid-borne mosaic virus (CABMV)
Blackeye cowpea mosaic virus (BICMV)
Cowpea mosaic virus (CPMV)
Cowpea severe mosaic virus (CSMV)
Southern bean mosaic virus (SBMV)
Cowpea mottle virus (CPMoV)
Cowpea golden mosaic virus (CGMV)
Cowpea chlorotic mottle virus (CCMV)
Nematodes
Root knot nematode – <i>Meloidogyne incognito</i> ; <i>M. javanica</i>
Cyst nematode (<i>Heterodera spp</i>)

Source: Olotuah and Akerele, 2011; OECD, 2015; Van, 1999

2.11 Effect of Plant Extracts in Plant Diseases Control

To mitigate the challenge of diseases in plant, there have been a major use of pesticides and chemicals throughout the world. After the great justified alarm in the early 60s about its dangerous consequences to man and environment in the area of phytotoxicity, there is an urgent need for alternative method of plant disease control (Suleiman and Emua, 2009). This scenario necessitates the search for and the development of ecologically sustainable diseases control method which are effective against the target species but create minimal adversity for non-target species. Historical successes have been recorded in the use of azadirachtin (from neem) and similar alkaloids, flavonoids, terpenoids from aloe, ginger, bitter kola, among other plant extracts as biopesticides (Suleiman and Emua, 2009). In the study of Oparaeke *et al.*, (2005), the insecticidal efficacy of aqueous extracts of five Nigerian spices (*Piper guineense* Schum and Thonn., *Aframomum melegueta* (Roscoe), *Xylopia aethiopica* (Dunal) A. Rich., *Zingiber officinale* L. and *Capsicum annum* L.) was tested in a field study for the control of two important post-flowering insect pests, *Maruca vitrata* Fab. (Lepidoptera: *Pylalidae*) and *Clavigralla tomentosicollis* Stal. (Hemiptera: *Coreidae*) of cowpea. The extracts were applied at 10% (w/v) and sprayed every week for 4 weeks. *P. guineense*, followed by *A. melegueta*, significantly reduced ($P < 0.01$) abundance of the pests and decreased the damage to cowpea pods. Grain yields were significantly higher in plots treated with *P. guineense* and *A. melegueta* extracts compared to plots treated with other extracts. Furthermore, in the study of Nahunnaro and Ibayaso (2011), evaluated the effect of two plant extracts, *Ricinus communis* and *Chromolaena odorata* on the control of the early blight pathogen, *Alternaria solani*. Three concentrations (25%, 50%, and 100%) of each plant extract were determined for inhibitory activity of *A. solani* growth. From the radial growth results, it revealed that *Ricinus communis* at 100 % concentration was recorded for the lowest radial growth rates of 1.43 cm, 2.00 cm and 2.72 cm at 24, 48 and 72 hours were recorded, respectively. It was concluded that the plant extracts used at different concentrations showed promising prospects for control *Alternaria solani* growth in vitro. Additionally, in the study of Suleiman and Emua (2009), on the use of ginger (*Zingiber officinale*), aloe (*Aloe vera*), bitter kola (*Garcinia kola*) and neem (*Azadirachta indica*) extracts in the control of root rot of cowpea caused by *Pythium aphanidermatum* was carried out in vitro and in the field (in vivo). They were evaluated for their antifungal activity over *P. aphanidermatum*, a rot fungus of many economic crops. Vegetative growth values over the fungus at 40, 60, 80 and 100% concentrations were generally low compared with the control, complete inhibition of fungal mycelia growth was exhibited at all concentrations in ginger extract. Aloe at 60% completely inhibited mycelial growth; this was followed by bitter kola with only retardation of mycelial growth while neem was the least effective. Highest mycelial dry weight was noticed on bitter kola extract but more sporangia formation in neem after a prolonged incubation. However, the extracts were sparingly effective for a short period on the field experiment. A statistical variance showed a significant difference between mycelial radial growths values recorded on the various plant extracts concentrations used compared with the control. Conclusively, this technology is cheap, safe, environmentally friendly and easy to adopt by limited resource farmers in third world countries.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The in-vitro assessment of this study will be carried out in the laboratory of Crop Science and the field study will be carried out at the teaching and research farm of faculty of agriculture, Akwa Ibom State University, Obio Akpa Campus, Oruk Anam Local Government Area, Akwa Ibom State respectively. The area lies between the humid tropical rainforest zone of south southern Nigeria with latitude $4^{\circ}30'N$ and $5^{\circ}00'N$ and longitudes $70^{\circ}30'E$ and $80^{\circ}00'E$. It records annual rainfall of 2000-2600mm. monthly sunshine of 3.13 hours and the mean annual temperature of $27^{\circ}C$ (SLUS-AK, 1989). The areas have a relative humidity of 88%, wind speed of 0.4m/s and evaporation rate of 2.3cm. The rainfall pattern is bimodal with long (march to July) and short (September to November) with a short period of relative moisture stress in August traditionally known as August break. The soil is sandy loam (SLUS-AK, 1989).

3.2 Experimental Design and Treatments

The field experiment will be laid out in a randomized complete block design (RCBD) with treatments and three replications. Each sub-plot measured of 3m X 8m and consisted of 3 rows as shown in figure 1, having a net plot of 2m x 5m and maximum 4m x 15 m with planting distance of 1m X 1m.

The treatments to use include; plant extract from neem leaf, siam weed and bush cane leaf extract. It consists of 21 experimental units. The control unit will be standard fungicides.

3.3 Soil Sampling

Prior to planting the soil will be randomly sampled at the depth of 0.15cm at three different location or spots in the area. The soil samples will be bulked together to obtain a representative sample, the representative sample will be air dried and sieved with 2mm sieve before being taken to the laboratory for analysis.

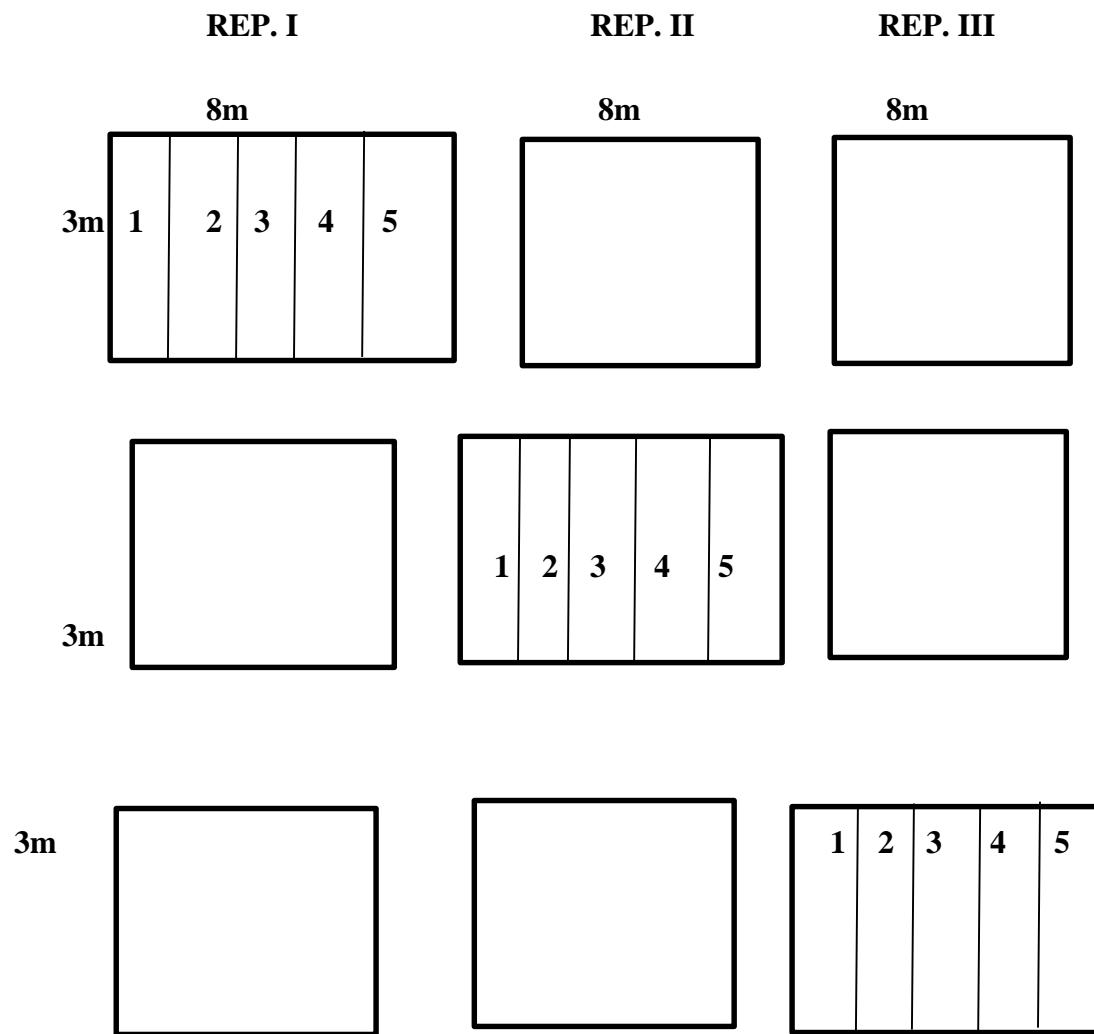
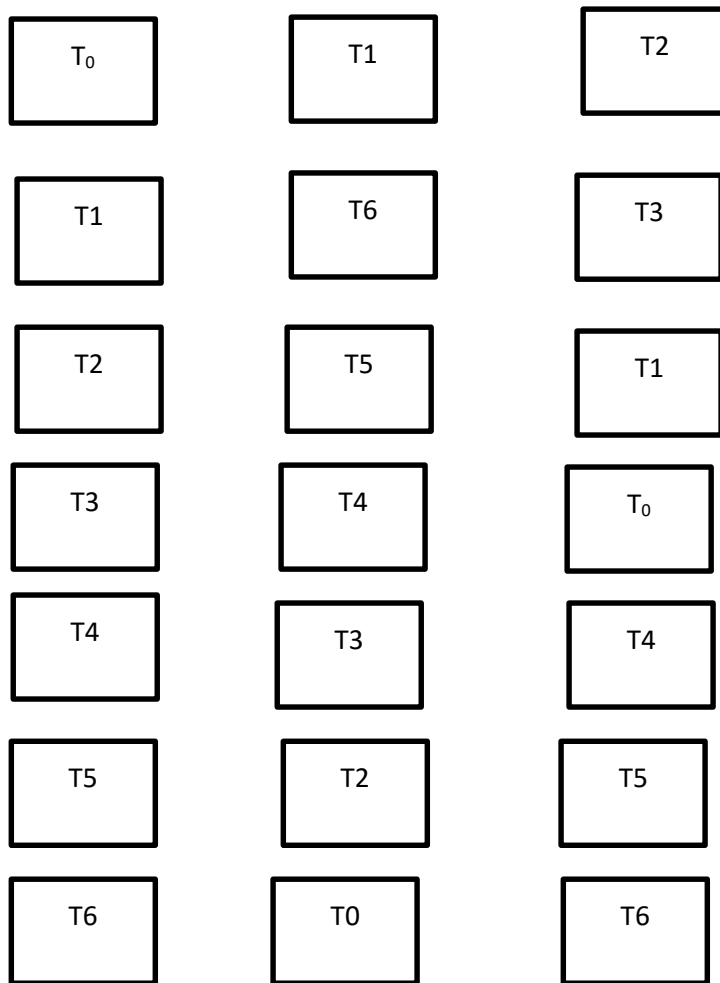


Figure 1; Field layout of the experiment



T₀ – Standard fungicides; T₁- 3% Siam leaf extract; T₂ – 5% Siam leaf extract; T₃ – 3% Bush cane leaf extract; T₄ – 5% Bush cane leaf extract T₅ – 3% Neem leaf extract; T₆ – 5% Neem leaf extract

Figure 2; Layout of the Treatments

3.4 Agronomic Practices

3.4.1 Land Preparation

The field will be cleared manually using cutlass and tilled with spade. Stumping (if any) and beds making will also be done manually through the use of spade.

3.4.2 Planting

Planting material will be done using cowpea seeds purchase from Udua Abak with a planting distance of 1m x 1m.

3.4.3 Fertilizer application

Organic manure in the form of poultry dung will be used alongside Compound Fertilizer (N: P: K 15:15:20) will be applied at the rate of 50 g/vine (500 kg/ha) by ring application, 12 days after planting.

3.4.4 Weeding

Weeding will be done manually using hoe at 2 week intervals.

3.5 In-vitro Assessment Laboratory Procedure

Based on previous biological activities, leaves of neem, bush cane and siam leaves will be collected for this study.

The fungus (*Cercospora canescens*) will be isolated from infected leaves of cowpea from the study areas. Pathogenicity test will be carried out according to Koch's postulate. Fresh matured leaves and seeds from each of the plants will be plucked, thoroughly rinsed in running tap water before they will be air-dried in the laboratory and pounded in a mortar to facilitate extraction.

Hot water extraction will be obtained by infusing 40, 60, 80 and 100 g each of the plant leaf extract separately in 100 ml of sterile distilled water, using a 250 ml Erlenmeyer's flask in a water bath set at 100°C for 30 min. This will be allowed to cool and the crude extract obtained from the infusion by filtration through 4 folds of sterile cheese cloth, to give concentrations of 40, 60, 80 and 100% respectively as described by Wokocha and Okereke (2005).

Each of the extract concentration will be kept aseptically in 150 ml conical flasks. The contents in the flasks will be exposed to U/V light for further sterilization. The linear growth will be carried out on extracts of neem leaf, bush cane leaves and siam leaf on potato dextrose agar. Mycelia A disc of 5 mm diameter (using a sterile cork-borer) of the fungus will be placed on the thin film formed on the PDA just at point of experiments had distilled water in place of plant extract respectively. The treatments and control will be incubated for 5 days at room temperature ($27 \pm 2^\circ\text{C}$). The diameter of the radial growth of the fungi will be measured at the end of incubation period and then used to determine the inhibition of each extract using the formula:

$$\text{Mycelial growth inhibition (\%)} = [(dc-dt) / dc] \times 100$$

Where dc = average diameter of fungal colony in the control

dt = average diameter of fungal colony in treatment group.

For the in vivo experiment, seeds of cowpea will be sown at the rate of 3 seeds in each plastic pot containing sterilized field soil. Potted plants will be randomly arranged in 3 groups and watered twice daily with tap water. Plants in the first group will be drenched inoculated with the sporangia suspension (3×10^4 sporangia/ml distilled water) 2 days after the plant extracts will be applied as soil drench, going by the method described by Amadioha (2003). Plants in the second group will be inoculated with sporangia suspension 2 days before application of plant extracts, a modified method of Fernando and Linderman (1993). The 3 replicated pots per treatment and the control will be set up in a completely randomized block design. The disease incidence will be determined using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected plants}}{\text{Total Number of plants}} \times 100$$

The Disease severity of Cercospora leaf spot is rated in the scale of 1-4.99. 1.0-1.99 rating no infection, 2.0-2.99 rating Low infection, 3.0-3.99 rating Moderate Infection and 4.0-4.99 rating severe infection. The disease severity will be determined using the formula:

$$\text{Disease Severity (\%)} = \frac{\text{Sum (Rating Number X Number of plants in the rating)}}{\text{Total Number of plants X Highest Rating}} \times 100$$

3.6 Data Collection

3.6.1 Growth Parameters

➤ Plant height (cm)

The plant height will be measured from the base of the plant to the terminal growing point using a measuring rule. The average plant height will be worked out and expressed in centimeters.

➤ Number of leaves per plant

The number of leaves per plant will be measured physically by counting the leaves at interval.

➤ Number of branches per plant

The number of branches per plant will be counted for each plants.

3.6.2 Yield Parameters

➤ **Total number of fruits**

The total numbers of fruits from the plants will be counted and the average total numbers of fruits will be worked out.

➤ **Fruit weight (g /fruit)**

The numbers of fruits from each treatment will be weighed and worked out for single fruit weight and expressed in grams.

➤ **Fruit yield (t/ha)**

The fresh fruit yield from the net plot area will be taken to calculate the unit yield per hectare.

3.6 Statistical Analysis and Interpretation of Data

The data on various parameters were subjected to Fischer's method of analysis of variance and interpretation of data will be done as described by Gomez and Gomez (1984). The level of significance used in F and t-test will be 0.05. Critical difference (CD) values were calculated whenever the F-test will be significant.

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