UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

EVALUATION OF PLANT EXTRACTS FOR THE MANAGEMENT OF CERCOSPORA LEAF SPOT OF GROUNDNUT (*Arachis hypogaea* L.)

MOSES NEINDOW

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BY

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July, 2017

DECLARATION

Student

I, Moses Neindow hereby declare that this thesis is based on my own research and that no part of it has been submitted for another degree in this university or elsewhere.

Candidate's Signature: ……. Date …….

Name: Moses Neindow

Supervisor

I hereby declare that the preparation and presentation of the thesis was supervised in accordance with the guidelines on supervision of thesis laid down by the University for Development Studies.

Supervisor's Signature:…… Date:…….

Name: Prof. Elias N. K. Sowley

DEDICATION

This thesis is dedicated to my lovely wife, Ruth Abdulai and my children - Godswill Wuniyubu Neindow, Gloria Wunifaako Neindow and Gracious Nasara Neindow.

ABSTRACT

Cercospora Leaf Spot (CLS) caused by *Cercospora arachidicola* and *Cercosporidium personatum* of groundnut (*Arachis hypogaea* L.) is a major constraint against groundnut cultivation. In the wake of rising cost of chemical control, ecological-friendly methods of curbing CLS were studied. The main objective of the study was to determine the effectiveness of Desert date seed extract (DDSE), Neem seed extract (NSE), Jatropha seed extract (JSE) and Tobacco leaf extract (TLE) for the control of CLS disease of groundnut. The study comprised field survey, laboratory studies, green house and field experiments. Multi-stage sampling technique was used for the field survey. The field experiment employed a factorial experiment consisting of 18 treatments laid out in a Randomised Complete Block Design with four replications per treatment for two consecutive years. Farmers' responses during the field survey showed CLS as a major constraint to groundnut production in Northern Region of Ghana. Farmers described the disease incidence as well as the disease severity to be above 50 %. *In vitro* studies indicated that aqueous DDSE, NSE, JSE and TLE at 100 g/l significantly inhibited mycelial growth of both *Cercospora arachidicola* and *Cercosporidium personatum* by 90.3 %, 80.8 %, 75.6 %, 54.5 % and 84.9 %, 73.3 %, 67.3 %, 59.4 % respectively. Pod yield was significantly more enhanced in plants treated with JSE, NSE, DDSE and Topsin-M, than those treated with TLE and the negative control plants for 2014 and 2015 cropping seasons with values ranging from 729 to 1095 and 931 to 1322 kg/ha respectively. For most of the parameters, DDSE produced similar results as Topsin-M followed by NSE and JSE. The adoption of DDSE, NSE and JSE as alternatives and better remedies to CLS disease control is recommended.

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LIST OF ACRONYMS

DDSE …… Desert date seed extract

CLS …… Cercospora Leaf Spot

CRD ……..…. Complete Randomised Design

CRIG ……. Cocoa Research Institute of Ghana

ELS ……. Early leaf spot

JSE …… Jatropha seed extract

LLS …….. Late leaf spot

NCERC ……Nuclear Chemistry and Environmental Research Centre

NNRI …….. National Nuclear Research Institute

NSE ……. Neem seed extract

GAEC ……. Ghana Atomic Energy Commission

PAS …….. Presbyterian Agricultural Services

RCBD …….. Randomised Complete Block Design

SARI …….. Savannah Agricultural Research Institute

TLE ……. Tobacco leaf extract

Topsin-M …….. Thiophanate methyl

WAP …… Week after planting

UDS …… University for Development Studies

UK …….. United Kingdom

USA ……. United States of America

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CHAPTER ONE

1.0 INTRODUCTION

Groundnut (*Arachis hypogaea* L.) which is widely cultivated as a staple food in tropical and sub-tropical developing countries is a valuable source of protein (25 − 28 %) and oil (48 – 50 %) (Nigam *et al.*, 1983; Janila *et al.*, 2013). It is also a rich source of dietary fibre, minerals and vitamins. The seed may be chewed raw, boiled or roasted. Groundnut hay (haulms) is a nutritious animal feed, particularly for the dry season when green forage is not available (Tshilenge-Lukanda *et al.*, 2012). In addition, groundnut seed and hay are often sold in local markets, providing income to the resource-poor farmers especially rural women (Tsigbey *et al*., 2003; Naab *et al.*, 2005; Nutsugah *et al.*, 2007).

According to Kombiok *et al*. (2012), groundnut remains the most popular and widely cultivated legume in Ghana because of its adaptation to a wide range of climatic conditions as well as limited field pest problems. In 2011, Ghana was ranked 10th in production volume (530,887 MT of in-shell groundnuts) in the world and 4th in Africa, after Nigeria, Senegal and Sudan (Ibrahim *et al*., 2012). It is an important cash crop in subsistence and commercial farming systems, as well as an important food source for the people in Northern Ghana (Tsigbey *et al*., 2003; Izge *et al*., 2007). It has become a cash crop for many agricultural communities in Northern Ghana where more than 90 % of farm families cultivate groundnuts (Tsigbey *et al.*, 2003). Also, being a legume crop, groundnut helps in improving soil health and fertility by fixing N_2 and organic matter in the soil (Janila *et al.*, 2013).

In spite of its importance, groundnut production in Africa has fluctuated greatly, although it never exceeded 8 % of the world's output over the last decade (Ambang *et al*., 2011). Yields per hectare are low, because of a combination of biotic and abiotic stresses. Abiotic stresses such as low temperature during the crop's germination and vegetative stages, high temperature during the pod filling and maturation stages and soil moisture deficit stress at various stages of the crop growth hamper the crop's productivity (Joshi, 2005; Ambang *et al*., 2011). Others include non-irrigated cultures; use of low-yielding seed varieties and increased cultivation on marginal lands (Ambang *et al*., 2011)**.** Among the biotic stresses, foliar fungal diseases (Early leaf spot, Late leaf spot, rust), viral diseases (rosette, bud and stem necrosis); soil borne diseases (stem rot, collar rot and pod rot complexes), insect pests such as defoliators (*Spodoptera, Helicoverpa*, red hairy caterpillar and leaf miner), and sucking pests (jassids, aphids, thrips) are the major ones that limit groundnut production and productivity (McDonald *et al.*, 1985; Subrahmanyam *et al.*, 1992; Hagan, 1998; Joshi, 2005; Nutsugah *et al.*, 2007). In addition, the pre- and post-harvest aflatoxin contamination in the kernels and meal also reduces the quality as well as export value (Joshi, 2005).

In Ghana, average yield is 800 kg/ha compared to developed countries with more than 3,000 kg/ha (Kombiok *et al*., 2012). This low yield is generally attributed to diseases such as Cercospora Leaf Spot (CLS), rust and rosette (Tsigbey *et al.*, 2003; Nutsugah *et al*., 2007; Ambang *et al*., 2008). Leaf spot can cause yield losses of 50 – 70% in West Africa and up to 50% worldwide as reported by Tshilenge-Lukanda *et al.* (2012). Both early and late leaf spots diseases are widely distributed and occur in epidemic proportions in Northern Ghana (Nutsugah *et al*., 2007). Combined attack of CLS and

rust can cause more than 80 % leaf defoliation of the groundnut crop during growth with associated pod losses (Tsigbey *et al.,* 2001). In Northern Ghana, pod losses due to CLS and rust can reach 78 and 23% respectively and high defoliation also affects hay quality which is fed to animals after harvest (Tsigbey *et al.*, 2003).

Most groundnut farmers in Northern Ghana do not practice any disease control measures on their fields and they often see leaf defoliation as a sign of the crop maturity (Tsigbey *et al.,* 2003; Nutsugah *et al.,* 2007). Many attempts have been made to develop groundnut cultivars that are resistant to CLS. Although researchers have developed and disseminated improved groundnut varieties to farmers, 50 % of farmers in the region still cultivate and produce highly CLS-susceptible cultivars such as 'Chinese' (Ibrahim *et al.,* 2012). Beside this, other control measures have also been used to check these foliar diseases. These include improved cultural practices on the farm and chemical control using fungicides which are found to be effective against leaf spot of groundnut (Nustugah *et al.,* 2007; Akinbode, 2010). The cultural practices include crop rotation, burning and burying of crop residues after harvest, removal of volunteer groundnuts, deep turning of crop debris which are seldom applied by smallholder farmers for a number of reasons; such as inadequate land size, lack of information especially in carrying out crop rotation and labour intensiveness (Wilber, 2014). Effective chemical control is heavily reliant upon multiple fungicide applications which are costly for resource poor farmers in Ghana (Nutsugah *et al.,* 2007; Akinbode, 2010; Jordan *et al*., 2012). Chemical control is expensive, adding to labour, equipment and mechanical damage to plants during applications, all greatly increases the overall cost of production (Hagan, 1998; Akinbode, 2010). Furthermore, chemical control also raises environmental and health concerns (Akinbode, 2010;

Jordan *et al*., 2012). Generally, pathogens on their own, also build up resistance to fungicides.

Various strategies have been suggested for the control of Cercospora leaf spot disease of groundnut. The chemical method using fungicide is considered as an effective way of controlling the disease. The problems associated with the use of synthetic fungicides are well known today. Increasing concerns about environmental hazards caused by excessive use of fungicides, development of fungicide – tolerant pathogens strains, non-availability of both fungicides and their application technology to resource – limited farmers necessitates the development of more economical and ecological – friendly alternative methods of curbing the disease. Similarly, the cost of these chemicals is increasing every year resulting in high cost of production and thereby low net profit. In order to minimise the cost of production and also the toxic hazards of chemicals there are some indigenous plants possessing phytochemical elements against field pests and pathogens. Therefore, biofungicide has been proposed as a replacement of chemical control against plant disease. Plants extracts used to control plant pathogens can be obtained from a collection of plants which show geographical differences in the content of biologically active compounds. Various plants in Ghana need to be tested for their efficacy against certain field diseases of economic importance. The use of effective plant extracts can offer an economical, safe and easily available alternative method for the management of leaf spot disease of groundnut.

The study sought to;

i. assess farmers' knowledge and management of leaf spot disease of groundnut.

ii. determine the incidence and severity of Cercospora leaf spot disease on groundnut fields.

iii. isolate and identify Cercospora leaf spot pathogens from infected plants.

iv. determine the chemical constituents of *Azadirachta indica* (A) seed, *Jatropha curcas* (L) seed, *Balanites aegyptiaca* (L) seed and *Nicotiana tabacum* (L) leaf extracts, and

v. determine the efficacy of *A. indica* (A) seed, *J. curcas* (L) seed, *B. aegyptiaca* (A) seed and *N. tabacum* (L) leaf extracts for the control of Cercospora leaf spots of groundnut.

CHAPTER TWO

2. 0 LITERATURE REVIEW

2.1 Botany, origin, diversity and distribution of groundnuts

The cultivated groundnut (*Arachis hypogaea* L.), a self-pollinated legume with cleistogamous flowers, belongs to the genus *Arachis* in the family *Leguminosae*, sub-family *Fabaceae*, tribe *Aeschynomeneae*, subtribe *Stylosanthenae* (Krapovickas and Gregory, 1994; Janila *et al*., 2013). The genus *Arachis* is derived from the Greek word "arachos", meaning a weed, and hypogaea, meaning underground chamber, that is in botanical terms, a weed with fruits produced below the soil surface (Prasad *et al*., 2010). The genus *Arachis* originates from South America with 80 known species (Kochert *et al*., 1996; Valls and Simpson, 2005). These species were assembled into nine sections (*Arachis, Caulorrhizae, Erectoides, Extranervosae, Heteranthae, Procumbentes, Rhizomatosae, Trierectoidse and Triseminatae*) according to morphology, geographic distribution and crossability data (Krapovickas and Gregory, 1994; Valls and Simpson, 2005). The section *Arachis* has the widest geographical distribution with 31 species known but only *Arachis hypogaea* and *Arachis monticola* are tetrapliods (Kochert *et al*., 1996). The remaining species of section *Arachis* are diploid and grouped into three genomes (A, B and D), each having 20 chromosomes, with exception of three species which have 18 chromosomes (Kochert *et al*., 1996; Lavia, 2000). The cultivated groundnut is a tetraploid, arising from hybridization between A and B diploid species (*A. duranensis and A. ipaensis*) followed by rare spontaneous duplication of the chromosomes (Kochert *et al*., 1996).

Six centres of diversity of *A. hypogaea* have been identified in South America (Holbrook and Stalker, 2003). Africa is also an important secondary centre with large genetic variation (Gibbons *et al*., 1972; Hammons, 1982). Archaeological records indicate that *A. hypogaea* was first domesticated in Peru, dated ca.1500 (Banks *et al*., 1993). However, latest molecular data indicated its origin as South America (Kochert *et al*., 1996). The crop was cultivated in many parts of South America, as well as in the Caribbean and Mexico. During the 16th and 17th centuries, early Spanish and Portuguese explorers found indigenous people of Central and South America cultivating groundnut. As a result of explorations by the Spanish and Portuguese, groundnut cultivation spread quickly from America to Africa and Asia (Stalker, 1997). Groundnut is grown on six continents and in over 100 countries (Nwokolo, 1996).

The morphology, anatomy and reproductive development of groundnut has been described by many workers (Rao, 1988; Holbrook and Stalker, 2003). All members of the genus *Arachis* are distinguished from other plants by flowering above the ground and producing fruits below the ground (Holbrook and Stalker, 2003). The cultivated groundnut is an annual herb with two subspecies. The subspecies *hypogaea* has been characterized by absence of flower on the main stem and alternate vegetative and reproductive nodes. It includes two botanical varieties of *hypogaea* and the less-frequently cultivated *hirsuta.* The *fastigiata* subspecies is typified by flowers on the main stem and sequential reproductive nodes. It has four botanical varieties, *fastigiata* (Valencia type), *vulgaris* (Spanish type), *peruviana*, and *aequatoriana* (Krapovickas and Gregory, 1994). Only *A. hypogaea* has been domesticated although several species have been cultivated for their edible seed (*A. Villosulicarpa* Hoehne and *A. Stenosperma* Krapov. and W. C. Gregory) or forage (*A. repens* Handro, *A. pintoi*

Krapov. & W. C. Gregory and *A. glabrata* Benth) (Coffelt and Simpson, 1997; Valls and Simpson, 2005).

2. 2 Groundnut production and economic importance in the world

Groundnut is grown worldwide mainly as a low input, small scale subsistence oilseed crop (Burns, 2010). Presently, it is the fifth most important oil seed crop in the world. Groundnut oil is versatile and has been widely used as a bio-fuel, in cooking, and as a food constituent. However, in Ghana and other parts of the world such as United States, groundnut is used primarily as a food product for direct consumption, for example, groundnut butter, dry roasted nuts, and flour (Burns, 2010). Nutritionally, groundnut is high in protein, as well as mono- and poly-unsaturated fats (e.g. linoleic and oleic acids) (Mali and Bodhankar, 2009). In many developing countries, groundnut serves as a crucial dietary component for the indigenous people (Burns, 2010). The crop is an important cash crop for small scale farmers of developing countries, hence an important source of income for farmers who sell groundnut seed and hay (Naab *et al*., 2005; Nutsugah *et al*., 2007; Debele and Ayalew, 2015). During the dry season when green forage is not available, groundnut hay (haulm) serves as a nutritious animal feed (Tshilenge- Lukanda *et al*., 2012).

Groundnut is produced in over 100 countries in the semi-arid tropical and subtropical regions of the world between 40^{o}N and 40^{o}S (Khan *et al*., 2014; Debele and Ayalew, 2015). China, India, Nigeria, USA and Myanmar are the leading groundnut producing countries in the world. Asia and Africa hold 11.6 million hectares (47.15 %) and 11.7 million hectares (47.56%) respectively as maximum global area under groundnut cultivation. Developing countries in Asia, Africa and South America account for over

97 % of world groundnut area and 95 % of total production. However, the productivity of Asia 2217 kg/ha and Africa 929 kg/ha is very poor as compared to Americas (3632 kg/ha (Ajeigbe *et al*., 2014). China led the world in groundnut production and value (13,079,363 metric tons (MT), Interest $6,112,785,000, respectively), followed by India (9,182,500 MT, Interest $4,205,879,000), Nigeria (estimated 3,835,600 MT, estimated Interest $1,778,082,000), and the USA (1,696,728 MT, Interest $778,851,000) (FAO, 2010).

Groundnut is usually grown as a smallholder crop in the semi-arid tropics under rainfed conditions. It is an important crop in many countries, especially in Sub Sahara Africa, where it is a good source of protein (25 – 34 %), cooking oil (48 – 50 %) and vitamins (Ajeigbe *et al*., 2014).

2. 3 Groundnut production in Ghana

Groundnuts are grown in many agro-environments. The agro-ecological zones in which groundnuts are grown are primarily classified as Guinea Savannah and Sudan Savannah areas, located primarily in the Northern, Upper East and Upper West regions of Ghana and some parts of the Forest Transitional zone (Figure 2.1).

These regions account for about 94 % of the country's groundnut production, which is normally cultivated under uni-modal rainfed conditions starting in April/May and ending in September/October, with a total annual precipitation varying between 900 and 1100 mm, followed by a period of dryness for post-harvest operations and

marketing (Tsigbey *et al.*, 2003; Masters *et al.*, 2013). Generally, groundnuts mature between 90 to 120 days depending on the variety.

Figure 2.1: Groundnut distribution map of Ghana. Source: (Tsigbey *et al.*, 2003)

Groundnut cultivation is both a commercial and subsistence farming venture for the people of Ghana more especially in the Northern, Upper East and Upper West regions, where almost everyone in farming communities cultivate it. Tsigbey *et al.* (2003) reported that more than 90 % of farm families of typical farming communities in northern Ghana farm groundnuts. This shows that groundnut is a major cash crop for the inhabitants of northern Ghana. Groundnut can be planted in rows, ridges, staggered on fields and sometimes on mounds (Tsigbey *et al.*, 2003). Field preparation is mostly carried out using tractors in large holdings whereas in smaller holdings bullock plough or hand hoeing is the preferred method (Tsigbey *et al.*, 2003). Planting is by hand and in most cases farmers use seed from their own stock or purchase from the local market.

In Ghana, groundnut is cultivated under rainfed conditions with low inputs from resource – limited farmers (Tsigbey *et al.*, 2003; Janila *et al.*, 2013). Farmers are restricted to mainly few cultivars and the selection of any is dependent on the rainfall regime in that location (Tsigbey *et al.*, 2003). Other production factors that limit yields of groundnut in Ghana include; small-scale traditional farming with little mechanization and increased cultivation on marginal lands as well as outburst of pest and diseases (Pazderka and Emmott, 2010).

2. 4 Diseases of groundnut

Every year, substantial crop damage is caused by various diseases and among them fungal disease is very common. According to Hewitt (2000), about 10 to 20 % of staple foods and cash crops are destroyed by plant pathogens and groundnut crop is no exception. Groundnut production is adversely affected by a large number of fungal,

viral and bacterial diseases. Most of these are widespread, but only a few of them are economically significant. Fungi cause seed rots and seedling diseases such as root rot, stem rot, wilts, blights and pod rot. The major foliar fungal diseases in Ghana include Early leaf spots (ELS), Late leaf spot (LLS), and rust.

2. 4. 1 Rust disease

Rust caused by *Puccinia arachidis* Speg. is one of the important foliar diseases that reduces seed quality and causes substantial losses to groundnut production worldwide. Combined attack of Late Leaf Spot and rust can cause more than 80 % leaf defoliation of the groundnut crop during growth with associated pod losses in the northern parts of Ghana (Tsigbey *et al.*, 2001). Pod losses due to rust in Northern, Upper East and Upper West regions is as high as 23 % (Tsigbey *et al*, 2003).

Rust is easily identified by the appearance of orange pustules (uredinia) on the abaxial (lower) surface of leaves and reddish-brown urediniospores (uredospores). Symptoms are mainly confined to leaflets but pustules can be seen on all the aerial parts of a plant except the flower. On rupturing, they produce masses of powdery, orange spores. In contrast to the rapid defoliation associated with leaf spots, leaves infected with rust become necrotic and dry up but tend to remain attached to the plant (Hagan, 1998). Groundnut rust can be equally as destructive as leaf spots disease when left uncontrolled. Epidemics of groundnut rust develop faster than those of Cercospora leaf spots. Spores of the groundnut rust fungus are short-lived and do not survive from year to year on groundnut crop debris as that of Cercospora leaf spots (McDonald *et al.*, 1985; Subrahmanyam *et al.,* 1992; Hagan, 1998).

2. 4. 2 Early and Late leaf spots Diseases

The groundnut crop is susceptible to both Early and Late leaf spot, caused by *Cercospora arachidicola* S. Hori (teleomorph: *Mycosphaerella arachidis* Deighton) and *Cercosporidium personatum* (Berk. & Curt) Deighton (teleomorph: *Mycosphaerella berkeleyi* Jenk.), respectively which can be found wherever groundnut is grown, making them the most significant of all groundnut pathogens (McDonald *et al.*, 1985; Zhang *et al*., 2001; Chaube and Pundhir, 2009). Infections of Early and Late leaf spots reduce the photosynthetic area by causing intense lesions on leaves, petioles, and stems that often lead to premature defoliation, loss of integrity of the peg, and hence yield loss. Pattee and Young (1982) reported reduction of leaf area index by 80 %, carbon dioxide uptake by 85 % and canopy carbon exchange rate by 93 % due to severe leaf spot damage. Photosynthesis of diseased canopies was reduced by defoliation and inefficient fixation of carbondioxide by diseased leaves. Globally, yield losses have been reported to be 50 % or more from early or late leaf spots if fungicides are not used (McDonald *et al.*, 1985; Zhang *et al*., 2001; Tshilenge-Lukanda *et al.*, 2012). Subrahmanyam *et al.* (1992) reported yield reductions of 20 to 100 % in South Africa and other parts of the world. Leaf spots in Uganda have been reported to cause over 60 % yield losses (Mugisha *et al*., 2004). Yussif (2010) reported 40-60% yield loss due to leaf spots of groundnut in Ghana. Both Early and Late leaf spots diseases are widely distributed and occur in epidemic proportions in Northern Ghana (Nutsugah *et al*., 2007). Regardless of past cropping history, Early leaf and Late leaf spots are significant threat to every field of groundnuts in Northern Ghana (Tsigbey *et al*., 2003; Nutsugah *et al*., 2007). Singly or together leaf spots can cause losses in pod yield as high as 78 % in Northern Ghana (Tsigbey *et al.*, 2003).

Besides reducing the yield, the disease also has an adverse effect on seed quality and grade characteristics, and quality of fodder which renders it unsuitable as animal feed (Bdliya, 2007). Moreover, the control of these diseases through application of fungicides not only increases the cost of cultivation but also leads to environmental and health hazards (Smith and Littrell, 1980; Culbreath *et al.*, 2002). The use of plant extracts with antifungal activity offers economical, safe and easily available alternative method for the management of leaf spots diseases of groundnut (Rahman and Hossain, 1996; Akinbode, 2010; Hossain and Hossain, 2013).

2. 4. 2. 1. Identification and classification of *Cercospora arachidicola* and *Cercosporidium personatum*

During the early production years of groundnut, leaf spots were regarded as common and natural feature of the groundnut plant (Backman *et al*., 1977; Nustugah *et al.*, 2007). The first documented description of an organism causing groundnut leaf spot was by Berkley (1875). Berkley identified a single fungal species and proposed the name *Cladosporium personatum* as being the causal agent of leaf spot disease. Studies following the work of Berkley led to a highly variable nomenclature and classification system for leaf spot disease. Comparison of specimens and earlier reports by Woodroof (1933) led to the determination that the causal agent of leaf spot disease was actually due to two distinct fungal organisms. The two fungi were identified and then named, *Cercospora arachidicola* Hori and *Cercosporidium personata* (Berk. and Curt.). The sexual stages for each pathogen was later identified by Jenkins (1938) and named *Mycosphaerella arachidicola* as causal agent of Early leaf spot and *Mycoshaerella berkeleyii* Late leaf spot. *C. personata* was later re-classified by

Deighton (1967) as belonging to the genus, *Cercosporidium*. Deighton re-named the pathogen *Cercosporidium personatum*.

2. 4. 2. 2 Symptoms and signs of Leaf spot diseases

Leaf spots diseases symptoms are influenced by host genotype and environmental factors. Early leaf spot and Late leaf spot diseases are characterised by small chlorotic spots which appear on leaflets 10 days after infection. The spots then develop in about 5 days into mature, sporulating lesions that reduce light interception and photosynthesis (Boote *et al.*, 1983; McDonald *et al.*, 1985). Lesions caused by *C. arachidicola* are subcircular and from 1 to over 10 mm in diameter (McDonald *et al.*, 1985; Chaube and Pundhir, 2009). They are dark brown on the adaxial (upper) leaflet surface where most sporulation occurs, and a lighter shade of brown on the abaxial (lower) leaflet surface. Lesions caused by *Cercosporidium personatum* are usually smaller, more nearly circular, and darker in colour than those of *C. arachidicola* (Plate 2.1). On the abaxial surfaces, where most sporulation occurs, the lesions are black with a slightly rough appearance (Table 2. 1).

A yellow halo (frog eye) is commonly observed around *C. arachidicola* (Plate 2.1) but its presence and prominence is altered by host genotype and environmental factors (Chaube and Pundhir, 2009). Similar halos may be found around *Cercosporidium personatum* lesions; therefore the yellow halo is not always indicative of Early leaf spot; conclusive identification can only be made by microscopically examining conidiophores and conidia. In Early leaf spot, conidiophores form on the upper leaf surface within the lesion covered area and conidia are often sparsely present or not present at all. Late leaf spot disease, on the other hand, produces brown to black lesions

with rare halo ever being present (Hagan, 1998). However, similar to early leaf spot, conclusive identification can only be made by microscopic examination of conidiophores/conidia. The formation of *Cercospora personatum* conidiophores and conidia is far more prolific than *C. arachidicola*. Conidiophores of *Cercosporidium personatum* tend to be densely packed into lesions with numerous conidia being present (McDonald *et al.,* 1985). Symptoms of Cercospora leaf spots can be confused with injuries caused by soil-applied chemicals or herbicides more especially insecticides. However, in soil-applied chemicals, lesions are scattered along the margins of leaves (Hagan, 1998). The disease occurs on all above ground parts of the plant but more severely on the leaves. The leaf symptoms produced by the two fungi can be easily distinguished by appearance such as spot colour and shapes among others (Table 2.1).

Regardless of lesion appearance, lesions caused by the presence of either *C. arachidicola* or *Cercosporidium personatum* have the same effect of reducing photosynthetic activity in leaf tissue, as described above. The reduction of photosynthetic leaf area is the primary factor associated with loss of yield in groundnut. Pre-mature defoliation (due to early onset of senescence mechanisms), another symptom associated with both leaf spots fungi; further compounds the reduction of active photosynthetic area (Burns, 2010). The quality and yield of nuts are drastically reduced in severe infections.

Table 2. 1: Comparison of Early and Late leaf spots of groundnut

Character	***Cercospora arachidicola***	***Cercosporidium personatum***
Stage of occurrence	Early infection	Usually late infection
Shape of spot	Circular to irregular	Usually circular
Leaf surface on which most spores are produced and their arrangement	Upper surface random	Lower surface, in concentric rings
Colour of spot on upper leaf surface	Light brown to black, tending towards brown with some chlorotic yellow halo	Brown to black, tending towards black
Colour of spot on lower surface	Brown	Black

Source: McDonald *et al*. (1985); Chaube and Pundhir (2009); Ijaz (2011)

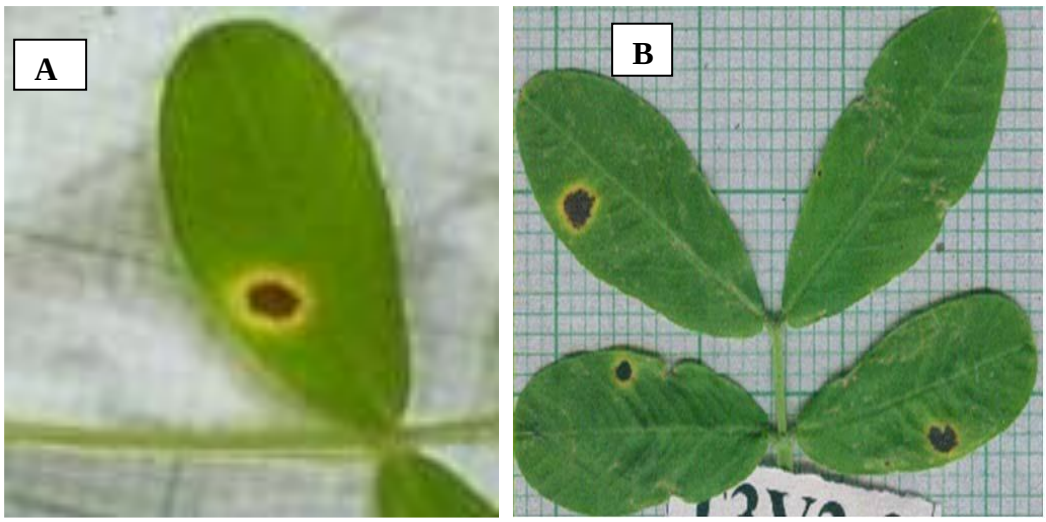

Plate 2. 1. Symptoms of Early leaf spots (A) caused by *Cercospora arachidicola* and symptoms of Late leaf spots (B) caused by *Cercosporidium personatum*. Source: (Ijaz, 2011)

2. 4. 3 Morphological Characteristics of Leaf spot Fungi

2. 4. 3. 1 *Cercospora arachidicola*

The anarmorph stage of the fungus is *Cercospora arachidicola* whilst its perfect state or teleomorph is *Mycosphaerella arachidis*. Conidiophores are olivaceous brown or yellowish brown in colour, short, one or two septate, unbranched and geniculate and arise in clusters. Conidia are sub-hyaline or pale yellow, obclavate, often curved 3-12 septate, 35 _ 110 x 2.5 _5.4 µm in size with rounded to distinctly truncate base and sub-acute tip (McDonald *et al.*, 1985; Chaube and Pundhir, 2009).

The perfect stage of the fungus produces perithecia as ascostromata. They are globose with papillate ostiole. Asci are cylindrical to clavate and contain eight ascospores. Ascospores are hyaline, slightly curved and two celled, apical cell larger than the lower cell (McDonald *et al.*, 1985).

2. 4. 3. 2 *Cercosporidium personatum*

The late leaf spot caused by *Cercosporidium personatum* is seen primarily in its imperfect state (Shokes and Culbreath, 1997). The perfect state (*Mycosphaerella berkeleyii* W. A. Jenkins) is classified under the ascogeneous fungi and both asci and spermatogonia occur on debris (Pattee and Young, 1982). Ijaz (2011) described the imperfect state as follows: conidiophores are fasciculate, one to three geniculate with conspicuous conidial scars, mostly arranged in concentric rings on lower surface and darker in colour. Conidia are olivaceous, obclavate, cylindrical and with one or more septa. The base is shortly tapered with conspicuous hilum and hyaline in colour.

2. 5 Epidemiology and Survival of Early and Late leaf spots pathogens

The fungi that cause Early leaf spots and Late leaf spots reproduce and infect by means of spores called conidia. Conidia may be detached from lesions at any time but peak release periods occur when leaf surfaces dry in the morning, and at the onset of rainfall (McDonald *et al.,* 1985). An attack by C. *arachidicola* normally precedes that of *C. personatum*, but both diseases may appear within three to five weeks after sowing (McDonald *et al.,* 1985).

High humidity and temperatures above 19 °C promote spore production (Shokes and Culbreath, 1997). Spores produced on infested groundnut residue in the soil during the growing season result in primary inoculum that causes initial leaf spot infection (McDonald *et al*., 1985; Shokes and Culbreath, 1997). When conditions are favourable, the spores develop into germination tubes that enter the plant cells directly via the epidermis or stomata, allowing intracellular mycelia growth into haustoria that obtain nutrients (Pattee and Young, 1982; Shokes and Culbreath, 1997). Host cells are killed in advance as the hyphae penetrates (Upadhyay *et al*., 2009). Lesions develop within 10-14 days and new spores are produced in spots on infected leaves (Melouk and Shokes, 1995; Shokes and Culbreath, 1997). These spores will subsequently infect plants and produce secondary infection after periods of extended leaf wetness and temperatures above 19 °C, and the cycle repeats (Shokes and Culbreath, 1997). Spores are spread by wind, splashing rain, insects and movement of infected crop debris or by movement of pods and seeds that are surface contaminated with conidia (McDonald *et al*., 1985; Shokes and Culbreath, 1997). According to Rao *et al.* (1993), conidia, ascopores and mycelia of *Cercosporidium personatum* could survive for a period of 30 - 60 days on infected groundnut debris buried under soil surface. However survival

could increase up to a year when debris is stored indoors. The pathogen of Late leaf spot is hemibiotroph of groundnut with no known alternative host (Upadhyay *et al*., 2009; Jordan *et al*., 2012), and may survive from season to season on volunteer groundnut plants and infected crop debris (McDonald *et al*, 1985; Subrahmanyam *et al.*, 1992).

Cercosporin is unique among fungal toxins in that it is activated by light and becomes toxic to plants by generating activated species of oxygen, particularly single oxygen. The generated active single oxygen destroys the membranes of host plants and provides nutrients for this intercellular pathogen (Daub and Ehrenshaft, 2000; Agrios, 2005). In addition to production of cercosporin, a biologically active red phytotoxin, *C. personata* produces cellulolytic and pectolytic enzymes that alter the starch, sugar and amino acid content of leaf tissue, resulting in reduced leaf efficiency and premature abscission (Pattee and Young, 1982). Mohapatra (1982) also reported association of higher quantities of reducing sugars in infected leaves than healthy ones. Horne *et al*. (1976) reported that the Late leaf spot fungus produced haustoria that penetrate individual plant cells. Leaves infected with the fungus showed a marked increase in respiration. Jyosthna *et al*. (2004) reported highest chlorophyll content in resistant cultivar, which decreased upon infection in all cultivars. There is serious reduction in yield due to intense spotting of leaves which lead to loss of photosynthesis in tissues (Gerlagh and Bokdam, 1974). Defoliation of leaves due to the disease is another factor that reduces the yield of groundnut crop. In a study of ethylene production and leaflet abscission of groundnut genotypes infected with *C. arachidicola*, 96 and 71.6 % defoliation was observed in control and surfactant treated plants, respectively as reported by Ijaz (2011). It is also possible that leaf defoliation

on the surface of the soil may increase the incidence of certain soil-borne disease such as Southern stem rot (Ijaz, 2011).

2. 6 Cercospora Leaf spot disease cycle

C. arachidicola and *C. personatum* are very similar in respect to their life cycles (Figure 2.2 and 2.3). Both pathogens produce conidia and mycelia that are capable of over seasoning in crop residue. They are necrophilic, thriving on the dead cells and tissues of the host. Although, the teleomorphs of both pathogens are known, they are rarely observed. Therefore, ascospores are not generally regarded as important sources of primary inocula (McDonald *et al.*, 1985). Conidial-spores and mycelia over seasoning in crop residue provide the inoculum source for the following season's initial infection (McDonald *et al*., 1985; Shokes and Culbreath, 1997).

Infection begins when conidial-spores germinate and form germ tubes that penetrate open stomata or lateral faces of epidermal cells. Following penetration, germ tubes form into networks of mycelia. These mycelia produce cellulolytic and pectolyic enzymes (dothistromin and/or cercosporin) which diffuse and degrade, host cell wall and middle lamellae constituents (Stoessl, 1984). Intercellular hyphae of *C. arachidicola* have been shown to kill host cells in advance of hyphal penetration as reported by Burns (2010). Conversely, *C. personatum* does not kill prior to penetration, but instead develops into haustoria. As mycelia spread into host tissues and enzymatic degradation occurs, cells collapse and produce necrotic lesions (Burns, 2010).

Sporulation of these fungi is characterized by the formation of long, thin multicellular conidia on short, darkly pigmented conidiophores (Agrios, 2005). Conidia and conidiophores for both organisms are very similar in appearance (Figure 2.2 and 2.3). Conidia are easily detached and can be dispersed by wind, water, or any other mechanical movement. *C. arachidicola* and *Cercosporidium personatum* growth and development favour warm temperatures. Temperatures ranging from 25-30^0 C and high relative humidity favour disease infection and development. The first lesions normally develop on the oldest leaves near the soil surface and the conidia produced on them are carried by wind, rain splash, and insects to the later-formed leaves and to adjacent plants (McDonald *et al.*, 1985). *C. arachidicola* is a necrotroph, as intracellular hyphae are only found in cells that have been killed by the pathogen (Figure 2.2). *C. personatum*, however, remains intercellular, and is known to produce haustoria in living cells (Figure 2.3) (Jekins, 1938; Yussif, 2010).

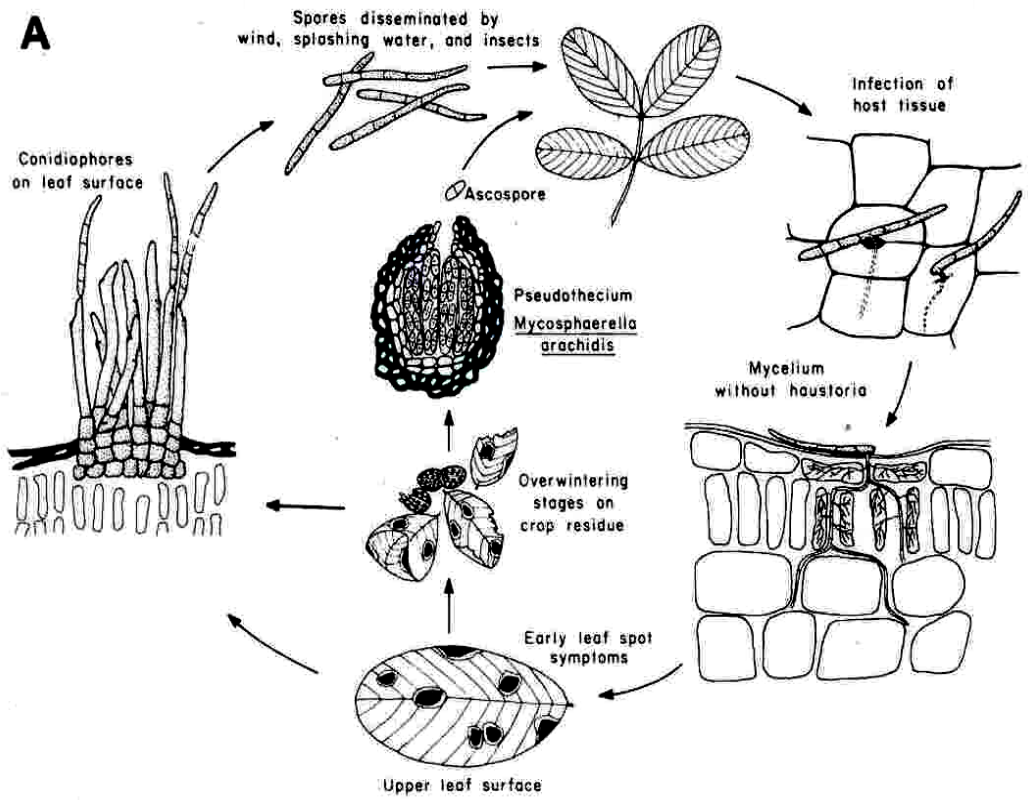

Figure 2. 2: Disease cycles of *Cercospora arachidicola* (A); Source: McDonald *et al.* (1985).

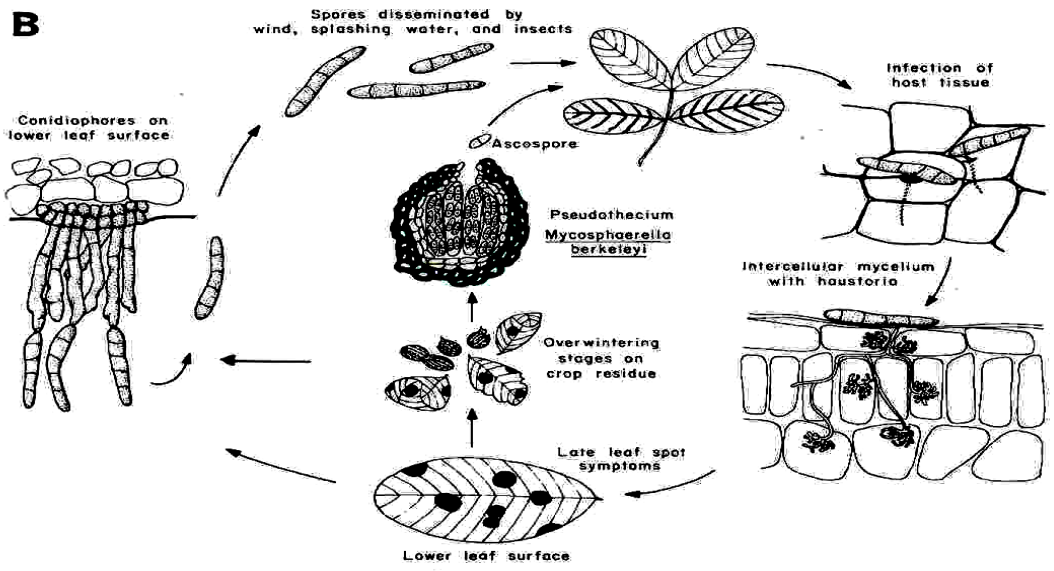

Figure 2 .3: Disease cycle of *Cercosporidium personata* (B); Source: McDonald *et al.* (1985).

2. 7 Management of Cercospora leaf spot Diseases of Groundnut

2. 7. 1 Host plant resistance

A resistant plant is one which possesses qualities that hinder the development of a given pathogen (Yussif, 2010). Host plant resistance is an important tool to control diseases of major food crops in developing countries, especially rice, cassava, groundnuts and cowpea (Khoury and Makkouk, 2010). The use of resistant varieties which are environment-friendly and do not require additional cost are very much welcomed by resource-limited farmers.

Host plant resistance to Early and Late leaf spots is an important component of disease management programmes. This involves heritable changes in the plant that will render it resistant or immune to diseases (Yussif, 2010). Cercospora leaf spot is one of the most destructive foliar disease of groundnut worldwide. Host plant resistance has been used as one control strategy to develop CLS resistant varieties (Khoury and Makkouk, 2010). Field trials conducted in some parts of the world have shown that resistant cultivars of groundnut can yield about 55 – 60 % more than local cultivars and severity level is also significantly lower than the local cultivar (Khoury and Makkouk, 2010). This implies that for an economic and a feasible mode of the disease management, groundnut farmers need to cultivate approved disease resistance cultivars.

However, in some crops, genetic resistance is often based on limited number of major genes that are readily overcome by evolving pathogen races. For instance, with the reduction of genetic diversity in wheat cultivars planted over large areas globally, serious rust epidemics are being recorded whenever new aggressive virulent rust races emerge. A typical example is the yellow rust epidemics that spread from East Africa

to Central and South Asia and North Africa during the 1980's and 1990's. In recent past, the breakdown of Yr27, a gene used to replace Yr9, and the emerging stem rust race Ug99 are threatening 80-90 % of commercial wheat varieties grown worldwide as reported by Khoury and Makkouk (2010). Groundnut varieties differ in reaction to leaf spot but levels of resistance in groundnut alone are not sufficient to provide adequate disease control. Spanish varieties are most susceptible, Virginia types are intermediate and runner varieties are partially resistant (McDonald *et al.,* 1985).

Pathogens in general build resistance when resistant cultivars are planted in endemic areas more especially when the same source of resistance is used whenever groundnuts are planted. According to McDonald *et al.* (1985), there is also the problem in resistance breeding of incorporating resistance to all three diseases (Early leaf spot, Late leaf spot and rust) into an agronomically acceptable cultivar. Besides this, many farmers have also relied on resistant varieties as a sole means of controlling these pathogens. Continuous use of one resistant variety generally results in a change in the pathogen population's ability to attack "resistant varieties", referred to as a 'race shift' as stated by Koenning and Dunphy (2000).

Many attempts have been made to develop groundnut cultivars that are resistant to Cercospora leaf spots in Ghana. However, upon improved groundnut varieties developed and disseminated by the researchers to groundnut farmers, 50 % of farmers still cultivate the Chinese cultivar while 38 % cultivate Mani-Pintar in the Northern Region (Ibrahim *et al.,* 2012). The commercial groundnut cultivar (Chinese) is very susceptible to Cercospora leaf spots. Generally, characteristics that a disease resistant groundnut variety or cultivar should have to ensure ready adoption and productivity in

tropical cropping systems are pathological, entomological, agronomic, and socio-economic in nature.

2. 7. 2 Cultural Methods of Disease Control

Cultural practices such as cultivation techniques, mulching, intercropping, plant density, planting date, crop rotation, strip farming, time of harvest, barrier crops, crop mixtures, roguing, healthy planting material, soil solarization, soil amendments and fertilizer management, and water management have been used singly and in combination as tools for disease management. For some crops in developing countries, such control practices may be the only economically viable methods available (Khoury and Makkouk, 2010). It is best to avoid plant diseases by using long rotations. It is good to be aware that adding a new crop to your usual rotation has the potential to increase or decrease the risk of disease in groundnut (Anon., 2010). It is also good to plant rotational crops that are not hosts of Cercospora leaf spot of groundnut pathogens to decrease the risk of disease problems. Cultural control methods not only serve in promoting the healthy growth of the crop, but are also effective in directly reducing inoculum potential (pruning, roguing, crop rotation and ploughing) and in enhancing the biological activities of antagonists in the soil (solarization, crop rotation and mulching).

2. 7. 3 Biological control

Biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens (Pal and Gardener, 2006). Success in using

microorganisms against plant pathogens started with the control of crown gall with *Agrobacterium radiobacter* K84 (Kerr, 1980), and that of seedling blights caused by *Pythium* and *Rhizoctonia* with *Trichoderma harizanum* (Björkman *et al*., 1998).

However, the use of naturally occurring bio-control agents (antagonists) of plant pathogens can be traced back to many centuries through the traditional practice of crop rotations that primarily permit the reduction of pathogens' inoculum potential in the soil below injury level. This approach is still the most important single component, in both developed and developing countries used to manage root pathogens. This process is often accelerated by adding composts or manures, which enrich the soil with antagonistic microflora (Baker and Cook, 1974).

2. 7. 4 Chemical control

It is estimated that 10-15 % of the already low yields in developing countries is lost due to disease attack, and losses can be higher if post-harvest diseases are considered (Khoury and Makkouk, 2010). It is also estimated that globally, these yield losses amount to between 60–525 billion US dollars annually (Agrois, 2005; Sygenta, 2012). This is a significant loss, considering that in developing countries at present more than 800 million people do not have enough food, and around 1.3 billion live on less than one dollar a day (FAO, 2004; Khoury and Makkouk, 2010).

In a bid to control these devastating attacks, fungicides are employed. For many decades fungicides played an important role in the management of plant diseases. In the 1960s, mercury-containing compounds were banned and systemic fungicides

started gradually to replace the older non-systemic chemicals with more effectiveness and specificity in disease control (Agrios, 2005; Khoury and Makkouk, 2010). Without controversy, fungicides contribute to yield increases in crop production. Edema and Adipala (1994) reported that the application of Mancozeb and Dithane M-45 significantly improved the yield of cowpeas in Uganda. Bennett (2005) also reported that 1.5 billion pounds of onion bulbs were harvested from onion plants cared for with less than 1 million pounds of fungicides in the United State of America (USA). Thiophanate methyl (Topsin-M) significantly lowered severity of Leaf spot disease of groundnut which resulted in greater biomass and pod yield (Nutsugah *et al*., 2007)

Fungicides play a very active role in production of high value crops with uniform appearances and quality (Biobank, 2009). Highly intensive and developed crop farming as practiced in the USA and Europe, involve use of highly-bred crop varieties to maintain uniform crop height, crop canopy, fruit size and shape as well as overall appearance and quality of produce in mechanized farms (Enyiukwu *et al*., 2014). According to the same source, without fungicides and other pesticides it will be difficult to grow such crops of high horticultural characteristics in large monocultures given serious potential pathogenic challenges in the environment. However, the high intensity of chemical pesticide applications and/or their inappropriate applications in agriculture have become a serious cause of concern in recent years.

Several demerits obviously associated with use of these synthetic fungicides in agriculture and pest control programmes have been reported such as pathogen resistance, pathogen resurgence, effects on non-target species, and ecological and human health concerns among others (Enyiukwu *et al*., 2014). Resistance to chemical

agents is a very serious matter. According to Enyiukwu *et al*. (2014) about 150 fungal pathogens exhibit resistance to fungicides. Some authorities asserted that evolution of races and biotypes of pathogens to previously effective chemical agents occurred 5-10 years post-introduction of the agent (Oreskes and Conway, 2010; Pallant, 2010). Besides, synthetic pesticides leave undesirable residues in the treated food materials and the environment. Some of these residues retain their toxic properties for a long time in the food chain; impairing metabolic processes when consumed by non-target species (Awurum *et al*., 2005; Okwu *et al*., 2007; Amadioha, 2012). These safety and environmental concerns have resulted in more stringent Federal environmental regulations in Nigeria that have limited the number of fungicides available to the farmer (Jordan *et al*., 2012). These and many other factors gave impetus for alternative use of natural enemies of the pathogen, and use of botanical pesticides such as neem (Azatin, Bioneem, Tomco and Mangosan) and extracts of other higher plants to combat challenges from phyto-pathogenic organisms (Enyiukwu *et al*., 2014).

2. 7. 5 Use of plant extracts in diseases management

Achieving food sufficiency in a sustainable manner is a major challenge for farmers, agro-industries, researchers and governments (Schillhorn van Veen, 1999). The intensification of agriculture to fulfill food needs has increased the number of phyto-pathogens attacking different crops and as a result the annual production losses of the standing crops. In the past, synthetic pesticides played a major role in crop protection programmes and have immensely benefited mankind (Agrios, 2005). Concurrent with greater awareness towards the use of synthetic chemicals in agricultural practice, the application of integrated pest management programmes has also increased. The use of

synthetic fungicides in plant disease control has been successful in improving agricultural output. However, several of these fungicides have been found to exhibit side-effects in the form of carcinogenicity, detrimental effects, other residual toxicities and development of fungicide-tolerant pathogen strains (Kishore *et al*., 2001; Akinbode, 2010). Non-availability of appropriate fungicides and their application technology to resource-limited farmers has also necessitated the development of more economical and eco-friendly alternative components of disease management.

The alternative choice therefore would be the use of botanical fungicides, which are found to be largely non-phytotoxic, systematic and easily biodegradable in nature (Akinbode, 2010; Gurjar *et al*., 2012; Enyiukwu *et al*., 2014). Plant-derived compounds are regarded as a substantial source for novel lead structures to develop bio-pesticides (Yazdani *et al*., 2011; Gurjar *et al*., 2012). Several plant species have been screened for antifungal activity and extracts or purified compounds from these plants were found to have a broad spectrum of antimicrobial substance against a wide array of microorganism (Table 2.2). Several studies also have conclusively asserted the fungi-toxic properties of plant-based extracts for management of phyto-fungal diseases (Table 2.2).

Plant extracts have been reported to have the merits of being readily available in farming localities of the tropics, cheap, eco-compatible, less harmful to non-target organisms and useable in Integrated Disease Management (IDM) programmes for smallholder, resource-limited farmers (Khoury and Makkouk, 2010). They are also reported to provide sustainable disease management solutions especially in organic farming where synthetic pesticides are non-tolerable.

Table 2.2: **Botanicals produced by plants having antimicrobial activity**

Common name	Scientific name	Compound	Class	Activity
Apple	*Malus pumila* Mill.	Phloretin	Flavonoid derivative	General
Blue gum tree	*Eucalyptus globulus* Labill.	Tannin	Polyphenol	Fungi, Bacteria, Viruses
Onion	*Allium cepa* Linn.	Allicin	Sulphuroxide	Fungi, Bacteria
Neem/Margosa tree	*Azadirachta indica* A.Juss	Azadirachtin	Terpenoides	Fungi, Bacteria
Garlic	*Allium sativum* Linn.	Allicin	Sulphuroxide	Fungi, Bacteria
Black pepper	*Piper nigrum* Linn.	Piperine	Alkaloid	Fungi
Castor bean	*Ricinus communis* Linn.	Ricinine Ricininoleic	Alkaloids	Fungi

Source: Gurjar *et al*. (2012)

Ambang (2011) reported that the application of *Thevetia peruviana* (Pers.) seed extract (at a rate of 3.8 kg/ha) lowered the epidemics and severity of CLS of groundnut. It was also observed that an increase in concentration of *T. peruviana* seed extracts resulted in a decrease in rate of spread of the disease. Neem (*A. indica*) extract which is also ecologically friendly and medicinal, can be used for the control of plant diseases. A study also showed that the mycelial growth of three fungi (*Aspergillus viridae*, *Penicillium digitatum* and *Rhizopus* sp.) decreased with increase concentrations of leaves extract of neem (Suleiman, 2011). An insignificant reduction was also observed when a foliar spray of 4 % aqueous leaf extract of *A. indica* was applied to *Cercospora* sp. of groundnut (Kishore *et al.,* 2001). However neem oil was able to reduce the incidence of leaf spot disease of groundnut (Kishore *et al*., 2001). Neem leaves extract demonstrated a strong ability against the development of many disease causing fungi

through its addition to the soil or by its direct application (Tewari and Nayak, 1991; Locke, 1995). Neem leaf extract reduced the growth of the fungi *Curvularia lunata*, and the germination of some pathogenic spores and succeeded in resisting fruit rotting in *Cucurbitaceae* caused by the fungus *Fusarrium equisitifolium* and *F. semitectum* and also in reducing tomato rotting caused by the fungus *Aspergillus flavus and A. niger* (Krishna and Ojha, 1986; Sinha and Saxena, 1987; Al-Hazmi, 2013). The aqueous leaf extract of Neem was found very effective against the fungal disease late leaf spot and rust of groundnut plants caused by fungi *Puccinia arachidis* (Speg.) and *Mycosphaerella berkeleyi* (Ghewande, 1989).

Aqueous neem seed and leaf extracts strongly inhibited *Alternaria alternata* growth at the highest concentrations Al-Hamzi, 2013). Amadioha, (2000) also reported that neem seeds and leaf extracts reduced the growth of the fungi *Pyricularia oxyzae* in rice. According to Hossain and Hossain (2013), water extract of 23 plant materials which included neem seed, neem leaves, leaves of tomato and ginger rhizome gave a considerable reduction in disease incidence and increase in growth parameters, pod and haulm yield compared to control. They also found that these plant materials decreased spot number per leaf, defoliation per plant, incidence of leaf spot, and number of infected leaf per plant by 35.45 -60.07, 42.06-72.20, 51.97–63.58, and 38.33 to 46.89 % and increased pod yield and haulm yield by 64.37-111.41 and 32.35-74.71 % respectively.

A study by Makun *et al*. (2011) showed that the fungi-toxic effect *of Jatropha curcas* was due to the presence of the active principle curcin. In that study, *J. curcas* crude extracts and de-oiled castor seed extracts significantly reduced the rot index of yam as

compared to other plant extracts. The inhibition was due to the fungi-toxic activities of the plant extracts. From the same work, it was observed that, castor oil seed crude extracts was highly fungi-toxic due to the presence of ricin which is the active ingredient that inhibits mycelial growth both *in vitro* and *in vivo*. It lowered mycelial growth of *Fusarium verticilliodes* and *Aspergillus flavus in vitro* (Makun *et al*., 2011). Aqueous leaf extracts of *Blumea bifoliata, Eucalyptus globules, Ocimum sanctum* and *Pongamia pinnata*, and ethanol leaf extracts of neem inhibited the conidial germination by more than 90 % when was evaluated (Makun *et al*., 2011).

Tobacco (*Nicotiana tabacum*) has been reported to be ecologically friendly in controlling of plant diseases (Olufolaji, 1999). The study also showed that, tobacco as a medicinal plant, possess potential antifungal properties which completely inhibit fungal mycelial growth at 60 % concentration on *Aspergillus* and *Penicillium* cultures (Suleiman, 2011). The efficacy of leaf extracts of four plants (*Gliricidia sepium, Tithonia diversifolia, Phyllanthus amarus* and *Morinda lucida*) suppressed the growth of maize leaf spot pathogen (*Curvularia* lunata) *in vitro* (Akinbode, 2010). Also, Akinbode (2010) observed that all the extracts at 100 % concentration significantly suppressed the growth of *C. lunata*. At all concentrations, *P. amarus* was most efficacious of all the plants extracts followed by extract of *T. diversifolia* and *M. lucida*. Extract of *G. sepium* was the least effective of all the plant extracts against *C. lunata* (Akinbode, 2010). There is no adequate data reporting on the effects of Desert date seed/kernel on crop diseases. However, Khalil *et al*. (2016) reported that aqueous extract of Desert date fruits has antidiabetic and antioxidant effects on diabetic rats. The root of Desert date is used for the treatment of render pest and antrax (Chothani and Vaghasiya, 2011).

2. 8 Antimicrobial secondary metabolites in plants extracts

Plants, as long-lived stationary organisms, must resist attackers over their lifetime, so they produce and exude constituents of the secondary metabolism which plays an important role in their defense mechanisms (Chaube and Pundhir, 2009). Plants produce several secondary metabolite compounds including alkanoids, cyanogenic glycosides, glucosinolates, flavanoids, saponins, phlobatinnins, anthraquinones, steroids, terpenoids, tannins and phenolic compounds, to protect themselves from the continuous attack of naturally occurring pathogens, insect pests and environmental stresses (Ebel, 1986; Kishore *et al*., 2001).

Table 2.3: Mode of action of phytochemicals

Class	**Sub-class**	**Mechanism**
Phenolics	Simple phenols	Membrane disruption, substrate deprivation
Phenolic acids	Phenolic acids	Bind to adhesins, complex with cell wall, inactivate enzymes
Terpernoids essential oils		Membrane disruption
Alkaloids		Intercalate into cell wall
Tannins		Bind to proteins, enzyme inhibition, substrate deprivation
Flavonoids		Bind to adhesins, complex with cell wall, Inactivate enzymes
Coumarins		Interaction with eucaryotic DNA
Lectins and polypeptides		Form disulfide bridges

Source: Gurjar *et al*. (2012)

However, according to Gurjar *et al*. (2012), there are six broad chemical groups which are flavonoides and isoflavonoides, saponins, steroides, tannins, phenolic and phenolic acids—chlrogenic acid, proto- catechuic acid, ferulic acid, caffeic acid, coumarins and pyrones. These compounds with antimicrobial activity may be specific against a

particular pathogen or may have a broad spectrum and can be used for control of fungal disease in crop plants (Table 2.3). Several phenolic compounds, tannins, and some fatty acid such as dienes pre-existing in high concentrations in cells have been implicated for the resistance of young tissues of parasitic fungi such as *Botrytis* (Chaube and Pundhir, 2009). Several other types of secondary metabolites that act as pre-formed compounds such as saponins tomatine in tomato, and avenacin in oats, have antifungal membranolytic activity against many fungi (Agrios, 2005; Chaube and Pundhir, 2009). Saponins are glycosides with soap-like properties that can disrupt membranes.

2. 8. 1 Essential oil components of plant extracts

Essential oils (EOs) are volatile, natural, complex compounds characterized by a stench odour and are formed as plant secondary metabolites by aromatic plants belonging to different families. These chemical volatiles have functions in chemical defense, acting as insecticides, acaricides, avoiding bacterial or fungi phytopathogen colonization (Iacobellis *et al*., 2005; Karamanoli *et al*., 2005; Bakkali *et al*., 2008; Yadav, *et al*., 2008). Terpenes form structurally and functionally different classes of compounds that are formed by coupling different numbers of isoprene units (5-carbon-base; C5), while terpenoids represent terpenes containing oxygen. When a molecule is optically active the enantiomers are present in different plants or in some cases they are both present in a racemic form (Bakkali, *et al*., 2008). EOs are heterogeneous mixtures of single substances and biological actions are primarily due to these components in a very complicated concert of synergistic or antagonistic activities. Several factors such as phenological age of the plant, percent humidity of the harvested

material, and the method of extraction have been identified as possible sources of variation for the chemical composition, toxicity and bioactivity of the extracts (Lahlou, 2004). Essential oils affect several targets at the same time, because of their great number of constituents; this fact decreases the target organisms' resistance or adaptation (Table 2.3). Also, EOs induce cytotoxicity, damage the cellular and organelle membranes, act as pro-oxidants on proteins and DNA and produce reactive oxygen species (ROS) (Table 2.3). Such activity is mostly induced by phenols, aldehydes and alcohols. In some cases essential oils and their components have demonstrated nuclear and cytoplasmic mutagenicity, acting on mitochondria and the respiratory system (Bakkali *et al*., 2008). While these substances are generally active against a broad spectrum of pathogens, interspecific toxicity of individual oils and compounds is highly distinctive (Table 2.3).

Antifungal activity of volatile components extracted from leaves, stems and flowers of *Lantana camara, Malvaviscus arboreus* and *Hibiscus rosa-sinensis* were tested against *Alternaria solani, Botrytis cinerea, Fusarium solani f. sp. cucurbitae, F. oxysporum f. sp. niveum, Pythium ultimum, Rhizoctonia solani* and *Verticillium dahlia* (Boughalleb *et al*., 2005; Yazdani *et al*., 2011). The results demonstrated that volatile components from flowers have stronger antifungal activity than extracts from stems or leaves against all fungi tested, except for *P. ultimum.* Volatile components extracted from the flowers of *L. camara* at concentration of 100 mg/ml, showed the strongest antifungal effect (38 %) against tested fungi. However, *P. ultimum* was not affected by the extracts of any of the four plants tested (Boughalleb *et al*., 2005; Yazdani *et al*., 2011).

Tzortzakis and Economakis (2007) investigated the antifungal activity of lemongrass (*Cymbopogon citratus*) oil against *Colletotrichum coccodes, Botrytis cinerea, Cladosporium herbarum, Rhizopus stolonifer* and *Aspergillus niger*. The results showed that fungal spore production was inhibited up to 70 to 100 % at 25 to 500 ppm of lemongrass oil concentration. However, lemongrass oil (up to 100 ppm) accelerated spore germination for *A. niger* (Yazdani *et al*., 2011). Ranasinghe *et al*. (2002) reported that essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum* at concentrations of 0.03 to 0.11 % (v⁄v) exhibited strong antifungal activity against *F. proliferatum, Lasiodiplodia theobromae* and *Colletotrichum musae*, the causal agents responsible for crown rot and anthracnose of banana.

Screening of essential oil from 30 species of higher plants against *Penicillium italicum* causing blue mould rot of mandarins was carried out by Dixit *et al*. (1995). The essential oil of *Ageratum conyzoides* exhibited the strongest effect against mycelial growth of *P. italicum*. Chang *et al*. (2008) investigated the antifungal activity of essential oil and its constituents from *Calocedrus macrolepis* var. *formosana* on the growth of plant pathogenic fungi. Their experiments showed that sesquiterpenoid components were more effective than monoterpenoid components of the leaf oil. These results revealed that T-muurolol and a-cadinol possess antifungal activities against a broad spectrum of tested plant pathogenic fungi (Yazdani *et al*., 2011). These two compounds strongly inhibited the growth of *Rhizoctonia solani* and *Fusarium oxysporum*. These compounds also efficiently inhibited the mycelial growths of *Colletotrichum gloeosporioides, Pestalotiopsis funerea, Ganoderma australe* and *F. solani* (Chang *et al*., 2008; Yazdani *et al*., 2011).

In another experiment reported by Dikbas *et al.* (2008), antifungal activity of essential oil from *Satureja hortensis* were also tested against *Aspergillus flavus*. The results of *in vitro* assay indicated that the oil of *S. hortensis* at 6.25 µl/ml had fungicidal effect against *A. flavus*. The results of *in vivo* assay on lemon fruits under storage conditions showed, the concentrations of 6.25 µl/mL applied before 8 days of pathogen inoculation had significant antifungal activity even at the end of the 20th days (Dikbas *et al*., 2008). Gurjar *et al*. (2012) reported that essential oil extracted from lemon grass (*Cymbopogon* spp.) was able to control post-harvest anthracnose of mango fruit. Wilson *et al*. (1997) evaluated 49 essential oils for their antifungal activity against *B. cinerea.* Of all the essential oils tested, *Cymbopogon martini*, *Thymus zygis*, *Cinnamomum zeylanicum* and *Eugenia caryophyllata* demonstrated the most antifungal activity against *B. cinerea* as reported by Yazdani *et al*. (2011).

Clove oil, cinnamon oil, and five essential oil components (citral, eugenol, geraniol, limonene, and linalool) were tested for growth inhibition of 14 phyto-pathogenic fungi. Citral completely inhibited the growth of *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium moniliforme*, *F. pallidoroseum*, and *Phoma sorghina* in paper disc agar diffusion assays (Kishore *et al*., 2007). Cinnamon oil, citral, and clove oil as low as 0.01% (v/v) inhibited the spore germination of *Cercospora arachidicola*, *Phaeoisariopsis personata*, and *Puccinia arachidis* by more than 90 % *in vitro* (Kishore *et al*., 2007). Clove oil (1 % v/v) applied as a foliar spray 10 min before *Phaeoisariopsis personata* inoculation reduced the severity of Late leaf spot of groundnut up to 58 % (Kishore *et al*., 2007). In the same work, seed treatment with the test compounds had no effect on the incidence of crown rot of groundnut in *Aspergillus niger*-infested soil. However, soil amendment with 0.25 % (v/w) clove oil

and cinnamon oil reduced the pre-emergence rotting by 71 and 67 % and post emergence wilting by 58 and 55 %, respectively, compared with the non-treated control (Kishore *et al*., 2007).

2. 8. 2 Methods of plant extract preparation and choice of solvent

The activity of plants secondary metabolites compounds also depend on the method and solvent used for extraction, its concentration and structure. According to Gurjar *et al*. (2012), extraction methods involve separation of medicinally active fractions of plant tissue from inactive or inert components by using selective solvents and extraction technology (Table 2.4).

Table 2. 4: Solvent used for active component extraction from plants

Water	Ethanol	Methanol	Chloroform	Dichloro-methanol	Ether	Acetone
Tannins	Alkaloids	Terpenoids	Terpenoids	Terpenoids	Alkaloids	Flavonols
Saponins	Tannins	Saponins	Flavonoids	-	Terpenoids	-
Terpinoides	Terpinoides	Tannins	-	-	Coumarins	-
-	Flavonol	Flavones	-	-	-	-

Source: Cowan (1999)

Generally, plant materials either dry or wet are crushed into fine particles to increase the surface area for extraction which increases the rate of extraction. In a study by Eloff (1998), 5 min extraction of very fine particles of diameter 10 µm gave higher quantities than values obtained after 24 h in a shaking machine with less finely ground

material. Earlier studies reported that solvent-to-sample ratio of 10:1 (v/w) solvent to dry weight ratio has been used as ideal (Green, 2004). The extraction method that has been widely used by researchers is plant tissue homogenization in solvent (Parekh *et al*., 2005). Dried or wet fresh plant parts are ground in a blender to fine particles, put in a certain quantity of solvent and shaken vigorously for 5 - 10 min or left for 24 h after which the extract is filtered (Gurjar *et al*., 2012). The filtrate then may be dried under reduced pressure and re-dissolved in the solvent to determine the concentration. Some researchers however, centrifuged (approximately 20,000 × g, for 30 min) the filtrate for clarification of the extract (Gurjar *et al*., 2012). Another common method is serial exhaustive extraction which involves successive extraction with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted (Green, 2004).

The choice of solvent in the extraction of a phytochemical constituent is important. This is because successful determination of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions include low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action and inability to cause the extract to complex or dissociate (Gurjar *et al*., 2012). As the end product in extraction will contain traces of residual solvent, the solvent should be non-toxic and should not interfere with the bioassay (Ncube *et al*., 2008). The choice will also depend on the targeted compounds to be extracted (Table 2.4). Initial screening of plants for possible antimicrobial activities typically begins by using the crude or alcohol extractions and can be followed by various organic solvent extraction methods (Gurjar *et al*., 2012). Water is a universal solvent,

used to extract plant products with antimicrobial activity. Also water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics is only important as antioxidant compound (Gurjar *et al*., 2012). A study reported that extraction of tannins and other phenolics were better in aqueous acetone than in aqueous methanol (Harmala *et al*., 1992; Gurjar *et al*., 2012). Chloroform is another solvent which was found to be the best solvent among the 20 different solvents evaluated, for the extraction of non-polar biological active compounds (Harmala *et al*., 1992). According to Gurjar *et al*. (2012), nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds which are often obtained through initial ethanol or methanol extraction. Thus the most commonly used solvents for preliminary investigations of antimicrobial activity in plants are methanol, ethanol and water. The other solvents used by researchers are dicholro-methane, acetone, and hexane (Gurjar *et al*., 2012).

CHAPTER THREE

3. 0 MATERIALS AND METHODS

3. 1 Experimental site

The study consisted of field survey, laboratory studies, green house and field experiments. A field survey was conducted in communities within the Tamale Metropolis, Kumbungu, Tolon and East Gonja Districts in the Northern Region of Ghana during the 2014 cropping season (Appendix 1). These districts are the operational areas of the Presbyterian Agricultural Services, 'Mile 7' (PAS-Mile 7). Laboratory studies were conducted in the Spanish laboratory at the Nyankpala campus of the University for Development Studies, Tamale whereas phytochemical anaylsis was conducted at Nuclear Chemistry and Environmental Research Centre (NCERC) of the National Nuclear Research Institute (NNRI) at Ghana Atomic Energy Commission (GAEC) during the 2014 and 2015 cropping seasons. The green house study was conducted in a green house at Fooshegu which belongs to the Presbyterian Agriculture Services, 'Mile 7' (PAS- Mile 7). Fooshegu is found in the southern part of Tamale Metropolis. It is about seven (7) miles away from Tamale Central.

The field study was conducted in 2014 and repeated in 2015 on the experimental field of the Faculty of Agriculture at the Nyankpala campus of the University for Development Studies. Nyankpala is located at latitude 9° 25′ 41″ N and longitude 0° 58′ 42″ W with an altitude of 200 m (SARI, 2014). The experimental field is located within the Guinea Savannah zone of Ghana. It experiences moderate unimodal rainfall from May to October each year with the peak occurring between August and September. The mean annual rainfall is 118.64 mm while the mean monthly maximum

rainfall is 10.8 mm. Mean monthly minimum temperatures of 22.4 ^{0}C and maximum of 33.6 °C have been recorded (SARI, 2014). The mean monthly minimum relative humidity is 80 % (SARI, 2014). The soil is moderately brown and drained sandy loam. The area is characterized by natural vegetation dominated with few shrubs.

3. 2 Field survey

3. 2. 1 Assesment of farmers' knowledge, perception and management of Cercospora leaf spot (CLS) disease

The survey was conducted by administering questionnaire to groundnut farmers in four administrative districts of the northern region of Ghana, namely Tamale Metropolis, East Gonja, Tolon and Kumbungu (Appendix 1). The districts were purposively selected based on the operational areas of the Presbyterian Agricultural Station-Mile 7 (PAS-Mile 7) which is promoting the production and marketing of groundnut among smallholder farmers. A multiple-stage sampling technique was used to select the respondents for the study. First, a total of 20 communities, consisting of five from each district were randomly selected through the assistance of field staff from PAS-Mile 7. In the second stage, using the list of farmers in the institution as the sampling frame, ten farmers were randomly selected from each community, which resulted in a total of 200 respondents (Appedix 2). A semi-structured questionnaire designed in a closed- and open-ended manner was used to elicit information on farmers' knowledge, perception and management of *Cercospora* Leaf Spot (CLS) disease. The questions were developed on the following key aspects: farmer's demographic information, knowledge of CLS disease and management strategies. A pilot test was conducted with 30 groundnut farmers at Gbabshie and Gbulahigu which

were not included in the sample, a month before the study. After the pilot test, minor changes were made in the questionnaire to enhance clarity.

Data were collected using face-to-face interview combined with field observations, from June to August, 2014. The field staff of Presbyterian Agricultural Station (PAS) -'Mile 7' assisted in administering the questionnaires to the selected farmers. The parameters which were given particular emphasis were the incidence and severity of CLS disease since this was the main aim of the study. In addition to this, the survey gathered information on farmers' practices that could affect the disease incidence and severity. Each interview lasted for about 30 min. Dagbani which is mostly spoken by the farmers was used throughout the interactions with respondents. A total of 200 farmers were used in the analysis; consisting of 100 female farmers and 100 male farmers (Appendix 2).

3. 2. 2 Determination of the incidence and severity of Cercospora Leaf Spot (CLS) on farmers' field

This was done to ascertain the extent of the incidence and severity of the CLS in the study area. Therefore, 10 groundnut farmers were selected in each of the four districts, using the multi-stage sampling technique and their farms examined. In total, 40 groundnut farms were examined. Assessment of disease incidence was done by walking diagonally across the farm and scoring groundnut plants for the presence or absence of CLS symptoms. Samples of leaves were also collected at every tenth pace along the diagonal walk. These leaves were used to assess severity of CLS using Florida 1 to 10 scale system (Appendix 3). The descriptive keys were used to

determine the severity of the disease. Mean % incidence was calculated by the formula:

Disease Incidence (%) = $\frac{\text{(Number of farms with disease)}}{\text{(Total number of farms surveyed)}} X100$ (Ijaz, 2011)

3. 3 Determination of efficacy of plant extracts for the control of Cercospora leaf spot disease

This was carried out in three different environments- laboratory, green house and field to test the efficacy of the various plant extracts for the control of Cercospora leaf spot of groundnut.

3. 3. 1 *In vitro* studies

3. 3. 1. 1 Plant extract preparation

3. 3. 1. 1. 1 Plant extract preparation for phytochemical analysis

Azadirachta indica and *Jatropha curcas* seeds as well as *Nicotiana tabacum* leaves were collected from Fooshegu and Tamale whilst *Balanites aegyptiaca* seeds were obtained from Jantong-Dashee in the East Gonja District. The plant materials were obtained from healthy plants. The seed and leaf samples were sent to the laboratory in separate well labelled polythene bags. Seeds of desert date, *J. curcas*, neem and leaves of tobacco were washed, air-dried at room temperature for 10 days and finely pulverised using a hammer mill (Thomas Scientific Model 4, USA) separately. The method of Kuberan *et al*. (2012) was used with some modification. The aqueous

extract were prepared by soaking 250 g of each plant material in 1000 ml of water in a conical flask which was covered and placed on a shaker (Orbital Shaker Lab-Line, USA) for 8 h. The extracts were filtered using a vacuum filtration system and concentrated to dryness using a rotary evaporator (Rotary evaporator RE 300, USA). Dry extracts were stored at 4 ^{0}C until they were required for the phytochemical analysis.

3. 3. 1. 1. 2 Plant extract preparation for *in vitro*, green house and field studies

The seeds of neem, *Jatropha curcas*, and desert date were removed from their shells and shade dried as well as tobacco leaves for 10 days on laboratory trays. All seeds coats were removed before pounding. The dried plant materials were pounded separately into powder with mortar and pestle. Moreso, seeds were pounded gently. The powders obtained were sieved through a screen with a mesh sizes of 0.4 mm to obtain fine powder.

For the plant extracts, the method of Kuberan *et al*. (2012) was used with some modification. Cold water extracts of the various ground plant materials were prepared in concentrations of 25, 50, 75 and 100 g in 1l beaker, stirred vigorously, allowed 24 h settling and the supernatant was filtered through folds of sterilised cheese cloth for the *in vitro* study.

For green house study, plant extracts were prepared as described above with some modification. Each pounded plant material was sieved and weighed into 25, 50, 75 and 100 g portions. Each sample was wrapped in a cotton cloth and soaked in 1 litre of

water for 24 h. The cloth was squeezed and the extract was filtered. To the filtrate, 2 g of an emulsifier ('key soap') was added to each 1l of water. The emulsifier helps the extract to stick well to the leaf surface of plants. Based on the results of the *in vitro* and green house studies, the most effective concentration of the extract in inhibiting the pathogens (100 g/l) was used for the field study.

3. 3. 1. 2 Media preparation and amendment with plant extracts

3. 3. 1. 2. 1 Media preparation

Potato Dextrose Agar (PDA) media was prepared according to the manufacturer's (Rapid Labs Ltd, Colchester, UK) instructions. Thirty nine (39) g of PDA powder was suspended in one 1l of distilled water in an Erlenmeyer flask and 250 mg of chloramphenicol added to suppress bacterial growth. The Erlenmeyer flask was plugged with non-absorbent cotton wool and sterilised in an autoclave at 121^0 C and a 1.03 Kg/cm^2 for 15 min. Twenty millilitres (20 ml) of the melted PDA medium was poured into a 9cm Petri dish and allowed to solidify.

3. 3. 1. 2. 2 Amendment of PDA with plant extracts and Topsin-M

Five millilitres (ml) of each extract concentration (25 g/l, 50 g/l, 75 g/l and 100 g/l) was dispensed into Petri dishes (9cm diameter) using a sterile pipette. To this, 20 ml PDA was added, agitated and allowed to solidify. For the positive controls, 5 ml of Topsin-M prepared at the recommended rate (1 g/l) as well as 2 and 3 g/l were used for the amendment. Five millilitres of sterilised distilled water was used for negative controls.

3. 3. 1. 3 Isolation and identification of *Cercospora arachidicola* and *Cercosporidium personatum*

3. 3. 1. 3. 1 Isolation of *Cercospora arachidicola* and *Cercosporidium personatum*

Infected groundnut leaves collected from farmers' fields during the survey were kept in sterile brown envelopes, labelled and sent to the laboratory for isolation of CLS pathogens. The infected leaves were washed twice with tap water and rinsed twice with sterilised distilled water for one minute.

The pathogens, *Cercospora arachidicola* and *Cercosporidium personatum* were isolated from infected groundnut leaves by the tissue segment method described by Rangaswami and Mahadevan (2006). The affected portion along with a portion of healthy tissue were cut with a sterilised scalpel into 1 x 1cm^2 pieces. The leaf fragments were soaked in sterilised distilled water for 1 min and surface sterilised in 5 % Sodium hypochlorite (1 % available chlorine) for 5 min, rinsed in three changes of sterilised distilled water; blot dried with sterilised tissue paper in a Lamina flow hood and plated on PDA in a 9cm Petri dish. Four of the cut sections were placed in each Petri dish containing about 25 ml PDA. The plates were sealed with Para film, incubated at 25 ^{0}C and cultures observed for seven consecutive days. The mycelia that grew were sub-cultured onto fresh PDA. Further sub-culturing was carried out until pure cultures of the pathogens were obtained.

3. 3. 1. 3. 2 Identification of *Cercospora arachidicola* and *Cercosporidium personatum*

Identification of pathogens was based on morphological and cultural characteristics as described by Barnett and Hunter (2006). Slides of pure cultures obtained were prepared on glass slides and observed under a compound microscope (CELESTRON LCD Digital microscope, Model number 44340, UK).

3. 3. 1. 4 Maintenance of stock cultures

Stock cultures of the test fungi *Cercospora arachidicola* and *Cercosporidium personatum* grown on slants of PDA on 9 cm Petri plates were stored in a refrigerator at 4 ^{o}C and were sub-cultured every two weeks.

3. 3. 1. 5 Determination of the inhibitory effect of the aqueous plant extracts on mycelial growth of *Cercospora arachidicola* and *Cercosporidium personatum*

There were 40 treatments made up of 25 g/l, 50 g/l, 75 g/l and 100 g/l of neem seeds, desert date seeds, *J. curcus* seed and tobacco leaf extracts with water and Topsin-M at 1 g/l, 2 g/l and 3 g/l as controls. The pathogen isolates were *Cercospora arachidicola* and *Cercosporidium personatum*. The experiment was laid out in a Completely Randomised Design. Each treatment was replicated five times. There were 200 experimental units.

The radial growth rate method was used to determine the inhibitory effect of the various plant extracts (neem seed, *J. curcas* seed, desert date seeds and tobacco leaves) on *Cercospora arachidicola* and *Cercosporidium personatum.* Petri plates containing PDA media amended separately with 25 g/l, 50 g/l, 75 g/l and 100 g/l concentrations

of the plant extracts and Topsin-M at 1 g/l, 2 g/l and 3 g/l were used to test the inhibitory effect of the treatments on mycelial growth of both fungi. Each Petri dish contained 5 ml of extract and 20 ml of sterilised PDA while Topsin-M at 5 ml amended with 20 ml of sterilised PDA served as positive controls and PDA plates not amended with extracts served as negative control. Mycelial disc (3mm diameter each) from one week-old pure culture of each fungus were removed using a sterile cork borer (3mm diameter) from the edge of an actively growing colony. One mycelial disc was placed at the centre of the plate after obtaining the point of intersection of two perpendicular lines drawn at the bottom of the plate as the centre. This was done using a sterile inoculation pin and the plates incubated at 28 ± 2 ^{o}C. The mycelial growth was determined by measuring the colony diameter with a transparent rule daily after inoculation for seven days. The percentage inhibition of mycelial growth was calculated as follows:

$$I = \frac{C - T}{C} \times 100 \text{ (Begum } et\ al., 2010)$$

Where; I = Percentage inhibition, C = Radial growth in control, T =Radial growth in treatment.

3. 3. 1. 6 Detection of phytochemicals in the plant extracts

3. 3. 1. 6. 1 Test for alkaloids

Five millilitres of each plant extract was placed into a test tube and diluted with 5 ml distilled water and two to three drops of Mayer's reagent were added. The mixture was shaken vigorously for 2 min. The appearance of a cream-coloured precipitate indicated the presence of alkaloids (Edeoga *et al*., 2005; Kareru *et al*., 2008).

3. 3. 1. 6. 2 Test for saponins

The method described by Wall *et al*. (1954) and Kareru *et al.* (2008) were used. About 0.5 g of each plant extract was mixed with about 5 ml of sterilised distilled water in a test tube. The mixture was shaking vigorously for 2 min. Frothing which persisted for about 15 min was taken as a preliminary evidence for the presence of saponins.

3. 3. 1. 6. 3 Test for Tannins and Phenolic compounds using ferric chloride test

The method described by Sabri *et al*. (2012) was used. Two millilitres of each plant extract was added to 2 ml of sterile distilled water and two drops of 0.1 % Ferric chloride solution was added in a test tube. The appearance of a blue–green colour confirmed the presence of tannins and phenolic compounds in the sample.

3. 3. 1. 6. 4 Test for Phlobatinnins

About 0.5 g of each plant extract was boiled with 5 ml of 1 % aqueous Hydrochloric acid. A deposition of a red precipitate was taken as evidence for the presence of phlobatannins (Trease and Evans, 1978).

3. 3. 1. 6. 5 Anthraquinones using Borntrage's tests

About 0.5 g of each extract was mixed with 5 ml Benzene. Each mixture was then filtered and 5 ml of 10 % ammonia solution added to the filtrate. The mixture was shaken for 2 min and the presence of a pink, red, or violet colour indicated the presence of anthraquinones (Trease and Evans, 1978).

3. 3. 1. 6. 6 Test for cardiac glycosides using Keller-Killani test

Five milliltres of each extract was each mixed with 2 ml of glacial acetic acid containing one drop of ferric chloride solution before adding two drops of concentrated sulphuric acid in a test tube. The appearance of greenish colour was used to confirm the presence of cardiac glycosides.

3. 3. 1. 6. 7 Test for steroids and terpenoids (Salkowski test)

About 2 ml of each test solution was mixed with 2 ml chloroform in a test tube and 2 ml sulphuric acid was added to it. Each mixture was vigorously shaken for 2 min and allowed to settle for about 3 min. The appearance of a red colour in the lower layer indicated the presence of steroids and the formation of a yellow layer indicated the presence of terpenoids.

3. 3. 2 Green house study

There were 20 treatments made up of 25 g/l, 50 g/l, 75 g/l and 100 g/l of the neem seed, *J. curcus* seed and tobacco leaf extract, with water and Topsin-M at 1 g/l, 2 g/l and 3 g/l as controls. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications in the green house. Each treatment unit consisted of five groundnut plants. Soil was obtained in a field where the main field experiment was conducted. The field was divided into different homogenous units based on visual observation and experiences of field technicians. Soils from these different units in a zig-zag pattern were brought together and mixed thoroughly. Two hundred black polythene bags measuring 15 x 30 cm^2 were perforated at the bottom and each filled with the soil. Only the susceptible “Chinese” cultivar was used in this

study. Data on disease severity index, disease incidence, plant height (cm) and pod weight were taken.

3. 3. 3 Pathogenicity test of *Cercospora arachidicola* and *Cercosporidium personatum*

A modified method of Eman (2011) was used for the test. Seedlings of a susceptible groundnut cultivar "Chinese" were used under greenhouse condition. The seedlings were raised on loamy soil contained in black polythene bags (with perforated holes at the bottom) measuring 15 x 30 cm^2. The pathogenicity test was conducted by atomising sterilised distilled water containing mycelia bits of *Cercospora arachidicola* and *Cercosporidium personatum*. This method is similar to that used by Subrahmanyam *et al*. (1990) to study the pathogenicity of *Cercospora arachidicola* on groundnut plants in which they used conidia suspension. When the seedlings were three weeks old, they were sprayed with the conidia suspension (*ca*. 10^5 conidia/ml) prepared except the control plants. The spraying was done using a 1 l compression sprayer. Five seedlings were inoculated with *Cercospora arachidicola* inoculum while another five were inoculated with *Cercosporidium personatum* conidia. Five seedlings which served as control were sprayed with sterile distilled water. Afterwards, the inoculated plants were covered with polythene bags for five days to maintain a high humidity greater than 90 % in order to facilitate infection. After this period, the polythene bags were removed and the seedlings left to grow under normal humidity in the greenhouse. The inoculated plants were then observed regularly for appearance and development of symptoms.

Both pathogens were re-isolated on PDA from leaves that showed symptoms of CLS of groundnut. The pure cultures obtained were compared with the originals to confirm their identity according to Koch's postulate.

3. 3. 4 *In vivo* studies

The main field studies were conducted under rain fed conditions for two consecutive cropping seasons in 2014 and 2015. The experimental field was cleared of existing vegetation using cutlass. It was ploughed, harrowed and demarcated using garden lines and pegs. The field experiment was a 6 x 3 factorial laid out in a Randomised Complete Block Design (RCBD) with four replications per treatment. Each replication consisted of 18 experimental plots measuring 4 x 5 m^2. A total land size of 2,400 m^2 was marked out (48 x 50 m^2) for the study. Alleys of 1.5 and 2 m were left between the plots and replicates respectively to prevent treatment drift to adjacent plots. The factor levels comprised three cultivars of groundnut namely; Chinese, Mani-Pinta and Bugla; and four plant extracts (Desert date seed extract, Neem seed extract, Jatropha seed extract and Tobacco leaf extract) with Topsin-M and water as positive and negative controls respectively. One seed each of the groundnut was sown per hole at a depth of about 5 cm. The inter- and intra-row distances were 50 and 20 cm respectively. Each plot consisted of 10 rows and the four median rows were used for disease assessment and yield records.

The 100 g/l concentration of the extracts used *in vitro* and in the green house proved effective in all the plant extracts hence selected for field application. Cold water extract of the various ground plant materials were prepared in a concentration of 100 g and was soaked in 1 l of water for 24 h. For the positive control, 2 g/l was used since it

performed relatively better in green house than the manufacturer's recommended rate. The negative control was only made up of water. It was included in the research for the real analysis for any possible differences in extract application and the positive control. Each treatment contained 2 g of an emulsifier ('key soap') per each 1 l of water except the positive control.

Treatments were applied every 2 weeks from 2 to 13 weeks after planting (WAP) using a 15 l knapsack sprayer. The spray volume used was 150 l/ha. Three cultivars combined with four levels of plant extracts, Topsin-M (positive control) and negative control (water) produced 18 treatments. The treatments used were as follows;

T1= Neem seed extract (NSE) + Chinese

T2= Neem seed extract (NSE) + Mani-Pintar

T3= Neem seed extract (NSE) + Bugla

T4= Desert date seed extract (DDSE) + Chinese

T5= Desert date seed extract (DDSE) + Mani-Pintar

T6= Desert date seed extract (DDSE) + Bugla

T7= Tobacco leaf extract (TLE) + Chinese

T8= Tobacco leaf extract (TLE) + Mani-Pintar

T9= Tobacco leaf extract (TLE) + Bugla

T10= Jatropha seed extract (JSE) + Chinese

T11= Jatropha seed extract (JSE) + Mani-Pintar

T12= Jatropha seed extract (JSE) + Bugla

T13= Topsin-M + Chinese

T14= Topsin-M + Mani-Pintar

T15= Topsin-M + Bugla

T16= Water + Chinese

T17= Water + Mani-Pintar

T18= Water + Bugla

All groundnut cultivars were obtained from farmers in Fooshegu and Gbabshie both in the Tamale Metropolis. These cultivars were confirmed by the Seed Unit of Savanna Agricultural Institute (SARI) as Chinese, Mani-pintar and Bugla cultivars.

3. 4 Agronomic practices

Land clearing was done manually with a cutlass. A single tractor ploughing and harrowing operations were then carried out before sowing in July, 2014 and June, 2015 cropping seasons. Groundnut seeds were planted in both years when the soil was not soggy as the crop does not do well in waterlogged soil. Groundnut cultivars seeds obtained from farmers were unshelled for the 2014 cropping season and was only shelled a few days before planting. Unshelled groundnuts from 2014 cropping season experimental field were preserved for the 2015 cropping season. The pods were shelled a week before sowing and only good quality seeds were selected for sowing. A fill-in after groundnut emergence was done to ensure required plant density.

Groundnut fields were weeded promptly during the early stages of growth. Two manual weeding operations were carried out at each site using a hoe. The first weeding was done at two weeks after planting whiles the second was carried out at the onset of flowering. Earthing up was done at the time of weeding to encourage pegging, or penetration of young nuts into the soil. Hand weeding was used after the start of pegging to avoid disturbing the growing nuts or damaging the flowers up to 6 WAP.

Groundnuts mature from 90-130 days depending on the variety or cultivar. Harvesting was done at 95 and 110 days after planting by hand – pulling for the 'Chinese' cultivar and hoe for the other two cultivars (Mani-Pinta and Bugla). A sample digging was done to determine the maturity of the crop. Mature nuts were firm and brown on the outside. The inside of the pods were grey and produce a rattling sound when shakened. The nuts were removed fresh from the plants. They were dried on mats at the Spanish laboratory for 13 days, to obtain a moisture content of 10 %. Also, all weights were measured with a Sartorius scale balance (Sartorius AG Gottingen, Germany).

3. 5 Data Collected

Data were collected on mycelial growth, disease incidence, disease severity index, leaf defoliation, plant height, pod weight, seed weight, dry pod yield and dry seed yield. Techniques used during the data collection were field interviews, observations, isolation and identification of *C. arachidicola* and *C. personatum*, pathogenicity tests and measurement of growth and yield parameters.

3. 5. 1 Measurement of crop variables

3. 5. 1. 1 Plant height

Five plants of each treatment were randomly selected and tagged. Heights (cm) of these selected plants were recorded every two weeks from 5 to 13 WAP with a tape measure. Measurement was done from the ground level to the last terminal leaf of groundnut plants after each spray. The average height of five plants was then taken as the height for each treatment.

3. 5. 1. 3 Hundred pod weight

One hundred pods from each treatment were randomly picked and weighed using a Sartorious scale balance. This was replicated five times and the average pod weight (g) determined. The average weight of five counts was then taken as the weight of 100 pods for each treatment.

3. 5. 1. 4 Mean of 100 seed weight

One hundred seeds from each treatment were randomly picked and weighed. This was replicated five times and the average seed weight determined. The average weight (g) of five counts was then taken as the weight of 100 seeds for each treatment.

3. 5. 1. 5 Dry pod yield and seed yield

The total weights of groundnut from the respective treatments were recorded before shelling and further drying to reducing moisture content. The weights of groundnuts harvested from each plot were extrapolated to total pod yield per hectare basis.

3. 5. 2 Measurement of disease parameters

3. 5. 2. 1 Disease incidence

Five plants were randomly selected and tagged for disease assessment per treatment during 2014 and 2015 cropping seasons. Disease incidence was recorded on these five plants in every treatment before treatment application. Mean % incidence was calculated with the formula;

Disease Incidence (%) $=\frac{\text{Number of infected plants}}{\text{Total number of plants}}$ x 100 (Chaube and Pundhir, 2009)

3. 5. 2. 2 Disease severity and disease severity index (%)

Five plants of each treatment were randomly selected and tagged. These plants were used to assess the severity of CLS using the Florida scale system of 1 – 10, where 1 = no leaf spot and 10 = plants completely defoliated and killed by leaf spots (Chiteka *et al*., 1988). The descriptive keys were used to determine the severity of the disease (Appendix 3).

Disease Severity Index (DSI) was then calculated using the equation proposed by Kobriger and Hagedorn (1983) below;

$$DSI = \sum \frac{(\text{severity} * \text{number of plants in the class}) * 100}{(\text{Total number of plants rated}) * (\text{Number of class} - 1)}$$

The evaluation of early and late symptoms of CLS was done after every 14 days starting from the 3rd WAP.

3. 5. 2. 3 Defoliation

Defoliation data were obtained by counting the number of empty nodes on a plant and considered as defoliated leaves. Empty nodes where branches emerged were not considered as defoliated. Five plants were randomly selected and tagged per treatment in each replication. These data were collected at 2 weeks intervals until the crops were harvested. Mean % defoliation was calculated by the following formula;

$$\text{Defoliation (\%)} = \frac{\text{Number of abscised leaves on the main axis}}{\text{Total number of leaflets on the main axis}} \times 100$$ (Komegay *et al*., 1980)

3. 6 Data analysis

The data were subjected to analysis of variance using Genstat Discovery (4th Edition). Count data and percentages were transformed using log /√ and arc sine respectively to

homogenize the variance before subjecting them to analysis of variance. Treatment means were separated using Least Significance Difference (LSD) at 5 % significant level. The Statistical Package for Social Scientists (SPSS) version 16 was used to analyse the association of the responses between male and female by employing the Chi-square.

CHAPTER FOUR

4.0 RESULTS

4.1 Field survey

4. 1. 1 Farmers' knowledge and perception of Cercospora leaf spot (CLS) diseases of groundnut

A significantly higher (p = 0.005) number of farmers (87.5 %) were aware of CLS disease of groundnut (Table 4.1). Forty-seven percent (47 %) of farmers who affirmed their awareness of the disease were males whilst the rest (40.5 %) were females. Among farmers who had not heard of the disease, 3 % were male whilst 9.5 % were female.

Majority (84.5 %) of the farmers knew the symptoms of the disease while the rest (15.5 %) were ignorant. A significantly higher (p = 0.032) percentage of male farmers (45 %) knew the symptoms of the disease. Most of the farmers (84.5 %) who claimed to know the disease could identify the symptoms of the disease on their groundnut farms. More male farmers (45 %) could identify the CLS disease symptoms than their female counterparts (39.5 %). Most of the farmers who could not identify the symptoms of the CLS were female. All the farmers who claimed that they knew the symptoms of CLS could actually identify them on their farms (Plate 4.1).

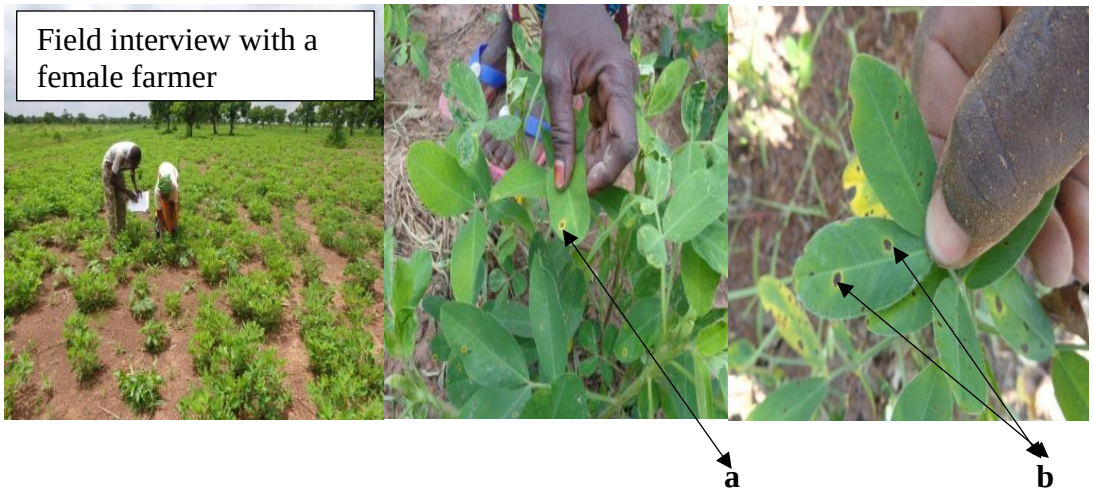

Plate 4.1: Female farmer (a) and male farmer (b) in East Gonja identifying CLS on their farms

Although more male farmers (40.5 %) could identify the disease symptoms than the females (38.5 %) the difference was not significant ($p > 0.05$). Most farmers (91 %) attributed the cause of the disease to poor soil fertility, high rainfall, wind or air and herbicides application while the rest (9 %) attributed it to insects and drought. A significantly higher ($p < 0.05$) percentage of male farmers (47.5 %) attributed the cause of the disease to poor soil fertility, high rainfall, wind or air and herbicides application.

Majority (84.5 %) of the farmers reported leaf spot disease incidence in their fields to be 50 % and above whilst the rest (15.5 %) reported the disease incidence to be 20 - 49 %. Female farmers recorded a significantly ($p < 0.05$) higher disease incidence than their male counterparts (Table 4. 1). A significant percentage of farmers (61 %) observed the appearance of the disease from 1 – 3 weeks after planting (WAP) whilst the rest (39 %) observed it at 4 WAP. A significantly higher ($p < 0.020$) percentage of female farmers (34.5 %) claimed that they observed the disease earlier (1-3 WAP) (Table 4.1).

Table 4.1: **Farmers' knowledge and perception on the existence of CLS of groundnut**

Factor	Farmer Responses	Sex of respondents		Chi-square	*P*-value
		Male (%)	**Female (%)**		
Whether farmer has heard of leaf spot disease before	Yes	47.00	40.50	7.726	0.005
	No	3.00	9.50		
Whether farmer is aware of the disease symptoms	Yes	45.00	39.50	4.619	0.032
	No	5.00	10.50		
Whether farmer can show diseased samples or examples	Yes	45.00	39.50	4.619	0.032
	No	5.00	10.50		
If yes, on which plant part do you observe the disease	Whole plant with symptoms	9.50	11.50	0.482	0.487
	Leaves with symptoms	40.50	38.50		
Farmer's believe of the cause of *Cercospora* leaf spot.	Low soil fertility, high rainfall, wind /air and herbicides	47.50	43.50	3.907	0.048
	Insects and drought	2.50	6.50		
Farmer's description of the incidence of the disease in his/her field	Low (20-49 %)	11.50	4.00	8.589	0.003
	High (50 % and above)	38.50	46.00		
What time and stage of growth farmer encounters the disease	1-3 weeks after planting	26.50	34.50	5.380	0.020
	4 weeks and above	23.50	15.50		
How often farmer encounter the disease	Every season	42.50	41.00	0.327	0.568
	Every year	7.50	9.00		
Whether farmer is aware of the effects of the disease on yield	Yes	40.50	43.00	0.907	0.341
	No	9.50	7.00		
Farmer estimates on the severity of the disease on a scale of 5	Not severe (1-3)	18.00	7.50	11.607	0.001
	Very severe (4-5)	42.50	32.00		
How farmer determines the maturity of groundnut	Defoliation and brown spots	33.50	26.00	4.669	0.031
	sample digging	16.50	24.00		

Farmers also reported that the disease was encountered any time they cultivated groundnut. There was no significant ($p > 0.05$) differences among farmers who encountered the disease every season or every year. Most of the farmers (74.5%) reported the disease severity to be above 50 % whilst the rest (25.5 %) described the disease severity to be less than 50 %. Generally, female farmers (32 %) experienced significantly ($p < 0.001$) lower disease severity compared to male farmers (42.5 %). During the field survey, it was observed that farms belonging to females were either an acre or less, free from weeds and intercropped mostly with vegetables. However, male farmers' farms were mostly more than one acre, weedy and sole cropped. Some female farmers also reported that, they sprayed aqueous neem leaf or seed extracts on their plants to prevent pest and disease from attacking their crops. All of the farmers could determine when their groundnut crops reached maturity and were ready for harvest. Farmers who used defoliation or brown spots of the groundnut crop to determine its maturity were significantly more ($p = 0.031$) compared to those who used sample digging. Male farmers (33.5 %) who used defoliation or brown spots as a sign of maturity were significantly more ($p=0.031$) than their female counterparts (26 %) (Table 4.1).

4. 1. 2 Cercospora leaf spot disease management practices

Farmers who used their own methods (62 %) such as intercropping and crop rotation of managing leaf spot disease were significantly more ($p < 0.001$) than those who used recommended methods including the use of chemicals (38 %), for example, the use of resistant varieties and addition of phosphorus and potassium fertilizer to the soil (Table 4.2). Other management strategies proposed by farmers were

fertilizer/manure application, spraying with recommended fungicides/botanicals and reporting the disease situation to MoFA.

Table 4. 2: Farmers' disease management practices in groundnut farms

Factor	Farmer Responses	Sex of respondents		Chi-square	*P*-value
		Male (%)	Female (%)		
Farmers' management practices on the disease	Farmers' own Methods	38. 00	24.00	16.638	<0.001
	Recommended methods including the use of chemicals	12.00	26.00		
Other ways forward to minimising leaf spot disease as proposed by farmers	Spray with botanicals / fungicides	29.50	32.00	0.528	0.467
	Fertilizer /Manure application and reports to MoFA	20.50	18.00		

4. 1. 3 Disease incidence and severity survey of CLS in the study area

Leaf spot disease incidence was 100 % on farms in the area surveyed. Tolon and Kumbungu Districts had high significantly ($p < 0.001$) lower disease severity scores from 4 to 8 WAP than Tamale Metro and East Gonja district (Figure 4.1). East Gonja District had significantly ($p < 0.001$) higher disease score than the other Districts (Figure 4.1). East Gonja District recorded the highest disease severity scores which ranged from 7 – 10 from 8 to 12 WAP (Figure 4.1). This situation led to high defoliation of groundnut plants. Some of the farmers in this district even harvested their groundnut farms before maturity due to high defoliation since most farmers use brown spots and defoliation as a sign of maturity.

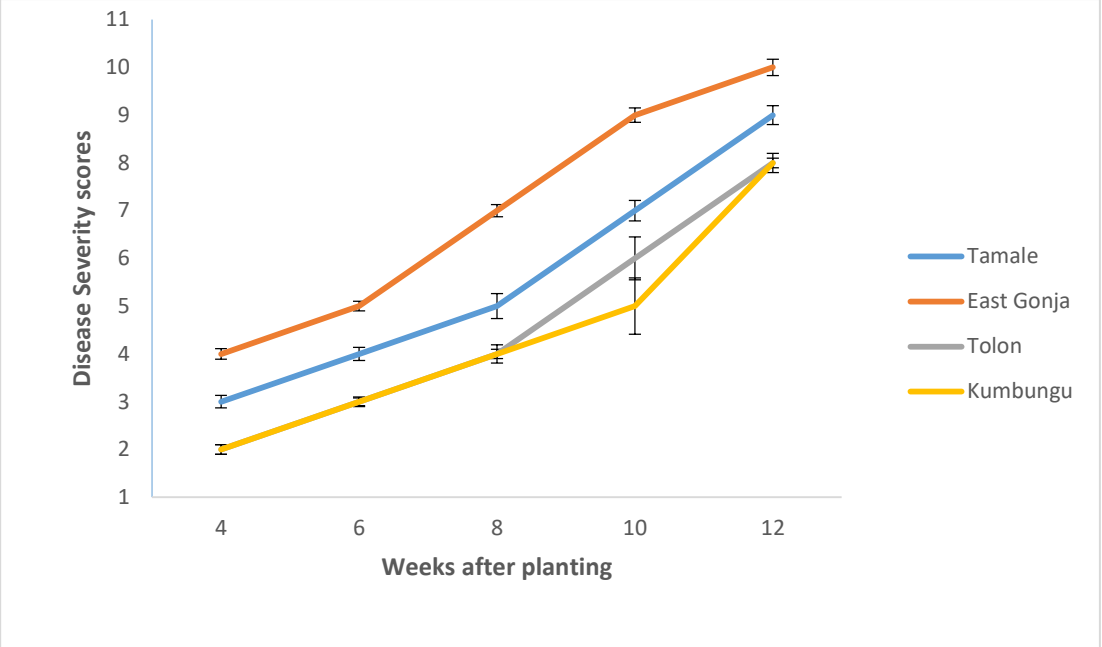

Figure 4.1: CLS disease severity in Tamale Metro, East Gonja, Kumbungu, and Tolon Districts during 2014 cropping season

4. 2 *In vitro* studies

4. 2. 1 Isolation and identification of Cercospora leaf spot (CLS) causing agents

The fungal pathogens isolated from leaves of three groundnut cultivars Bugla (A), Mani-Pinta (B) and Chinese (C) (Plate 4.2) and identified as the causative agents of Cercospora leaf spot diseases of groundnut in this study were: *Cercospora arachidcola* (Plate. 4.3) and *Cercosporidium personatum* (Plate. 4.4).

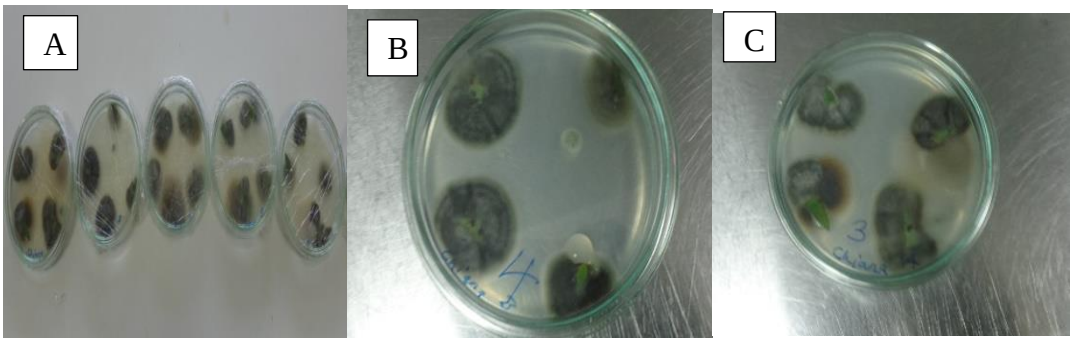

Plate. 4.2: Cultures from infected leaves of groundnut in Petri plates

The conidium of *Cercospora arachidicola* below is sub hyaline or pale yellow, obclavate or cylindrical and septate with rounded base and sub-acute tip (Plate 4. 3).

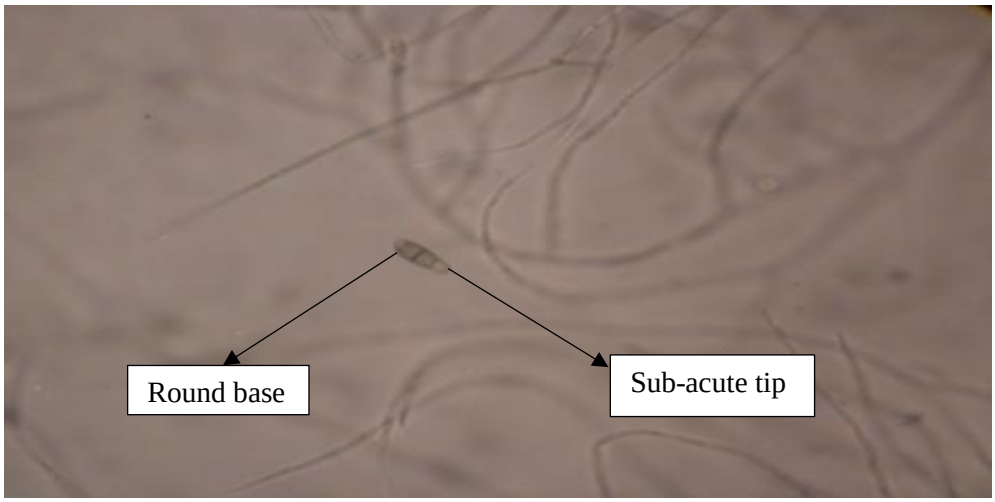

Plate 4.3: Conidium of *Cercospora arachidicola*

However, in the case of *Cercosporidium personatum* conidium was obclavate or cylindrical and light coloured. The base is shortly tapered with a conscipicous hilum (Plate 4.4)

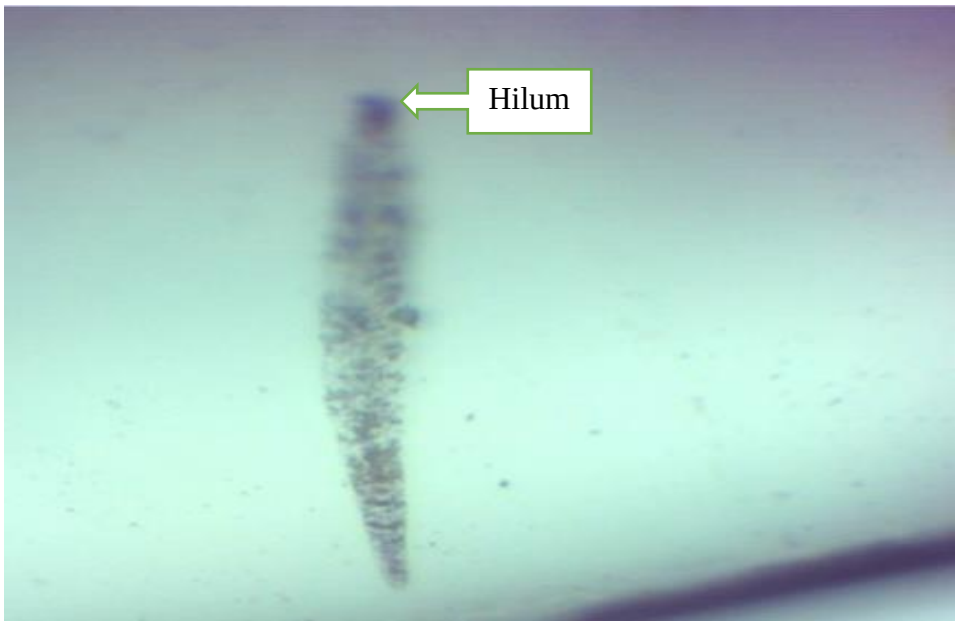

Plate. 4.4: Broken conidium of *Cercosporidium personatum* with distinct hilium at base

4. 2. 2 Pathogenicity test of *Cercospora arachidicola* and *Cercosporidium personatum*

Groundnut seedlings that were inoculated with a suspension of *C*ercospora *arachidicola* and *Cercosporidium personatum* showed symptoms of CLS of groundnut on their leaves (Plate 4.5). The control seedlings did not show symptoms of the disease (Plate 4.5).

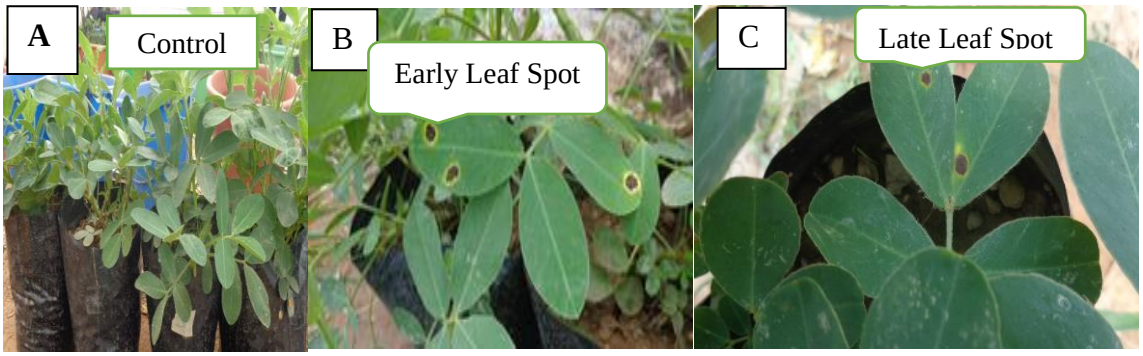

Plate. 4.5: Pathogenicity test of *Cercospora arachidicola* and *Cercosporidium personatum* on susceptible groundnut cultivar Chinese under greenhouse condition. A = Control; B = *Cercospora arachidcola* and C = *Cercosporidium personatum*

For both *Cercospora arachidicola* and *Cercosporidium personatum*, small chlorotic spots appeared on leaflets 10 and 12 days after inoculation respectively. These spots then developed into mature, sporulation lesions for both diseases. The observed spots caused by *Cercospora arachidicola* were sub-circular to irregular in shape, dark brown and surrounded by yellow halo (Plate 4.5b) whilst that of *Cercosporidium personatum* were more nearly circular in shape and brown turning towards black in colour (Plate 4.5c). Also, the yellow halo was more conspicuous and spreading in *Cercospora arachidicola* spots (Plate 4.5b) but dull and limited to margins of spots in *Cercosporidium personatum* (Plate 4.5c). The pure cultures of the pathogens that were

re-isolated from the lesion areas had the same characteristics as the original cultures used in inoculating the seedlings in the green house thereby proving the Koch's postulates.

4. 2. 3 Phytochemical analysis

Alkaloids, tannins and phenolic compounds were detected in all the plant extracts used in this study (Table 4.3). Desert date seeds, neem seeds and tobacco leaves contained saponins. Cardiac glycosides are a constituent part of desert date seed and tobacco leaves. Terpenoids were detected in neem seeds and tobacco leaves. Neem seeds also contained steroids. Phlobatinnins and anthraquinones were not detected in any of the four plant extracts used.

Table 4.3: **Phytochemical constituents of plant extracts**

Phytochemical constituent	**Jatropha seed**	**Desert date seed**	**Neem seed**	**Tobacco leaf**
Alkaloids	+	+	+	+
Saponins	-	+	+	+
Tannins and Phenolic compounds	+	+	+	+
Phlobatinnins	-	-	-	-
Anthraquinones	-	-	-	-
Cardiac glycosides	-	+	-	+
Steroids	-	-	+	-
Terpenoids	-	-	+	+

+ = Present; - = Absent

4. 2. 4 Efficacy of plant extracts for the control of *Cercospora arachidicola* and *Cercosporidium personatum* disease under in vitro and green house conditions

4. 2. 4. 1 Growth inhibition

Desert date seed extract at 25, 50, 75 and 100 g/l significantly ($p < 0.001$) suppressed mycelial growth compared to Tobacco leaf extract (TLE) and the negative control (Table 4.4). DDSE at 100 g/l significantly ($p < 0.001$) suppressed mycelial growth of *Cercospora arachidicola* and *Cercospora personatum* with percentage growth inhibition values of 90.33 and 84.96 % respectively compared to all the plant extracts. The highest dose of Desert date seed extract (100 g/l) was significantly ($p < 0.001$) superior to all the plant extracts used and water (negative control). DDSE at 75 g/l also recorded relatively high growth inhibition percentage values of 82.16 and 78.30 % for *Cercospora arachidicola* and *Cercospora personatum* respectively than NSE at 100 g/l. DDSE at 75 g/l significantly ($p<0.001$) reduced mycelial growth of both fungi compared to Jatropha seed extract (JSE) at 100 g/l. It was also observed that the inhibition effect of DDSE on mycelial growth of both fungi increased with increasing concentration. Topsin-M (positive control) at 1, 2 and 3 g/l completely inhibited the growth of both fungi (Table 4.4).

Neem seed extract at 25, 50, 75 and 100 g/l significantly ($p < 0.001$) reduced mycelial growth of *Cercospora arachidicola* and *Cercosporidium personatum in vitro.* NSE at 25, 50, 75 and 100 g/l reduced the mycelial growth of *Cercospora arachidicola* better than all the Tobacco leaf extract (TLE) concentrations and water (negative control) (Table 4. 4).

Table 4.4: Effects of plant extracts on mycelia growth of the fungi

Treatment	% Growth inhibition	
	Cercospora arachidicola	***Cercosporidium personatum***
Topsin-M (1 g/L)	100.00^{a}	100.00^{a}
Topsin-M (2 g/L)	100.00^{a}	100.00^{a}
Tops-M (3 g/L)	100.00^{a}	100.00^{a}
DDSE (25 g/L)	73.43^{ef}	71.61^{de}
DDSE (50 g/L)	77.94^{de}	75.06^{cd}
DDSE (75 g/L)	82.16^{cd}	78.30^{c}
DDSE (100 g/L)	90.33^{b}	84.96^{b}
JSE (25 g/L)	56.88^{ij}	49.92^{i}
JSE (50 g/L)	60.56^{hi}	59.47^{gh}
JSE (75 g/L)	68.71^{fg}	62.91^{g}
JSE (100 g/L)	75.66^{ef}	67.28^{ef}
NSE (25 g/L)	58.47^{i}	60.20^{g}
NSE (50 g/L)	64.35^{gh}	64.63^{fg}
NSE (75 g/L)	70.15^{fg}	70.65^{def}
NSE (100 g/L)	80.88^{c}	73.32^{cd}
TLE (25 g/L)	49.34^{l}	54.01^{hi}
TLE (50 g/L)	50.57^{kl}	56.46^{hi}
TLE (75 g/L)	51.53^{kl}	57.59^{h}
TLE (100 g/L)	54.50^{jkl}	59.38^{gh}
Control (Water)	0.00	0.00
F (pr)	**<0.001**	**<0.001**
LSD (0.05)	**6.461**	**6.583**

Means with different letters within the same column are significantly different at 5 %.

Neem seed extract (NSE), Desert dates seed extract (DDSE), Jatropha seed extract (JSE) and Tobacco leaf extract (TLE).

NSE at 25, 50 and 75 g/L also suppressed mycelial growth of *Cercosporidium personatum* more than all the TLE concentrations but they were insignificant ($p > 0.05$) compared to TLE at 100 g/l. The highest dose of NSE (100 g/l) significantly ($p < 001$) lowered mycelial growth of both fungi compared to NSE at 75, 50 and 25 g/l (Table 4.4). NSE at 100 g/l also significantly ($p < 0.001$) suppressed mycelial growth of both fungi compared to JSE and TLE at 100 g/l. NSE at 100 g/l reduced the mycelial growth of *Cercospora arachidicola* and *Cercospora personatum* with percentage growth inhibition values of 80.88 and 73.32 % respectively. Apart from the Desert date seed extracts, Neem seed extract was the next best in suppressing mycelial growth of both fungi. However, it was observed that higher concentration of NSE resulted in reduced mycelial growth (Table 4.4).

Jatropha seed extract at 25, 50, 75 and 100 g/l significantly ($p < 0.001$) reduced mycelial growth of *Cercospora arachidicola* compared to Tobacco leaf extract (TLE) concentrations and water (negative control) (Table 4.4). Aqueous JSE at 75 and 100 g/l reduced mycelia growth of *Cercospora arachidicola* better than JSE at 25 and 50 g/l (Table 4.4). Jatropha seed extract at 100 g/l significantly ($p < 0.001$) suppressed the mycelial growth of *Cercosporidium personatum* compared to JSE at 25, 50 and 75 g/l (Table 4:4). Also, JSE at 100 g/l reduced mycelia growth of *Cercosporidium personatum* more than TLE at 25, 50, 75 and 100 g/l (Table 4.4).

Aqueous Tobacco leaf extract at 25, 50, 75 and 100 g/l significantly ($p < 0.001$) suppressed the mycelial growth of *Cercospora arachidicola* and *Cercosporidium personatum in vitro* (Table 4.4). Higher concentrations of aqueous TLE had higher inhibitory effects on the mycelial growth of both fungi. However, aqueous TLE at 100

g/l was statistically not different ($p > 0.05$) from 25, 50 and 75 g/L. Generally, the effect of Tobacco leaf extract on both fungi showed that the suppressive effect in vegetative growth was not significantly different ($p > 0.05$) with increase in concentrations.

4. 2. 4. 2 Disease severity index (DSI)

All plants treated with Topsin-M, Desert date seed extract (DDSE), Neem seed extract (NSE) and Tobacco leaf extract (TLE) at their various concentrations significantly ($p = 0.048$) lowered the disease severity index compared to the negative control plants (Table 4.5). Topsin-M at 2 g/l performed relatively better than the manufacturer's recommended rate of 1 g/l. Plants treated with Topsin-M at 2 g/l and aqueous DDSE at 100 g/l performed similarly and had the same transformed mean percentage value of 5.87 %. Control plants recorded the highest mean percentage value of 8.08 %. However, there was no significant difference ($p > 0.05$) between treatments.

Table 4.5: Effects of plant extracts on disease severity index, plant height and dry pod yield

Treatments	Disease Severity Index (%)	Plant height (cm)	Dry pod weight (g)
Topsin-M (1g/l)	6.11^{a}	13.87^{bc}	20.94^{a}
Topsin-M (2 g/l)	5.87^{a}	15.35^{abc}	21.90^{a}
Topsin-M (3 g/l)	5.98^{a}	11.87^{c}	17.53^{a}
DDSE25 (g/l)	5.98^{a}	11.90^{c}	18.01^{a}
DDSE50 (g/l)	6.22^{a}	13.59^{bc}	15.59^{a}
DDSE75 (g/l)	6.22^{a}	15.32^{abc}	25.18^{a}
DDSE100(g/l)	5.87^{a}	18.32^{a}	30.80^{a}
JSE25 (g/l)	6.11^{a}	12.00^{c}	18.16^{a}
JSE50 (g/l)	6.11^{a}	16.27^{abc}	16.16^{a}
JSE75 (g/l)	6.22^{a}	14.60^{abc}	18.07^{a}
JSE100 (g/l)	6.11^{a}	16.42^{abc}	25.25^{a}
NSE25 (g/l)	6.11^{a}	16.50^{abc}	25.09^{a}
NSE50 (g/l)	6.22^{a}	12.75^{c}	19.10^{a}
NSE75 (g/l)	6.11^{a}	14.25^{bc}	15.18^{a}
NSE100 (g/l)	6.11^{a}	17.25^{ab}	25.15^{a}
TLE25 (g/l)	6.22^{a}	11.96^{c}	20.71^{a}
TLE50 (g/l)	6.22^{a}	15.00^{abc}	16.32^{a}
TLE75 (g/l)	6.22^{a}	14.69^{abc}	22.39^{a}
TLE100 (g/l)	6.22^{a}	13.42^{bc}	23.12^{a}
Control 0	8.08^{b}	12.23^{c}	15.10^{a}
F (pr)	**= 0.048**	**= 0.032**	**= 0.889**
LSD(0.05)	**0.98**	**4.09**	**22.95**

Means with different letters within the same column are significantly different ($p \leq 0.05$). Neem seed extract (NSE), Dates seed extract (DDSE), Jatropha seed extract (JSE), Tobacco leaf extract (TLE).

4. 2. 4. 3 Plant growth and yield

Plants treated with Desert date seed extract (DDSE) at 100 g/l were significantly ($p < 0.05$) taller than those treated with DDSE 25, DDSE 50, Topsin-M (1 g/l), Topsin-M (3 g/l), Tobacco leaf extract (TLE) at 100 g/l and water (negative control) (Table 4.5). There was no significant difference ($p > 0.05$) between height of plants treated with DDSE at 100 g/l and those treated withTopsin-M 2 g/l. Plants treated with Jatropha seed extract (JSE) at 100 g/l were relatively taller than those treated with JSE 25, JSE 50 and JSE 75. Also, plants treated with Neem seed extract (NSE) at 100 g/l were significantly ($p < 0.05$) taller than those treated with NSE at 50 g/l and negative controls (Table 4.5). There was no significant ($p > 0.05$) difference among the various concentrations of Tobacco leaf extract (TLE) and even when compared to water (negative control) (Table 4.5). Generally, plants treated with aqueous DDSE 100 g/l, NSE 100 g/l, JSE 100 g/l and Topsin-M at 2 g/l were taller with values of 18.32, 17.25, 16.42 and 15.35 cm respectively.

Heavier dry pod weight was recorded in plants treated with Desert date seed extract (DDSE) at 100 g/l with a mean value of 30.80 g followed by Jatropha seed extract (JSE) and Neem seed extract (NSE) at 100 g/l (Table 4.5). All plants treated with aqueous plant extracts at 100 g/l and Topsin-M at 2 g/l had heavier dry pod weight compared to the other plant extract concentrations (Table 4.5). However, there was no significant ($p > 0.05$) difference between treatments.

4. 3 Field experiment

4. 3. 1 Disease incidence

Groundnut plants sprayed with Topsin-M and aqueous Desert date seed extract had high significantly ($p < 0.001$) lowered disease incidence compared to other treatments from 3 to 7 weeks after planting (Figure 4.2). Topsin-M and DDSE had disease incidence percentage values of 18 and 21 % respectively in 2014 cropping season which was followed by Neem seed extract with disease incidence of 49 %. Neem seed extract significantly ($p < 0.001$) lowered disease incidence compared to Jatropha seed extract (87 %), Tobacco leaf extract (98 %) and control (99 %) from 3 to 7 WAP (Fig. 4.2). Plants treated with Jatropha seed extract lowered the disease incidence compared to plants sprayed with Tobacco leaf extract and control plants from 3 to 7 WAP which was highly significant ($p < 0.001$).

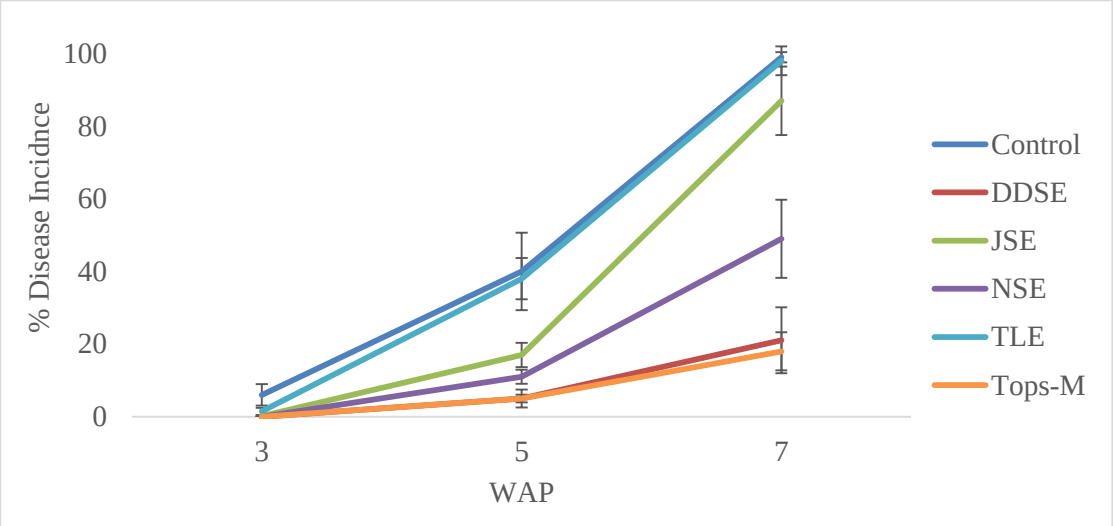

Figure 4.2: Effects of plant extracts on disease incidence of CLS of groundnut in 2014 cropping season. Neem seed extract (NSE), Date seed extract (DDSE), Jatropha seed extract (JSE), and Tobacco leaf extract (TLE).

Plants treated with Topsin-M, DDSE and NSE had highly significant ($p < 0.001$) lower disease incidence with values of 11, 14 and 22 % respectively compared to plants treated JSE (69 %) at 7 WAP, TLE (100 %) and control plants (100 %) at 5 to 7 WAP in 2015 (Figure 4.3). Generally, there was a reduction in disease incidence within treatments in 2015 compared to 2014 cropping seasons except in TLE and control plots (Figure 4.2; Figure 4.3).

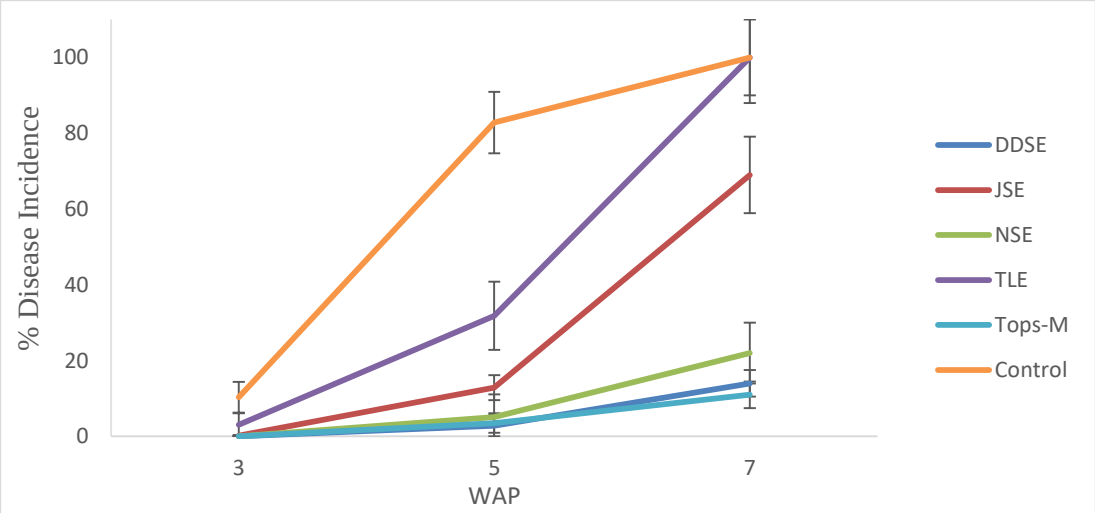

Figure 4.3: Effects of plant extracts on disease incidence of CLS of groundnut in 2015 cropping season. Neem Seed Extract (NSE), Date Seed Extract (DDSE), Jatropha Seed Extract (JSE), Tobacco Leaf Extract (TLE), 2015 cropping season.

4. 3. 2 Disease severity index

4. 3. 2. 1 Early leaf spot caused by *Cercospora arachidicola*

Mani-Pinta, Bugla and Chinese cultivars treated with Topsin-M and Desert date seed extract significantly ($p < 0.001$) lowered severity of Early leaf spot disease compared to Mani-Pinta and Chinese cultivars treated with Tobacco leaf extract and control in 2014 cropping season (Table 4.6). Plants of the Chinese cultivar treated with Jatropha seed extract and Neem seed extract had lower disease severity indices. Similarly,

Mani-Pinta cultivar plants treated with Neem seed extract had significantly ($p < 0.001$) lower disease severity index compared to negative control plants in 2014.

In 2015, Mani-Pinta, Bugla and Chinese cultivars plants sprayed with Topsin-M and DDSE recorded high significantly ($p < 0.001$) lower disease severity indices compared to Mani-Pinta, Bugla and Chinese cultivars plants sprayed with TLE and the negative control plants (Table 4.6). Chinese plants sprayed with DDSE and Topsin-M had lower ELS disease severity indices than Chinese plants sprayed with JSE in 2015 cropping season. Plants treated with NSE had relatively lower disease severity indices than JSE treated plants in both seasons. Conversely, Chinese and Mani-Pinta cultivar plants sprayed with NSE recorded high significantly ($p < 0.001$) lower disease severity index compared to Chinese plants treated with TLE and Mani-Pinta and Chinese plants under control in 2014 cropping season (Table 4.6). TLE treated plants and their control had relatively similar ELS disease severity values but they were not significantly ($p > 0.05$) different.

4. 3. 2. 2 Late leaf spot caused by *Cercosporidium personatum*

Groundnut cultivars treated with Topsin-M (positive control), Desert date seed extract and Neem seed extract had significantly ($p < 0.001$) lower Late leaf spot disease severity indices compared to all groundnut cultivars in the control plots during 2014 and 2015 cropping seasons (Table 4.6). Cultivars treated with Topsin-M, DDSE and NSE had significantly ($p < 0.001$) lower disease severity indices compared to Chinese cultivar treated with Tobacco leaf extract (TLE) in 2014 cropping season (Table 4.6).

Table 4:6 Effects of plant extracts on severity of ELS and LLS disease on three cultivars of groundnut in 2014 and 2015 cropping seasons

Treatment		Disease severity index (%) cropping seasons			
		C. arachidicola (ELS)		*C. personatum* (LLS)	
Plant Extract	**Cultivars**	**2014**	**2015**	**2014**	**2015**
DDSE	Mani-Pinta	22.00^{ab}	23.08^{a}	20.42^{a}	21.42^{ab}
	Bugla	21.42^{a}	22.75^{a}	21.08^{ab}	21.67^{ab}
	Chinese	21.75^{ab}	23.5^{a}	20.00^{a}	21.75^{ab}
JSE	Mani-Pinta	26.5^{abcd}	29.92^{abc}	26.08^{abc}	29.42^{abcd}
	Bugla	25.08^{abcd}	28.08^{ab}	27^{abcd}	29.75^{abcd}
	Chinese	28.5^{bcde}	32.33^{bcd}	26.50^{abc}	30.83^{bcde}
NSE	Mani-Pinta	23.42^{abc}	26.92^{ab}	24.17^{ab}	26.00^{abc}
	Bugla	23.08^{abc}	25.83^{ab}	24.42^{ab}	25.58^{abc}
	Chinese	25.42^{abcd}	29.17^{abc}	24.00^{ab}	27.00^{abc}
TLE	Mani-Pinta	29.58^{cde}	36.33^{cde}	28.17^{abcd}	35.25^{cde}
	Bugla	28.75^{bcde}	32.58^{bcd}	28.33^{abcd}	33.83^{cde}
	Chinese	36.08^{ef}	40.58^{ef}	30.58^{bcd}	38.75^{def}
Topsin-M (positive control)	Mani-Pinta	19.92^{a}	22.83^{a}	19.25^{a}	20.67^{a}
	Bugla	20.33^{a}	22.33^{a}	19.00^{a}	20.33^{a}
	Chinese	21.83^{ab}	24.00^{a}	19.67^{a}	21.00^{ab}
Water (negative control)	Mani-Pinta	30.50^{de}	39.17^{de}	35.08^{de}	39.83^{ef}
	Bugla	29.08^{cde}	35.92^{cde}	33.42^{cde}	37.17^{def}
	Chinese	39.83^{f}	47.28^{f}	42.58^{e}	47.92^{f}
Fr (p)		**<0.001**	**<0.001**	**<0.001**	**<0.001**
LSD (0.05)		**7.001**	**7.754**	**9.920**	**10.379**

Means with different letters within the same column are significantly different at 5 %

However, Topsin-M and DDSE treated plants recorded lower values of disease severity index percentages followed by NSE and JSE. Conversely, cultivars sprayed with Tobacco leaf extract were not significantly ($p > 0.05$) different from negative control except the Chinese cultivar.

Also, DDSE and Topsin-M had significantly ($p < 0.001$) lower LLS disease severity indices compared to cultivars sprayed with TLE in 2015 cropping season (Table 4.6). Cultivars sprayed with NSE had a relatively lower severity of LLS disease better than cultivars sprayed with JSE (Table 4.6). Cultivars such as Mani-Pinta and Bugla treated with JSE had a significantly ($p < 0.001$) lower severity of LLS disease compared to Mani-Pinta and Chinese cultivars plants in negative control. Apart from TLE treated plants, all aqueous plant extracts drastically reduced disease severity of LLS compared to control plants consistently over the two years with Desert date seed extract being the best and followed by NSE.

4. 3. 3 Defoliation

Cultivars such as Mani-Pinta and Bugla treated with Desert date seed extract had a significantly ($p < 0.001$) lower defoliations compared to Chinese cultivar plants treated with Tobacco leaf extract and their negative controls both in 2014 and 2015 cropping seasons (Table 4.7). Cultivars sprayed with Neem Seed Extract (NSE) and Jatropha Seed Extract (JSE) had a higly significant ($p < 0.001$) lower defoliations compared to 'Chinese' plants in control plots which recorded leaf defoliation percentages of 66.31 and 64.50 % in 2014 and 2015 cropping seasons repectively.

All cultivars sprayed with Topsin-M, DDSE and NSE had highly significant ($p < 0.001$) lower defoliations compared to negative control plants in 2015 cropping season (Table 4.7). Plant extracts effectively lowered leaf defoliation in 2015 better than in 2014. Cultivars treated with Topsin-M recorded the least percentage defoliation followed by DDSE.

Table 4.7: Effects of plant extracts on defoliation of three groundnut cultivars

Treatments		**% Defoliation in cropping seasons**	
Plant Extract	**Cultivars**	**2014**	**2015**
DDSE	Manipinta	33.72^{ab}	19.50^{ab}
	Bugla	37.66^{abc}	20.23^{ab}
	Chinese	48.56^{bc}	24.33^{abc}
JSE	Manipinta	41.46^{abcd}	27.01^{abcd}
	Bugla	38.51^{abc}	29.96^{bcde}
	Chinese	49.78^{bcd}	34.49^{ef}
NSE	Manipinta	40.33^{abcd}	25.74^{abc}
	Bugla	36.08^{abc}	24.03^{abc}
	Chinese	48.12^{bcd}	31.58^{cd}
TLE	Manipinta	34.52^{abc}	33.60^{de}
	Bugla	43.38^{bcd}	29.74^{bcde}
	Chinese	55.05^{de}	49.98^{f}
Topsin-M (positive control)	Manipinta	26.30^{a}	15.01^{a}
	Bugla	33.79^{abc}	16.20^{a}
	Chinese	39.50^{abcd}	18.29^{ab}
Water (negative control)	Manipinta	50.49^{cde}	48.36^{f}
	Bugla	46.84^{bcd}	43.74^{ef}
	Chinese	66.31^{e}	64.50^{g}
Fr (p)		**<0.001**	**<0.001**
LSD (0.05)		**16.706**	**12.895**

Means with different letters within the same column are significantly different at 5 %.

4. 3. 4 Plant height

Plants treated with Topsin-M were significantly ($p < 0.05$) taller than those treated with Neem seed extract, Jatropha seed extract, Tobacco leaf extract and the negative control plants in 2014 cropping season (Table 4.8). However, Topsin-M treated plants produced statistically similar results with DDSE-treated plants in 2014 cropping season (Table 4.8). Desert date seed extract treated plants were significantly ($p < 0.05$) taller than the control plants in 2014 cropping season (Table 4.8). Plants sprayed with DDSE were relatively taller than those sprayed with NSE, JSE and TLE. However, NSE-, JSE- and TLE-treated plants were relatively taller than negative control plants in 2014 cropping season (Table 4.8).

Conversely, plants sprayed with DDSE produced highly significant ($p < 0.001$) taller plants than NSE-, JSE-, TLE-treated plants and Topsin-M (positive control)-treated plants during the 2015 cropping season (Table 4.8). Topsin-M sprayed plants were highly significant ($p < 0.001$) taller than plants treated with JSE and TLE in 2015 cropping season. Similarly, Topsin-M-treated plants were not significantly ($p > 0.05$) different compared to those treated with NSE in 2015 cropping season (Table 4.8). NSE and JSE treated plants were not significantly ($p > 0.05$) different. However, plants treated with NSE and JSE were taller than those treated with TLE and negative control plants in 2015 cropping season (Table 4.8). TLE sprayed plants were relatively taller than control plants but was not significant in 2015 cropping season. DDSE, NSE and JSE effectively reduced disease incidence, severity and defoliation thereby increasing plant growth.

4. 3. 5 One hundred pod weight

Topsin-M (positive control)-treated plants had highly significant ($p < 0.001$) heavier pods than Neem seed extract, Jatropha seed extract and Tobacco leaf extract treated plants and control plants for 2014 and 2015 cropping seasons (Table 4.8). However, Topsin-M (positive control)-treated plants produced relatively heavier pods than Desert date seed extract-treated plants in both cropping seasons but was not significantly ($p > 0.05$) different (Table 4.8). Plants treated with DDSE had highly significant ($p < 0.001$) heavier pods than plants treated with JSE, TLE and negative control plants in 2014 cropping season (Table 4.8). DDSE-sprayed plants produced heavier pods than NSE sprayed plants which was not significant ($p > 0.05$) in 2014 cropping season.

Plants sprayed with aqueous DDSE and Topsin-M had highly significant ($p < 0.001$) heavier pods than those sprayed with NSE, JSE, TLE and negative control plants in 2015 cropping season (Table 4.8). Also, plants treated with NSE produced highly significant ($p < 0.001$) heavier pods compared to plants treated with JSE, TLE and control plants in 2015 cropping season (Table 4.8). JSE-sprayed plants had highly significant ($p < 0.001$) heavier pods compared to plants sprayed with TLE and control plants in 2015 cropping season (Table 4.8). However, plants sprayed with TLE produced statistically similar pod weight compared to negative control plants but was not significantly ($p > 0.05$) differently (Table 4.8).

4. 3. 6 One hundred seed weight

Plants treated with Topsin-M produced highly significant ($p < 0.001$) heavier seeds than those treated with plant extracts and the control plants in 2014 cropping season (Table 4.8). However, plant extracts treated plants had highly significant ($p < 0.001$) heavier seeds than control plants in 2014 cropping season (Table 4.8). Desert date seed extract sprayed plants had relatively heavier seed than those treated with NSE, JSE and TLE in 2014 cropping season.

Topsin-M (positive control) and DDSE treated plants had highly significant ($p < 0.001$) heavier seeds than those treated with NSE, JSE, TLE and negative control plants during the 2015 cropping season (Table 4.8). NSE-treated plants produced highly significant ($p < 0.001$) heavier seeds than those treated with JSE, TLE and control plants in 2015 cropping season (Table 4.8). Also plants treated with JSE produced heavier seeds than those treated with TLE and negative control plants which was highly siginificant ($p < 0.001$) in 2015 cropping season (Table 4.6). However, there was no significant ($p > 0.05$) difference between TLE treated plants and control plants in 2015 cropping season (Table 4.8).

Effects of plant extracts on plant height, 100 pod weight, 100 seed weight, dry pod yield and seed yield in 2014 and ing season

ract	Plant height (cm)		Dry pod yield (kg/ha)		Dry seed yield (kg/ha)		100 pod weight (g)		100 seed weight (g)	
	2014	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
)ate Seed	31.40^{ab}	33.7^{a}	931.00^{b}	1275.00^{b}	751.00^{b}	992.00^{a}	87.90^{ab}	87.57^{a}	39.50^{b}	49.82^{a}
seed	27.82^{bc}	26.85^{c}	729.00^{c}	931.00^{c}	546.00^{c}	698.00^{b}	75.40^{cd}	56.39^{c}	36.70^{b}	32.86^{c}
ed Extract	25.71^{bc}	28.53^{cb}	875.00^{b}	1004.00^{c}	688.00^{b}	786.00^{b}	85.30^{bc}	67.07^{b}	37.50^{b}	37.31^{b}
leaf	25.94^{bc}	25.36^{cd}	626.00^{c}	692.00^{d}	504.00^{c}	570.00^{c}	74.50^{d}	49.86^{d}	37.20^{b}	30.19^{d}
I (positive	32.57^{a}	30.03^{b}	1095.00^{a}	1322.00^{a}	922.00^{a}	1045.00^{a}	96.80^{a}	88.23^{a}	46.70^{a}	50.72^{a}
gative	25.21^{c}	25.17^{d}	426.00^{d}	581.00^{d}	306.00^{d}	430.00^{d}	45.70^{e}	49.86^{d}	23.60^{c}	27.67^{d}
FF (p)	**= 0.032**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
LSD (0.05)	**5.78**	**3.24**	**103.6**	**140.9**	**80.3**	**124.9**	**10.46**	**5.397**	**5.21**	**3.75**

Means with different letters within the same column are significantly different

4. 3. 7 Dry pod yield

Topsin-M (positive control)-treated plots had highly significant ($p < 0.001$) heavier pod yield (kg/ha) than those treated with Desert date seed extract, Neem seed extract, Jatropha seed extract and Tobacco leaf extract both 2014 and 2015 cropping seasons with values of 1095 and 1322 (kg/ha) respectively followed by DDSE with values of 931 (kg/ha) in 2014 and 1275 (kg/ha) in 2015 (Table 4.8). Plants treated with DDSE and NSE produced highly significant ($p < 0.001$) heavier pod yield than those treated with JSE, TLE and negative control plants (Table 4.8). Plants treated with JSE and TLE were not significantly ($p > 0.05$) different but JSE- and TLE-treated plants were highly significant ($p < 0.001$) heavier in pod yield than control plants in 2014 cropping season (Table 4.8).

Plants sprayed with DDSE produced highly significant ($p < 0.001$) pod yield with a value of 1275 kg/ha compared to those sprayed with NSE, JSE and TLE and negative control plants in 2015 cropping season (Table 4.8). NSE-treated plants had hghly significant ($p < 0.001$) pod yield with a value of 1004 kg/ha than JSE, TLE and negative control plantts in 2015 cropping season (Table 4.8). However, in 2015 cropping season, plants treated with JSE yielded more dry pods than plants treated with TLE (Table 4.8). Though JSE treated plants produced more pod yield than TLE-treated plants but there was no significant ($p > 0.05$) difference between them in 2014 cropping season. Plant treated with NSE produced relatively more dry pods than JSE but there was no significant ($p > 0.05$) difference between them in 2015 cropping season (Table 4.8).

4. 3. 8 Dry seed yield

Topsin-M (positive control)-treated plants had highly significant ($p < 0.001$) seed yield (ka/ha) than those treated with DDSE, NSE, JSE and TLE and control plants in 2014 cropping season (Table 4.8). However, plants treated with Topsin-M and DDSE produced highly significant ($p < 0.001$) seed yield than those treated with NSE, JSE, TLE and negative control plants in 2015 cropping season (Table 4.8). NSE-treated plants recorded significantly ($p < 0.001$) heavier seeds compared to plants treated with JSE, TLE and negative control plants in 2014 cropping season but had statistically similar seed yield with JSE in 2015 cropping season (Table 4.8). Plants sprayed with JSE produced significantly ($p < 0.001$) higher seed yield compared to those sprayed with TLE and control plants in both 2014 and 2015 except TLE treated plants in 2014 where their yields were not significantly ($p > 0.05$) different (Table 4.8).

CHAPTER FIVE

5.0 DISCUSSION

5. 1 Field Survey

5. 1. 1 Farmers' knowledge, perceptions and mangement of Cercospora leaf spot (CLS) disease of groundnut

Majority (87.50 %) of the groundnut farmers in the study area knew that CLS is a disease. This means that more farmers are aware of the disease in their farms and its devastating effects. More males (47.00 %) were aware of the disease than females (40.50 %). The greater awareness could be due to their role as family heads who are mostly in charge of farming. It could also be that males are more resourced than females and have easy access to information on agronomics, pests and disease management. This confirms the report by Quisumbing *et al*. (1995), that although they provide 60 to 90 % of the farm work as females, they usually lack technical knowledge, and often have poor access to current information, markets and credit to enable them engage in cash crop farming.

Majority of the respondents (84.5 %) knew the symptoms of the CLS disease. This means that more farmers could identify the symptoms of the disease. The findings in this study confirm an earlier report that traditional rural farmers are able to successfully detect plant diseases through observation informed by their farming experiences in the absence of a scientific process and equipment to conduct such assessment (Adam *et al*., 2015). Most of the farmers (79 %) were able to identify the symptoms on the leaves of groundnuts on their farms. This clearly indicates that farmers in the northern sector

have observed the disease for a very long time. It also shows that the disease is common in all groundnut growing areas and also commonly found on the leaves of the crop. The report that the Cercospora Leaf Spot (CLS) disease is commonly found wherever groundnut is grown is true (Zhang *et al*., 2001; Nutsugah *et al*., 2007; Chaube and Pundhir, 2009). Majority (91 %) of the smallholder farmers attributed the disease to poor soil fertility, high rainfall, wind or air and herbicides applications. This implies that farmers have critically observed the disease for a very long time in order to determine the factors that cause or increase the incidence and severity of the disease. However, it also shows that farmers may not be able to distinguish between herbicides injury to groundnut plants and CLS disease. Herbicides injury to plants is normally due to wrong time of application, wrong dosage and application under unfavourable environmental conditions.

Farmers in the Northern Region (84.5 %) rated CLS diseases incidence on their fields to be 50 % and above which confirms an earlier report that both early and late leaf spots diseases are widely distributed and occur in epidemic proportions in Northern Ghana (Nutsugah *et al*., 2007). Female farmers recorded higher percentage of disease incidence than male farmers. This can be attributed to the fact that most women are restricted to continuous cultivation on marginal lands and old groundnut fields where there is a build-up of inoculum and loss of nutrients. This supports Pazderka and Emmott (2010) report that factors that limit yields of groundnut in Ghana include increased cultivation on marginal lands and outburst of pest and diseases. Female farmers also reported that the disease is often encountered at the early stage of vegetative growth which probably is an indication of early leaf spot.

Most farmers (83.5 %) in the Northern region of Ghana encountered this disease any season groundnut was planted and they were aware of its detrimental effects leading to significant yield losses. This agrees with the report that CLS is widely spread and causes pod loses of about 78 % in Northern region of Ghana (Tsigbey *et al.*, 2003; Nutsugah *et al*., 2007). Farmers observed highly significant disease severity on their fields. Even though female farmers experience higher (46 %) incidence of the disease their farms had a lower disease severity (32 %) than those of the males. It implies that female farmers practiced better crop management than their male counterparts. Good crop management strategies can help reduce the severity of a disease.

Most of the farmers (60 %) used leaf defoliation and brown spots to determine the maturity of the groundnut crop which confirms reports that farmers use the leaf defoliation as a sign of groundnut maturity (Tsigbey *et al.*, 2003; Nutsugah *et al.*, 2007). Disease management increases the quantity and improves the quality of the plant products available for use. More farmers (62 %) relied solely on non-chemical methods for the control of the disease. This confirms Bently and Thiel (1999) report that farmers in developing countries have been using their own knowledge in managing plant diseases.

Most of the non-chemical methods mentioned were crop rotation, spacing, and mixed cropping among others. Farmers reported that more research should be carried out on other control measures to help reduce the negative impact of this disease. This is an indication that most of the measures are old and do not help much in reducing the disease incidence and severity on their groundnut fields.

5. 1. 2 Incidence and severity of Cercospora leaf spot surveyed on selected farms in the sudy area

Cercospora leaf spot was prevalent in all farms surveyed. Tolon and Kumbungu Districts recorded significantly lower disease severity as compared to Tamale Metro and East Gonja District. The reason may be that due to the proximity of Savanna Agricultural Research Institute and University for Development Studies– Faculty of Agriculture to farmers in the Tolon and Kumbungu Districts benefited from their research findings. This is also an indication that the levels of disease severity differ from locality to locality, district to district and ecology to ecology due to differences in environmental conditions as indicated by Nutsugah *et al.* (2007).

5. 2 *In vitro* Studies

5. 2. 1 Phytochemical analyses

Alkaloids, tannins and phenolic compounds were detected in all the plant extracts used. This confirms the report by Gurjar *et al*. (2012) that plant extracts contain phytochemicals such as phloretin, tannins, allicins, azadirachtin which have antimicrobial properties. Desert date seeds, neem seeds and tobacco leaves contained saponins. Cardiac glycosides were detected in desert date seed and tobacco leaves. Terpenoids were detected in neem seeds and tobacco leaves. Neem seeds also contained steroids. Gurjar *et al*. (2012) also noted that plant extracts with antimicrobial property can be specific or broad spectrum in action against pathogens.

5. 2. 2 Isolation and identification of fungal pathogens

The fungal pathogens isolated and identified from infected groundnut leaves were *Cercospora arachidcola* and *Cercosporidium personatum* which are the causative agents of Cercospora leaf spot diseases of groundnut. The conidium of *Cercospora arachidicola* was sub-hyaline or pale yellow, obclavate or cylindrical and septate with rounded base and sub-acute tip. The morphological characteristics were similar to those reported by McDonald *et al*. (1985). However, the conidium of *Cercosporidium personatum* was obclavate or cylindrical, light coloured and the base was shortly tapered with a conscipicous hilum. This morphological description is similar to that described by Ijaz (2011). *Cercospora arachidcola* and *Cercosporidium personatum* are significant threat to groundnut farms in the Northern Region of Ghana (Nutsugah *et al*., 2007).

5. 2. 3 Pathogenicity test of *Cercospora arachidicola* and *Cercosporidium personatum*

Groundnut seedlings that were inoculated with a suspension of mycelia of *Cercospora arachidicola* and *Cercosporidium personatum* showed symptoms of CLS of groundnut on their leaves, which indicated that groundnut is susceptible to these pathogens. The observed lesions caused by *Cercospora arachidicola* were sub-circular to irregular in shape, dark brown and surrounded by a yellow halo whilst that of *Cercosporidium personatum* were more nearly circular in shape and brown black described by McDonald *et al*. (1985) as well as Hagan (1998). The yellow halo was more conspicuous and spreading in *Cercospora arachidicola* spots but dull and limited to margins of spots in *Cercosporidium personatum* as reported by Ijaz, (2011). The spots of *Cercospora arachidicola* were also bigger in size than *Cercosporidium*

personatum. This is in line with the description that *Cercospora archidicola* lesions are usually larger than that of *Cercosporidium personatum* (Chaube and Pundhir, 2009). Koch's postulate was proved since the pure cultures of the pathogens that were re-isolated from the lesion areas had the same characteristics as the original cultures used in inoculating the seedlings in the green house.

5. 3 Effect of aqueous plant extracts on mycelial growth of *Cercospora arachidicola* and *Cercosporidium personatum* under in vitro and green house conditions

5. 3. 1 Growth inhibition

Desert date seed extract at all concentration levels progressively suppressed mycelial growths of both *Cercospora arachidicola* and *Cercosporidium personatum* due to the phytochemical constituents present in the seed. It is realised that efficacy of plant extracts increases as concentration increases. Therefore, DDSE at 100 g/l recorded the highest mycelia percentage growth inhibition of 90.33 and 84.96 % in *Cercospora arachidicola* and *Cercosporidium personatum* respectively. DDSE at 100 g/l drastically suppressed the mycelia growth of both fungi and retarded their vegetative growth. The inhibition of mycelia growth in both fungi is attributed to the phytochemicals (alkaloids, tannins, phenolics and saponins) contained in the desert date seed extract (Table 4.3). This confirms the findings of Akinbode (2010) who observed that all plant extracts at 100 % concentration significantly suppressed the growth of *Curvularia lunata*. Aqueous extract of Desert date seed at 75 g/l was comparable to NSE 100 g/L in both ELS and LLS suppression. This showed that the level of efficacy depends on the type of plant extract use. DDSE at 75 g/l also

significantly inhibited the mycelial growth of the two pathogens (*Cercospora arachidicola* and *Cercosporidium personatum)* compared to all concentrations in Tobacco Leaf Extract (TLE) and Jatropha Seed Extract (JSE) for both ELS and LLS (Table 4.4). Similarly, Ambang *et al*. (2011) also observed lower epidemics and severity of CLS of groundnut when *Thevetia peruviana* seed extract was applied at higher concentration.

Neem seed extract at 100 g/L highly lowered mycelial growth of both *Cercospora arachidicola* and *Cercosporidium personatum* compared with its concentrations of 25, 50 and 75 g/l. Neem seed extract at all concentrations progressively retarded vegetative growth of both fungi due to the azadirachtin and other phytochemical constituents such as alkaloids, saponins, steroids, terpenoids, tannins and phenolic compounds present in the seed. Apart from DDSE at 100 and 75 g/l, NSE 100 g/l was the next best with percentage mycelia inhibition of 80.88 and 72.32 % in both *Cercospora arachidicola* and *Cercosporidium personatum* respectively. This observed inhibition was due to the fungi-toxic activity of the aqueous neem seed extract. This confirmed the findings that aqueous neem seed extract strongly inhibited *Alternaria alternata* growth at the highest concentration (Al-Hazmi, 2013). Neem seed and leaf extracts also reduced the growth of the fungi *Pyricularia oryzae* in rice (Amadioha, 2000).

Jatropha seed extract at 100 g/l suppressed mycelial growth compared to JSE at 25, 50 and 75 g/l with mycelia inhibition percentages of 75.66 and 67.28 % in *Cercospora arachidicola* and *Cercosporidium personatum* respectively. The fungi-toxic effect of

Jatropha curcas was due to the presence of the active ingredient curcin and other phytochemicals such as alkaloids, tannins and phenolic compounds (Makun *et al*., 2011). JSE also caused significant reduction in vegetative growth with increasing concentration. This agrees with the findings that the phytochemicals present in *Jatropha curcas* seed extract significantly reduced the rot index of yam caused by *Fusarium verticilliodes* and *Aspergillus flavus* (Makun *et al*., 2011). The effect of JSE on the fungi showed that vegetative growth decreased with increase in concentrations.

Different concentrations of Tobacco leaf extract at 25, 50, 75 and 100 g/l reduced mycelial growth of both fungi. This is attributed to the presence of the following phytochemicals in TLE; saponins, cardiac glycosides and terpenoids which affect the growth of *Cercospora arachidicola* and *Cercosporidium personatum*. This confirms that tobacco possess potential antifungal properties which completely retarded fungal mycelial growth at 60 % concentration (Suleiman, 2011). However, TLE was not as effective compared to DDSE, NSE and JSE in fungi-toxic activity against Cercospora leaf spot diseases.

5. 3. 2 Disease severity index

The plant extracts lowered the disease severity index with Desert date seed extract at 100 g/l recording the least severity index percentage which was statistically similar to Topsin-M at 2 g/l due to their fungi-toxic activities. Kishore *et al*. (2001) similarly observed that neem oil reduced the incidence of groundnut leaf spot. Hossain and Hossain (2013) also reported that plant extracts decreased spot number per leaf, defoliation per plant, incidence of leaf spot and number of infected leaf per plant of groundnut.

5. 3. 3 Plant growth and yield

Vigorous vegetative growths was observed in plants treated with DDSE 100 g/l, NSE 100 g/l and JSE 100 g/l. It was realised that plants treated with DDSE 100 g/l were the tallest between plant extracts including the positive control. This observation could be attributed to the fact that they were able to reduce the disease incidence and severity and therefore prevented stunting. Hossain and Hossain (2013) also observed that water extract of 23 plant materials which included neem seed, neem leaves, leaves of tomato and ginger rhizome gave considerable reduction in disease incidence and increased growth parameters compared to the control.

Plants treated with DDSE 100 g/l, NSE 100 g/l, JSE 100 g/l and TLE 100 g/l produced heavier pod weight which may due to the phytochemicals contained in these extracts were more and reduced the negative impact of the disease and increased yields. Hossain and Hossain (2013) concluded that the application of plant extracts increased pod weight and haulm yield by 64.37-111.41 and 32.35-74.71 %, respectively.

5. 5 Field Experiment

5. 5. 1 Effects of aqueous plant extracts on disease incidence and severity caused by *Cercospora arachidicola* and *Cercosporidium personatum*

In 2014 cropping season, there was a reduction in disease incidence with plants treated with Desert date seed extract better than the other plant extracts. Similary, in 2015 cropping season, plants treated with DDSE, Neem seed extract and Jatropha seed extract reduced disease incidence except in Tobacco leaf extract treated plants. The

performances of DDSE and NSE were outstanding compared to all plant extracts. The decreased in disease incidence in plants treated with DDSE and NSE could be due to the antifungal properties of the extracts. This is in accordance with Ambang (2011) who observed that an increase in concentration of *T. peruviana* seed extracts resulted in a decrease in rate of spread of Cercospora Leaf Spot of groundnut. This also confirmed the findings that neem leaf extract reduced the growth of *Curvularia lunata* and succeeded in resisting fruit rotting in Cucurbitaceae caused by *Fusarrium equisitifolium* and *F. semitectum* (Al-Hamza, 2013). The assertion that neem leaves extract demonstrated a strong ability against the development of many disease causing fungi is true (Tewari and Nayak, 1991; Locke, 1995).

Early leaf spot severity caused by *Cercospora arachidicola* greatly reduced with the application of plant extracts compared to the negative control across the two cropping seasons. This observed reduction in Early leaf spot severity with the application of DDSE, NSE and JSE is due to their fungicidal effects, which lowered the spread of the early leaf spot pathogen (*Cercospora arachidicola*). This agrees with Hossain and Hossain (2013) report that aqueous neem seed and leaves extracts gave a considerable reduction in disease incidence, spot number per leaf, defoliation per plant and number of infected leaf per plant by 35.45 -60.07 and 42.06-72.20 % respectively. The Chinese cultivar recorded the highest value of disease incidence in all treatments. It has been noted to be very susceptible to Cercospora leaf spots, which is the reason it had a highest value of disease incidence. According to McDonald *et al*. (1985), groundnut varieties / cultivars differ in their resistance to leaf spot disease.

DDSE-sprayed plants had the lowest disease severity percentage of Late leaf spot compared to NSE, JSE and TLE in 2014 and 2015 cropping seasons. The positive control and the plant extracts were active enough to reduce the severity of *Cercosporidium personatum* which is the causal organism of late leaf spot due to the presence of phytochemical and / or antifungal properties of the extracts and fungicide. Probably, the presence of alkaloids, saponins, cardiac glycosides, tannins and phenolic compounds contained in DDSE might have called for its outstanding consistent performance. Also, Mani-Pinta, Bugla, and Chinese cultivars treated with Topsin-M and DDSE had disease severity percentage values of 20.67 - 21 and 21.42 - 21. 75 % respectively in 2015 cropping season. Control plants especially Chinese recorded the highest disease severity percentage value of 47.92 %. This is in accordance to McDonald *et al*. (1985) who reported that groundnut varieties/cultivars differ in reaction to leaf spot in which some of the varieties/cultivars are most susceptible.

5. 5. 2 Effects of plant extracts on growth parameters

Desert date seed extract greatly reduced defoliation in Manipinta and Bugla during the 2014 and 2015 cropping seasons. However, there was more reduction in defoliation in 2015 cropping season compared to 2014. This might be due to differences in temperature and relative humidity in both years which might have led to infection and development of the disease hence defoliation. This confirms the finding that temperatures ranging from 25-30 ^{0}C and high relative humidity favour CLS disease infection and development (McDonald *et al*., 1985; Shokes and Culbreath, 1997).

Plants treated with aqueous Desert date seed extract produced average plant height value of 31.40 cm which was comparable to 32.57 cm of the positive control in 2014

cropping season. Plants treated with DDSE, NSE, JSE and TLE were taller than negative control plants in the 2014 cropping season. Generally, plants sprayed with DDSE were taller than positive control, NSE-, JSE- and TLE-treated plants and negative control plants in 2015 cropping season. Aqueous DDSE- and NSE-treated plants increased plant performance over the two cropping seasons. The vigorous vegetative growth caused by plant extracts could be attributed to the positive effect of the phytochemicals contained in them, which strongly inhibited the growth, reproduction, spread, incidence and severity of the fungi leading to better groundnut plant establishment. This is in agreement with Culver *et al*. (2012) who reported that plant materials such as Moringa increased the height of tomato.

5. 5. 3 Effect of plant extracts on yield parameters

Topsin-M treated plants recorded the heaviest pods among treatments with values of 96.8 g and 88.23 g in 2014 and 2015 cropping season respectively. These were statistically not different from plants treated with aqueous Desert date seed extract. DDSE-treated plants were also not statistically different from plants treated with NSE. Plants treated with DDSE, NSE and JSE produced heavier pods than TLE. This could be attributed to the antifungal properties which retarded and inhibited the activity of the fungi leading to a decreased in spot number per leaf, defoliation per plant, incidence of leaf spot and number of infected leaf per plant. This could lead to an increase in photosynthetic activity which enhanced vegetative growth, net assimilation and dry matter accumulation, subsequently 100 pod weight. These results support the finding of Hossain and Hossain (2013), who observed that plant materials which included aqueous neem seed and leaf extracts caused significant increase in 100 pod weight compared to control.

In one hundred seed weight, all plants sprayed with Desert date seed extract, Neem seed extract, Jatropha seed extract and Tobacco leaf extract produced heavier seeds than all negative control plants in 2014 and 2015 cropping seasons. DDSE, NSE, JSE and TLE recorded statistically similar results in 2014 cropping season. In 2015 cropping season, DDSE-treated plants produced heavier seeds than NSE-, JSE- and TLE-treated plants. This observation can be attributed to the fungicidal effects of the plant extracts which decreased disease incidence and severity thereby increasing seed weight. This is confirmed of the study of Hossain and Hossain (2013) that plant extracts considerably reduced disease severity thereby increasing yield in groundnut.

Topsin-M (positive control)-, DDSE-, NSE-, JSE- and TLE-treated plants produced more pod yield in both 2014 and 2015 cropping seasons with values ranging from 626 to 1095 and 692 to 1322 kg/ha respectively. In 2014 cropping season, DDSE- and NSE-treated plants produced considerable dry pod yields. Plants sprayed with DDSE had dry pod yield value of 1275 kg/ha in 2015 cropping season. NSE-treated plants produced a better yield of 1004 kg/ha compared to JSE, TLE and negative control plants in 2015 cropping season. DDSE-, NSE- and JSE-treated plants yielded more than negative control plants and this can be attributed to their antifungal properties which suppressed the activities of the fungi leading to decreased spot number per leaf, defoliation per plant, incidence of leaf spot and number of infected leaf per plant. This could have led to the increase in photosynthetic activity, faster groundnut plant establishment, and subsequently dry pod yield. This is in line of the findings by Nahunnaro and Tunwari (2012) that plant extracts and benlate (chemical fungicides) used for the control of Cercospora leaf leaf spot of sesame significantly had 40.71 and 38.22 % higher yields than unsprayed plots.

Plants treated with DDSE had more seed yield than NSE-, JSE- and TLE-treated plants in 2015 cropping season. In 2014 cropping season, aqueous NSE-treated plants yielded more seeds than JSE- and TLE-treated plants. This observation could be attributed to the fungicidal effects of the plant extracts which decreased disease incidence and severity, promoted growth parameters and increased yield. This confirms the report that aqueous plant extracts such as neem seed extract decreased spot number per leaf, defoliation per plant, incidence of leaf spot, and number of infected leaf per plant and increased pod yield (Hossain and Hossain, 2013).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6. 1 Conclusion

- The study revealed that farmers were aware of the Cercospora leaf spot (CLS) disease and its devastating effects, and perceive it as a major constraint to groundnut production in Northern Region of Ghana. Farmers described the disease incidence as well as the disease severity on their farms to be above 50 %.
- A notable finding from this study was that farmers were not able to distinguish between herbicide injuries to plants and Cercospora leaf spots symptoms. It has also been realised that most farmers used defoliation and brown spots as signs of groundnut maturity.
- The study also showed that leaf spots disease severity differ from one locality to another depending on environmental factors and control measures adopted by farmers.
- Farmers also expressed various opinions as the future management strategies for lessening CLS problem in the area which included spraying with effective plant extracts. Farmers in Northern Region of Ghana mostly relied solely on non-chemical methods for minimising the effects of CLS disease and they are likey to adopt plant materials that are effective in the management of the disease.

- Phytochemical analyses of plant extracts used for the management of the disease revealed the presence of alkaloids, tannins and phenolic compounds.

Desert date seeds, neem seeds and tobacco leaves had other phytochemical constituents such as glycosides, terpenoids and steroids. All the phytochemicals identified were found to be fungicidal or fungitoxic.

- *Cercospora arachidcola* and *Cercosporidium personatum* were identified as the causal agents of Cercospora leaf spot disease symptoms on groundnut and their pathogenicity proofed using Koch's postulates.

- Preliminary experiments conducted under laboratory and green house conditions showed that aqueous Desert date seed, Neem seed, Jatropha seed, and Tobacco leaf extracts at 100 g/l were effective against *Cercospora arachidicola* and *Cercosporidium personatum.* However, DDSE was consistent in its performance against CLS diseases under both conditions followed by NSE and JSE. DDSE at 100 g/l recorded the highest inhibition percentages for both *Cercospora arachidicola* and *Cercosporidium personatum* with values of 90.33 and 84.96 % respectively. Plants treated with DDSE at 100 g/l produced the heaviest pods. It has been realized from the study that efficacy increases as concentrations of plant extracts increases and the level of efficacy also depends on the type of plant material use.

- DDSE, NSE and JSE consistenly reduced disease severity of both *Cercospora arachidicola* and *Cercosporidium personatum* than TLE and negative control. However, the most effective plant extract was aqueous Desert date seed extract which was as potent as the positive control, Topsin-M in 2014 and 2015

cropping seasons followed by NSE and JSE. Plants sprayed with DDSE were taller than Topsin-M (positive control)-, NSE-, JSE- and TLE-treated plants and negative control plants. Topsin-M treated plants had heavier dry pod yield in both 2014 and 2015 cropping seasons with values of 1095 and 1322 kg/ha respectively followed by DDSE with values of 931 kg/ha in 2014 cropping season and 1275 kg/ha in 2015 cropping season. Generally, the plant extracts positively influenced the yield of groundnut. However, tobacco leaf extract was inconsistent in its effectiveness against CLS disease.

6. 2 Recommendations

(a) Farmers need to be educated by Ministry of Food and Agriculture and Non-governmental organisations on the practices that increase incidence and severity of the disease, how to distinguish the symptoms from herbicides injury and integrated management approach which may include the use of plant extracts since the disease is widely distributed and endemic in the study area.

(b) The performance of some plant extracts tested is comparable to the synthetic fungicide Topsin-M, and therefore this can give farmers an ample opportunity to try many alternatives that are user friendly. For most of the parameters, Desert date seed extract produced the best results after Topsin-M, followed by Neem and Jatropha Seed Extracts. Since these plants are common in the study area, more especially Desert dates and neem, they can be used by farmers to control Cercospora leaf spot disease of groundnut.

(c) Aqueous Desert date seed extract, Neem seed extract and Jatropha seed extract at 100 g/l are recommended to farmers for use in the control of CLS.

(d) Tobacco leaf extract contained the same phytochemicals as Desert date seed extract but was not potent. Therefore, in future research, quantitative data should be obtained for various phytochemicals elements and the mode of operation. Also, different methods of extraction that will detect more phytochemical elements should be studied.

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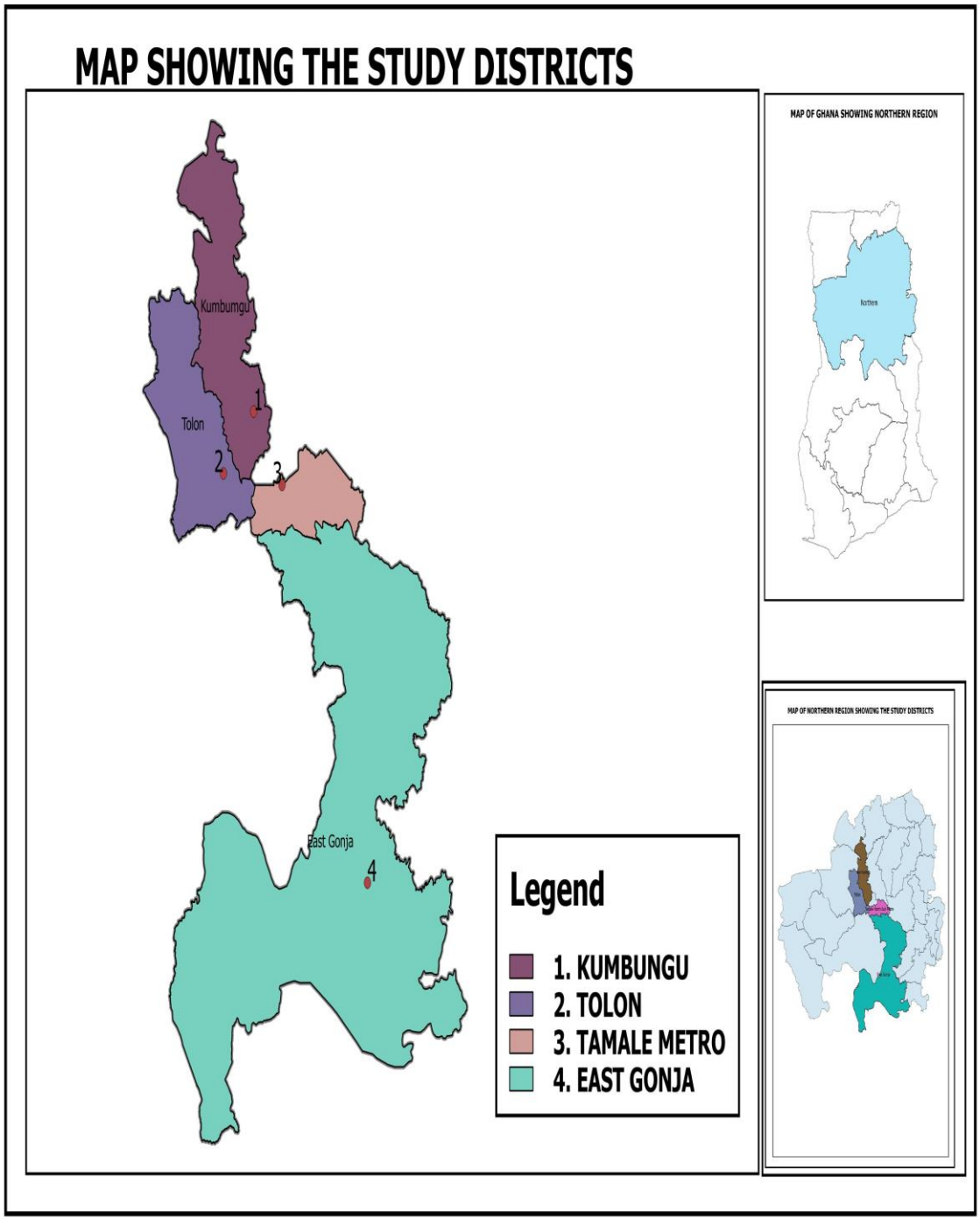
Appendices

Appendix 1: Map showing the study Districts/Metropolis



Appendix 2: Study districts and number of groundnut farmers interviewed in Northern Region

Districts/Metropolis	Communities	No. of groundnut farmers interviewed
Tamale	Fooshegu	10
	Kotingli	10
	Bagli-Dakpemyili	10
	Dalogyili	10
	Bagliga	10
East Gonja	Sakpalua	10
	Jantong-Daashee	10
	Jangyili	10
	Kpinchila	10
	Jantong-Wulanyili	10
Tolon	Golinga	10
	Tingoli	10
	Kpana	10
	Tali	10
	Waribogu	10
Kumbungu	Cheyohi	10
	Gizaa-Gundaa	10
	Gbuling	10
	Kunkuling	10
	Tonjing	10
Total		**200**

Appendix 3: Florida 1 to 10 scale system for groundnut

Scale	Interpretation
1	no leaf spot
2	very few lesions on the leaves, none on the upper canopy
3	few lesions on the leaves, very few on the upper canopy
4	some lesions with more on the upper canopy, 5% defoliation;
5	lesions noticeable even on upper canopy, 20% defoliation;
6	lesions numerous and very evident on upper canopy, 50% defoliation;
7	lesions numerous on upper canopy, 75% defoliation
	upper canopy covered with lesions, 90% defoliation
9	very few leaves remaining and those covered with lesions, 98% defoliation; and
10	plants completely defoliated and killed by leaf spot

Source: Chiteka *et al*. (1988)

Appendix 4: Whether farmer had heard of leaf spot disease analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	7.726[a]	1	.005*		
Continuity Correction[b]	6.583	1	.010		
Likelihood Ratio	8.070	1	.005		
Fisher's Exact Test				.009	.005
N of Valid Cases[b]	200				

Significant (p=0.005)

Appendix 5: Whether farmer is aware of the disease symptoms analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.619[a]	1	.032*		
Continuity Correction[b]	3.818	1	.051		
Likelihood Ratio	4.706	1	.030		
Fisher's Exact Test				.049	.025
N of Valid Cases[b]	200				

Significant (p = 0.032)

Appendix 6: Whether farmer can show some diseased samples or examples analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.619[a]	1	.032*		
Continuity Correction[b]	3.818	1	.051		
Likelihood Ratio	4.706	1	.030		
Fisher's Exact Test				.049	.025
N of Valid Cases[b]	200				

Significant (p = 0.032)

Appendix 7: What time and stage of growth farmer encounters the disease analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	5.380[a]	1	.020*		
Continuity Correction[b]	4.729	1	.030		
Likelihood Ratio	5.410	1	.020		
Fisher's Exact Test				.029	.015
N of Valid Cases[b]	200				

Significant (p = 0.020)

Appendix 8: Farmer's believe the cause of the disease analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	3.907[a]	1	.048*		
Continuity Correction[b]	2.991	1	.084		
Likelihood Ratio	4.035	1	.045		
Fisher's Exact Test				.081	.041
N of Valid Cases[b]	200				

Significant (p = 0.048)

Appendix 9: Farmer's description of the incidence of the disease in his/her farm analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	8.589[a]	1	.003*		
Continuity Correction[b]	7.482	1	.006		
Likelihood Ratio	8.905	1	.003		
Fisher's Exact Test				.006	.003
N of Valid Cases[b]	200				

Significant (p = 0.030)

Appendix 10: **How farmer determines the maturity of groundnut analysis**

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	4.669[a]	1	.031*		
Continuity Correction[b]	4.067	1	.044		
Likelihood Ratio	4.690	1	.030		
Fisher's Exact Test				.043	.022
N of Valid Cases[b]	200				

Significant (p = 0.031)

Appendix 11: Farmers' management practices on the disease analysis

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	16.638[a]	1	.000**		
Continuity Correction[b]	15.471	1	.000		
Likelihood Ratio	16.940	1	.000		
Fisher's Exact Test				.000	.000
N of Valid Cases[b]	200				

Highly Significant ($p < 0.001$)

Appendix 12: Analysis of variance on disease severity at 4 WAP for survey

ANOVA					
	s.s.	d.f.	m.s.	F.	Sig.
Between Groups	135.495	3	45.165	78.436	<0.001**
Within Groups	112.860	196	.576		
Total	248.355	199			

Highly Significant ($p < 0.001$)

Appendix 13: Analysis of variance on disease severity at 6 WAP for survey

ANOVA					
	s.s.	d.f.	m.s.	F.	Sig.
Between Groups	173.135	3	57.712	96.072	.000**
Within Groups	117.740	196	.601		
Total	290.875	199			

Highly Significant ($p < 0.001$)

Appendix 14: Analysis of variance on disease severity at 8 WAP for survey

ANOVA					
	s.s.	d.f.	m.s.	F.	Sig.
Between Groups	222.660	3	74.220	83.547	.000**
Within Groups	174.120	196	.888		
Total	396.780	199			

Highly Significant ($p < 0.001$)

Appendix 15: Analysis of variance on percentage mycelia inhibition of ELS disease

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	19	36539.08	1923.11	71.06	<.001**
Residual	112	3030.99	27.06		
Total	131	39570.07			

Highly Significant ($p < 0.001$)

Appendix 16: Analysis of variance on percentage mycelia inhibition of LLS disease

Source of variation	d.f	s.s	m.s	v.r	F pr.
Treatment	19	30951.17	1629.01	57.97	<.001**
Residual	112	3147.10	28.10		
Total	131	34098.27			

Highly Significant ($p < 0.001$)

Appendix 17: Analysis of variance on disease severity index, greenhouse

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	2	0.593	0.593	0.48	
Plant_Extracts	19	39.145	2.060	1.65	0.048*
Residual	179	222.928	1.245		
Total	199	262.666			

Significant (p = 0.048)

Appendix 18: Analysis of variance on plant height under greenhouse condition

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	1	24.12	24.12	1.12	
Plant_Extracts	19	714.35	37.60	1.75	0.032*
Residual	179	3850.98	21.51		
Total	199	4589.44			

Significant (p = 0.032)

Appendix 19: Analysis of variance for % disease incidence 3 WAP, field experiment, 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	4.400	1.467	1.03	
Plant_Extracts	5	283.292	56.658	39.75	<.001**
Variety	2	82.789	41.395	29.04	<.001**
Plant_Extracts.Variety	10	272.567	27.257	19.12	<.001**
Residual	51	72.689	1.425		
Total	71	715.737			

Highly Significant (p < 0.001)

Appendix 20: Analysis of variance for % disease incidence 5 WAP, field experiment, 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1066.72	355.57	3.59	
Plant_Extracts	5	15201.19	3040.24	30.67	<.001**
Variety	2	564.02	282.01	2.84	0.067
Plant_Extracts.Variety	10	749.91	74.99	0.76	0.669
Residual	51	5055.52	99.13		
Total	71	22637.36			

Highly Significant ($p < 0.001$)

Appendix 21: Analysis of variance for % disease incidence 7 WAP, field experiment, 2014

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	1649.8	549.9	2.96	
Plant_Extracts	5	84993.8	16998.8	91.63	<.001**
Variety	2	726.9	363.4	1.96	0.151
Plant_Extracts.Variety	10	582.8	58.3	0.31	0.974
Residual	51	9461.5	185.5		
Total	71	97414.7			

Highly Significant ($p < 0.001$)

Appendix 22: Analysis of variance for % disease incidence, field experiment, 2015

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Rep stratum	3	623.4	207.8	1.81	
Plant_Extracts	5	107508.9	21501.8	187.65	<.001**
Variety	2	815.5	407.7	3.56	0.031*
Week	2	92411.9	46206.0	403.26	<.001
Plant_Extracts.Variety	10	472.3	47.2	0.41	0.939
Plant_Extracts.Week	10	58951.7	5895.2	51.45	<.001**
Variety.Week	4	189.0	47.2	0.41	0.800
Plant_Extracts.Variety.Week	20	955.8	47.8	0.42	0.987
Residual	159	18218.5	114.6		
Total	215	280147.0			

Highly Significant ($p < 0.001$), Significant ($p = 0.031$)

Appendix 23: Variance on disease severity of ELS on the main field 2014

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Rep	6.29	3	2.10	288.0	0.101
Plant_extracts	31.64	5	6.33	288.0	<0.001**
Variety	6.42	2	3.21	288.0	0.042
Rep.Plant_extracts	6.18	15	0.41	288.0	0.975
Rep.Variety	1.57	6	0.26	288.0	0.954
Plant_extracts.Variety	3.19	10	0.32	288.0	0.976
Rep.Plant_extracts.Variety	3.42	30	0.11	288.0	1.000

Highly Significant ($p < 0.001$)

Appendix 24: Variance on disease severity of LLS on the main field 2014

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Rep	0.47	3	0.16	288.0	0.926
Plant_extracts	37.08	5	7.42	288.0	<0.001**
Variety	0.18	2	0.09	288.0	0.912
Rep.Plant_extracts	0.47	15	0.03	288.0	1.000
Rep.Variety	0.02	6	0.00	288.0	1.000
Plant_extracts.Variety	1.47	10	0.15	288.0	0.999
Rep.Plant_extracts.Variety	0.64	30	0.02	288.0	1.000

Highly Significant ($p < 0.001$)

Appendix 25: Variance on disease severity of ELS on main field 2015

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Rep	1.39	3	0.46	360.0	0.707
Plant_extracts	108.72	5	21.74	360.0	<0.001**
Variety	10.18	2	5.09	360.0	0.007*
Rep.Plant_extracts	2.47	15	0.16	360.0	1.000
Rep.Variety	0.07	6	0.01	360.0	1.000
Plant_extracts.Variety	6.02	10	0.60	360.0	0.813
Rep.Plant_extracts.Variety	0.72	30	0.02	360.0	1.000

Highly Significant ($p < 0.001$); Significant ($p = 0.007$)

Appendix 26: Variance on disease severity of LLS on main field 2015

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Rep	0.20	3	0.07	360.0	0.978
Plant_extracts	79.02	5	15.80	360.0	<0.001**
Variety	2.54	2	1.27	360.0	0.282
Rep.Plant_extracts	0.82	15	0.05	360.0	1.000
Rep.Variety	0.13	6	0.02	360.0	1.000
Plant_extracts.Variety	3.49	10	0.35	360.0	0.967
Rep.Plant_extracts.Variety	0.53	30	0.02	360.0	1.000

Highly Significant ($p < 0.001$)

UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

FACULTY OF AGRICULTURE

QUESTIONNAIRE

TITLE: FARMERS' KNOWLEDGE AND PERCEPTIONS OF CERCOSPORA LEAF SPOT DISEASE OF GROUNDNUT AND THEIR MANAGEMENT IN NORTHERN REGION OF GHANA

Objective: Assessing the knowledge, perceptions and management of groundnut farmers regarding leaf spot disease of groundnut

Background of respondent

1. Name of farmer……

2. Age …… 3. Sex: Male [ ] Female [ ]

4. Educational level: (a) Non-formal [ ] (b) Primary –JHS [ ] (c) SHS/Technical [ ] (d) Tertiary [ ]

Groundnut cultivation experience

5. How long have you been cultivating groundnuts?

(a) 1-3 years (b) 4-6 years (c) 7-9 years (d) 10 years and above

6. How many acres do you normally cultivate?

a. less than an acre b. 1-3.5 acres c. 4-6.5 acres d. 7-10 acres e. 10 acres and above

7. Which groundnut cultivar /cultivars do you grow?

a. Chinese b. Manipinta c. Bugla d. others (specify) ……

8. Why do you cultivate that variety?

a. matures early b. marketability c. disease resistant d. others (specify) ……..

Respondent's knowledge / awareness of Cercospora leaf spot

9. Have you heard of leaf spot disease of groundnut before?

a. Yes b. No

10. Are you aware of the symptoms of the disease?

a. Yes b. No

11. (a) Can you show me some diseased samples /examples in this your field?

a. Yes b. No

(b) Observe the plant part the farmer is showing to you and indicate it below

a. Leaves b. whole plant c. other (specify) ……..

12. (a) Do you have any knowledge about the causal organism of the disease?

a. Yes b. No

(b) Write down what farmer thinks/believes is/are the cause(s) of the disease

……

13. How will you describe the incidence of the disease in your field?

a. low (less than 50 %) b. high (50 % and above)

14. What time and stage of growth do you encounter the disease?

a. 1-3 weeks after planting b. 4 weeks and above

15. Are you aware of the effect of the disease on yield?

a. Yes b. No

16. How will you estimate the severity of the disease on a scale of 5?

a. Not severe (1-3) b. Very severe (4-5)

17. How do you determine the maturity of groundnut?

a. brown spots and defoliation b. sample digging

18. Are you aware of how to control the disease?

a. Yes b. No

Management practices and way forward regarding Cercospora leaf spot

19. Which management practice (s) /control measures do you use?

……..

20. What else do you think can be done to minimize Leaf spot disease of groundnut?

…….