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Antifungal activity of selected pesticidal plants against phaeoisariopsis personata and phytochemical analysis, central Tanzania

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ANTIFUNGAL ACTIVITY OF SELECTED PESTICIDAL PLANTS AGAINST *Phaeoisariopsis personata* AND PHYTOCHEMICAL ANALYSIS, CENTRAL TANZANIA

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A Thesis Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Life Sciences of the Nelson Mandela African Institution of Science and Technology

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ABSTRACT

The late leaf spot (LLS) caused by *Phaeoisariopsis personata* L., is the most serious fungal disease of groundnut worldwide. The LLS causes considerable damage to groundnuts leading to leaf defoliation and consequently reduces pod yields by more than 50%. Research was carried out to evaluate the antifungal activity of *Parthenium hysterophorus*, *Azadirachta indica* and *Jatropha curcas* against *P. personata* and analyse the possible compounds exhibiting antifungal properties at the Tanzania Plant Health and Pesticide Authority (TPHPA)-Arusha, Tanzania. The field survey results indicated that the LLS disease of groundnuts prevailed in the central zone of Tanzania with overall disease incidences between (54.65-84.70%) and severities (4.9-7.00). This finding provides alerts to the global plant health regulators suggesting a proper management skill. The evaluation the selected pesticidal plants revealed that all the plant extracts showed antifungal activities against *P. personata* under *in vitro* and *in vivo* (screen-house) conditions. The methanolic leaf extracts of all tested plants offered higher antifungal activities with >75% inhibition of mycelial growth of *P. personata* compared to control (0% inhibition). Similarly, the application of methanolic leaf extracts of selected plant extracts on groundnuts seedlings grown in pots under screen-house condition at the highest concentration (0.5 mg/ml) exhibited antifungal potentials against *P. personata* by reducing the disease incidence being 14.30% and severity of 2.22 compared to chloroform (17.44%, 4.07) and ethyl acetate (20.56%, 4.26) leaf extracts. Subsequently groundnut seed yield/plant was greater 45.09 g for crop treated with the most effective plant extract (*A. indica* at 0.5 mg/ml) as compared to untreated 7.76 g. Furthermore, the phytochemical analysis of the selected pesticidal plants by GC-MS identified important phytocompounds with antifungal properties from the tested plant extracts mainly; hexadecanoic acid ethyl ester, methyl salicylate, phytol, phenol 2,4-bis (1,1-dimethylethyl), *n*-hexadecanoic acid, hexadecanoic acid methyl ester and hexadecane. The presence of these antifungal phytocompounds in the selected plants is associated with their effect on the *P. personata*. A further study under field condition is recommended on the tested plants (*P. hysterophorus*, *A. indica* and *J. curcas*) on possibility of developing bio fungicides for the management of groundnut LLS disease in Tanzania.

DECLARATION

I, Magreth Francis, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

Signed……Date……

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Dr. Ernest Mbega (Supervisor 1)

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Prof. Patrick A. Ndakidemi (Supervisor 2)

Signed…….. Date……

Dr. Musa Chacha (Supervisor 3)

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Nelson Mandela African Institution of Science and Technology a Thesis entitled: "Evaluation of selected botanicals as insecticides against cabbage insect pests in Tanzania" and recommend for examination in fulfillment of the requirements for the degree of Doctor of Philosophy in Environmental Science and Engineering of the Nelson Mandela African Institution of Science and Technology.

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DEDICATION

This work is dedicated to Almighty God who arms me with strength and makes my way perfect (Psalms 23:1).

To my beloved husband, Augustine Malima, and sons- Samuel Augustine Malima, Nathan Augustine Malima and David Augustine Malima and to all family members.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
ANOVA	Analysis of Variance
cm	Centimeter
CRD	Complete Randomised Design
CREATES	Centre for Research, Agricultural Advancement, Teaching Excellence and Sustainability
DAAD	Deutscher Akademischer Austauch Dienst
DAI	Days After Inoculation
DAS	Days After Sowing
DMSO	Dimethyl Sulphoxide
ELS	Early Leaf Spot Disease
FAO	Food and Agriculture Organization of the United Nations
Fig.	Figure
g	Gram
GC-MS	Gas Chromatography Mass Spectroscopy
h	Hour
LLS	Late Leaf Spot Disease
LSD	Least Significant Different
$NaOCl_2$	Sodium hypochlorite
NIST	National Institute Standard and Technology
NM-AIST	Nelson Mandela African Institution of Science and Technology
ºC	Degree centigrade
Rh	Relative humidity
SDW	Sterile Distilled Water
TPHPA	Tanzania Plant Health and Pesticide Authority
µl	Microliter

CHAPTER ONE

INTRODUCTION

1.1 Background of the problem

Groundnut (*Arachis hypogaea* L.) is the vital oil kernel crop in the tropics and subtropics countries and used as food, cash crop and animal feed (Pasupuleti *et al*., 2013). The crop is self-pollinated and belongs to fabaceae family. It is originated from Latin America and was introduced in Africa in 16th century (Hammons *et al.*, 2016). Currently, groundnut is cultivated in many countries between latitudes 40 ˚N and 40 ˚S in semiarid tropics and subtropics (Kayondo *et al*., 2014). Groundnut cultivated in Sub-Saharan Africa covers 40% of the harvested area worldwide (ICRISAT, 2012). Groundnut production plays a great role in developing and developed nations in improving the economic status (Tsigbey *et al*., 2003).

In Tanzania, the crop is cultivated mainly by smallholder farmers in Tabora, Mtwara, Dodoma, Singida, Shinyanga, Mwanza, Songwe, Geita and Kigoma regions (Akpo *et al*., 2020). Groundnuts are locally consumed uncooked/raw, roasted or boiled also used as important ingredient in preparing porridge flour for pregnant women and babies (Osei *et al*., 2013; Kemoring, 1998).

Despite the importance of groundnut in Tanzania, its average yield is still low being 960 kg/ha related to the predictable yield potential of 1500 kg/ha in developing countries (Nutsugah *et al*., 2007; Philipo & Nchimbi-Msolla, 2019). Groundnut production is mainly constrained by drought stress, insect pest attack, low level of inputs (fertilizer, pesticides), viral (rosette disease) and foliar fungal diseases. The foliar fungal diseases namely; early leaf spot (ELS) caused by *Cercospora arachidicola*, late leaf spot (LLS) caused by *Phaeoisariopsis personata* and rust caused by *Puccinia arachidis* are among the most destructive diseases which accounts for a great economic yield loss (Naidu *et al*., 1999). Each disease can cause substantial yield loss. However, when they occur together can lead to more than 70% pod yield losses (Monfort *et al*., 2004; Pande *et al*., 2003). The epidemics of early leaf spot, late leaf spot (LLS) and rust on susceptible groundnut genotypes causes' high leaf defoliation leading to great grain yield loss (Waliyar *et al*., 2000). The late leaf spot disease caused by *Phaeoisariopsis personata* is one among three diseases opted due to its importance being distributed wherever groundnut is cultivated worldwide, causing about 50% yield loss (Kokalis-Burelle *et al*., 1997). The current study aimed to look for alternative sustainable

remedies from plant origins that will minimize the yield losses caused by the LLS in Tanzania, furthermore analyses their possible phytocompounds exhibiting antifungal properties.

1.2 Statement of the problem

The late leaf spot disease caused by a pathogen *Phaeoisariopsis personata* (Berk & Curt. Arx) is one among the major constraints in groundnut growing areas in Tanzania, causing a considerable yield loss of about 10-50% (Branch & Culbreath, 2013). When the disease occurs, it may lead to two to three-week earlier maturing pods, smaller seeds and increased pod detachment during harvesting (Shokes & Melouk, 1995). In addition, LLS causes leaf damage consequently reducing photosynthetic area and premature leaf abscission (McDonald *et al*., 1985).

However, there is confusion on identifying the causal pathogen for the late leaf spot since there symptoms resembles to the one which causes early leaf spot disease. Furthermore, the LLS disease can be managed by a combination of methods such as use of synthetic fungicides, host plant resistance, biological control agents and cultural practices (Mondal *et al*., 2014). Moreover, synthetic fungicides have been prioritized due to its effectiveness (Harris *et al*., 2001; Monyo *et al*., 2009). Nevertheless, due to regular disease problems, farmers do practice multiple application of fungicides leads to high production cost, environmental pollution, low quality produces due to chemical contamination, adverse effects to farmers and possibility of pathogen resistance against fungicides (De Rodríguez *et al*., 2011; Daudi *et al*., 2021).

Thus, this justifies the search for alternative management strategies which are eco-friendly in order to enhance groundnut production. Three pesticidal plants *Parthenium hysterophorus*, *Azadirachta indica* and *Jatropha curcas* were selected on the basis of available ethno medicinal information traditionally used for treatment of various diseases, including those caused by bacterial, virus, fungi, nematodes (EL-Kamali & EL-Amir, 2010; Grayer & Harborne, 1994).

Pesticidal plants possess important bioactive compounds with interesting medicinal activities such as antibacterial, antifungal, antioxidant anticancer and anti-inflammatory (Ammal & Bai, 2013). Despite the importance of pesticidal plant in controlling fungal diseases, the scientific study about phytochemicals responsible for antifungal effects is lacking. Screening

for phytochemical compounds in pesticidal plants is an important pre-requisite in establishing lead compounds which can be further developed into potential botanical products for treatment of several diseases (Bohlin & Bruhn, 1999). In this regard, the current study identified the causal pathogen for LLS, described the LLS disease status in the central Tanzania, evaluated the antifungal activity of the selected plant species and analysed the possible phytochemical compounds exhibiting antifungal properties using GC-MS technique.

1.3 Rationale of the study

In Tanzania, groundnut production is done by smallholder farmers normally as a source of food and income (Akpo *et al*., 2020). However, the LLS fungal diseases caused by *Phaeoisariopsis personata* attacking groundnut is a major destructing factor leading to more than 50% yield loss (Kokalis-Burelle *et al*., 1997).

Development of effective and justifiable control measure depends on proper identification of the pathogen. Thus, correct diagnosis of the causal pathogen is the most important aspects towards developing effective management measure (Riley *et al*., 2002). The study chose three pesticidal plants namely *Parthenium hysterophorus*, *Azadirachta indica* and *Jatropha curcas* to test their efficacy against LLS. The selection of plants was based on the historical background being used as medicinal plants. Moreover, application of pesticidal plants is relatively safe, biodegradable, affordable and viable economically (Grzywacz *et al*., 2014). The pesticidal plants possess valuable bioactive compounds which are effective against bacteria, fungi, and nematodes (Basaid *et al*., 2021). Screening for phytochemical compounds in pesticidal plants is the prerequisite procedure for determining the leading compounds for developing potential botanical products for treating diseases (Kilonzo *et al*., 2017).

However, literature has little information related to the selected plants in managing the LLS on groundnuts also the scientific studies concerning the phytocompounds with antifungal effects. Moreover, the antimicrobial activity of the pesticidal plants depends on solvent type used for extraction of phytocompounds. The current study evaluated the antifungal activity of the selected plant species extracted through different solvents also analysed the possible phytochemical compounds exhibiting antifungal properties using GC-MS technique.

1.4 Research objectives

1.4. 1 General objective

To evaluate antifungal effects of selected pesticidal plants to lay a strong basis of developing plant based-fungicide for managing LLS disease of groundnut in Tanzania.

1.4. 2 Specific objectives

(i) To determine the current status of groundnut LLS disease in central zone of Tanzania

(ii) To evaluate the *in vitro* efficiency of the selected pesticidal plants in inhibiting the mycelial growth of *Phaeoisariopsis personata*

(iii) To assess the *in vivo* effect of the selected pesticidal plants on managing the LLS disease

(iv) To examine the phytochemical constituents of the selected pesticidal plants that exhibit antifungal efficacy against LLS disease

1.5 Research questions

(i) What is the status of groundnut LLS disease in central zone of Tanzania?

(ii) Which plant among the selected pesticidal plants will inhibit mycelial growth of P. *personata* under *in vitro* condition?

(iii) Which plant(s) among the selected pesticidal plants manage LLS disease incidence and severity under screen-house condition?

(iv) Does the selected pesticidal plant possess phytochemical constituents with antifungal properties?

1.6 Significance of the study

The study is significant since it aimed to determine the status of LLS disease of groundnut in central zone of Tanzania and evaluate the effectiveness of the selected pesticidal plants against *P. personata*. Likewise, the study assessed the phytochemical analysis of the selected plants for compounds with antifungal properties. Groundnut is an important crop being

cultivated as a food and cash crop. Therefore, the results of the current study will be helpful since:

(i) It provides the status of LLS disease in central Tanzania by alerting the effect of the disease occurrence hence measures for management should be undertaken to reduce the yield losses associated by the disease

(ii) Evaluated the effectiveness of the selected plants against the *P. personata* on PDA media by measuring the percent inhibition of the mycelial growth which provided scientific evidence for their efficacy against the pathogen

(iii) Determined the antifungal activity of the selected plants against LLS disease under screen-house condition

(iv) Confirmed the presence of phytochemical constituents with antifungal properties on the selected pesticidal plant species using GC-MS.

Thus, the study offers preliminary suggestion of using bio-control alternative for management of LLS disease to farmers and other stakeholders aiming to improve crop production in Tanzania.

The information on LLS disease status, deduced effective plant extracts against the *P. personata* is the most valuable facts to researchers, extension officers and farmers towards developing the sustainable management of the disease for improvement of groundnut production in Tanzania.

1.7 Delineation of the study

The LLS disease status was only conducted to Dodoma and Singida regions. Other groundnut growing regions were not investigated. The antifungal activity of the selected pesticidal plants was tested against *P. personata* under *in vitro* and *in vivo* (screen-house) conditions, the study did not evaluate their efficacy under the field condition due to shortage of fund and time.

CHAPTER TWO

LITERATURE REVIEW

2.1 Significance of groundnut

Groundnut (*Arachis hypogaea* L.) is a significant oil seed cash crop used for subsistence and commercial purposes (Izge *et al*., 2007). The crop is the 13th vital food crop in term of production, 4th oil kernel crop and 3rd important on basis of protein provision worldwide (Naawe & Angyiereyiri, 2020). Groundnut seeds contain about 12-15% carbohydrates, 25-30% protein and 40-50% fats (Saeed & Hassan, 2009). Additionally, groundnut contains vitamin, dietary fibres and minerals (niacin, magnesium, iron, phosphorus, calcium, zinc and riboflavin) (Pande *et al*., 2003; Izge *et al*., 2007; Tshilenge-Lukanda *et al*., 2013). Groundnut haulms are used as animal feeds and compost making (Indira *et al*., 2010). Groundnut as a leguminous plant it improve the fertility status of the soil by converting the atmospheric nitrogen to fixed forms such as nitrates, ammonia and organic nitrogen (Pasupuleti *et al*., 2013).

The groundnut plant requires a minimal well distributed amount of rainfall ranging between 500-1200 mm, during the vegetative period of growth (Aulakh, 2003). According to Tweneboah (2000), groundnuts prefer light-textured, deep, well-drained soils which allow penetration of the sharp point of the ovary. Groundnuts are cultivated at largest scale in India and China followed by African countries (Sub-Saharan), Tanzania inclusive (Johansson & Ives, 2001). In Tanzania, groundnut is among the important oil seed crops cultivated by smallholder farmers, as a source of food and income generating commodity (Monyo & Varshney, 2016). The groundnut with other crops i.e., sesame, sunflower, bananas, vegetables, and fruits contribute 3.2% of Gross Domestic Product (GDP) in Tanzania (FAOSTAT, 2014).

2.2 Distribution of groundnut LLS

In Tanzania, the LLS disease is severe in areas growing groundnuts (Philipo & Nchimbi-Msolla, 2019). The LLS infections cause intense lesions on stems, leaves and petioles this interferes photosynthetic process consequently leading to high yield loss (Pretorius, 2006). According to Ijaz (2011) when LLS disease attacks the groundnut plant it reduces canopy carbon exchange rate and carbon uptake by about 80 and 93%, respectively. The LLS disease

also causes adverse effects on seed and fodder quality becoming unsuitable for animal feed. The report has indicated that LLS disease causes over 50% of yield loss wherever groundnut is cultivated (Nath *et al*., 2013). Since the LLS disease is severe and there is necessity to look for better management option in order to reduce the yield loss.

2.3 Identification and classification of *P. personata*

The literature shows that this fungus has undergone numerous tremendous changes. According to Nutsugah *et al*. (2007) the leaf spots were considered as natural and common features of groundnut plants signifying the maturity stage. The description of the causing organism was first documented by Berkeley (1874), who identified the causal organism of late leaf spot disease as a single fungal species named C*ladosporium personatum*. However, through several studies the leaf spot diseases nomenclature and classification are highly variable. The comparison of specimens study determined the two distinct fungal organisms, *Cercospora arachidicola* and *Phaeosariopsis personata* (Fávero *et al*., 2009). Later the sexual stage for *Mycosphaerella arachidicola* was identified as a causal agent for early leaf spot and *Mycoshaerella berkeleyii* for late leaf spot (Jenkins, 1938; Fávero *et al*., 2009). Basing on conidionatal structure and position on the host plant and the type of scars on the conidiogenous cells and conidia, twenty-three genera were enumerated. The *P. personata* was proposed based on the synnemata formation (small), thickened and darkened scars (Fávero *et al*., 2009).

The LLS fungal pathogen *P. personata* is normally seen in its imperfect state (Kokalis-Burelle *et al*., 1997). According to Ijaz (2011), the perfect state was described as follows: conidiophores are fasciculated one to three geniculate with conspicuous conidial scars, mostly arranged in concentric rings on lower surface and darker in colour. The conidial are cylindrical having one or more septa (Jenkins, 1938).

2.4 Epidemiology, pathogenicity, and symptoms of LLS

The pathogen reproduces and infects by the means of microscopic spores called conidia (Wambi, 2014). Spore production is promoted by high humidity above 85% and temperature above 19 ˚C (Shokes *et al*., 1997). The primary inoculum that causes initial leaf infections originates from spore infested groundnut residue from the soil (McDonald *et al*., 1985). Under favourable condition spores develop in germinate tubes which enter the plant cells via stomata/ epidermis, which allows intracellular mycelia growth by obtaining nutrients through

haustoria (Ijaz, 2011). Spores are produced on leaves and disease lesions within 10-14 days under favourable wetness and temperature (Shokes & Melouk, 1995; Shokes *et al*., 1997). The pathogen does not have alternate hosts than the genus *Arachis* (Shew *et al.,* 1995). It survives season after season through volunteer plants (groundnut) and infected crop debris. The LLS lesions are circular in shape, darker brown in color without a definite chlorotic halo (Ijaz, 2011). Lesions are black on abaxial side of the leaflets, later the spots spread to petioles and stem (Jyosthna *et al*., 2004).

Plate 1 : Symptoms of LLS on groundnut leaves. Source: Science alert: imgres.htm

2.5 Management of groundnut LLS disease

The late leaf spot disease can be managed by integrated management options which include the use of host plant resistance, judicious fungicides and cultural practices (Ijaz, 2011; Shew *et al.,* 1995).

2.5.1 Cultural practices

The *P. personata* pathogen always survives on the plant debris and under favourable condition causes LLS disease repeated (Shokes *et al*., 1997). Cultural practices are the simplest approach for management of LLS diseases. Early sowing of crop in the season, maintaining preferable spacing between plants aims to take gain advantage against high disease incidence and severity (Subrahmanyam *et al*., 2002). Intercropping groundnuts with other cereals i.e. finger millet, cowpea, sorghum, maize and beans have proven positive effect

in destructing disease infection (Naidu *et al*., 1998). Crop rotation is also useful if groundnuts are grown once after 3-4 years to prevent initial infection by leaf spot and stem debris from previous crop (Shew *et al.*, 1995). Volunteer groundnut plants in the field should be removed immediately after harvest since they possess primary inoculum sources (Shew *et al*., 1995).

However, cultural practices done by smallholder farmers are not successful since farmers are reluctance to adopt them by ignoring to remove volunteer groundnut plants immediately after harvest which possess primary inoculum sources of *P*. *personata* which enhance LLS disease spreading (Shew *et al*., 1995). Moreover, farmers don't have other income generating activities rather than cultivating groundnut thus crop rotation practices aiming to disrupt the disease cycle cannot be adopted. Moreover, small-land holdings and unpredictable climate makes early sowing technique ineffective in lowering the groundnut LLS disease (Waliyar *et al*., 2007). Furthermore, the cultural practices should be integrated with other management options for effective results.

2.5.2 Use of resistant varieties

The use of host resistant is the effective management option for LLS disease (Kishore *et al*., 2001). Lately, more efforts have been concerted to exploit genetic resistance of foliar diseases including LLS due to its importance in Tanzania (Waliyar *et al*., 2007). The following resistant varieties i.e., Florida MDR98', 'Georgia Green', 'Georgia-03L', 'Southern runner', 'Florida MDR98', 'York' and Georgia-07W' have been released from Worldwide (Shew *et al.,* 1995).

Despite the merits of resistant varieties, there is no variety with complete immunity to fungal disease (Shew *et al.,* 1995). However, in order to obtain the optimum yield is recommended the use of partially resistant varieties integrated with fungicides (Culbreath *et al*., 2010). However, the adoption of the released varieties resistant to LLS is still a challenge since sometimes such improved varieties do not have qualities preferred by farmers such as low crop yield. These limitations open a door to explore for other management option for LLS on groundnut.

2.5.3 Synthetic chemical pesticides

The LLS can be controlled with multiple applications of fungicidal sprays according to Culbreath *et al*. (1995). Bowen *et al*. (1997) reported four spray of tebuconazole have

detrimental effect on incidence of ELS, LLS and stem rot and lead to increased groundnut yield. Hexaconazole (6.2%) fungicides sprayed twice on 60- and 75-day old plants lead to reduction of LLS severity and improved groundnut pod yield, similarly hexaconazole application increased 71% pod yield and 87% folder yield (Jadeja *et al*., 1999). A mixture of benomyl and Chlorothalonil were found effective against groundnut late leaf disease (Culbreath *et al*., 1995).

The application of nativo and triazole controlled LLS disease subsequently increased groundnut pod yield (Khan *et al*., 2014). Also, according to Chandra *et al*. (1998), carbendazim and mancozeb sprayed once on 40-50 days old plant managed LLS disease of groundnut. Likewise, tubeconazole (0.15%) reduced the LLS disease intensity to 67% as compared to 39% decrease increase by tubeconazole (0.10%)(Nath*et al*.,2013). Additionally, Chlorothalonil and benomyl was effective against *P. cercospora* and *P. personata* (Culbreath *et al*., 1995).

Synthetic fungicides are an effective option for reducing the LLS disease but they have been reported to have unspecific mode of action to a very broad spectrum of organisms i.e., higher plants and animals (Apple, 1977). In addition, intensive application of fungicides leads to environmental risk and residual toxicity in food chain for long periods (Amadioha, 2002). This opens a gap to seek for other management options as alternative aimed at management of LLS disease of groundnut.

2.5.4 Use of botanical pesticides

The substantial costs associated with the synthetic fungicides, applications risks, growing pathogen population being resistant to fungicides and failure of adoption of using resistant varieties has made botanical pesticides become a suitable alternative for management of LLS on groundnuts. Biopesticides unlike synthetic are less toxic to human and environment, easily degradable, easily available and leave less residual effects on produce (Srijita, 2015). There are more than 250,000 higher plant species on earth that can be evaluated for their phytochemicals against different pests and pathogens (Sofowora, 1993). According to Shiberu and Getu (2017), Africa, Tanzania inclusive has a good source of pesticidal plants with variety of antimicrobial effects.

Kalaskar *et al*. (2012) confirmed that the neem seed kernel extract at 5% was effective against *P. personata*. It also *A. indica* inhibited the conidial of *C. arachidicola* and *P.*

personata by 90.7% (Hossain & Hossain, 2013). Likewise, the conidial germination of *P. personata* was completely inhibited when treated with ethanolic leaf extract of *Sphaerathus indicus* (Kishore *et al*., 2001). The aqueous leaf extracts of *Prosopis juliflora* and *Lycopersicon esculentum* was found effective against groundnut LLS and rust (Kishore & Pande, 2005).

Neem and Datura leaf extracts completely managed foliar leaf spot diseases of groundnut than untreated (Hasan *et al*., 2014). Similarly, according to Ihejirika *et al*. (2006), low LLS disease severity of groundnuts were recorded to plants treated with *Azadirachta indica* and *Ocimum viride* compared to control (untreated) which had the highest disease severity. The chloroform extract of the three fern plants lowered the ELS and LLS disease severity (Sahayaraj *et al*., 2009).

The *J. curcas* possesses important phytocompounds such as flavonoids, sterols, steroids, terpenes and saponins which play a great antifungal activity (Nwosu & Okafor, 1995; Campa *et al*., 2008; Saetae & Worapot, 2010). Several researches confirm the antifungal potential of *J. curcas* for management fungal diseases. A study by Thangavelu *et al*. (2004) found that *J. curcas* was effective in managing banana anthracnose disease. In addition, according to Garcia and Lawas (1990), *J. curcas* leaf extracts were found effective in managing *Sclerotium* spp. causal pathogen for *Azolla* disease. Also, according to Saetae and Worapot (2010), the *J. curcas* ethanolic seed extract was found effective against several fungal pathogens on plants.

According to Kushwaha and Maurya (2012), *Parthenium hysterophorus* leaves contain several important chemical constituents with antifungal properties. Rajiv *et al*. (2013), reported the highest zone of inhibition in 25 µg/ml of 27±5 nm size zinc oxide nanoparticles (Parthenium) against *Aspergillus niger* and *Aspergillus flavus*. Also, methanolic extract of *Parthenium hysterophorus* showed antifungal effectiveness against *Candida kefyr, Aspergillus niger* and *Candidia albicans* (Malarkodi & Manoharan, 2013). Likewise, Bajpai *et al.* (2012) reported the invasive alien plants species were effective against the following fungal pathogens *Phytophthora infestans, Puccinia recondite, Magnaporthe oryzae, Rhizoctonia solani, Colletotrichum cocodes* and *Botrytis cinerea*.

In this study the following plant species; *Jatropha curcas, Parthenium hysterophorus* and *Azadirachta indica* leaves extracted through chloroform, ethyl acetate and methanol were

assessed for their antifungal efficacy against LLS disease of groundnut. The selection of plant species was based on their historical background been used for medicinal purposes.

2.6 Plant extraction methods

Plant contains numerous bioactive compounds i.e. tannins, alkaloids, fixed oils, resins, volatiles oils, steroids, phenols, glycosides and flavonoids which are deposited in a specific part of plants (Kalimuthu *et al*., 2010). Such plant parts include leaves, flowers, fruits, seeds, roots and barks. These bioactive compounds help plant to survive and overcome local challenges on their surroundings (Harborne, 1998). Bioactive compounds possess antifungal, anti-inflammatory, antioxidant, antibacterial, antidiabetic and antiarthritic (Khan & Wassilew, 1987; Wong *et al*., 2009; Rungsung *et al*., 2015).

Preparation of botanical extracts for biological testing involves better selection of plant (whole) or part and standard extraction procedures (Abubakar & Haque, 2020). Generally, extraction of plant extract involves maceration, digestion, infusion, decoction, soxhlet extraction, superficial extraction, percolation, ultrasound-assisted and microwave-assisted extractions (Handa *et al*., 2008; Azwanida, 2015). The plant part and nature of phytochemical compounds normally determine the type of solvent to be used for extraction (Kothari *et al*., 2009). For instance, the commonly used polar solvents are water and ethanol whereas hexane, chloroform and dichloromethane for non-polar compounds and ethyl acetate for mid-polar compounds (Pandey & Tripathi, 2014). Considering the polarity nature of the phytochemical compounds this study opted chloroform for extracting non-polar compounds, ethyl acetate for mid-polar and methanol for polar compounds from selected plant species.

2.7 Screening for phytochemical constituents

Recently, there is increasing concern for searching and developing new bioactive compounds mainly from plant based materials which fight against diseases pathogenic microorganisms i.e. bacteria, fungi, nematodes, viruses (Kumar *et al*., 2021). Screening for phytochemical compounds of plants is paramount procedures which aim to explore the composition of plant based materials identifying potential bioactive compounds (Altemimi *et al*., 2017). According to Jimoh *et al*. (2019) and Saxena *et al*. (2013), the quality, quantity, and biological activities of the phytochemical compounds depend on the plant developmental stage, plant parts, and the solvents used for the extraction and isolation. Generally, the phytochemical screening

technique is a quick, simple, and less expensive technique which provides various secondary metabolites from plant origins (Sasidharan *et al*., 2011).

The fractionation and purification of phytochemical compounds can be done by using different chromatographic separation techniques i.e. gas chromatography, paper chromatography, thin-layer chromatography or high-performance liquid chromatography (Doughari, 2012). The phytocompounds are used to determine the structure and biological activity. Currently, several analytical methods have been developed, in order to facilitate structural determination of the pure bioactive compounds i.e. gas chromatography mass spectrometry (GC/MS), HPLC, TLC, ion spray mass spectrometry (MS), LC/electrospray ionization tandem mass spectrometry (MS/MS), nuclear magnetic resonance and capillary electrophoresis (Gad *et al*., 2013). Gas chromatography mass spectrometry (GC-MS) technique has been used by most researchers to analyse bioactive compounds of relatively low molecular weight (Frank *et al*., 2004). The MS spectra offers specific chemical information related to the chemical structure such as accurate mass, isotope distribution patterns for elemental formula determination and characteristic fragment of molecular ions for structural elucidation (Kind & Fiehn, 2007). Thus, the effective selected pesticidal plants against *P. personata* were analysed for the possible phytochemical compounds using GC-MS technique.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the site

The study was done in Central zone of Tanzania, particularly in Singida (Manyoni District Council (DC) and Singida rural) and Dodoma (Bahi DC and Chamwino DC) regions in 2018-2019. These regions are characterized by a single rain season (Masika) between November-April (Myeya, 2021). These regions receive mean annual average rainfall of 564 mm for Dodoma and 700 mm for Singida (Chidodo, 2017) and temperature ranges between 15 ˚C - 30 ˚C. These regions were chosen based on their history of groundnut production and reported frequent infestation of LLS disease (Kongola, 2018). The antifungal effects of the selected pesticidal plants against *P. personata* done under *in vitro* (amended with PDA media) and *in vivo* (screen-house) conditions where groundnut seedlings were foliar sprayed with plant extracts and the phytochemical analysis of the plants by GC-MS were done at Tanzania Plant Health and Pesticide Authority (TPHPA), Arusha.

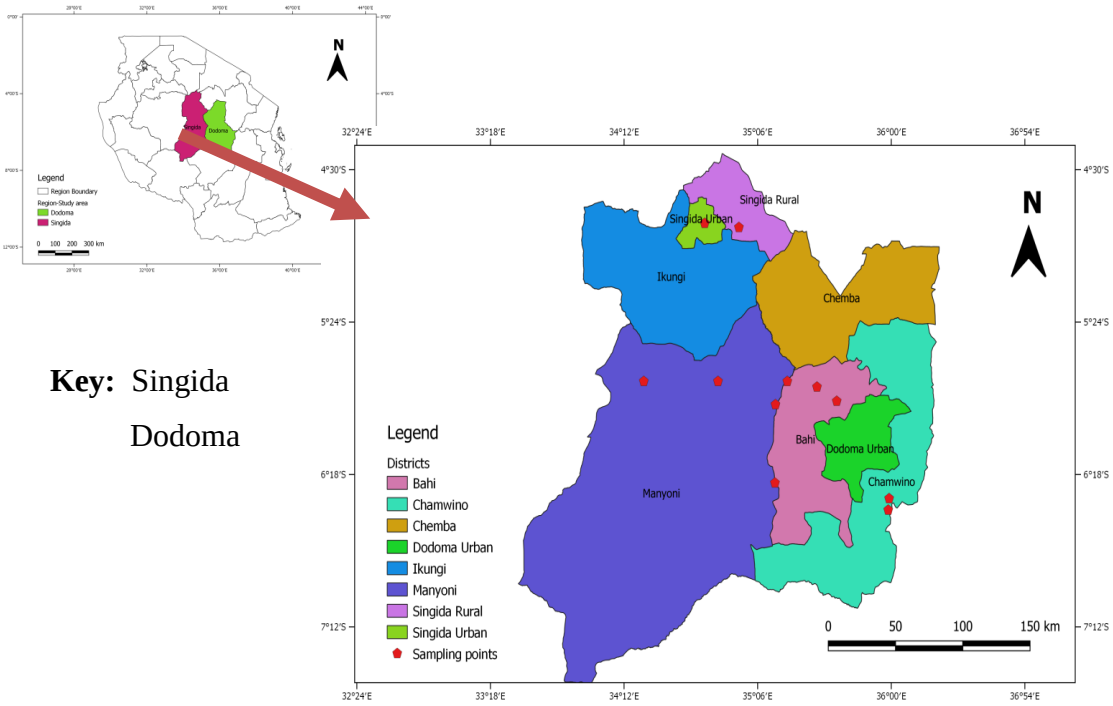


Figure 1: Map indicating surveyed areas

Dodoma and Singida region in upper left and districts in respective regions in the lower right.

3.2 Field survey in Singida and Dodoma regions

To determine the major foliar fungal disease limiting groundnut production in Singida and Dodoma, a total of 20 villages from both regions were surveyed. The survey covered a total of one hundred and sixty (160) groundnut farmers' fields from both regions. In each village, six (8) fields were randomly selected and LLS disease incidence and severity data were collected in a zig zag style by tossing a quadrat box with 2mx2m plot in triplicate. The disease occurrence was determined by counting total number of plants with infected leaves per plot divided by the total number of plants per plot x 100%. The disease severity of LLS was measured using a disease score scale (1-9) (Appendix 1) (Chiteka *et al*., 1988).

Table 1: List of villages/ districts of the surveyed regions in Central Tanzania

Village	District	Region
Chonde	Singida rural	Singida
Makanda	Singida rural	Singida
Msisi	Singida rural	Singida
Lamaiti	Singida rural	Singida
Azimio	Singida rural	Singida
Chonde	Manyoni DC	Singida
Makanda	Manyoni DC	Singida
Msisi	Manyoni DC	Singida
Lamaiti	Manyoni DC	Singida
Azimio	Manyoni DC	Singida
Chonde	Chamwino DC	Dodoma
Makanda	Chamwino DC	Dodoma
Msisi	Chamwino DC	Dodoma
Lamaiti	Chamwino DC	Dodoma
Azimio	Chamwino DC	Dodoma
Chonde	Bahi DC	Dodoma
Makanda	Bahi DC	Dodoma
Msisi	Bahi DC	Dodoma
Lamaiti	Bahi DC	Dodoma
Azimio	Bahi DC	Dodoma

3.3 Assessing the efficacy of plant extracts against *P. personata*

3.3.1 Agar infusion assay

i) *Phaeoisariopsis personata* isolation and identification

The *P. personata* was isolated from infected groundnuts leaves by scheduled technique with some modifications (Kishore *et al*., 2001). The diseased leaves were collected randomly from the field, kept on envelopes and were sent to TPHPA laboratory, Arusha for isolation. The

diseased leaf lesion and healthy/uninfected tissue were cut into small portions (1x1 cm^2) with a pair of scissors; sterilized by sodium hypochlorite ($NaOCl_2$) solution for 5 minutes. Thereafter, diseased leaves were rinsed with sterile distilled water (SDW) then dried on a sterilized blotter paper before plated on Potato Dextrose Agar (PDA). Then petri dishes were kept at room temperature for 7 days. After the emergence of the fungi, were sub-cultured into new PDA plates then remained at standard room temperature for 7 days to obtain pure *P. personata* culture. The identification of The *P. personata* was based on morphological and cultural characteristics by a single spore method using a Compound microscope with magnification (40X) (Agrios, 2005).

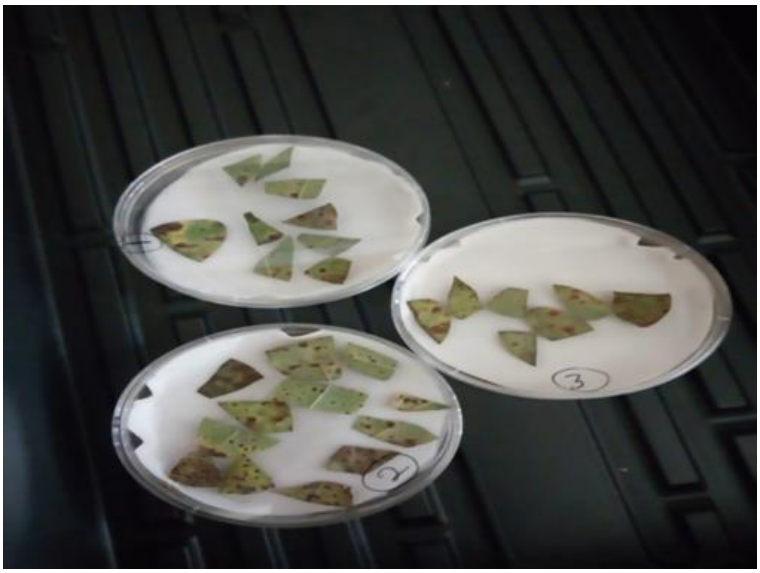


Plate 2: Infected groundnut leaves on blotter paper

Plate 3: Plating infected leaves on PDA media

(ii) Preparation of the selected plant leaf extracts

The *J. curcas*, *A. indica* and *P. hysterophorus* leaves were washed, air-dried, and ground into powder. The dried powdered leaf sample (0.5 kg), sequentially extracted using different solvents i.e. chloroform, ethyl acetate and methanol; based on their polarity aiming to ensure maximum extraction of different compounds. The ground particles were first soaked on chloroform for 48 hrs, and respective extracts were sieved by filter paper (Whatman no.1). Thereafter, the obtained filtrated samples were further sequentially soaked in ethyl acetate and methanol for 48 hrs in each solvent. Solvents from all collected filtrates were concentrated by rotary evaporator then kept in a closed glass vials at -4 °C and used for antifungal evaluation. The stock solutions were prepared by putting required amount of plant leaf extract in a 100 ml of PDA for *in vitro* test and 500 ml (SDW) for *in vivo* trial to prepare the preferred concentration of 0.1, 0.25 and 0.5 mg/ml.

(iii) Mycelial inhibition of *P. personata*

The *in vitro* activity of the selected plants against *P. personata* was determined by food poison technique with some modification (Kritzinger, 2005). The appropriate amount of each plant leaf extract (10, 25 and 50 mg) was added to 100 ml of PDA when preparing the required concentrations i.e. 0.1, 0.25 and 0.5 mg/ml then poured into Petri dishes. Plugs (5 ml diameter) of *P. personata* from 7-day-old fungal culture was placed at the midst of the Petri dishes with PDA amended individually with chloroform, ethyl acetate and methanolic leaf extracts of *A. indica*, *J. curcas* or *P. hysterophorus*. The plates without phytoextract served as negative control and plates along with Chlorothalonil (2.1 L/ha) as positive control. Treatments were arranged in a complete randomized design (CRD) replicated thrice. The experiment was conducted twice; 1st trial from 14th March - 9th April and 2nd from 21st April - 29th May, 2018. The inoculated petri dishes were incubated at room temperature and the radial growth was recorded when the fungus reached at the edge of the petri plates. The mycelial growth inhibition of *P. personata* was calculated by comparing with mycelial growth on plant extracts and control following a standard proposed formula by Begum *et al*. (2010).

$$\mathrm{I} = \frac{\mathrm{C}-\mathrm{T}}{\mathrm{C}} x\ 100 \qquad (2)$$

Where; I = percent inhibition, C = the diameter of fungal colony of the negative control (PDA only), T = diameter of fungal colonies grown in the presence of plant leaf extracts or standard fungicide.

3.3.2 Screen-house antifungal assay

The experimentation was done to assess the efficacy of *J. curcas*, *A. indica* and *P. hysterophorus* leaf extracts against LLS on groundnuts under screen-house condition in 2018 – 2019 at the Post Entry Plant Quarantine Station (TPHPA) Arusha, Tanzania. The susceptible groundnut variety Pendo was grown in plastic pots filled with a combination of sand, farmyard manure and black soil in ratio of 1:1:3. Then, 30 days after sowing (DAS) groundnut plants were sprayed with fresh *P. personata* conidial suspension using a hand sprayer. After disease symptoms development, groundnut plants were foliar sprayed four times at two weeks interval by chloroform, ethyl acetate and methanol leaf extract of *J. curcas*, *P. hysterophorus* and *A. indica* at 0.1, 0.25 and 0.5 mg/ml concentration from 48 DAS continued until 15 days before harvest. To prepare required concentrations (0.1, 0.25 and 0.5 mg/ml) of plant extracts were prepared adding 50, 125 and 250 mg on 500 ml of water. Control pots were sprayed with SDW and Chlorothalonil (2.1 L/ha) as negative and positive controls, respectively. Irrigation, weeding and other cultural practices were done whenever it was necessary. The treatments were arranged in a randomised completely block design (RCBD), replicated three times and the experiment was done twice: 1st trial from 14th July - Oct 2018 and 2nd Dec - March 2019.

The LLS disease incidence was evaluated on each plant following this formula

$$\text{Disease incidence } (\%) = \frac{\text{Number of infected leaves per plant}}{\text{Total number of leaves per plant}} \text{X } 100 \qquad (1)$$

Late leaf spot disease severity was evaluated using a nine-point (ICRISAT, 2009) based on lesion density and leaf necrosis condition; where (1 = no disease symptoms, and 9 => 80% disease symptoms) at 14, 21 and 28 days after inoculation (DAI) (Chiteka *et al*., 1988; Subrahmanyam *et al*., 1995). Growth parameters i.e. shoot length and number of leaves per plant and yield parameters (number of pods/plant, number of seeds/pod, 100-kernel weight and seed yield/plant) were recorded.

3.4 Phytochemical analysis

The phytochemical compounds of the selected pesticidal plant extracts were carried out using gas chromatography mass spectrometry (GC-MS) with specification; Agilent technologies 7890A GC connected to Agilent 5975 MSD (Agilent technology, USA). The inert gas helium (99.999%) was used as carrier gas with the flow rate of 1.2 ml/min. The GC was equipped with capillary column (HP 5) length of 30 meters, film 0.25 µm and internal diameter 0.250 mm and temperature limit 50 °C to 340 °C (360 °C) was used. The oven temperature rose from 50-280 °C with the rate of 10 °C/min rise in temperature. The sample size of 1 µl was injected through the injector. The mass spectrometer operated in electron ionization mode with an ionizing energy of 70 eV. The inlet temperature was 250 °C and the total GC-MS running time was 35 minutes.

3.5 Statistical analysis

The disease data i.e. percent inhibition of *P. personata* mycelia growth, incidence, and severity, growth and yield components were statistically analysed by using STATISTICA program version (2010). Disease incidence (percentage) and severity (count) data were transformed using arc sine and square root respectively to harmonize the variance before subjecting them for analysis (Groth *et al*., 1999). The comparison of treatment means was done by the least significant difference (LSD) at ($P \leq 0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Late leaf spot disease status in central, Tanzania

The field survey was conducted during 2018/2019 cropping season in groundnut cultivating regions in Singida and Dodoma regions, Tanzania. The results of the fields surveyed revealed that the LLS disease pressure occurred in almost both regions at varying levels (Table 1). Disease incidence and severity differed significantly ($P < 0.001$) among districts in Singida and Dodoma. High disease incidence and severity was recorded in Manyoni District Council (DC) (84.70%, 7.00) DC and Chamwino DC (80.6%, 6.75), followed by Singida rural (60.55%, 5.25) and Bahi (54.65%, 4.90) (Table 1).

Disease incidence and severity also differed significantly ($P < 0.001$) across villages. The results showed that Sanza (89.7%, 7.7) and Heka (88.8%, 7.3) had high LLS disease incidence and severity (Table 2). The lowest LLS disease incidence and severity were recorded at Makanda (47.8%, 4.3) and Lamaiti (50.3%, 4.8) (Table 2).

Table 2: Groundnut LLS incidence and severity in Central Tanzania, 2018/2019

Region	District	Disease incidence (%)	Disease severity
Singida	Singida rural	60.55 ± 3.76^{b}	5.25 ± 0.47^{b}
Singida	Manyoni DC	84.70 ± 1.55^{a}	7.00 ± 0.33^{a}
Dodoma	Chamwino DC	80.60 ± 1.67^{a}	6.75 ± 0.35^{a}
Dodoma	Bahi DC	54.65 ± 4.35^{b}	4.90 ± 0.43^{b}
One way ANOVA F-Statistics			
LSD (P=0.05)		22.78***	6.94***

Means followed by the same letter(s) within the column are not significant at 5% level based on Least Significance Difference test (LSD). *, **, ***significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

Table 3: Groundnut LLS incidence and severity in surveyed villages, Central Tanzania

Region	Village	Mean LLS incidence (%)	Mean LLS severity
Singida	Heka	88.8 [a]	7.3 [ab]
Singida	Sanza	89.7 [a]	7.7[a]
Singida	Kikio	53.0 [de]	4.0 [d]
Singida	Mgori	53.5 [de]	5.8 [abcd]
Singida	Makotea	59.5 [cde]	5.3 [abcd]
Singida	Mkola	66.5 [bcde]	5.8 [abcd]
Singida	Surhana	70.3 [abcd]	5.5 [abcd]
Singida	Kitinku	81.0 [ab]	6.8 [abcd]
Singida	Salala	82.5 [ab]	7.3 [ab]
Singida	Malolo	81.5 [ab]	6.0 [abcd]
Dodoma	Makanda	47.8 [e]	4.3 [cd]
Dodoma	Lamaiti	50.3 [de]	4.8 [bcd]
Dodoma	Msisi	51.0 [de]	4.8 [bcd]
Dodoma	Azimio	57.3 [de]	4.8 [bcd]
Dodoma	Chonde	67.0 [bcde]	6.0 [abcd]
Dodoma	Makoja	77.8 [abc]	6.3 [abcd]
Dodoma	Muungano	80.5 [ab]	7.3 [ab]
Dodoma	Idifu	80.8 [ab]	7.0 [ab]
Dodoma	Ngaheleze	81.5 [ab]	5.8 [abcd]
Dodoma	Ikombolinga	82.5 [ab]	7.5 [a]
Mean (Singida)	-	72.63	6.15
Mean (Dodoma)	-	67.6	5.86
General Mean	-	70. 1	6.0
LSD (P=0.05)	-	3.93	1.45
P-value	-	<0.001	<0.01

Means followed by the same letter(s) within the column are not significant at 5% level based on the Least Significance Difference test (LSD). *, **, ***significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

4.1.2 *In vitro* assessment of selected pesticidal plant extracts against mycelial growth of *P. personata*

(i) Isolation and identification of the *P. personata*

The fungal pathogen isolated from infested groundnut leaves from Singida and Dodoma was identified based on the morphological characteristics i.e. colony and conidia using Compound microscope (40X) (Agrios, 2005) (Plate 4 and 5). The conidium of *P. personata* was cylindrical, with short base tapered with a conscipicous hilum.

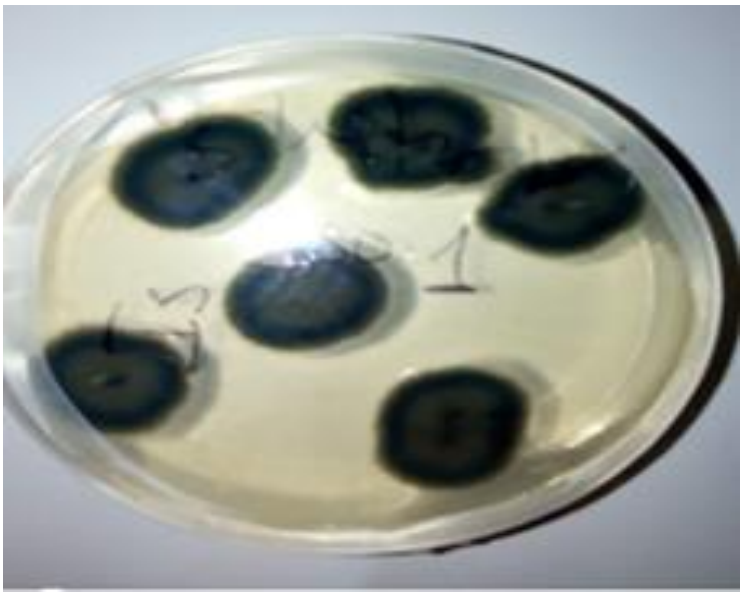


Plate 4: Fungal colony of *P. personata*

Plate 5: *Phaeosariopsis personata* conidium

(ii) Mycelial inhibition of *P. personata*

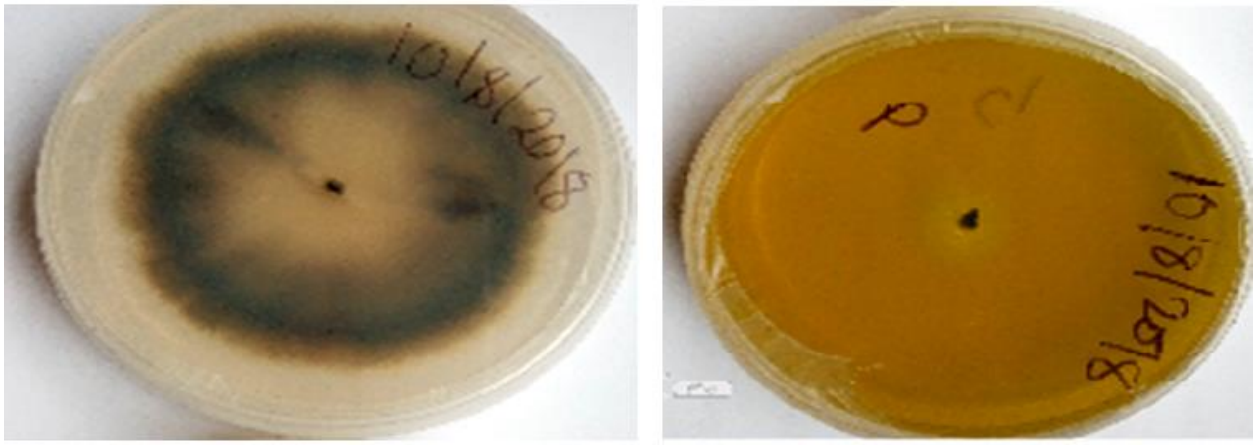
The antifungal effect of chloroform, ethyl acetate and methanol leaf extracts of *A. indica*, *J. curcas* and *P. hysterophorus* at three concentrations (0.1, 0.25 and 0.5 mg/ml) was measured by percent inhibition of mycelial growth *P. personata* as depicted in Table 4. All chloroform, ethyl acetate and methanol leaf extracts of *A. indica*, *J. curcas* and *P. hysterophorus* showed antifungal potential against *P. personata* tested compared to the control treatment. The leaf extracts of *A. indica* inhibited the mycelia growth with respect to their concentrations; at (0.5

mg/ml) by 88.78%, *A. indica* (0.25) by 88.11%, and *A. indica* (0.1 mg/ml) by 84.78%. The leaf extracts of *P. hysterophorus* showed maximum inhibition of 84.22% at concentration of 0.5 mg/ml and 79.89% at 0.25 mg/ml further, *J. curcas* inhibited the mycelial growth of *P. personata* by 84.11% at 0.5 mg/ml, 82.33% at 0.25 mg/ml and 81.22% at 0.1 mg/ml. Moreover, plant extract of the selected plants inhibited the mycelia growth of *P. personata* by more than 77% even at the lowest concentration (0.1 mg/ml). Furthermore, the percent inhibition did not differ significantly across different solvents similar results were observed. Therefore all plants extracted by the chosen solvents had similar effects on inhibiting the mycelial growth of *P. personata* (Table 4).

Table 4: The effect of selected plant extracts on inhibiting mycelial growth of *P. personata*

Treatments and solvents	Percent inhibition (Mean ± SE)
Treatments	
J. curcas (0.1 mg/ml)	81.22 ± 2.25^{def}
J. curcas (0.25 mg/ml)	82.33 ± 1.64^{de}
J. curcas (0.5 mg/ml)	84.11 ± 1.11^{cde}
A. indica (0.1 mg/ml)	84.78 ± 1.41^{abc}
A. indica (0.25 mg/ml)	88.11 ± 1.09^{abc}
A. indica (0.5 mg/ml)	88.78 ± 1.05^{bcd}
P. hysterophorus (0.1 mg/ml)	77.22 ± 1.71^{f}
P. hysterophorus (0.25 mg/ml)	79.89 ± 2.36^{ef}
P. hysterophorus (0.5 mg/ml)	84.22 ± 1.38^{abcd}
Chlorothalonil (2.1 L/ha)	89.56 ± 1.16^{a}
Control	0.00 ± 0.00^{g}
Solvents	
Chloroform	82.63 ± 1.17^{a}
Ethyl acetate	83.96 ± 1.07^{a}
Methanol	83.63 ± 1.13^{a}
2-way ANOVA (F-statistics)	
Treatments	4.49***
Solvents	0.47ns
Treatments*Solvents	0.41ns

Means followed by the same letter(s) within the column are not significant at 5% level based on the Least Significance Difference test (LSD). *, **, ***significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant



Control

Parthenium extract

Plate 6: Inhibition pictures on cultured media

4.1.3 Determination of antifungal efficacy of plant extracts against LLS disease under screen-house conditions

(i) The effect of treatments application on incidence and severity of LLS

The results showed that LLS disease incidence and severity across plant extract treatments and solvents differed significantly ($P \leq 0.001$) as compared with Chlorothalonil and SDW as positive and negative control respectively (Table 5). The lowest LLS incidence and severity were recorded to groundnut plants treated with *J. curcas* (0.5 mg/ml) had lower incidence and severity (2.33%, 1.56) respectively, compared to groundnut plants treated by chlorothalonil (standard fungicide) being (5.41%, 2.00). Plants treated by *J. curcas* leaf extracts showed antifungal effect by lowering LLS incidence and severity by 13.44%, and 3.56 at (0.25 mg/ml) and less activities was observed to plants treated by *J. curcas* leaf extracts (0.1 mg/ml) as (24.22%, 4.67). Groundnut plants treated by *P. hysterophorus* leaf extracts (0.5 mg/ml) showed lower LLS incidence and severity (5.67%, 2.22) and *P. hysterophorus* leaf extracts (0.25 mg/ml) showed incidence (16.56%) and severity (3.89) respectively. The leaf extract of *A. indica* showed antifungal activity against the tested pathogen by reducing the LLS disease incidence and severity by 15.56%, and 3.57 respectively at highest concentration (0.5 mg/ml), and less activities was reported by *A. indica* (0.25 mg/ml) (22.33%, 4.78) and *A. indica* (0.1 mg/ml) as (30.33%, 5.44) as LLS incidence and severity. Similarly, LLS disease incidence and severity differed significantly with respect to type of solvents used in extraction process, whereby selected plants leaves extracted by methanol had the lowest LLS

incidence (14.30%) and severity (3.33) compared with chloroform (17.44%, 4.07) and ethyl acetate solvents (20.56%, 4.26) respectively (Table 5).

Table 5: The effect of treatments and solvents on managing groundnut LLS disease

Treatments and solvents	Incidence (%)(Mean ± SE)	Severity (Mean ± SE)
Treatments		
J. curcas (0.1 mg/ml)	24.22 ± 1.28^{c}	4.67 ± 0.17^{bcd}
J. curcas (0.25 mg/ml)	13.44 ± 2.40^{d}	3.56 ± 0.41^{de}
J. curcas (0.5 mg/ml)	2.33 ± 1.60^{e}	1.56 ± 0.38^{f}
A. indica (0.1 mg/ml)	30.33 ± 1.94^{b}	5.44 ± 0.18^{b}
A. indica (0.25 mg/ml)	22.33 ± 1.99^{c}	4.78 ± 0.28^{bc}
A. indica (0.5 mg/ml)	15.56 ± 3.19^{d}	3.67 ± 0.53^{de}
P. hysterophorus (0.1 mg/ml)	26.44 ± 1.19^{bc}	5.22 ± 0.15^{b}
P. hysterophorus (0.25 mg/ml)	16.56 ± 1.19^{bc}	3.89 ± 0.42^{cde}
P. hysterophorus (0.5 mg/ml)	5.67 ± 0.18^{e}	2.22 ± 0.52^{f}
Chlorothalonil (2.1 L/ha)	5.41 ± 1.07^{e}	2.00 ± 1.00^{f}
Control	89.41 ± 0.92^{a}	9.00 ± 0.00^{a}
Solvents		
Chloroform	17.44 ± 1.68^{ab}	4.07 ± 0.25^{ab}
Ethyl acetate	20.56 ± 2.25^{ab}	4.26 ± 0.32^{ab}
Methanol	14.30 ± 2.22^{b}	3.33 ± 0.35^{b}
2-way ANOVA (F-statistics)		
Treatments	22.00***	14.64***
Solvents	7.34***	6.03***
Treatments*Solvents	1.23ns	0.89ns

Means followed by the same letter(s) within the column are not significant at 5% level based on the Least Significance Difference test (LSD). *, **, ***significant at P≤ 0.05, P ≤ 0.01, and P≤ 0.001, respectively; ns=not significant.

(ii) Growth attributes of groundnut with respect to treatments application

Growth attributes differed significantly (P ≤ 0.001) among different treatments and solvents (Table 6). Plants treated with Chlorothalonil had taller shoots (39.89 cm) and greater number of leaves per plant 9.33 followed by *P. hysterophorus* (0.5 mg/ml; 0.25 mg/ml; 0.1 mg/ml) by (34 cm, 32 cm and 26.67 cm), *J. curcas* (0.5 mg/ml) by 30.89 cm and *A. indica* (0.5 mg/ml) by 29.78 cm as compared with control 19.56 cm (Table 6). The shoot length (cm) and number of leaves per plant at flowering and maturity stage differed significantly (P ≤ 0.001) across different solvents. The chloroform and methanolic leaf extract of *J. curcas*, *P.*

hysterophorus and *A. indica* had the taller shoots and greater number of leaves as compared with ethyl acetate extracts (Table 6).

Table 6: The effect of treatments and solvents on growth attributes of groundnuts

Treatment and Solvent	Shoot length (flw) (cm)(Mean ± SE)	Number of leaves (flw) (Mean ± SE)	Shoot length (cm)(maturity) (Mean ± SE)	Number of leaves (maturity) (Mean ± SE)
Treatments				
J. curcas (0.1 mg/ml)	24.44 ± 1.55^{f}	5.56 ± 0.29^{fg}	32.78 ± 1.65^{d}	8.11 ± 0.54^{d}
J. curcas (0.25 mg/ml)	28.00 ± 1.72^{cdef}	6.67 ± 0.33^{cde}	36.11 ± 2.00^{cd}	9.56 ± 0.60^{bcd}
J. curcas (0.5 mg/ml)	30.89 ± 2.28^{bcd}	8.00 ± 0.47^{b}	39.33 ± 2.29^{bc}	10.78 ± 0.85^{bc}
A. indica (0.1 mg/ml)	24.89 ± 1.24^{f}	5.44 ± 0.88^{fg}	32.56 ± 1.02^{d}	8.11 ± 0.54^{d}
A. indica (0.25 mg/ml)	26.89 ± 1.68^{def}	5.78 ± 0.43^{ef}	35.56 ± 1.53^{cd}	9.11 ± 0.68^{cd}
A. indica (0.5 mg/ml)	29.78 ± 0.68^{cde}	7.00 ± 0.33^{bcd}	39.33 ± 0.83^{bc}	10.44 ± 0.63^{bc}
P. hysterophorus (0.1 mg/ml)	26.67 ± 1.56^{ef}	6.22 ± 0.40^{def}	35.22 ± 1.61^{d}	8.67 ± 0.53^{d}
P. hysterophorus (0.25 mg/ml)	32.00 ± 1.31^{bc}	7.00 ± 0.41^{bcd}	40.78 ± 1.00^{b}	10.44 ± 0.67^{bc}
P. hysterophorus (0.5 mg/ml)	34.00 ± 1.69^{b}	7.44 ± 0.44^{bc}	42.11 ± 0.86^{b}	11.11 ± 0.56^{b}
Chlorothalonil (2.1 L/ha)	39.89 ± 0.98^{a}	9.33 ± 0.44^{a}	48.33 ± 1.24^{a}	14.22 ± 0.64^{a}
Control	19.56 ± 0.82^{g}	4.67 ± 0.24^{g}	25.56 ± 1.12^{e}	4.33 ± 0.24^{e}
Solvents				
Chloroform	30.63 ± 0.85^{a}	7.04 ± 0.22^{a}	38.74 ± 0.90^{a}	10.48 ± 0.37^{a}
Ethyl acetate	25.41 ± 0.83^{b}	6.00 ± 0.19^{b}	33.85 ± 0.84^{b}	8.26 ± 0.19^{b}
Methanol	29.81 ± 1.17^{a}	6.67 ± 0.33^{ab}	38.67 ± 1.09^{a}	10.04 ± 0.47^{a}
2-way ANOVA (F-statistics)				
Treatments	8.67***	8.00***	9.89***	6.78***
Solvents	19.22***	8.5***	19.64***	21.15***
Treatments*Solvents	3.30***	2.39**	2.64***	3.04**

Means followed by the same letter(s) within the column are not significant at 5% level based on Least Significance Difference test (LSD). *, **, ***significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

(iii) Yield attributes of groundnut with respect to treatments application

The groundnuts yield components differed significantly ($P \leq 0.001$) under different treatments. Plants treated by chlorothalonil had greater number of pods/plant (41.89), number of seeds/ plant (79) and greater seed weight/plant (48.08) followed by *A. indica* (0.5 mg/ml), by (36.11) number of pods/plant, (66.67) number of seeds/plant, and 35.22 g seed weight/plant; *P. hysterophorus* (0.5 mg/ml), by (36.11) as number of pods/plant, 75.78 number of seeds/plant, and 40.35 g seed weight/plant as compared to control treatment. However, no significant effect was observed on yield components to plants treated by different solvents similar results were observed (Table 7).

Table 7: The influence of treatments and solvents on grain yield of groundnut

Treatment and Solvent	No. pods/plant	No. seeds/plant	100 seed wt (g)	Seed yield/plant (g)
Treatments				
J. curcas (0.1 mg/ml)	28.44 ± 1.86^{f}	51.78 ± 3.65^{f}	47.56 ± 4.35^{a}	24.04± 2.06^{g}
J. curcas (0.25 mg/ml)	33.11 ± 1.65^{de}	61.11 ± 3.08^{de}	47.56 ± 2.96^{a}	29.34 ± 2.58^{efg}
J. curcas (0.5 mg/ml)	37.33 ± 1.14^{bc}	70.56 ± 2.14^{bc}	51.89 ± 4.75^{a}	36.54 ± 3.50^{cd}
A. indica (0.1 mg/ml)	30.56 ± 2.07^{ef}	55.67 ± 4.26^{ef}	51.11 ± 2.40^{a}	28.51 ± 2.57^{fg}
A. indica (0.25 mg/ml)	36.11 ± 1.23^{cd}	66.67 ± 2.69^{cd}	53.00 ± 2.90^{a}	35.22 ± 2.23^{cde}
A. indica (0.5 mg/ml)	41.56 ± 1.02^{a}	77.22 ± 1.55^{ab}	58.44 ± 2.06^{a}	45.09 ± 1.71^{ab}
P. hysterophorus (0.1 mg/ml)	30.67 ± 0.94^{ef}	56.33 ± 1.73^{ef}	47.56 ± 1.87^{a}	28.80 ± 1.26^{fg}
P. hysterophorus (0.25 mg/ml)	35.78 ± 0.72^{cd}	67.67 ± 1.67^{cd}	48.67 ± 3.88^{a}	32.67 ± 2.21^{def}
P. hysterophorus (0.5 mg/ml)	39.89 ± 0.70^{ab}	75.78 ± 1.18^{ab}	53.33 ±3.02^{a}	40.35 ± 2.26^{bc}
Chlorothalonil (2.1 L/ha)	41.89 ± 1.52^{a}	79.00 ± 2.11^{a}	60.89 ± 2.85^{a}	48.08 ± 2.78^{a}
Control	14.22 ± 0.92^{g}	20.22 ± 1.60^{g}	39.22 ± 2.60^{a}	7.76 ± 0.54^{h}
Solvents				
Chloroform	35.56 ± 1.02^{a}	66.00 ± 2.06^{a}	50.07 ± 2.12^{a}	33.14 ± 1.81^{a}
Ethyl acetate	34.70 ± 1.18^{a}	64.19 ± 2.32^{b}	51.41 ± 1.87^{a}	33.38 ± 1.96^{a}
Methanol	34.22 ± 1.11^{a}	64.07 ± 2.26^{ab}	51.56 ± 1.79^{a}	33.00 ± 1.64^{a}
2-way ANOVA (F-statistics)				
Treatment	10.08***	10.95***	1.24ns	8.94***
Solvents	0.69ns	0.47ns	0.19ns	0.02ns
Treatments*Solvents	0.65ns	0.71ns	1.09ns	1.38ns

Means followed by the same letter(s) within the column are not significant at 5% level based on Least Significance Difference test (LSD). *, **, ***significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

(iv) Interactive effects of treatments and solvents on growth of groundnuts

There was significant interaction influence between treatments and solvents in number of leaves/plant and shoot length at flowering and maturity stage of groundnuts grown under screen-house condition. All plants treated by plant extract treatments extracted by each solvent influenced the growth of groundnut (Figs. 3, 4, 5 and 6).

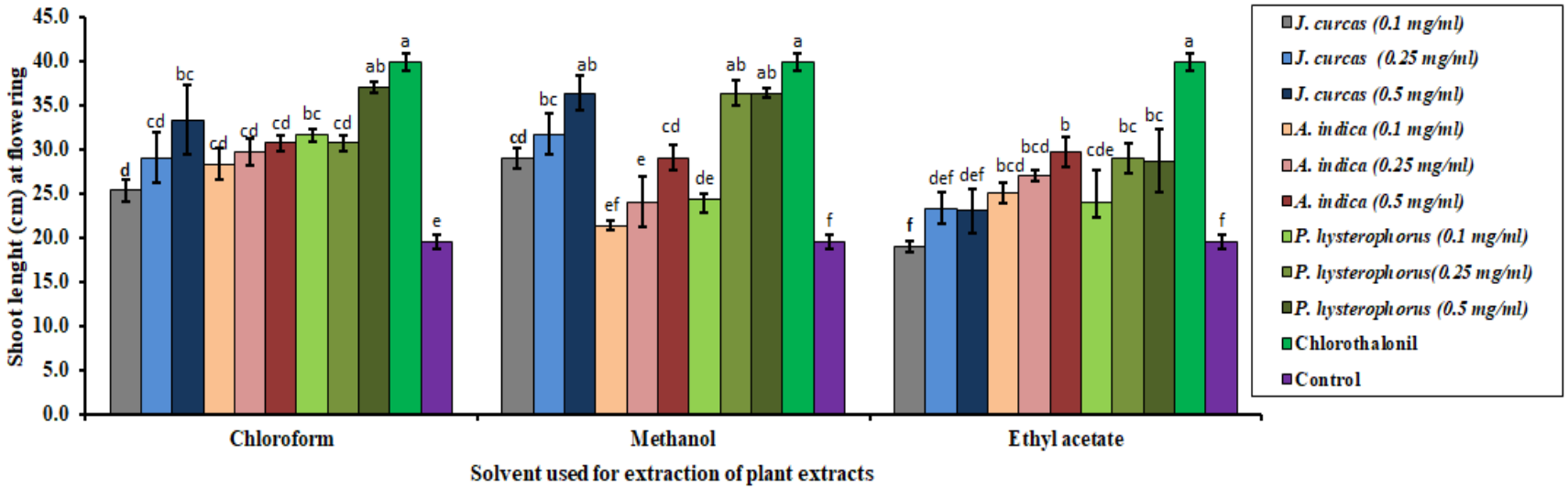


Figure 2: Interactive effect of treatments and solvents on shoot length at flowering of groundnut grown under screen-house condition

Blocks followed by similar letter(s) do not differ significantly from each other. *, **, ***significant at P ≤ 0.05, P ≤ 0.01, and P≤ 0.001, respectively; ns=not significant

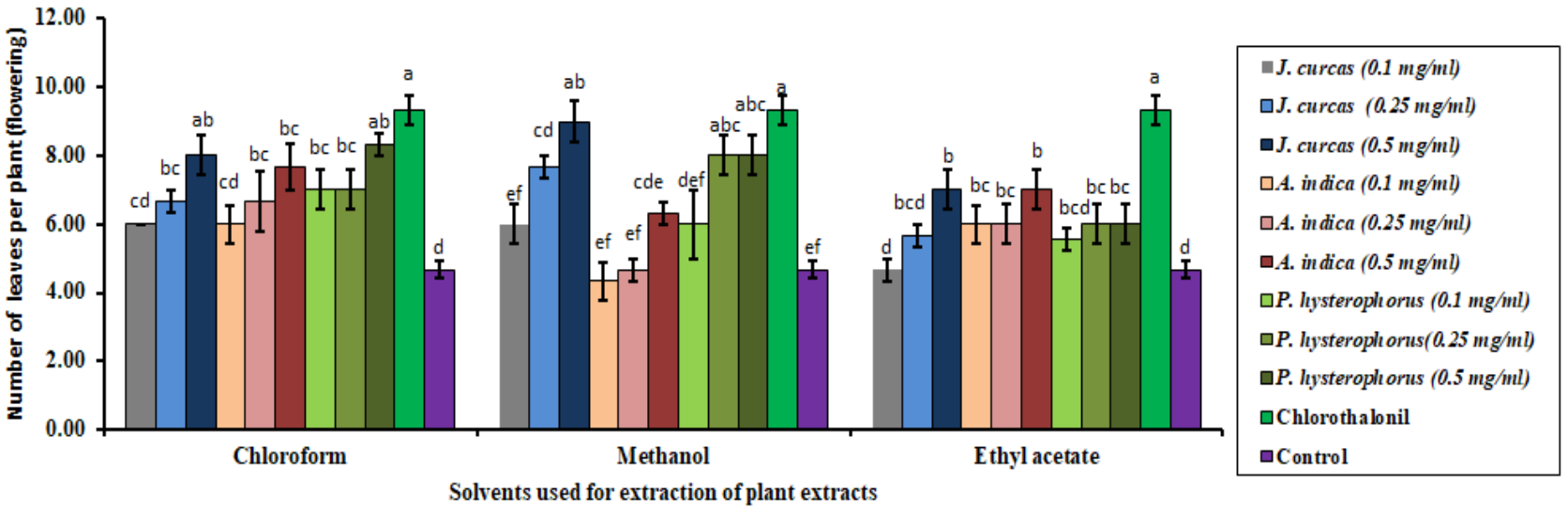


Figure 3: Interactive effect of treatments and solvents on number of leaves at flowering of groundnut grown under screen-house condition

Blocks followed by similar letter(s) do not differ significantly from each other. *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

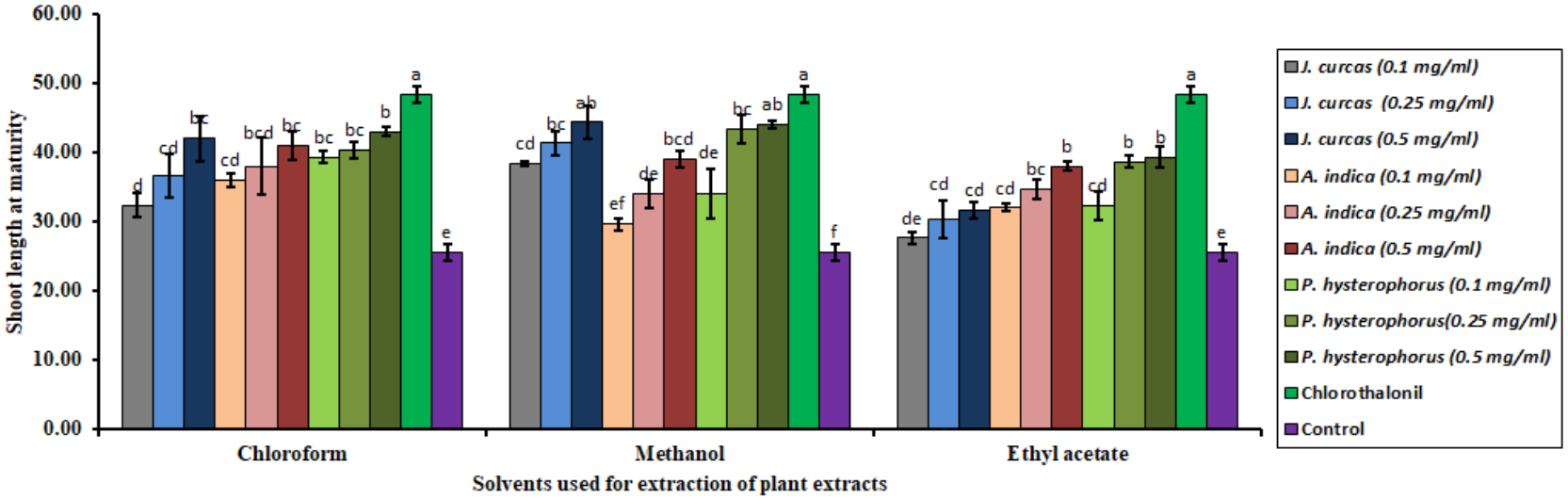


Figure 4: Interactive effect of treatments and solvents on shoot length at maturity of groundnut grown under screen-house condition

Blocks followed by similar letter(s) do not differ significantly from each other. *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

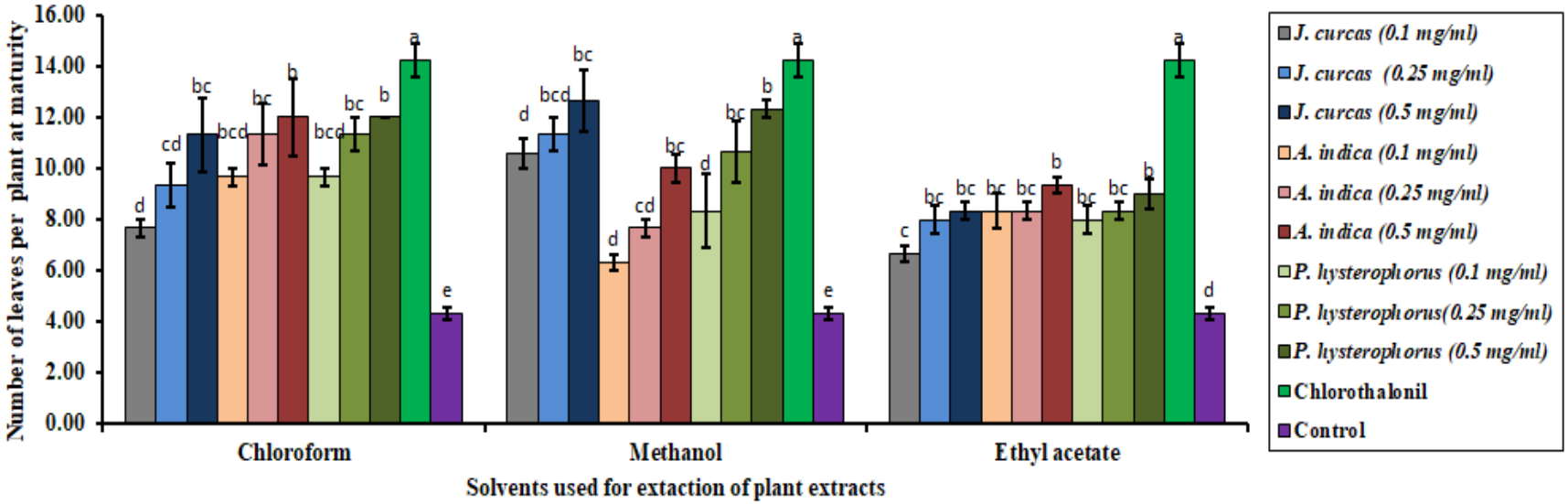


Figure 5: Interactive effect of treatments and solvents on number of leaves per plant at maturity of groundnut grown under screen-house condition

Blocks followed by similar letter(s) do not differ significantly from each other. *, **, *** significant at $P \leq 0.05$, $P \leq 0.01$, and $P \leq 0.001$, respectively; ns=not significant

4.1.4 Chemical composition of leaf extracts

The GC-MS results identified different phytocompounds from chloroform, ethyl acetate and methanolic leaf extracts of *J. curcas*, *P. hysterophorus* and *A. indica*. The mass spectra of the detected phytocompounds from the selected pesticidal plants were compared with the known compounds' spectra found in the NIST library. The name of compounds, retention time, molecular weight and molecular formula of the compounds are presented in Table 8-16.

The following phytoconstituents; dodecane, 2,6,11-trimethyl-2-tetradecene, tetradecane, pentadecane, octacosane, sulfurous acid butyl decyl ester, heneicosane, phenol 2,4-bis (1, 1-dimethylethyl), 2-bromo dodecane, hexadecane, heptadecane, 9-octyl-, heptacosane, 2,4-dimethyldodecane, pentadecane, ethanol 2-(octadecyloxy), octacosane, hentriacontane, geranylgeraniol, octadecane, octadecane, 12-methyl-E-E-2 13-octadecadien-1-ol, tetradecanal and cyclotetracosane with antifungal properties were identified from chloroform leaf extract of *J. curcas* by GC-MS (Table 8). Among them major phytoconstituents were *n*-hexadecanoic acid (7.89%), phenol 2,4-bis (1,1-dimethylethyl) (4.04%), cyclotetracosane (1.23 %), hexadecane (1.20%) and octacosane (1.02%) (Table 8).

The following phytoconstituents with antifungal effects were identified from ethyl acetate leaf extract of *J. curcas*; 1,2,3-ropanetriol, monoacetate, 2,5-pyrrolidinedione, hexadecane, methyl salicylate, triacetin, heptadecane, 8-hexadecenal, 14-methyl-, (Z)-, undecane, phenol, 2,4-bis(1,1-dimethylethyl), 1-naphthalenol, 2,6,10,14,18,22-tetracosahexaene, hexadecane, heptadecane, 1H-indene 1-ethylideneoctahydro-7 a-methyl- cis-, E-14-hexadecenal, 1-tetradecene, tetramethyl-2-hexadecen-1-ol, *n*-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, 9,12-octadecadienoic acid (Z,Z)-, 5-eicosene, (E)-, hexadecanoic acid ethyl ester, 2-methyl-Z,Z-3,13-octadecadienol, 9,17-octadecadienal, (Z)-, phyto, 9,12,15-octadecatrienoic acid ethyl ester, (Z,Z,Z)-, heptadecanoic acid ethyl ester and eicosane (Table 9). The dominant phytoconstituents were phytol (9.31%), thiomorpholine (4.83%), hexadecanoic acid ethyl ester (3.97%), phenol 2,4-bis (1,1-dimethylethyl) (3.37%), 9,12,15-octadecatrienoic acid ethyl ester, (Z,Z,Z)- (2.75%), 5-eicosene, (E)- (2.11%) and 1-heneicosyl formate (1.92%) (Table 9).

Also, the GC-MS analysis identified the following phytocompounds with antifungal properties; 1,2,3-propanetriol monoacetate, methyl salicylate, 2-undecanone, indole, decanoic acid methyl ester, 2-methoxy-4-vinylphenol, tert-hexadecanethiol, phenol, 2,6-dimethoxy-,

phenol, 2,6-dimethoxy-,tetradecane, cyclotetradecane, pentanoic acid ethyl ester, 2-propenoic acid 3-phenyl- methyl ester, diphenyl ether, pentadecane, tridecane, hexadecane, heptadecane, 17-pentatriacontene, 1-nonadecene, E-15-heptadecenal, 8-hexadecenal 14-methyl-, cyclopentadecane, hexadecanoic acid methyl ester, 1-octadecene, 2-methyl-Z, Z-3, 13-octadecadienol, oleic acid, 9,17-octadecadienal, (Z)-, 2-methyl-Z,Z-3,13-octadecadienol, 9, 12-octadecadienoic acid (Z,Z)-methyl ester, phytol, octadecanoic acid methyl ester, behenic alcohol, octadecanoic acid ethyl ester, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 9,17-octadecadienal, (Z)-, eicosan and docosanoic acid methyl ester from methanolic leaf extract of *J. curcas* (Table 10). Phytol (26.75%), hexadecanoic acid methyl ester (14.32%), octadecanoic acid methyl ester (2.79%), and 9,12-octadecadienoic acid (Z, Z)- methyl ester (2.33%) were identified as major phytoconstituents (Table 10).

The phytoconstituents with antifungal properties identified from chloroform leaf extracts of *P. hysterophorus* using GC-MS were; 1,2,3-propanetriol monoacetate, benzoic acid, .alpha.-cubebene, hexadecane, geranyl tiglate, heptacosane, 1-nonadecene, octadecane, benzyl benzoate, heptadecane, hexadecane, hexadecanoic acid methyl ester, *n*-hexadecanoic acid, hexadecanoic acid ethyl ester, 9,12-octadecadienoic acid (Z,Z)-, pentadecanoic acid methyl ester, 9,17-octadecadienal (Z), 9,12-octadecadienoic acid (Z,Z)- methyl ester, phytol, 17-pentatriacontene, octadecanoic acid methyl ester, 9,12,15-octadecatrienoic acid (Z,Z,Z)-, eicosanoic acid methyl ester, 12-methyl-E,E-2,13-octadecadien-1-ol, ethanol 2-(octadecyloxy)-, nonadecane and eicosane (Table 11). The dominant phytocompounds were phytol (24.14%), *n*- hexadecanoic acid (7.52%), hexadecanoic acid methyl ester (6.48%), 9,12,15-octadecatrienoic acid (Z,Z,Z)- (5.46%) and octadecanoic acid methyl ester (1.43%) (Table 11).

The phytocompounds found from ethyl acetate extract of *P. hysterophorus* were 1-cyclohexene-1-carboxaldehyde, tridecane, alpha.-cubebene, gamma.-elemene, dodecane, alpha farnesene, phenol 2,4-bis(1,1-dimethylethyl), octatriacontyl pentafluoropropionate, 5-octadecene, (E)-, benzyl benzoate, 1,4 eicosadiene, 1-tetradecene, hexadecanoic acid methyl ester, isophytol, trans-13-octadecenoic acid, 2-methyl-Z,Z-3,13-octadecadienol, 9,12-Octadecadienoic acid (Z,Z), 3-eicosene, (E), eicosane and heptacosane (Table 12). The major compounds were phenol, 2,4-bis (1,1-dimethylethyl) (2.73%), 1,4-eicosadiene (2.68%), heptacosane (2.35%), dibutyl phthalate (2.24%), alpha. –cubebene (1.91%), 4-amino-2,6-dihydroxypyrimidine (1.76%), cyclohexadecane (1.75%) (Table 12).

The following phytoconstituents with antifungal effects; 1,3,5-triazine-2,4,6-triamine, tert-hexadecanethiol, 1,2-benzenediol, methyl salicylate, *n*-aminopyrrolidine, l-[-]-4-hydroxy-1-methylproline, 2-undecanone, gamma.-elemene, pentadecane, phytol, 3-deoxy-d-mannoic lactone, falcarinol, methyl tetradecanoate, cyclotetradecane, 17-pentatriacontene, 1-docosene, ethanol 2-(octadecyloxy)-, octadecane, ethanol 2-(octadecyloxy), octadecane, benzyl benzoate, 7-hexadecenoic acid methyl ester, (Z)-, hexadecane, 2,6,10,14-tetramethyl-, 9-hexadecenoic acid methyl ester (Z)-, hexadecanoic acid methyl ester, *n*-hexadecanoic acid, 3-eicosene (E)-, hexadecanoic acid ethyl ester, 9,17-octadecadienal, (Z)-, 9,12-octadecadienoic acid (Z,Z)-, octadecanoic acid methyl ester, eicosane and 2-methyl-Z,Z-3,13-octadecadienol were found from methanolic leaf extract of *P.* hysterophorus using GC-MS (Table 13). The dominant compounds were hexadecanoic acid methyl ester (9.45%), octadecanoic acid methyl ester (2.09%), 9,12-octadecadienoic acid (Z,Z)- (1.97%) and *n*-hexadecanoic acid (1.31%) (Table 13).

The following phytoconstituents with antifungal properties were identified by GC-MS from chloroform leaf extracts of *A. indica*; sulfurous acid hexyl pentadecyl ester, pentadecane, tetradecane, eicosane, phenol 2,4-bis (1,1-dimethylethyl), 1-octadecene, ethanol 2-(octadecyloxy), hexadecanoic acid methyl ester, phytol, *n*-hexadecanoic acid, hexadecanoic acid ethyl ester, heptadecane, 9,12-octadecadienoic acid (Z,Z)-, heptacosane and hexadecane (Table 14). 9,12-octadecadienoic acid (Z,Z)- (3.63 %), *n*-hexadecanoic acid (3.54%), hexadecane (2.78%), tetradecane (2.58%), phenol 2,4-bis (1,1-dimethylethyl) (2.40%) were the major compounds (Table 14).

Methyl salicylate, piperazine, alpha.-cubebene, pentadecane, phenol 2,4-bis (1,1-dimethylethyl), hexadecanoic acid ethyl ester, hexadecane, tetracosane, 1-nonadecene, hexadecanoic acid methyl ester and phytol were phytoconstituents with antifungal potential identified by GC-MS from ethyl acetate leaf extracts of *A. indica* (Table 15). The dominant compounds identified were phytol (32.12%), phenol 2,4-bis (1,1-dimethylethyl) (4.92%), 1-nonadecene (4.45%), hexadecanoic acid methyl ester (2.00%), hexadecane (1.86%) and methyl salicylate (1.04%) (Table 15).

The GC-MS identified the presence of the following phytoconstituents with antifungal effects; methyl salicylate, octadecanoic acid methyl ester, 4-hydroxy-3-methyl-2-butenyl, cyclopropane, 2-methoxy-4-vinylphenol, .alpha.-cubebene, heptadecane, caryophyllene oxide, 5-eicosene, (E), benzyl benzoate, (R)-(-)-14-methyl-8-hexadecyn-1-ol, hexadecanoic

acid methyl ester, 2-methyl-Z,Z-3,13-octadecadienol, 9,12-octadecadienoic acid (Z,Z)-, 9,12-octadecadienoic acid (Z,Z)- methyl ester, phytol, octadecanoic acid methyl ester, 9,17-octadecadienal (Z), cyclotrisiloxane hexamethyl-,1,2-benzene dicarboxylic acid diisooctyl ester and 1-bromoeicosane from the methanolic leaf extract of *A. indica* (Table 16). Phytol (35.96%), octahydropyrrolo [1,2-a] pyrazine (8.00%), 1,2-benzenedicarboxylic acid, diisooctyl ester (2.97%), hexadecanoic acid methyl ester (2.38%), octadecanoic acid 3-hydroxy- methyl ester (1.99%) and octadecanoic acid methyl ester (1.26%) were the major phytoconstituents.

Table 8: Phytochemical compounds with antifungal activity identified from chloroform leaf extract of *J. curcas* detected using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
10.629	dodecane, 2,6,11-trimethyl-	$C_{15}H_{32}$	212.41	Zhang *et al*. (2015)
11.745	2-tetradecene, (E)-	$C_{14}H_{28}$	196.37	Shirani *et al*. (2017)
11.905	tetradecane	$C_{14}H_{30}$	198.39	Yuan *et al*. (2012)
12.460	pentadecane	$C_{18}H_{38}$	254.49	Yuan *et al*. (2012)
12.958	octacosane	$C_{28}H_{58}$	394.76	Zhang *et al*. (2015)
13.192	sulfurous acid butyl decyl ester	$C_{16}H_{34}O_3S$	306.50	Sharma and Saini (2019)
13.267	heneicosane	$C_{21}H_{44}$	296.57	Ebrahimabadi *et al*. (2016)
13.461	phenol 2,4-bis (1, 1-dimethylethyl)	$C_{14}H_{22}O$	206.32	Manikandan *et al*. (2017)
14.011	2-bromo dodecane	$C_{12}H_{25}Br$	249.23	Manikandan *et al*. (2017)
14.503	hexadecane	$C_{16}H_{34}$	226.44	Zhang *et al*. (2015)
15.041	heptadecane, 9-octyl-	$C_{25}H_{52}$	352.68	Musa *et al*. (2015)
15.401	heptacosane	$C_{27}H_{56}$	380.73	Bouzabata *et al*. (2018)
16.002	2,4-dimethyldodecane	$C_{14}H_{30}$	198.38	Dhouib *et al*. (2019)
17.009	ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.50	El-Din and Mohyeldin (2018)
18.067	octacosane	$C_{28}H_{58}$	394.76	Zhang *et al*. (2015)
18.142	hentriacontane	$C_{31}H_{64}$	436.84	Ruban and Gajalakshmi (2012)
18.457	geranylgeraniol	$C_{20}H_{34}O$	290.48	Ashraf *et al*. (2017)
18.542	octadecane	$C_{18}H_{38}$	254.49	Zhang *et al*. (2006)
18.869	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Omoruyi *et al*. (2014)
19.584	12-methyl-E-E-2, 13-octadecadien-1-ol	$C_{19}H_{36}O$	280.00	Vijayabaskar and Elango (2018)
20.013	tetradecanal	$C_{14}H_{28}O$	212.37	Passos *et al*. (2003)
29.037	cyclotetracosane	$C_{24}H_{48}$	336.64	Bughio *et al*. (2017)

Table 9: Phytochemical compounds with antifungal activity identified from ethyl acetate leaf extract of *J. curcas* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.539	1,2,3-ropanetriol, monoacetate	$C_5H_{10}O_4$	134.13	Teoh and Mashitah (2012)
8.460	2,5-pyrrolidinedione	$C_8H_{13}NO_2$	331.32	Takayama *et al*. (1982)
8.826	hexadecane	$C_{16}H_{34}$	226.44	Adeleye *et al*. (2011)
9.273	methyl salicylate	$C_8H_8O_3$	152.15	Pawar and Thaker (2006)
11.321	triacetin	$C_9H_{14}O_6$	218.21	Osuntokun and Olajubu (2014)
11.813	heptadecane	$C_{17}H_{36}$	240.5	Zhang *et al*. (2015)
11.899	8-hexadecenal, 14-methyl-, (Z)-	$C_{17}H_{32}O$	252.4	Osuntokun and Olajubu (2014)
12.952	undecane	$C_{11}H_{24}$	156.31	Peng *et al*. (2013)
13.467	phenol, 2,4-bis(1,1-dimethylethyl)	$C_{17}H_{30}OSi$	278.50	Ma *et al*. (2018)
13.993	1-naphthalenol	$C_{10}H_8O$	144.17	Kumar *et al*. (2012)
14.337	2,6,10,14,18,22-tetracosahexaene	$C_{24}H_{38}$	326.6	Joseph *et al*. (2016)
14.503	hexadecane	$C_{16}H_{34}$	226.41	Oliveira *et al*. (2014)
15.658	heptadecane	$C_{17}H_{36}$	240.48	Zhang *et al*. (2015)
16.591	1H-indene, 1-ethylideneoctahydro-7 a-methyl-	$C_{12}H_{22}$	166.30	Wang *et al*. (2013)
16.889	E-14-hexadecenal	$C_{16}H_{30}O$	238.41	Joseph *et al*. (2016)
17.106	1-tetradecene	$C_{14}H_{28}$	196.37	Tayung and Jha (2014)
17.896	tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.50	El-Din and Mohyeldin, (2018)
18.868	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Tyagi and Agarwal (2017)
18.983	9,12-octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	280.40	El-Din and Mohyeldin (2018)
19.109	5-eicosene, (E)-	$C_{20}H_{40}$	280.50	Adibe *et al*., 2019)
19.172	hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.47	El-Din and Mohyeldin (2018)
19.338	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.50	Adibe *et al.* (2019)
20.179	9,17-octadecadienal, (Z)-	$C_{18}H_{32}O$	264.40	Adibe *et al.* (2019)
20.413	phytol	$C_{20}H_{40}O$	296.54	Pejin *et al.* (2014)
21.186	heptadecanoic acid ethyl ester	$C_{19}H_{38}O_2$	298.50	Bashir *et al.* (2019)
23.869	eicosane	$C_{20}H_{42}$	282.50	El-Naggar *et al.* (2017)

Table 10: Phytochemical compounds with antifungal activity identified from methanolic leaf extract of *J. curcas* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.539	1,2,3-propanetriol monoacetate	$C_5H_{10}O_4$	134.13	Teoh and Mashitah (2012)
9.273	methyl salicylate	$C_8H_8O_3$	152.15	Ebrahimabadi *et al.* (2016)
10.549	2-undecanone	$C_{11}H_{22}O$	170.29	Bisht and Chanotiya (2011)
10.841	indole	C_8H_7N	117.15	Sumiya *et al.* (2017)
10.898	decanoic acid methyl ester	$C_{11}H_{22}O_2$	186.29	Belakhdar *et al.* (2015)
11.121	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	Guo *et al.* (2008)
11.287	tert-hexadecanethiol	$C_{16}H_{34}S$	258.50	Yang *et al.* (2016)
11.653	phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154.16	Yang *et al.* (2016)
11.813	tetradecane	$C_{14}H_{30}$	198.39	Dhouib *et al.* (2019)
11.905	cyclotetradecane	$C_{14}H_{28}$	196.37	Afrouzan *et al.* (2018)
11.991	pentanoic acid ethyl ester	$C_7H_{14}O_2$	130.18	Sumiya *et al.* (2017)
12.248	2-propenoic acid 3-phenyl-, methyl ester	$C_{10}H_{10}O_2$	162.18	Umaiyambigai *et al.* (2017)
12.334	diphenyl ether	$C_{12}H_{10}$	170.21	Zhang *et al.* (2015)
13.198	pentadecane	$C_{15}H_{32}$	212.41	Zhang *et al.* (2006)
13.272	tridecane	$C_{13}H_{28}$	184.36	Yuan *et al et al.* (2012)
14.503	hexadecane	$C_{16}H_{34}$	226.44	Oliveira *et al.* (2014)
16.706	heptadecane	$C_{17}H_{36}$	240.47	Musa *et al.* (2015)
16.797	17-pentatriacontene	$C_{35}H_{70}$	490.93	Zhang *et al.* (2015)
16.889	1-nonadecene	$C_{19}H_{38}$	266.50	Asong *et al.* (2019)
17.015	E-15-heptadecenal	$C_{17}H_{32}O$	252.43	Dhouib *et al.* (2019)
17.192	8-hexadecenal 14-methyl-,	$C_{17}H_{32}O$	252.40	Aja *et al.* (2014)
17.787	cyclopentadecane	$C_{15}H_{30}O$	210.40	Nakashima *et al.* (2014)
18.474	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	Belakhdar *et al.* (2015)
18.777	1-octadecene	$C_{18}H_{36}$	252.48	Omoruyi *et al.* (2014)
18.868	2-methyl-Z, Z-3, 13-octadecadienol	$C_{19}H_{36}O$	280.49	Phatangare *et al.* (2017) Adibe *et al.* (2019)
19.486	9,17-octadecadienal, (Z)-	$C_{18}H_{32}O$	264.40	Adibe *et al.* (2019)
19.836	oleic acid	$C_{18}H_{34}O_2$	282.46	Adibe *et al.* (2019)
20.288	9, 12-octadecadienoic acid (Z,Z)-methyl ester	$C_{19}H_{34}O_2$	294.47	Ojinnaka *et al.* (2015)
20.413	phytol	$C_{20}H_{40}O$	296.0	Hema *et al.* (2011)
20.556	octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.50	Banaras *et al.* (2017)
21.129	behenic alcohol	$C_{22}H_{46}O$	326.60	Chandrasekaran *et al.* (2011)
21.186	octadecanoic acid ethyl ester	$C_{20}H_{40}O_2$	312.53	El-Din and Mohyeldin (2018)
21.380	3,7,11,15-tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	El-Din and Mohyeldin (2018)
22.096	9,17-octadecadienal, (Z)-	$C_{18}H_{32}O$	264.40	Adibe *et al.* (2019)
23.875	eicosane	CH	282.50	Shirani *et al.* (2017)
24.241	docosanoic acid methyl ester	$C_{23}H_{46}O_2$	354.61	Shobier *et al.* (2016)

Table 11: Phytochemical compounds with antifungal activity identified from chloroform leaf extract of P. hysterophorus using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.545	1,2,3-propanetriol monoacetate	$C_5H_{10}O_4$	134.13	Teoh and Mashitah (2012)
8.426	benzoic acid	$C_7H_6O_2$	122.12	Krátký and Vinšová (2012)
11.338	.alpha.-cubebene	$C_{15}H_{24}$	204.35	Costa *et al.* (2011)
12.958	hexadecane	$C_{16}H_{34}$	226.44	El-Din and Mohyeldin (2018)
14.337	geranyl tiglate	$C_{15}H_{24}O_2$	236.35	Chavez *et al.* (2018)
15.659	heptacosane	$C_{27}H_{56}$	380.73	Bouzabata *et al.* (2013)
16.059	1-nonadecene	$C_{19}H_{38}$	266.50	Asong *et al.* (2019)
16.929	octadecane	$C_{18}H_{38}$	254.49	Omoruyi *et al.* (2014)
17.198	benzyl benzoate	$C_{14}H_{12}O_2$	212.24	Jantan *et al.* (1994)
18.073	heptadecane	$C_{17}H_{36}$	240.47	Musa *et al.* (2015)
18.142	hexadecane	$C_{16}H_{34}$	226.44	El-Din and Mohyeldin (2018)
18.474	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	Belakhdar *et al.* (2015)
18.880	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Omoruyi *et al.* (2014)
19.172	hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.48	Sudha *et al.* (2013)
19.263	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O2$	280.44	El-Din and Mohyeldin (2018)
19.538	pentadecanoic acid methyl ester	$C_{16}H_{32}O_2$	256.42	Belakhdar *et al.* (2015)
19.721	9,17-octadecadienal (Z)-	$C_{18}H_{32}O$	264.45	Adeyemi *et al.* (2017)
20.293	9,12-octadecadienoic acid (Z,Z)- methyl ester	$C_{19}H_{34}O_2$	294.47	El-Din and Mohyeldin (2018)
20.413	phytol	$C_{20}H_{40}O$	296.0	Hema *et al.* (2011)
20.505	17-pentatriacontene	$C_{35}H_{70}$	490.93	Zhang *et al.* (2015)
20.557	octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.50	Belakhdar *et al.* (2015)
20.774	9,12,15-octadecatrienoic acid (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.43	El-Din and Mohyeldin (2018)
22.468	eicosanoic acid methyl ester	$C_{21}H_{42}O_2$	326.56	Rahman *et al.* (2014)
22.925	12-methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280.49	Salem *et al.* (2016)
27.125	ethanol 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.50	El-Din and Mohyeldin (2018)
28.716	nonadecane	$C_{19}H_{40}$	268.52	Omoruyi *et al.* (2014)
33.179	eicosane	$C_{20}H_{42}$	282.55	Ahsan *et al.* (2017)

Table 12: Phytochemical compounds with antifungal activity identified from ethyl acetate leaf extract of *P. hysterophorus* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
9.668	1-cyclohexene-1-carboxaldehyde	$C_7H_{10}O$	110.15	Farzaei *et al*. (2014)
10.366	tridecane	$C_{13}H_{28}$	184.36	El-Din and Mohyeldin (2018)
11.338	alpha.-cubebene	$C_{15}H_{24}$	204.35	Martins *et al*. (2015)
12.552	gamma.-elemene	$C_{15}H_{24}$	204.35	Prieto *et al*. (2011)
12.609	dodecane	$C_{12}H_{26}$	170.33	Wijekoon *et al*. (2013)
12.883	alpha farnesene	$C_{15}H_{24}$	204.35	Bayan and Küsek (2018)
13.467	phenol, 2,4-bis (1,1-dimethylethyl)	$C_{14}H_{22}O$	206.0	Chandrasekaran *et al*. (2011)
16.059	octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	697.04	Govindarajan *et al*. (2016)
16.889	5-octadecene, (E)-	$C_{18}H_{36}$	252.48	Adeyemi *et al*. (2017)
17.215	benzyl benzoate	$C_{14}H_{12}O_2$	212.24	Jantan *et al*. (1994)
17.896	1,4 eicosadiene	$C_{20}H_{38}$	278.51	Ojinnaka *et al*. (2015)
18.016	1-tetradecene	$C_{14}H_{28}$	196.37	Bughio *et al*. (2017)
18.474	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	Belakhdar *et al*. (2015)
18.651	isophytol	$C_{20}H_{40}O$	296.53	Zhang *et al*. (2013)
19.097	dibutyl phthalate	$C_{16}H_{22}O_4$	278.34	Ahsan *et al*. (2017)
20.974	trans-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.46	El-Din and Mohyeldin (2018)
21.260	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.49	Adeyemi *et al*. (2017)
21.306	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.45	Ojinnaka *et al*. (2015)
22.994	3-eicosene, (E)-	$C_{20}H_{40}$	280.53	Adibe *et al*. (2019)
23.875	eicosane	$C_{20}H_{42}$	282.55	Ahsan *et al*. (2017)
28.722	heptacosane	$C_{27}H_{56}$	380.73	Bouzabata *et al*. (2013)

Table 13: Phytochemical compounds with antifungal activity identified from methanolic leaf extract of *P. hysterophorus* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
7.236	1,3,5-triazine-2,4,6-triamine	$C_3H_6N_6$	126.12	Baldaniya and Patel (2009)
8.844	tert-hexadecanethiol	$C_{16}H_{34}S$	258.5	Zhang *et al*. (2015)
8.992	1,2-benzenediol	$C_6H_6O_2$	110.11	Oramahi *et al*. (2018)
9.267	methyl salicylate	$C_8H_8O_3$	152.15	Zhang *et al et al*. (2006)
9.593	*n*-aminopyrrolidine	$C_4H_6N_2O_2$	114.10	Anisha and Radhakrishnan (2017)
9.799	l-[-]-4-hydroxy-1-methylproline	$C_6H_{11}NO_3$	145.16	Ojinnaka *et al*. (2015)
10.549	2-undecanone	$C_{11}H_{22}O$	170.29	Bisht and Chanotiya (2011)
12.551	gamma.-elemene	$C_{15}H_{24}$	204.35	Costa *et al*. (2011)
13.198	pentadecane	$C_{15}H_{32}$	212.41	Zhang *et al*. (2006)
15.035	phytol	$C_{20}H_{40}O$	296.53	Phatangare *et al*. (2017)
15.121	3-deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	Bhardwaj *et al*. (2019)
15.544	falcarinol	$C_{17}H_{24}O$	244.37	Santos *et al*. (2013)
16.196	methyl tetradecanoate	$C_{15}H_{30}O_2$	242.39	Jumina *et al.* (2019)
16.540	cyclotetradecane	$C_{14}H_{28}$	196.37	Afrouzan *et al*. (2018)
16.797	17-pentatriacontene	$C_{35}H_{70}$	490.93	Zhang *et al*. (2015)
16.889	1-docosene	$C_{22}H_{44}$	308.59	Vimalavady and Kadavul (2013)
16.980	ethanol, 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.50	El-Din and Mohyeldin (2018)
17.112	octadecane	$C_{18}H_{38}$	254.49	Khan *et al*. (2016)
17.192	benzyl benzoate	$C_{14}H_{12}O_2$	212.24	Jantan *et al*. (2008)
17.283	7-hexadecenoic acid methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.43	El-Din and Mohyeldin (2018)
17.993	hexadecane, 2,6,10,14-tetramethyl-	$C_{20}H_{42}$	282.55	Govindarajan *et al*. (2016)
18.273	9-hexadecenoic acid methyl ester, (Z)-	$C_{17}H_{32}O_2$	268.43	El-Din and Mohyeldin (2018)
18.479	hexadecanoic acid methyl ester	$C_{17}H_{34}O_2$	270.45	Belakhdar *et al*. (2015)
18.886	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Omoruyi *et al*. (2014)
19.109	3-eicosene (E)-	$C_{20}H_{40}$	280.53	Adeyemi *et al*. (2017)
19.172	hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.47	Sudha *et al*. (2013)
19.258	9,17-octadecadienal, (Z)-	$C_{18}H_{32}O$	264.45	Adeyemi *et al.* (2017)
20.293	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	Ojinnaka *et al.* (2015)
20.562	octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.50	Ojinnaka *et al.* (2015)
25.838	eicosane	$C_{20}H_{42}$	282.55	Ahsan *et al.* (2017)
26.559	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.48	Adeyemi *et al.* (2017)

Table 14: Phytochemical compounds with antifungal activity identified from chloroform leaf extract of *A. indica* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
9.730	sulfurous acid hexyl pentadecyl ester	$C_{21}H_{44}O_3S$	376.60	Chen *et al.* (2017)
9.919	pentadecane	$C_{15}H_{32}$	212.41	Yuan *et al.* (2012)
11.813	tetradecane	$C_{14}H_{30}$	198.39	Yuan *et al.* (2012)
11.899	eicosane	$C_{20}H_{42}$	282.55	Ahsan *et al.* (2017)
13.467	phenol, 2,4-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206.32	Dharni *et al.* (2014)
16.894	1-octadecene	$C_{18}H_{36}$	252.48	Belakhdar *et al.* (2015)
18.142	ethanol 2-(octadecyloxy)-	$C_{20}H_{42}O_2$	314.5	El-Din and Mohyeldin (2018)
18.473	hexadecanoic acid methyl ester	$C_{17}H_{34}O2$	270.45	El-Din and Mohyeldin (2018)
18.651	phytol	$C_{20}H_{40}O$	296.0	Hema *et al.* (2011)
18.851	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	Manikandan *et al.* (2017)
19.177	hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.48	Sudha *et al.* (2013)
19.503	heptadecane	$C_{17}H_{36}$	240.47	Zhang *et al.* (2015)
20.757	9,12-octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	Ojinnaka *et al.* (2015)
25.838	heptacosane	$C_{27}H_{56}$	380.73	Bouzabata *et al.* (2013)
28.722	hexadecane	$C_{16}H_{34}$	226.44	Zhang *et al.* (2015)

Table 15: Phytochemical compounds with antifungal activity identified from ethyl acetate leaf extract of *A. indica* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
9.278	methyl salicylate	$C_8H_8O_3$	152.15	Zhang *et al. (*2006)
9.610	piperazine	$C_4H_{10}N_2$	86.14	Thamban Chandrika *et al.* (2018)
11.338	alpha.-cubebene	$C_{15}H_{24}$	204.35	Tolouee *et al.* (2010)
13.192	pentadecane	$C_{15}H_{32}$	212.41	Ma *et al.* (2018)
13.467	phenol 2,4-bis (1,1-dimethylethyl)	$C_{17}H_{30}OSi$	278.5	Guo *et al.* (2008)
13.581	hexanoic acid ethyl ester	$C_{10}H_{18}O_4$	202.25	Tyagi and Agarwal (2017)
14.502	hexadecane	$C_{16}H_{34}$	226.44	Oliveira *et al.* (2014)
16.242	tetracosane	$C_{24}H_{50}$	338.7	Dandekar *et al.* (2015)
6.894	1-nonadecene	$C_{19}\ H_{38}$	266.51	Balachandar *et al. (*2018)
18.479	hexadecanoic acid methyl ester	$C_{17}H_{34}O$	270.45	Rajeswari and Rani (2015)
20.413	phytol	$C_{20}H_{40}O$	296.54	Pejin *et al.* (2014)

Table 16: Phytochemical compounds with antifungal activity identified from methanolic leaf extract of *A. indica* using GC-MS

Retention time (min)	Compound name	Molecular formula	Molecular weight (g/mol)	References
9.261	methyl salicylate	$C_8H_8O_3$	152.15	Vidhyasekaran (2007)
9.879	octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.50	Saadabi *et al.* (2012)
10.251	4-hydroxy-3-methyl-2-butenyl-	$C_{13}H_{16}O_2$	204.26	Soberón *et al.* (2015)
10.474	cyclopropane	C_3H_6	42.08	Pohl *et al.* (2011)
11.126	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	Guo *et al.* (2008)
11.332	.alpha.-cubebene	$C_{15}H_{24}$	204.35	Costa *et al.* (2011)
14.508	heptadecane	$C_{17}H_{36}$	240.47	Musa *et al.* (2015)
15.212	caryophyllene oxide	$C_{15}H_{24}O$	220.35	Sarpietro *et al.* (2015)
16.889	5-eicosene, (E)-	C_20H	280.53	Naragani *et al.* (2016)
17.197	benzyl benzoate	$C_{14}H_{12}O_2$	212.24	Jantan *et al.* (1994)
17.272	(R)-(-)-14-methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252.44	El-Din and Mohyeldin (2018)
18.473	hexadecanoic acid methyl ester	$C_{17}H_{34}O$	270.0	Sudha *et al.* (2013) Agoramoorthy *et al.* (2007)
18.548	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	Subashini *et al.* (2015)
18.971	cis-11-hexadecenal	$C_{16}H_{30}O$	238.41	Vimalavady and Kadavul (2013)
19.172	hexadecanoic acid ethyl ester	$C_{18}H_{36}O_2$	284.0	Sudha *et al.* (2013)
19.875	9,12-octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294	Lima *et al.* (2011)
20.121	2-methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280	Subashini *et al.* (2015)
20.293	9,12-octadecadienoic acid (Z,Z)- methyl ester	$C_{19}H_{34}O_2$	294.5	El-Din and Mohyeldin (2018)
20.419	phytol	$C_{20}H_{40}O$	128.17	Pejin *et al.* (2014)
20.556	octadecanoic acid methyl ester	$C_{19}H_{38}O_2$	298.5	Chandrasekaran *et al.* (2008) Lima *et al.* (2011)
20.671	9,17-octadecadienal (Z)-	$C_{18}H_{32}O$	264.4	Adeyemi *et al*. (2017)
24.327	cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222.46	Pamila and Karpagam (2017)
24.470	1,2-benzenedicarboxylic acid diisooctyl ester	$C_{38}H_{56}O_8S_2Sn$	823.7	Rahman and Anwar (2006)
28.710	1-bromoeicosane	$C_{20}H_{41}Br$	361.4	El-Naggar *et al*. (2017)

4.2 Discussion

The present study determined the LLS disease status in two groundnut growing regions; Singida and Dodoma. The result confirmed the widespread of LLS disease occurrence and severity in all the groundnut-growing areas covered. The LLS disease incidence and severity were high in both regions being 72.63% and 6.15 for Singida; and 67.6%, 5.86 for Dodoma. The possible reasons for high disease incidence and severity are common farming practices, nature of the pathogen and preferable conditions favored groundnut LLS disease development and existence of its reservoirs. This agrees with the findings by Shokes *et al*. (1997) who reported that *P. personata* survives on crop debris. The field survey study observed farmers in both regions cultivate groundnut continuously year after year also presence of volunteering groundnut plants. These ensure the inoculum source for disease development from crop debris is present. This agrees with the findings by Shew *et al*. (1995) who reported that the failure of rotating crops and presence of the volunteer groundnut plants in the fields assist the movement of infected pods, seeds or crop debris or seeds consequently spread the LLS disease.

Furthermore, crop is cultivated during rain seasons where both regions experiences high humid condition above >75% (Rh) and temperature above 27 ˚C (Shokes *et al*., 1997). According to Wadia and Butler (1994); Nutsugah *et al*. (2007), those conditions affect each step of disease development from propagule germination, germtube penetration, host colonization which allows disease spreading to other hosts. Also, it agrees with Nutsugah *et al*. (2007), who described that the disease incidence and severity of foliar fungal diseases is directly proportional to favorable weather condition.

Thus, the field survey results revealed the prevalence of the LLS disease in central zone and it alerts global plant health regulators to suggest proper management skills. The commonly recommended management option i.e. use of synthetic fungicides is necessarily associated with multiple applications which lead to hazardous impacts to human and environment (De Rodríguez *et al*., 2011). Hence, this justified the need for developing an alternative approach involving the use of as a natural remedy against LLS disease.

To ascertain the pathogen causing LLS disease the fungal pathogen was isolated from diseased groundnut leaves then cultured on the PDA media. The isolated *P. personata* pathogen was identified by using Compound microscope (40X) basing on morphological

characteristics (Ijaz, 2011). The identified pathogen was used in the tests with plant extracts used in the current study.

The selected plant extracts hindered the mycelia growth of *P. personata* even at the lowest concentration confirming their antifungal potential. This finding agrees with the findings by several authors where the same plant species were effective against various pathogenic fungi. For instance, the mycelial growth of *C. gloesporioides* of rubber tree was inhibited by the *J. curcas* leaf extract (Muklesur *et al*., 2011). According to Shokes and Melouk (1995) the methanolic extracts of *P. hysterophorus* inhibited *Fusarium solani* pathogen causing Fusarium wilt on potatoes. In addition, *Azadirachta indica* aqueous leaf extract suppressed the growing *A. solani* pathogen (Hassanein *et al*., 2008). The selected plant extracts possess phytocompounds with antifungal properties which inhibit growth of the tested pathogen *P. personata*. Thus, a farmer can opt one among the tested plant species basing on the availability to their localities.

The methanolic leaf extract of the selected plants (*A. indica*, *J. curcas* and *P. hysterophorus*) effectively inhibited the mycelial growth of *P. personata*. This concurs with Sharma and Saini (2019), where the methanolic fraction of *J. curcas* were effective against *Candida albicans* ATCC 90028, *Candida krusei* ATCC 6258, *Candida tropicalis* ATCC 750 and *Candida parapsilosis* ATCC 22019. Also, the methanolic root extract of *J. curcas* was effective against *Rhizoctonia* (Ingle *et al*., 2017). Likewise, the methanolic leaf extract of *P. hysterophorus* suppressed the growth of *Fusarium solani* (Zaheer *et al*., 2012). Similarly, the methanolic seed extract of *A. indica* inhibited the growth of *Fusarium oxysporum* and *Aspergillus niger* (Mahmoud *et al*., 2011).

The antifungal potentiality of the selected plant extracts increased as the concentration increases. This finding agrees with the observation by Agbenin and Marley (2006), where the neem leaf extract at highest concentration reduced mycelial growth of *Fusarium oxysporum*. Also, according to Goel and Sharma (2013) as the plant extract concentration increases percent inhibition of fungi growth increases. Therefore, the antimicrobial activity of fungicide treatments to the pathogen depends on their toxicity and concentration of plant extracts.

The preliminary findings from *in vitro* trial was supplemented by the comprehensive *in vivo* study under controlled screen-house environment aiming to practically assess the effect of the

same plants in managing LLS disease. Low LLS disease incidence and severity was attained with all selected plant species. This corresponds to the findings by Thangavelu *et al*. (2004), where the *J. curcas* leaf extract was effective against *Colletotrichum musae* and *Sclerotium* sp, the causal agents for anthracnose disease in banana and azolla, respectively. According to Gupta *et al*. (2010), *J. curcas* leaf extract revealed its antifungal potential against *Aspergilus niger*, *Saccharromyces cerevisiae*, *Candida albicans* and *Candida oryzae*. Similarly, *P. hysterophorus* has been reported by different authors to have antifungal property against *Fusarium solani* (Shafique & Shafique, 2012) and *Alternaria alternate* (Barsagade & Wagh, 2010).

The methanolic plant leaf extracts (selected plants) showed high antifungal effect as compared with ethyl acetate and chloroform solvents. Concurring with de Boer *et al*. (2005), reporting that the antimicrobial activity of the plant based resources depends on the type of solvents used for extraction of phytocompounds. This agrees with Devkota and Sahu (2017) where the *J. curcas* methanolic leaf extract had greater antifungal effect as compared with aqueous leaf extract. Also, the methanolic extract of *A. indica* seed coat proved its antifungal property against *A. niger* and *Curvularia lunata* (Verma *et al*., 1998). Correspondingly, according to Shukla *et al*. (2002) the methanolic neem seed extract proved effectiveness against *Trichoderma resii* and *Fusarium oxysporum.* Likewise, both *A. indica* and *J. curcas* methanolic leaf extracts proved their antifungal against *Fusarium* sp, *T. rubrum* and *T. mentagrophyte* (Ndam *et al*., 2018). In addition, some polar compounds are more effective against a variety of microbes than intermediate and non-polar solvents (Bassey *et al*., 2013).

The selected plant extracts influenced the groundnut shoot length and number of leaves per plant both at flowering and maturity. Plants treated with *P. hysterophorus* (0.5 mg/ml; 0.25 mg/ml; 0.1 mg/ml), *J. curcas* (0.5 mg/ml) and *A. indica* (0.5 mg/ml) had taller shoots and greater number of leaves/plant as compared to control plants. Similarly, the plants treated by selected plant leaves extracted by chloroform and methanolic had greater number of leaves per plant and taller shoots than ethyl acetate leaf extracts. This agrees with the findings by Gayatri and Sahu (2014), the *A. indica* has a potential source been reported to have positive effect on growth parameters. Generally, plants possess important metabolites such as amino acids (tryptophan and phenylalanine) which play a great role for growth and defense to crops (Hussain *et al*., 2011; Pretali *et al*., 2016). Also, the study revealed that growth parameters differed significantly under different concentrations of plant extract treatments where the

higher concentration had taller shoots and greater number of leaves per plant. Likewise plants possess important secondary metabolites which support the growth, which includes terpenoids, saponins, steroids and phenolic (Hashem *et al*., 1997).

Likewise, the study showed a significant interactive effect between treatments and solvents on growth attributes (shoot length and number of leaves/plant) both at flowering and maturity stage. The selected plant extracts by methanol had taller shoots and greater number of leaves per plant compared with chloroform and ethyl acetate extracts. This signifies that the polar compounds extracted from selected pesticidal plants by the methanol solvent had positive impact to groundnut growth compared to other solvents. This concur with the findings by Pylak *et al*. (2019), who suggested the use organic polar solvents (e.g. ethanol, methanol) allows extraction of more substances (aromatic or saturated organic compounds), which exhibit better activity. Furthermore, according to Bulgari *et al*. (2015), the biological activity of plant extracts depends on presence of the natural plant hormones which regulates plant growth. Generally, plant extracts constitute a rich source of the biologically active compounds that play beneficial effect on growth of crop plant (Godlewska *et al*., 2021).

The study showed the significant effect of treatments to groundnut yield. Plants treated by *P. hysterophorus*, *J. curcas* and *A. indica* leaf extracts had greatest number of pods/plant, number of seeds/plant and greater seed yield (g/plant) more similar to chlorothalonil (standard fungicide) treatment. Possibly this is attributed by their antifungal potentials which retarded disease development subsequently facilitated photosynthesis activity by enhancing vegetative growth and increased dry matter accumulation hence increased yield. This finding agrees with Hossain and Hossain (2013), who reported that the plant materials neem leaf aqueous extracts increased yield significantly as compared with control. *A. indica* extracts were found effective against insect pest and subsequently improved grain yield (Ogah, 2013).

The antifungal effects of the selected pesticidal plants can be ascribed by the possession of different phytocompounds present in their respective extracts which act either alone or synergistically (Field *et al*., 2006; Giordani *et al*., 2008; Rongai *et al*., 2012). According to Ghani *et al*. (2008), there is association between the antifungal efficacy of plant extracts and its phytochemical constituents. Thus, the study evaluated the phytochemical constituents of the selected pesticidal plant extracts using GC-MS.

The study identified the presence of 86 different phytocompounds from *J. curcas* leaf extracts, 79 phytocompounds from *P. hysterophorus* leaf extracts and 50 phytocompounds from *A. indica* leaf extracts with antifungal activities. The qualitative differences of phytochemical constituents observed in this study may be attributed by different solvents employed for extraction. This observation corresponds to findings by Kordali *et al*. (2003), who suggested that the solubility of phytochemicals depends to the type of solvent used for extraction.

The methanolic leaf extracts of the selected plant species had greater number of phytoconstituents as compared with other solvents used i.e., chloroform and ethyl acetate. This corresponds to the findings by Prabhat and Navneet (2010), where the methanolic plant extracts had more phytocompounds compared to other extracts. Also, Abubakar and Haque (2020) suggested that the methanol solvent has the ability to extract different antimicrobial phytocompounds (organic and inorganic) from plants. The lesser extraction observed in this study by mid-polar and non-polar indicates that the selected plant samples composed of more polar compounds since like solvents dissolve like solutes (Mann *et al*., 1997).

Generally, phytochemical investigation of the selected plant extracts revealed the possession of important phytoconstituents; phenol 2,4 bis (-dimethylethyl), hexadecane, heptadecane, hexadecanoic methyl ester and *n*-hexadecanoic acid which have been reported to have antifungal potentials (Amadioha, 2002; Kushwaha & Maurya, 2012: Dada *et al*., 2014). The revealed phytochemical compounds with antifungal properties possessed by the *J. curcas, P. hysterophorus* and *A. indica* proves their potential for controlling the LLS disease consequently improve the groundnut production in Tanzania.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The study findings identified groundnut LLS disease as the most destructive foliar fungal disease limiting groundnut production in central zone of Tanzania. The LLS disease incidence mean was above 70% and disease severity mean scored above 5. Hence, a study enforced to come up with a management option which is effective against *P. personata* and affordable to smallholder farmers. The selected plant extracts namely; *J. curcas*, *P. hysterophorus* and *A. indica* extracted by methanol were found effective against *P. personata* under *in vitro* and *in vivo* (screen house) conditions. *Jatropha curcas* and *P. hysterophorus* leaf extracts at highest concentration 0.5 mg/ml were more effective compared to mid and lowest concentrations.

Furthermore the phytochemical analysis of the selected plants by GC-MS proved the possession of important phytoconstituents with antifungal potentials against the tested pathogen. The identified phytocompounds namely; phenol 2,4 bis (-dimethylethyl), hexadecane, heptadecane, hexadecanoic methyl ester and *n*-hexadecanoic acid have been reported effective against numerous fungal pathogens. Hence, the use of the selected plant extracts is sustainable management approach and will improve groundnut production while ensuring the safety to human health, biodiversity, and the environment.

5.2 Recommendations

From the conclusion the following recommendations are suggested;

(i) This study found that, the LLS disease is prevalent throughout Singida and Dodoma regions, further studies are recommended to determine the LLS disease status to other groundnut growing regions to assess the LLS disease level in the whole country of Tanzania.

(ii) Further studies are recommended to determine the antifungal potential of the selected plants under field conditions to validate the results, as this study was conducted under *in vitro* and *in vivo* (screen house) condition only.

(iii) Extensive research is needed to isolate the phytochemicals with antifungal properties for developing plant-based bio fungicides for commercialization aimed at improving groundnut production in Tanzania.

(iv) There is a need of analyzing the phytochemical compounds of the selected pesticidal plants from other parts of Tanzania, other than Singida and Dodoma region covered in this study.

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APPENDICES

Appendix 1: Late leaf spot disease score scale

No:	Severity percentage
1	no disease
2	Sparsely distributed lesions, primarily on lower leaves between (1-5%)
3	Many lesions on the lower leaves with evident necrosis and very few lesions on middle and upper leaves (6-10%)
4	Numerous lesions on lower and middle and severe necrosis on lower leaves (11-20%)
5	Severe necrosis of middle and lower leaves and less severe lesions on top leaves (21-30%)
6	Extensive damage to lower leaves, lesions densely present on middle leaves with necrosis and lesions may be on top leaves as well (31-40%)
7	Severe damage to lower and middle leaves and lesions are densely distributed on top leaves (41-60%)
8	100% damage to lower and middle 25 leaves and lesions on top leaves with severe necrosis (61-80%)
9	Almost all leaves are withering and bare stems are present (81-100%).

RESEARCH OUTPUTS

Published papers

Francis, M., Chacha, M., Ndakidemi, P. A., & Mbega, E. (2021). Phytochemical analysis and *in vitro* antifungal evaluation of *Jatropha curcas* against Late Leaf Spot disease on groundnut. *Journal of Animal and Plant Sciences, 47(1)*, 8358- 8371

Francis, M., Chacha, M., Ndakidemi, P. A., & Mbega, E. (2020). Groundnut rust disease epidemiology and potential sustainable management strategies. *International Journal of Bioscience*, *16(4)*, 524-536

Francis, M., Chacha, M., Ndakidemi, P. A., & Mbega, E. Antifungal Effects against *Phaeoisariopsis personata* under Greenhouse Conditions and Phytochemical Analysis of *Jatropha curcas* Leaf Extracts. *International Journal of Agriculture and Biology, 26*(2), 231-240.

Poster presentation

Antifungal activity of selected pesticidal plants against *Phaeoisariopsis personata* and phytochemical analysis, Central Tanzania