

**EVALUATION OF ANTIFUNGAL EFFICACY OF SOME LEAF EXTRACTS OF
SOME PLANTS ON RED ROT PATHOGEN (*Colletotrichum falcatum*) OF SUGARCANE
(*Saccharum officinarum*)**

Botanical extracts have shown appreciable achievement in controlling plant diseases. This became evident in this study when the potency of leaf extracts from *Azadirachta indica*, *Carica papaya*, *Lawsonia inermis*, *Khaya senegalensis* and *Ziziphus spina-christi* were tested on the mycelia growth of *Colletotrichum falcatum*, the pathogen of red rot disease of sugarcane. The infected sugarcane used for isolation and identification were collected from National Cereal Research Institute (NCRI) Badeggi. And a total of sixty (60) healthy sugarcane setts planted were collected from farmers in Wuya rake along Bida Mokwa road in Niger state. The plant leaf materials used in this study were collected from the mountain area at Egubagi village and were identified at the herbarium in the Department of Plant Biology, Federal University of Technology, Minna. After collection, leaves were sterilised using 0.5% sodium hypochlorite and the extraction was done using Soxhlet extraction. Tested leaves were subjected to qualitative phytochemical screening. The isolation and identification of the fungus was done by cutting the transition zone between the rotten tissue and healthy side into 5mm, sterilised and inoculated into potato dextrose agar (PDA) medium. *In vitro* antagonistic effects of the plant extracts were done using food poison techniques at 75 %, 50 %, and 25 % concentrations while the controls are 0.5g/l (clot) and 0% (sterile distilled water) concentrations with three replicates each. After the *in vitro* evaluation, the best three plant extracts that had the highest mycelia inhibition were taken to the field to evaluate their effect on the fungus as it affects the growth parameters (plant girth, plant height, number of nodes and internodes length) on the growing sugarcane plants in the screen house (*in vivo*). The phytochemical analysis of the extractants revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins, terpenoids, steroids, anthraquinones and cardiac glycosides. The results of *in vitro* revealed that the percentage mycelia growth inhibition were 100.0±0.00 (*Lawsonia inermis*), 53.00±1.15 (*Azadirachta indica*), 78.00±0.00 (*Carica papaya*), 45.00±1.00 (*Ziziphus spina-christi*) and 60.60±0.00 (*Khaya senegalensis*) while controls were 100.0±0.00 (0.5 g/L) and 0.00±0.00 (sterile distilled water) positive and negative respectively. The results obtained from the field were promising at 75 % concentration of plant extracts used for the percentage disease reduction with 6.94 %, 15.28 % and 25.8 % *Lawsonia inermis*, *Carica papaya* and *Khaya senegalensis* were used in that order, while controls had 5.90±0.00 0.5 g/L (clot) 0 % (sterile distilled water). The percentage inhibition and reduction in all the treatments used were significantly different ($P < 0.05$). The efficacy of the ethanolic leaf extracts increase with the increase in concentrations, 75% concentrations of all the plant extracts showed the highest level of mycelial growth inhibition (*in vitro*) and percentage disease reduction (*in vivo*). Extracts from *Ziziphus spina-christi* (45.00±1.00 %) had the least effect on the percentage mycelia growth of *C. falcatum* (*in vitro*) while *Khaya senegalensis* had least (25.8–35.09 %) effect than other plants extracts used (*in vivo*) in suppressing the disease severity on sugarcane plants. The results were promising and the best plant extracts can go a long way in reducing the red rot disease of sugarcane on the field.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Sugarcane (*Saccharum officinarum*), is a perennial grass of the family Poaceae, primarily cultivated for its juice from which sugar can be processed. It is an important cash crop cultivated in tropical and sub-tropical regions of the world (Dacosta *et al.*, 2011). Its edible stem is rich in sucrose and used for the production of sugar, ethanol, and important renewable biofuel source (Menossi *et al.*, 2008 and Dacosta *et al.*, 2011). Globally, sugarcane is an important source of commercial sugar accounting for almost two thirds of the world sugar production (Menossi *et al.*, 2008). It is a tropical crop which attains maturity between 8 and 12 months. Matured cane may be green, yellow, purplish or reddish in color and it consider ripe when sugar content is at its maximum (Imolehin and Wada, 2008).

World production of sugarcane stood at 1.5 billion tonnes as of 2008 (Imolehin and Wada, 2008) Brazil, China, Cuba, Mexico, Pakistan, Thailand, USA, Colombia, Australia and Indonesia are the leading countries in sugarcane production, producing over half of the total world sugarcane production. Africa in the same reporting period has 1.2 million hectares with 72.1 million metric tonnes, respectively (Onwueme and sinha, 1991). Nigeria is one of the most important producers of the crop with a land potential of over 500, 000 hectares of suitable cane field capable of producing over 3.0 million metric tonnes of sugarcane. If processed, it will yield about 3.0 million metric tons of sugar (National Sugar Development Council Policy, 2003).

Nigeria is highly blessed with human, water and environmental potentials for commercial sugarcane. And it was pointed out that most of the areas in the Northern states where water for irrigation is available, sugarcane cultivation in large quantities are feasible. Sugarcane production

is widely affected by biotic (microbes) and abiotic factors (climate and drought), of which the loss to fungal pathogens (*Colletotrichum falcatum*) causing red rot disease is most critical (Wada *et al.*, 2016). Red rot pathogen (*C. falcatum*) of sugarcane is a dreadful fungi disease which infects the sugarcane stalk at both the initial and mature stages of growth, cause discoloration, reduction in cane weight, loss of sugar, sucrose content and juice (Sharma and Tanta, 2015).

Annual loss of revenues to *C. falcatum* infection is estimated to be between 500 -100 million USD (Viswanathan and Samiyappan, 2002). Chemical fungicides are used to curtail direct destruction caused by fungi on plants, but the use of these chemical fungicides is toxic to human and cause environmental pollution (Abu-Taleb *et al.*, 2011). Thus, alternative control such as use of botanicals are desired that are eco- friendly and cheap (Gurjar *et al.*, 2012). Botanical extracts are gaining popularity as some plant product are being used globally as green pesticides, in the context of agricultural pest and disease management, botanical pesticides are best suited for use in organic food production in industrialized countries but can play a much greater role in the production and post-harvest production of food products in developing countries (Malkhan *et al.*, 2012). Therefore, the efficacy of *Azadirachta indica*, *Khaya senegalensis*, *Carica papaya*, *Lawsonia innermis* and *Ziziphus spina-christi* were determined to ascertain their effectiveness in the control of red rot fungi (*C. falcatum*) disease of sugarcane.

1.2 Statement of the Research Problems

Red rot disease of sugarcane caused by *Colletotrichum falcatum* is one of the oldest, broadly distributed, and documented diseases of sugarcane in many countries and a threat to the production and cultivation of sugarcane (Viswanathan and Samiyappan, 2002; Viswanathan, 2012). The pathogen attacks the cane plant from seedling stage till maturity stage causing retard growth and

most times extermination of seedling (Viswanathan, 2012). It therefore, observed to be a part of obstacles hindering sugarcane growing in Nigeria according to the recent survey of some states in the country, the disease was observed in states like Niger, Katsina, Benue, Plateau, Adamawa and FCT, Abuja (Wada *et al.*,1998). The sugar industry in India suffer losses of more than 500 million dollars (US) every year due to red rot disease and this loss is due to the reduction in the sucrose contents and weight of the cane due to red rot disease (Viswanathan and Samiyappan, 2002). It is not only of a huge concern for the global export sector, but also has a great impact on the domestic production, as many locally preferred cultivars are also endangered (Sharma and Tanta, 2015).

Various methods are adopted for red rot disease management, including application of chemical fungicides, breeding and tissues culture methods. All of these have limitations and disadvantages. For instance, chemical methods increase cost of production and contaminate the environment. Whiles tissue culture , the selection of fungus -resistance cells (Mohanraj *et al.*, 2003; Sengar *et al.*, 2009) is most often associated with somaclonal variations.also breeding methods are laborious and time consuming (Agnihotri, 1996).

1.3 Justification for the Study

Biofungicide botanicals are gaining more significant as they have been used as alternative control in the management of fungal pathogens. The active ingredients in plants may either act on the pathogen directly or induce systemic resistance in the plant, resulting in reduction of disease development (Yulier *et al.*, 2015). They are considered not only as an alternative to chemicals but also less expensive, easily available and also for the eco-friendly mangement of various plant diseases including red rot (Imtiaj *et al.*, 2007 and Hafiz *et al.*, 2016). Plant extracts have opened

a new avenue for the control of plant diseases and much attention has been given to the use of secondary metabolites rich plant substances such as; phenols, alkaloids, terpenoids amongst others. These serve as chemical defence against plant disease (Tripathi and Singh, 2015). Plant extracts have also been investigated throughout the world for their antifungal activity against a wide range of fungi (Gupta *et al.*, 2008). Hence, the fungicidal and fungistatic potentials of *Lawsonia inermis*, *Ziziphus spina-christi*, *Carica papaya*, *Khaya senegalensis* and *Azadirachta indica* plant extracts on different plant pathogenic fungi previously reported by Hafiz *et al.* (2016) motivated this research.

1.4 Aim and Objectives of the Study

The aim of the study was to evaluate the antifungal potential of five plant extracts (*Ziziphus spina-christi*, *Azadirachta indica*, *Carica papaya*, *Khaya senegalesis* and *Lawsonia inermis*) on the red rot pathogen (*C. falcatum*) of sugarcane.

The objectives of the study are to:

- i. determine the phytochemical constituents of *Ziziphus spina-christi*, *Azadirachta indica*, *Carica papaya*, *Khaya senegalensis* and *Lawsonia inermis* extracts.
- ii. characterize the causative organisms of red rot disease of sugarcane.
- iii. determine *in vitro* antifungal efficacy of the plant extracts of *Ziziphus spina-christi*, *Azadirachta indica*, *Carica papaya*, *Khaya senegalensis* and *Lawsonia inermis* on mycelia growth of *C. falcatum*.
- iv. determine the efficacy of the selected plant extracts in the control of red rot disease of sugarcane under screen house conditions.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biology of Sugarcane

Sugarcane (*Saccharum officinarum*) is the world's economically important crop which is cultivated on 20.42 millions hectares across the world with an estimated total production of 1,333 million metric tons (Alwala *et al.*, 2006). *Saccharum* is complex genus as it consists of many species which can cross breed with each other, and with species in related genera to produce many different

species. Genus *Saccharum* consists of six species viz: *S. spontaneum*, *S. robustum*, *S. officinarum*, *S. barberi*, *S. sinense* and *S. edule*, (Alwala *et al.*, 2006). Four species like *Saccharum officinarum*, *Saccharum edule*, *Saccharum barberi* and *Saccharum sinense* are cultivated species while *S. spontaneum* and *S. robustum* are wild species which are mostly found in Indonesia and Asia. *Saccharum edule* is served as vegetable due to its aborted inflorescence (Irvine, 1999). *Saccharum officinarum* is the most domesticated sugar producing species, which is found only in native gardens. It is considered as derivative of *Saccharum robustum* (Wada *et al.*, 2016). The clones which are currently cultivated are derivatives of *S. officinarum* and *S. spontaneum* obtained by interspecific hybridization (Alwala *et al.*, 2006).

2.2 History and Taxonomy of Sugarcane

Sugarcane was originated in New Guinea about 6000 BC. Its cultivation extended progressively with the migration of human to India, Southeast Asia, East and Pacific since 1000BC. It proliferated westward to the Mediterranean between 600-1400AD through Arabs. The sugarcane disseminated to different countries like Syria, Cyprus, Crete and eventually reached the Spain by 715 AD. The crop was introduced to the New world; firstly to Madeira around 1420 by the Portuguese and then from the Madeira to Canary Islands, the Azores and West Africa. The crop was brought to the Central and South America in 1520 from India by one cultivar which was taken to the new world. It was called 'Creole' or "Cana Criolla" in Latin America. This sugarcane cultivar was grown in the new world for 250 years. Then the noble cane 'Otaheite' (Bourbon) replaced this cultivar at the end of these centuries (Robinson, 2007).

Kingdom: Plantae – Plants

Subkingdom: Tracheobionta – Vascular plants

Superdivision: Spermatophyta – Seed plants

Division: Magnoliophyta – Flowering plants

Class: Liliopsida – Monocotyledons

Subclass: Commelinidae

Order: Cyperales

Family: Poaceae – Grass family

Genus: *Saccharum* L. – sugarcane

Species: *Saccharum officinarum* L. – sugarcane

2.3 Economic Importance of Sugarcane.

Sugarcane is an important cash crop which also serves as food by providing required energy to humans and feed stock for industry (Martin, *et al.*, 2002). It consists of 70% water, 14% fibre, 13% sucrose and 2-7% soluble impurities. Sugarcane contributes significantly to the sugar production as 60% or more of the world's sugar is prepared by using raw material derived from sugarcane (Almanzan *et al.*, 1998). Every part of sugarcane are useful: the solid residue (bagasse) in which the juice were extracted are used in the production of steam, electricity, as fuel in boilers to produced energy and minimize disposal problems in industries (Viswanathan and Samiyappan, 2006). Excessive availability of bagasses, its richness in carbohydrate and less ligni content made it an ideal substrate for ethanol production and also as an influent solar energy reservoir (Martin *et al.*, 2002; Viswanathan and Samiyappan, 2006). Production of bagasse along with sugar is more than 200 million tonnes in all cane producing countries, majority of which (about 95%) is consumed as fuel in the mills thus saving about 40 million tonnes of (biofuel). The remaining 5 % (that is not used as fuel) is utilized in manufacturing other products including pulp, paper, boards, furfural and animal feeds. The most extensive use of bagasse around the world is for the production of pulp and paper (Martin *et al.*, 2002).

In addition, the efficient photosynthetic mechanism of sugarcane enables it to fix almost 2-3% of radiant solar energy and transform it into green biomass. This efficient photosynthetic capability also allows it to show a high coefficient of carbon dioxide fixation as compared to the moderate climate zone woods; thereby contributing to the decrease of the greenhouse effect (Alwala *et al.*, 2006). Many products (white sugar, ethanol and molasses) and by-products (bagasses, molasses etc.) can be obtained by processing the sugarcane starting from harvesting which are potential raw materials for the extractive. The molasses, one of the by-products of the sugarcane is widely used in alcohol synthesis, fertilizer and livestock feed (Martin *et al.*, 2002). A hundred tones of green matter per hectare can be obtained from sugarcane each year which is more than twice the yield of other crops. It is effectively used for animal feeding due to a good metabolic energy carrier and provides 75000 million calories each year which is equivalent to more than eight times the yield of fodder crops (Almazan *et al.*, 1998).

2.4 Causal Agent of Red Rot Disease of Sugarcane (*Colletotrichum falcatum*)

The morphological identification features of *C. falcatum* fungus are: its mycelium which is both intracellular and intercellular, asexual fruiting bodies known as acervuli (minute, velvety and formed on the surface of the host part), often with setae (dark-pigmented, unbranched, thick-walled, unicucleated sterile hyphae usually pointed at the tips), having hyaline, linear or club shaped conidiophores producing elongated, single celled, thin walled, uninucleate, cloudless, sickle shape (Falcate), slimy conidia having granular protoplasm with a large oil globular, thick walled, greenish black chlamydospores and the presence of appressoria (thick-walled swelling at the end of a hypha or germ tube for attaching the fungus to the host surface before penetration of the tissue), presence or absence of the telomorph, colony colour and growth, production of pigments and growth rate which are mostly used for genetic characterization (Kumar, 2010).

The fungus *C. falcatum* is a facultative saprophyte, known to produce a phytotoxic metabolite identified as an anthraquinone compound. It has been established that the toxic metabolite is host-specific and produces part of the disease symptoms (Malathi, 2002). Its sexual stage known as *G. tucumanensis* is responsible for the survival of the fungus on decaying leaves and formation of new virulent pathological races which are responsible for the frequent epidemics (Satyavir, 2003).

Ten races of *C. falcatum* have been reported based on host differentials (Satyavir, 2003). If a fairly large number of isolates of the fungus obtained from different cane varieties or geographic areas are studied on artificial culture media, considerable variation in the type of growth and colour of the fungus colony is seen. Some isolates or races are light gray and form a loose cottony colony, others are dark form a restricted velvety colony while some are intermediate in those respects. If they are inoculated into stalks of sugar cane, they are also different in pathogenicity and their ability to infect and rot the stalks (Malathi, 2002). The light race produced abundant spores and proved more virulent than the dark one. All the previous isolates of *C. falcatum* from India were dark type with sparse sporulation (Satyavir, 2003).

Division: Eumycota

Sub division: Deuteromycotina

Class: Coelomycetes

Order: Melanconiales

Family: Melanconiaceae

Genus: *Colletotrichum*

Species: *C. falcatum* (Ruchika and Sushama, 2015)

2.5 Symptomatology of Red Rots

Expression of the disease may vary depending upon nature of infection and prevailing environmental conditions (Satyavir, 2003). In the early stages of infection, it is difficult to recognize the presence of the disease in the field as redefining of the interrupted red and white patches the characteristics symptoms of the disease, develops on the stem only at later stages. Furthermore, latent infection occurs frequently, making visual diagnosis impossible (Nithya *et al.*, 2002).

The symptoms of the disease are seen when the vegetative growth of the plant is stopped and formation begins, after raining season. The pathogen, *C. falcatum* (Went), can attack any part of the sugarcane plant; be it stalk, leaf, buds or roots. *C. falcatum* complete its life cycle on the sugarcane leaf and usually the damage to leaf does not pose a serious threat to cane or cause much harm to the plant (Duttamajamber, 2008). Discolouration of the leaves is the first symptom in the field. The spindle leaves (3rd and 4th leaf) display drying which withers away at the tips along the margins. This discoloration from tip to the base is continued till all the leaves of the crown wilt (Agnihotri, 1996). Tiny reddish lesions occur on the upper surface of the lamina with minute red spots in both the directions of the upper mid rib. Infection also resulted in change in the colour of the leaves that becomes straw coloured in the center and dark reddish shown at the margins with the development of black acervuli. The infected leaves may break at the lesions and hang down.

The most damaging phase of this disease occur when the pathogen attacks the stalks. Depends on the age of the stalks, time of infection and susceptibility of the cane genotype, it produces different types of symptoms (Viswanathan, 2011). Typical symptoms of red rot are observed in the internodes of a stalk splitting it longitudinally. These includes the angles to the long axis of the stalk. Cross-wise white patches are the important diagnostic characters of the disease. The white spots may vary in size and number and sometimes they are so numerous to give the tissue a mottled

appearance (Viswanathan, 2011). With the advancement of the disease, the stalk becomes hollow and covered with white mycelia growth. Later on the rind shrinks longitudinal with the protrusion of minutes black, velvety fruiting bodies. The infected cane emits acidic-sour smell while its juice emits alcoholic smell. As sucrose gets converted to glucose and alcohol in disease cane, it does not set well upon boiling.

2.5.1 Mode of infection of red rot

The pathogen mainly infects canes through nodes and main portal of entry are leaf scar, growth ring, root primordial and buds (Viswanathan, 2011). The pathogen can also enter the stalk through root lets, growth cracks and cut ends of the sett (Wada *et al.*, 2016).

After the fungus invades the tissues of the stalk, the mycelium may spread from cell to cell leads to gum formation in moderately resistant genotypes. More rapid spread can occur through the vascular bundles. Infected internode tissues develop a rot with a characteristic red colour that often contains interspersed areas with normal colour known as "white spot" (Viswanathan, 2011).

2.6 Epidemiology of Red Rot

2.6.1 Source of infection

Red rot infects stalks and leaf mild ribs causes rot of planting material which results in substantial losses in crop yield and sugar quality (Rachika and Sushma, 2015). The perpetuation of red rot is through infected sett/canes, disease stubble /debris and by resting propagules in the soil. Annual recurrence of red rot in sugarcane is primarily due to infected seed cane and stubble through which the pathogen is carried over to subsequent crops (Viswanathan *et al.*, 2011). The role of soil-born inoculum in the recurrence of disease is perhaps negligible as the fungus is not a true soil borne

organism and does not survive in soil for more than 5-6 months (Wada *et al.*, 2016). The pathogen (*C. falcatum*) attacks the cane plant from germination (Wada *et al.*, 2016) and cause germination failure. In general, dormant mycelia present in the bud scales are responsible for post-germination infection of young emerging shoots (Viswanathan *et al.*, 2011).

2.6.2 Dissemination of red rot

Manifestation of red rot varies depending on the nature of infection, time of the season and the prevailing environment. Diagnostic symptoms are observed during monsoon period (Viswanathan, 2011). The secondary transmission of the fungus during monsoon is mediated through irrigation, rain water, rain splash and results in the infection of mid-rib, lamina, leaf sheath and stalk, while in winter air currents aid in the spread of the pathogen (Viswanathan, 2011). The conidia produced over the rind wash down with water and cause infection through nodes. Dissemination of inoculum by means of wind appears more difficult because of the mucilaginous nature of the spore mass. But the occurrence of the diseases in the upper portion of the canes provides an indication of an aerial mode of dispersal of the inoculum. Environmental conditions prevailing during the winter season do not favour the fungus to infect the cane crop and may not pose any serious threat to infect the cane crop (Satyavir, 2003) but leads to the development of incipient infections in the nodal region (buds, bud scales, root Pretoria). Such infection serves as primary infection when cane is used as seed (Viswanathan, 2011). Borders help in the secondary transmission of the pathogen (Satyavir, 2003).

2.6.3 Host resistance of *C. falcatum*

The factors responsible for determining host resistance against *C. falcatum* have not been fully understood. Two types of resistance against red rot pathogen have been recognized (Satyavir, 2003).

2.6.4 Morphological resistance

It refers to those structures or modifications in the plant tissue, which mechanically restrict or prevent the entry or spread of the pathogen in the host tissues. Various parameters viz, thickness of epidermis, cuticle, bud scales, rind, relative abundance of vascular bundles under the rind and the presence of septa in vascular bundles prevents the rapid migration of spores in plant tissue (Satyavir, 2003).

2.6.5 Biochemical resistance

A Brown gummy substance is formed in advance of the infection in resistance varieties which slows off further spread of the pathogen in adjoining tissue (Wada *et al.*, 2016). On the contrary, gum formation in susceptible varieties takes place after tissue has been infected to a lesser extent. This has been termed as hypersensitive gumming reaction (Satyavir, 2003). In resistance varieties it was observed that the level of total phenolics increased after infection and was maintained, while in susceptible varieties, the level of phenolic content dropped after an initial increase (Viswanathan, 2011). Srivastava and Solomon (1990), observed that higher activity of poly phenol oxidase (PPO) and flavone glycosides is linked with red rot resistance. Viswanathan *et al.* (1996) linked with red rot resistance to higher specific activity of phenylalanine ammonia lyase (PAL) and Tyrosine ammonia lyase (TAL). Viswanathan *et al.*, (1996) correlated the role of phytoalexin 3-deoxyanthocyanin with red rot resistance. As pathogen is highly variable in nature, the resistance sugarcane varieties get prone to red rot within a short time period, therefore detailed investigation is required to understand the true basis of disease resistance at molecular level (Satyavir, 2003).

2.7 Disease Management of Red Rot

Management of red rot have been a challenging area of work for pathologists and sugarcane breeders. The epiphytotic of the disease depends upon weather conditions, genotypes, presence of virulent pathogen and time for disease development. These factors must be studied in depth so as to achieve effective control of the disease. It has been observed that once the disease has appeared in the field it is impossible to control. Most of the recommended management practices hence are aimed at prophylactic measures to reduce pathogen build up in the field (Viswanathan, 2011). In view of diversity in the kinds of pathogens, a single method would not be useful to mitigate the losses from red rot (Agnihotri, 1996), hence Integrated Disease Management (IDM) should be practiced. The following methods in desirable combinations could be adopted for controlling the red rot disease.

2.7.1 Diagnostic measures

Correct diagnosis of pathogens is the primary requirement in any sound disease management practice. Disease diagnosis and pathogen identification by conventional methods involves isolating the pathogen and characterizing it by inoculation tests (Nithya *et al.*, 2012). The variability of red rot pathogen was first studied by (Kumar *et al.*, 2010). The characterization of pathogen has primarily been based upon variation in colour, conidial size and shape, appressoria, colony characters, host association and sporulation (Kumar *et al.*, 2010). But this phenotypic identification is time consuming, expertise specific and not always fully discriminative (Kumar *et al.*, 2010).

Over the past few decades, immunological methods have increasingly received attention as an alternative or complement to conventional methods (Nithya *et al.*, 2012). Polyclonal antisera were developed against the *C. falcatum* proteins and Western blot methods were standardized for

detection of *C. falcatum* in sugarcane (Viswanathan *et al.*, 1998). Hiremath and Naik (2004), developed a protocol for rapid diagnosis of sugarcane red rot infection by using Dot Immunobinding assay (DIBA) technique. But it is very much difficult to diagnose the dormant infections of the *C. falcatum* fungus in seed canes under field conditions as red colour develops on stem and leaves at a later stage (Viswanathan and Samiyappan, 2002). It is therefore, important to explore other possibilities for the management of red rot in sugarcane (Alvi *et al.*, 2008).

2.7.2 Resistant varieties of sugarcane

Effective control of red rot has been mainly through the use of resistant varieties. Even though genetics of inheritance of red rot resistance is not well established, considerable progress has been made in the production of red rot resistant varieties (Alwala *et al.*, 2006). In India, the breeding work is primarily focused on developing red rot resistant varieties. But as the pathogen is highly variable in nature, therefore, even if a disease resistant variety is released for cultivation, it gets susceptible to red rot disease within 8–10 years because of the development of new more virulent races of the pathogen (Viswanathan, 2011).

From the last few decades, molecular diagnostic tools have increasingly been used as an alternative to traditional techniques (Parida *et al.*, 2009). Molecular markers could be used as an effective means that can unfold the complex genetics of sugarcane and also aid the breeders in improving the genetic makeup of varieties (Parida *et al.*, 2009). So far, sugarcane diversity has been studied using ribosomal DNA (Glaszmam *et al.*, 1990), simple sequence repeats (Cordeiro *et al.*, 2000) amplified fragment length polymorphism (Buterfield *et al.*, 2001) restriction fragment length polymorphism (Coto *et al.*, 2002) and random amplified polymorphic DNA (Alvi *et al.*, 2008). Considerable efforts are going on to identify genes and develop markers associated with red rot resistance.

2.7.3 Legislation (Quarantine)

Quarantine regulations govern the introduction of plant material from high-risk areas. In India, seed cane is frequently taken from one state to another without any regulatory restriction (Agnihotri, 1996). Unrestricted movement of seed material has been largely responsible for the spread of red rot in different regions. The introduction of the disease in Karnataka and Maharashtra was because seed material of highly susceptible varieties was brought from red rot endemic areas (Agnihotri, 1996). Hence there is a need for restricting transport of cane from an infected zone to disease free zones. The seed material from outside is procured only from research stations with proper phytosanitary certificate (Satyavir, 2003).

2.7.4 Prophylaxis

Prevention is better than cure hence; healthy cultural practices should be adopted while planting the sugarcane. Uses of healthy seeds, crop rotation, field sanitation and efficient drainage have been recommended to reduce inoculum in the field and minimize losses due to the disease. The primary inoculum of the disease mainly comes through infected seed material (Satyavir, 2003) hence; disease free seed nursery should be established in each farm. In this context, seed from heat treated crop or certified nursery will serve the purpose. In addition to this, sanitary measures must be adopted in the field. Crop debris, trash and stubble should be burnt prior to planting (Agnihotri, 1996). Extremely dry and wet soils should be avoided. Ratooning should not be done in case of heavily infected plant crops. To minimise the soil borne inoculum, crop rotation should be adopted by growing some other crop for 2-3 years. Flow of irrigation water from diseased to healthy plants should be discouraged to avoid the spread of disease through water medium. Long setts should be used for planting. Three or four budded setts are very suitable seed setts for the control of soil born inoculums of red rot (Anwar *et al.*, 2010).

2.7.5 Chemotherapy

A number of fungitoxicants have been tried against the red rot but a little success is found in controlling this devastating disease. This may be because of impervious nature of rind, presence of fibrous nodes at the cut ends, low solubility of fungicides, lack of broad spectrum fungicides and presence of abundant nutrients in the sett (Agnihotri, 1996; Satyavir, 2003). Soaking of sugarcane setts in 0.25 % suspension of thiophanate methyl and its metabolite carbendazim for 24h before planting was found to be effective in controlling debris-borne infection (Malathi, *et al.*, 2004). Subhani *et al.* (2008) report that benomyl, foliar, radomil and tilet completely inhibited the growth of fungus (100 % inhibition) while minimum mycelial growth inhibition was observed in case of nimrod. In related development Khan *et al.*, (2009) recommend that treatment with topsin helps in protecting canes from red rot disease and improving plant yield. In an experiment conducted (Bharadwaj and Sahu, 2014), bavistin showed complete inhibition of mycelial growth of the *C. falcatum*.

2.7.6 Thermotherapy

Heat therapeutic measures have been used for controlling sett borne infection of red rot by various workers. According to the work of Comstock, (2014) reported complete elimination of sett-borne infection by hot air treatment (52 °C for 20 or 30 or 45 minutes). Some workers have used heat and chemotherapy in combination by incorporating the chemical into the hot water tank for adequate control of red rot (Agnihotri, 1983). Findings of various workers have proved that moist hot air therapy (MHAT) of seed cane at 54 °C for 4 hr (R.H. 95-100) was most effective against red rot (Comstock, (2014). Aerated steam treatment at 52 °C or the soaking of setts in cold running water for 48 h followed by hot-water treatment (50 °C for 150-180 min) also helps in eliminating the pathogen from infected setts (Joshi, 1954).

2.7.7 Biological control

Red rot disease of sugarcane was observed to be biologically controlled through *Trichoderma harzianum* and *T. viride* (Singh *et al.*, 2008). *T. harzianum* and *Pseudomonas* spp. possess the ability to protect the crop from soil borne inoculum of red rot and the efficacy is because of the chitinase enzyme produced by them (Singh *et al.*, 2008). The findings of Singh *et al.* (2013) clearly supported that each 42 gene of *Trichoderma* spp. is responsible for controlling the red rot incidence in sugarcane in an experiment conducted by Singh *et al.*, (2010) addition of SA (salicylic acid) has boosted the protection level significantly against red rot disease and helped *T. harzianum* for inducing systemic resistance in sugarcane. *Ocimum*, Ginger, Onion and Garlic were also found to inhibit the mycelial growth. It was also examined that the essential oils, viz. Peppermint oil, Mentha oil, Geranium oil, Patchouli oil and Palmaroza oil were effective in inhibiting the growth of mycelia of *C. falcatum* (Bharadwarj and Sahu, 2014). In sugarcane Vismiyappan and Samiyappan (1999) established PGPR (Plant growth promoting rhizobacteria) mediated ISR (Induced systemic resistance) against *C. falcatum* causing red rot disease. Viswanathan *et al.* (2005) indicates a possible role of PR (pathogenesis related) proteins in conferring red rot resistance in sugarcane. It was observed in an experiment that leaf extracts of *Curcuma domestica* and *Datura metel* inhibited the conidial as well as mycelial growth *C. falcatum*. Most interestingly it was observed that smoke of dhup (incense) and tobacco also showed inhibition against conidial germination (Imtiaj *et al.*, 2007).

2.8 Preparation of Plant Extracts

The health fresh plant materials was collected, washed with running tap water and then shade dried the leaves, flowers and seeds. These were then crushed into coarsely powdered nature in which 25 g of the different parts was subjected to successive extraction in 250 mL of methanol

solvent using soxhlet apparatus. A known weight of five gram (5 g) of powdered sample was extracted separately with 50 mL of some selected solvents, the powder was filled in the thimble and placed in Soxhlet apparatus and extracted for 24 hours and the obtained extracts were concentrated using rotary flask evaporator which were later preserved in airtight containers for further analysis (Gnanasekaran *et al.*, 2015). Plant samples collected were washed several times with distilled water, then oven dried at about 40 °C for one week and ground into powder using a medium kitchen blender. 100 grams of each powder sample was exhaustively extracted by soaking in 100 mL of distilled water for 12 hours; the extracts were filtered using Whatman filter paper No. 42 (125 mm) (Akinyeye and Olatunya, 2014). In related methodes Kawo and Kwa, (2011) extracted the leaves of *L. inermis* using distilled water and ethanol, where hundred grams (100 g) of the powdered, air dried leaves were percolated in a litre of the distilled water for one week with occasional shaking. And the extract was filtered using Whatmans No. 1 paper and the crude extract was evaporated to dryness using a water bath at 40 °C. The same procedure was done for the ethanol extraction except that it was dried (concentrated) using rotary evaporator at 40 °C. Fernando *et al.* (2013) collected plants, washed them with tap water and disinfected them with sodium hypochlorite (0.5 %) for 30 minutes to remove micro-organisms present on the surface, after which they were rinsed thrice with sterile distilled water to remove the remaining sodium hypochlorite. The plants were dried on paper towels to remove excess moisture and were later packed in paper bags and oven dried with air circulation at 45 °C for 09 hours. The dried samples were sliced and ground into powder, ten grams (10 g) of the dry powder was added in 100 mL of sterile boiling distilled water and left in an infusion at room temperature in darkness for 24 hours. It was collected after 24 hours and filtered in sterile gauze and Whatman filter paper (No. 1) to obtain crude aqueous extract.

2.9 Efficacy of Plant Extract (*In vitro*) Antimicrobial Susceptibility Testing (AST)

2.9.1 Diffusion test methods

Agar well diffusion

Agar disc diffusion

Food poison technique

Bio authography

2.9.2 Dilution methods

Agar dilution

Broth micro dilution assay

Broth macro dilution assay (Balourie *et al.*, 2016)

2.10 Evaluation of the Five Plants Used as Treatments

2.10.1 Description of *Cariaca papaya*

Carica papaya is an evergreen, tree-like herb, 2-10m tall, usually unbranched, although some times branched due to injury, containing the white latex in all parts. Stem is cylindrical, 10-30cm in diameter, hollow with prominent leaf scars and spongy-fibrous tissue. and has an extensive rooting system (Orwa *et al.*, 2009).

Leaves spirally arranged, clustered near the apex of trunk; petiole is up to 1m long, hollow, greenish-green; lamina orbicular, glabrous, prominently veined; lobes deeply and broadly toothed. Flowers are tiny, yellow, funnel-shaped, solitary or clustered in the leaf axils of 3 types; female flowers are 3-5cm long, large functional pistil, no stamens, ovoid-shaped ovary, male flowers on long hanging panicles, with 10 stamens in 2 rows, gynoecium absent except for a pistillode, hermaphrodite flowers larger than males, 5-merous ovary; occurrence depends on the season or age of the tree. Fruits large, cylindrical, with flesh orange pulp, hollow berry, thin yellowish skin

when ripe, varied (Orwa *et al.*, 2009). Fruits formed from female flowers are oblong, spherical, pear-shaped; from hermaphrodite flowers, long, obovoids or pyriform seeds numerous, small, black, round, covered with gelatinous aril. Small latex vessels extend throughout the tree and are particularly abundant in fruits that has reached full size but has not yet begun to ripen. (Orwa *et al.*, 2009).

Kingdom plantae
Phylum spermatophyta
Subphylum angiospermae
Class dicotyledonae
Order violales
Family caricaceae
Genus *Carica*
Species *Carica papaya* (Orwa, 2009).

2.10.1.2 Phytochemical constituents of *Carica papaya* extracts

The identification of phytochemical compounds of ethanolic extract on *Carica papaya* leaf, seed unripe and seed ripe were alkaloids, flavonoids, saponins and triterpenes (singh *et al.*, 2016). Similarly, Sheneni *et al.* (2018), reported the presence of flavonoids, saponins, tannins, terpenoids, glycosides, carbohydrates, anthroquinone, and alkaloids from *C. papaya* leaf using different solvents (methanol, ethanol, ethyl acetate, n-butanol and n-hexane). More also, extracts of *C. papaya* (dried) leaves contains compounds such as phenols, alkanoids, glycosides, saponins, anthroquinones while anthrocyanosides were not dictated in the sample (Ngozi *et al.*, 2010). Qualitative analysis of *Carica papaya* leaf extract does not showed steroids and tannins all the

possible photochemical constituents were present meanwhile, the extracts possessed carbohydrates, proteins, anthraquinones, flavonoids and alkaloids. (Ayoola and Adeyeye, 2010).

2.10.1.3 Antifungal activity of *Carica papaya* extracts

Essential oil of *papaya* seed possesses antifungal activity. He *et al.* (2017) filter paper disk diffusion method and broth dilution method were implemented. The obtained essential oil showed an inhibitory effect against all the tested *Candida* strains including *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. Chavez-Quintal *et al.* (2011) studied ethanolic extracts from *C. papaya* leaves which are potential source of secondary metabolites with antifungal properties. Crushed and boiled *papaya* extracts were tested for their antifungal against 6 saprophytic fungi (*Penicillium* sp., *Aspergillus flavus*, *A. niger*, *Fusarium* sp., *Rhizopus* and *Helminthosporium*), 5 dermatophytic fungi (*Microsporium canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes* and *T. tonsurans*), and 6 yeast species (*Candida albicans*, *Saccharomyces cerevisiae*, *C. glabrata*, *C. tropicalis* and *C. kusei*) the activity was found against the majority of fungi but was much better in case of crushed leaf extracts (Sherwani *et al.*, 2013). Singh and Ali (2011), showed that methanolic extracts of the seeds and 2, 3, 4-trihydroxytoluene (200mg.m/l) showed antifungal activity against *A. flavus*, *C. albicans* and *Penicillium citrinum*. It was found that *papaya* seed oil contain benzyl isothiocyanate (Vij and Prashar, 2015) and plant leaves exhibited carpaine and carposide as alkaloids types compounds (Barroso *et al.*, 2016) showing antifungal efficacy.

2.10.2 Description of *Azadirachta indica*

A. indica is a medium to large, deep-rooted, evergreen tree, 15 to 30m tall, with a round large crown of 10 to 20 m in diameter; branches spreading; boles branchless for up to 7.5 m, 90 m in diameter, sometimes fluted at base; bark moderately thick, with small, scattered tubercles, deeply fissured

and flaking in old trees, dark grey outside and reddish inside, with colourless, sticky foetid sap. Leaves alternates, crowded near the end of branches, simply pinnate, 20-40cm long, light green, with 2 pairs of glands at the base, glabrous; petioles 2-7 cm long, subglabrous; rachis channelled above; leaflets 8-19, petioluled, alternate and more or less posites distally, ovate to lanceolate, sometimes falcate, glossy, serrate; apex acuminate; base unequal. Inflorescense an axillary, many-flowered thyrus, up to 30 cm long; bracts minutes and caducouse; flowers are bisexual or male on same tree, actinomorphic, small, pentamerous, whites or pale yellow. Slightly sweet scented; calyx lobes imbricate, broadly ovate and thin, puberulous inside; petals free imbricates, spathulate, spreading, ciliolate insides. Fruit 1 (or 2)- seeded drupe, ellipsoidal, 1-2 cm long, greenish, greenish-yellow to yellow or purple when ripe; exocarp thin, mesocarp pulpy, endocarp cartilaginous; seed ovoid or spherical; apex pointed; testa thin, composed of a shell and a kernel (sometimes 2 or 3 kernels), each about half of the seed's weight (Orwa *et al.*, 2009).

Kingdom plantae

Phylum spermatophyta

Subphylum angiospermae

Class dicotyledonae

Order rutales

Family meliaceae

Genus *Azadirachta*

Species *Azadirachta indica* (Orwa, 2009).

2.10.2.1 Phytochemical constituents of *Azadirachta indica* extracts.

The presence of the phytochemical constituents mainly alkaloids, flavids, tannins and phenols compounds in the *Azadirachta indica* leaf extracts is thought to be responsible for the antifungal

activity. Numerous investigations have proved that medicinal plants contain diverse classes of bioactive compounds. The presence of the phytochemical constituents mainly alkaloids, flavonoids, tannins and phenolic compounds has been reported to be most important compounds in many other medicinal plants (Jayasree *et al.*, 2014). According to the preliminary phytochemical screening of aqueous and ethanolic extracts of *Azadirachta indica* leaf, and ethanolic extracts, alkaloids, saponins, tannins, phenols, flavonoids and terpenoids (Ruchi *et al.*, 2014). The results of phytochemical analysis of aqueous leaf extracts (ALE) and ethanolic leaf extracts (ELE) of neem, results indicate the presence of many phyto-components in both the extracts. Alkaloids, saponins, tannins, phenols, flavonoids, terpenoids, glycosides but absence of amino acids and terpenoids due to the reasons that the extract was treated with chloroform unlike the other extracts was treated with ethanols. Pandey *et al.* (2014) also reports the presence of alkaloids, saponins, tannins, phenols, flavonoids, terpenoids and amino acids respectively. More interestingly the results of phytochemical in the investigation of neem leaf report by some researchers had contains eight phytochemicals components. Abdullah *et al.* (2011); Mahapatra *et al.* (2014) report the presence of alkaloids, tannins, glycosides, anthraquinones, reducing sugar, polyphenol, terpenoids and steroids. The presence of three phytochemicals is the reasons neem leaf ethanol extracts have antifungal and antimicrobial activity.

2.10.2.2 Antifungal activity of *Azadirachta indica* extracts

Experiment was made to evaluate the efficacy of various extracts of neem leaf on seed born fungi *Aspergillus* and *Rhizopus* and results confirmed that growth of both the fungal species was significantly inhibited and controlled with both alcoholic and water extract. Furthermore, alcoholic extracts of neem leaf was more effective as compared to aqueous extracts for retarding the growth of both fungal species (Mondal *et al.*, 2009). Another finding showed the antimicrobial role of

aqueous extracts of neem cake in the inhibition of spore germination against three sporulating fungi such as *C. lunata*, *H. penneiseti* and *C. gloeosporioides* (Anjali *et al.*, 2013) and results of the study revealed that methanol and ethanol extracts of *Azadirachta indica* showed growth inhibition against *Aspergillus flavus*, *Alternaria solani* and *Cladosporium* (Shrivastava and Swarnkar, 2014).

Aqueous extracts of various parts of neem such as neem oil and its chief principles have antifungal activities and have been reported by earlier investigators (Natarajan *et al.*, 2003; Lloyd *et al.*, 2005). A study was undertaken to examine the antifungal activity of *Azadirachta indica* L. against *Alternaria solani* sorauer and results confirmed that ethyl acetate fraction was found most effective in retarding fungal growth with media concentration of 0.19 mg and this fraction was also effective than fungicides (metalaxy + mancozeb) as fungicide has media concentration of 0.78 mg (Jabeen *et al.*, 2013).

2.10.3 Description of *L. inermis* plant

L. inermis (Henna) is a tall shrub or small tree, 2.6 m high. It is glabrous; multi branched with spine tipped branchlets. Leaves are opposite, entire, glabrous, sub-sessile, elliptical, and broadly lanceolate (1.5–5.0 cm x 0.5–2 cm), acuminate, having depressed veins on the dorsal surface. Henna flowers have four sepals and a 2 mm calyx tube with 3 mm spread lobes. Petals are obvate, white or red stamens inserted in pairs on the rim of the calyx tube. Ovary is four celled, style up to 5 mm long and erect. Fruits are small, brownish capsules, 4–8 mm in diameter, with 32–49 seeds per fruit, and open irregularly into four splits Mastanaiah *et al.* (2011). Rao *et al.* (2016) described the genus *Lawsonia* to consist of one species, *L. inermis* (Henna). It has synonyms such as *Alba* and *Spinosa* belonging to family Lythraceae.

It is biennial dicotyledons herbaceous herb. It is grown as an ornamental and dye plant. It is much branched glabrous shrub or small tree (2 to 6 m) in height. Leaves are small, opposite in arrangement along the branches, sub-sessile, about 1.5 to 5 cm long, 0.5 to 2 cm wide, greenish brown to dull green, elliptic to broadly lanceolate with entire margin, petiole short and glabrous and acute or obtuse apex with tapering base. Young branches are green in colour and turn red with age. Bark is greyish brown, unarmed when young but branches of older trees are spine tipped. Inflorescence is a large pyramid shaped cyme. Flowers are small about 1cm across, fragrant, white or rose coloured with four crumbled petals. Calyx is 0.2 cm tube and 0.3 cm spread lobes. Fruit is a small brown coloured round capsule. Fruit opens irregularly and splits into four sections at maturity and is many seeded. Seeds are about 3mm across, numerous, smooth, pyramidal, hard, thick seed coat with brownish coloration.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Lythraceae
Genus	<i>Lawsonia</i>
Species	<i>inermis</i> (Lawson, 1947)

2.10.3.1 Phytochemical constituents dictated in *L. inermis* extracts

Literatures surveyed on the details of phytochemicals present in *L. inermis* (Henna) are reviewed below: Scientific researchers around the world have reported the different biological actions of *L. inermis* in vitro and in vivo test methods from the leaves, flower, seeds, stem bark and roots.

Mehadi *et al.* (2014), carried out the phytochemical analysis of ethanolic, chloroform, and acetone leaves extracts of *Lawsonia inermis*, they reported the presence of terpenoids, phenols, tannins, quinines, and cardioglycosides in the ethanol and acetone extracts while for chloroform, same components were reported except terpenoids. Wangini *et al.* (2014) reported that, in the qualitative screening of the leaves of *L. inermis* revealed the presence of 10 secondary metabolites viz alkaloids, flavonoids, glycosides, saponins, tannins, quinines, carbohydrates, resins, sterols and lipid/fat in the fractions of both Nigerian and Egyptian Henna. Rao *et al.* (2016) reported the extract of *L. inermis* to contain carbohydrates, proteins, flavonoids, tannins, phenolic compounds, alkaloids, terpenoids, quinones, coumarins, xanthenes, and fatty acids. Phytochemical screening of henna plant has revealed the presence of numerous chemicals including alkaloids, tannins, flavonoids, steroids, glycosides, saponins amongst others (Arun *et al.*, 2010).

2.10.3.2 Antifungal activity of *L. inermis* extracts

Abdelraouf *et al.* (2011) reported in his review that some studies suggested that *L. inermis* has a wide spectrum of antimicrobial activity including antifungal, antibacterial, antiviral, antiparasitic, molluscidal, and nematocidal activities amongst others. Certain antifungal activities of *L. inermis* are discussed below; Three medicinally important plants viz *L. inermis*, *Latana camara* and *Swertia angustifolia* were reported for their antifungal and antibacterial activities by Zarrin *et al.* (2013) and that amongst the three plants, *L. inermis* showed maximum zone of inhibition against three fungal strains such as *F. solani*, *Alternaria* and *mucor* with percentage inhibitions at 78.8, 65.3 and 71.1 % respectively.

Aqueous extract leaves of *L. inermis* were tested for the antifungal potential against eight important species of *Aspergillus* which were isolated from sorghum, maize and paddy seed samples. *A. flavus* recorded high susceptibility and hence solvent extracts viz., petroleum ether,

benzene, chloroform, methanol and ethanol extracts of the plant showed significant antifungal activity (Wangini *et al.*, 2014). Mansour *et al.* (2012) and Abdulyazid *et al.* (2013), reported fungicidal effect of *L. inermis* leaves extract against *Trichophyton mentagrophytes* and *Candida albicans*. *L. inermis* was assayed by Sharma and Sharma (2011) of antifungal activity on plant pathogenic fungi viz *Alternaria solani*, *Dreschlera halodes*, *D. gramineae*, *Rhizoctonia solani* (ITCC no. 4574), *S* (ITCC no. 2927), *Aspergillus flavus* (Navjot 4 NSt), *A. parasiticus* var. *globosus* (MTT No. 411), *Culvularia lunata* and three human fungi that is; *Trichopyhton rubrum* (MTCC 296), *Aspergillus fumigatus* (MTCC 2550) and *Candida albicans*. The results suggest that, of all the extracts assayed acetone extract was the most effective as it showed significant inhibition of all test fungi which was comparable with that of standards used, the methanol extract also showed significant activity against all plant pathogenic as well as human pathogenic fungi except *A. flavus* and *A. parasitica* against which petroleum ether and benzene extracts was more effective. Inhibition of *D. gramineae*, *C. lunata*, *A. fumigatus* and *C. albicans* were observed with petroleum ether and benzene fractions of the leaf.

Santosh *et al.* (2013) reported different works on the antifungal activities of *L. inermis* such as; the screening of barks of 30 plant species against *Microsporium gypseum* and *Trichophyton mentagrophytes*. Only *L. inermis* extract exhibited absolute toxicity. The extract showed broad fungitoxic spectrum when tested against 13 ring worm fungi. Further the fungitoxicity of the extract remained unaltered at high temperature on autoclaving and after long storage. The leaves of *L. inermis* were also found to exhibit strong fungi toxicity and non-phytotoxicity. The minimum effective dose against test organism was found to be 1000ppm.

Ethanol, methanol and aqueous extract of *L. inermis* leaves are involved in defensive mechanism against spore germination of *Drechslera oryzae*. *Lawsonia* isolated from the leaves of *L. inermis*

has shown significant antifungal antibiotic effect. Aqueous extract of leaves of *L. inermis* was tested for the antifungal potential against eight important species of *Aspergillus* which were isolated from sorghum, maize and paddy seed samples. *A. flavus* recorded high susceptibility and hence solvent extracts viz., petroleum ether, benzene, chloroform, methanol and ethanol extracts of the plant showed significant antifungal activity. Essential oil obtained by hydro-distillation from leaves of *L. inermis* growing in Iran were analysed by GC-MS and showed an antifungal activity. Ethanol leaves extract of *L. inermis* showed significant antifungal effect against phytopathogenic fungi. *L. inermis* extract inhibited the growth of fungi, *A. niger* and *Fusarium* where mild inhibition was observed and suggests that, the inhibition rate will be increased by increasing the concentrations.

This antimicrobial experiment revealed that *L. inermis* leaves are capable of inhibiting the growth of fungi pathogens (Rao *et al.*, 2016). Sharma & Sharma (2011) also demonstrated that *L. inermis* has fungicidal potential against *A. solani*, *D. halodes*, *R. solani*, *F. solani*, *C. lunata*, *D. gramineae*, *F. moniliformae*, *A. flavus*, *A. parasiticus* var. *globosus*, *Trichophyton rubrum*, *A. fumigatus*. *L. camara* fruit extract was mildly active against *Mucor* and *F. solani*. *Swertia angustifolia* extract showed mild activity against *Alternaria* and low activity against *F. solani*.

2.10.4. Description of *Z. spina-christi* plant

The genus *Ziziphus* belongs to the family Rhamnaceae in the order Rosales that consist of about 60 genera and more than 850 species. The genus is made up of about 100 species of deciduous or evergreen trees and shrubs throughout the world (Abalaka *et al.*, 2010). *Z. spina-christi* (Christi thorn jujube) is a shrub, sometimes a tall tree, reaching a height of 20 m and a diameter of 60 cm; bark light-grey, very cracked, scaly; trunk twisted; very branched, crown thick; shoots whitish, flexible, drooping; thorns in pairs, one straight, the other curved (Salehi, 2010). Leaves are

glabrous on upper surface, finely pubescent below, ovate-lanceolate or ellipsoid, apex acute or obtuse, margins almost entire, lateral veins conspicuous. Flowers are in cymes, subsessile, peduncle 1-3 mm. Fruit is about 1 cm in diameter.

This genus comprises of about 100 species of deciduous or evergreen trees and shrubs distributed in the tropical and subtropical regions of the world. They grow either as Spiny shrublets, shrubs or trees of about 3-10 m tall, with short spines positioned in pairs along branches which are used as hedge to form defensive fences for animals (Bukar *et al.*, 2015). The Bark of the tree is whitish-brown or pale grey, cracked. Trunk is twisted, leaves are alternate, oval more pointed at the tips. Flowers are small, greenish-yellow. Fruits are 1cm in diameter, red brown with stone in center. Some species, like *Z. mauritiana* Lam. and *Z. spina-christi* (L.) wild occur on nearly every continent. *Z. mauritiana* and *Z. spina-christi* have very nutritious fruits and are usually eaten fresh.

The majority of the rural population in Northern Nigeria use *Z. spina-christi* extensively for its medicinal and economic importance (WHO, 2010). *Z. spina-christi* is a tree with white branches, leaves larger, ovate-lanceolate with an acute or obtuse apex, 2.5-8.5 cm long and 1-3.5 cm wide. Margins slightly crenate, 3 strong veins from the base, lateral veins inconspicuous, numerous flowers per cyme, peduncle up to 1.5 cm and fruit is 2 cm in diameter; var. *Microphylla hochst* ex A. Rich. is a very bushy shrub, leaves are widely ellipsoid or ovate-ellipsoid, rounded at the tip, 1-3 cm long and just as wide, margins almost entire, basal veins not reaching the apex, 1-2 strong lateral veins on each side of the central vein; branches brown-reddish, and fruits up to 1 cm in diameter. The name '*Zizyphus*' is often erroneously written as *Zizyphus*. The generic name is derived from the latinized version of the Arabic vernacular name '*zizouf*' for *Z. jujuba*. The specific name is derived from its common name Christ thorn (Orwa *et al.*, 2009).

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Rosales
Family	Rhamnaceae
Genus	<i>Ziziphus</i>
Species	<i>spina-christi</i> (Ziziphus, 1754)

2.10.4.1 Phytochemicals reported on *Z. spina-christi* extracts

Literatures surveyed on the details of phytochemicals present in *Z. spina-christi* are reviewed below: Screening of seed oil of *Z. spina-christi* revealed the presence of some secondary metabolites which includes; tannins, glycosides, alkaloids and flavonoids (Bukar *et al.*, 2015). Hatil and Ahmed (2015), reported the presence of anthraquinones, terpenoids, aponins, tannins, monosaccharides, reducing sugars and carbohydrates from ethanol leaves extracts of *Z. spina-christi*. Glycosides, tannins, flavonoids, saponins, steroids and anthraquinones in the ethanol extract of *Z. spina-christi* (Abu-Taleb *et al.*, 2011). In the extracts of *Z. spina-christi* and *Z. mauritiana*, the isolated phytochemicals were cardiac glycosides, polyphenols, saponins and tannins from (Abalaka *et al.*, 2010). Phytochemical analysis revealed the presence of tannins, flavonoids, terpenoids, saponin glycosides and alkaloids in ethanolic extract of *Z. spina-christi* (Ads *et al.*, 2017). Seed extract showed the absence of steroids and terpenoids while their presence was revealed in the fruit extract (Alhakmani *et al.*, 2014). Flavonoids, alkaloids and saponins were the main phytochemicals reported from *Z. spina-christi* plant (Asgarpanah and Haghghat, 2012). Phytochemical investigation also showed that the bark of *Z. spina-christi* is rich in tannin and phenolic compounds, which have been shown to possess antimicrobial activities against a number

of microorganisms (Ads *et al.*, 2017). The phytochemical screening of *Z. spina-christi* extracts revealed the presence of alkaloids, tannins, saponins, and steroids (Dangogo *et al.*, 2012).

2.10.4.2 Antifungi activity of *Z. spina-christi* extracts

Z. spina-christi has been revealed for its antifungal, antibacterial, antiplasmodial or antiparasitic activities amongst others (Abalaka *et al.*, 2010; Waggas and Al-Hasani, 2010; Adzu *et al.*, 2011). Previous studies on the antifungi activity of *Z. spina-christi* are given below: *Z. spina-christi* was found to produce a diverse of bioactive compounds which were found to possess antimicrobial properties (Panduraju *et al.*, 2009; Abalaka *et al.*, 2010). Antimycotic activity of the ethanol extracts prepared from *Urtica dioica* L., *Citrullus colocynthis* L. Schrad, *Z. spina-christi* L. and *Nerium oleander* L. floral parts were noted against *Alternaria alternate*, *F. oxysporum*, *F. solani* and *I* using agar dilution bioassay (Hadizadeh *et al.*, 2009). Researches have shown that *Z. spina-christi* (L.) is potentially a good source of antimicrobial compound, reports on its uses in the application of crop protection is not as many as in medical field. *F. solani* were generally more sensitive to the extracts of *Z. spina-christi* and *Rumex vesicarius* than *D. biseptata*. *F. solani* failed completely to produce spores when treated with ethanolic extract of *Z. spina-christi* at 20 %. On the other hand, the growth of *D. biseptata* was generally more susceptible to plant extracts than that of *F. solani*. *Z. spina-christi* extracts induced high antifungal activity particularly against *D. biseptata* (Abu-Taleb *et al.*, 2011). Asgarpanah and Haghghat (2012), reviewed that the methanol extract of *Z. spina-christi* roots showed antifungal activity against dermatophytes, including *T. rubrum*, *T. mentagaphytes*, *Microsporum canis* and *A. fumigatus*. The fruits were also active against *C. albicans* (Pirbalouti *et al.*, 2009). Ads *et al.* (2017) reported antimicrobial activity of the bark of *Z. spina-christi* which was evaluated and showed antifungi activity against pathogenic fungi *A. fumigatus*, *Syncephalastrum racemosum*, *Geotricum candidum*, and *C. albicans*.

2.10.5. Description of *Khaya senegalensis* plant

Khaya senegalensis is a deciduous evergreen tree, 15-30 m high, up to 1 m in diameter, with a clean bole (8-16 m), buttresses not prominent or absent; bark dark grey, with small, thin, reddish-tinged scales; slash dark pink to bright crimson, exuding a red sap, leaves alternate, compound, stipules absent; petiole and rachis 13-33 cm long; leaflets 3-4 (max. 7) usually opposite pairs, oblong to narrowly oblong-elliptic, 4-12 x 2-5 cm, apex acute to shortly acuminate, base rounded, margins entire, pale green, lateral nerves 8-16, petiolules about 3.5 cm long (Orwa *et al.*, 2009). Inflorescence a lax, much-branched axillary panicle up to 17 cm long; flowers tetramerous, monoecious but with well-developed vestiges of those of the opposite sex with very little external differences between sexes. Calyx pale green, lobed almost to the base, lobes subcircular, about 1 x 1 mm, imbricate; petals cream, free, oblong-ovate, 4 x 2.5 mm, contorted in bud; orange disk around the ovary. Fruit an upright, almost spherical, woody capsule, 4-6 cm in diameter, opening by 4 valves from the apex (a distinction from *K. ivorensis*, which is closely related but has 5 valves). Seeds brown, 6 or more per cell, broadly transversely ellipsoid to flat, about 25 x 18 mm, margins narrowly winged (Orwa *et al.*, 2009).

Kingdom plantae

Phylum epermatophyta

Subphylum angiospermae

Class dicotyledonae

Order rutales

Family meliaceae

Genus *Khaya*

Species *Khaya senegalensis* (Orwa, 2009).

2.10.5.1 Phytochemical constituents of *Khaya senegalensis* extracts

Studies were reported on the phytochemical constituents from *Khaya senegalensis* extracts which are known to be biologically active compounds that were attributed to be responsible for antifungal activities (Abdelgalil *et al.*, 2004; Ademola *et al.*, 2004). Phytochemical screening of methanolic and ethanolic crude extracts from fresh and dry powder leaves, barks and roots samples of *Khaya senegalensis* revealed the presence of alkaloids, saponins, flavonoids and tannins in all the extracts (Kankia and Zainab, 2015). It also revealed the presence of steroids and terpenoids but not in all extracts. Flavonoids were rich in leaves extracts while they were not detected in bark, the biological function of flavonoids includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins and tumor (Okwu, 2004). These observations therefore support the use of *K. senegalensis* in herbal care remedies.

2.10.5.2 Antifungal activity of *Khaya senegalensis* extracts

Various reviews of *Khaya senegalensis* on some fungal pathogens. Abdulsalam *et al.* (2015), reported that different concentrations of *Khaya senegalensis* A. Juss plant extracts retarded the vegetative growth of the fungi responsible for the neck rot disease of onions. A similar observation but on a different organism that mahogany extract was highly effective on the control of root knot disease of tomatoes caused by nematodes (Liman *et al.*, 2010). According to Khare *et al.* (2004) that plant essential oil is a useful source of anti-fungal compounds and the effectiveness of *Khaya senegalensis* oil in controlling fungal pathogens could probably be due to constituents of secondary metabolites capable of controlling and inhibiting the pathogens. O'Bryne *et al.* (1997), also reported that the fresh and dried mahogany bark extracts have shown strong antimicrobial properties. Although, there are few reports on the use of *Khaya senegalensis* products in controlling plant pathogens, extracts from the plant have been extensively used in the control of insect pests of

crops, particularly cotton boll worm, apart from the insecticidal properties of *Khaya senegalensis* products, these products have also been reported to possess antifungal and bactericidal properties (Bamaiyi *et al.* (2006).

2.11 Generals Effects of Different Plant Extracts on the Growth of Various Fungi Pathogens in vitro and in vivo

Various works on the use of different botanical extracts on the growth of *Fusarium oxysporum* cubense and other plant pathogens are reviewed below: The anti-fungal activity of *Calotropis gigantia* L., *Centella asiatica* L., *O. sanctum* L., *Piper betle* L. and *Vintex negundo* L. plant extracts were studied against the plant pathogen *F. oxysporum* cubense. Among the five plants tested, *P. betle* L. plant extracts exhibited maximum antifungal activity against the tested *F. oxysporum* cubense (Gnanasekaran *et al.*, 2015). Lorenzetti *et al.* (2011) reported that *C. zeylanicum* and *S. aromaticum* show effects with reduction on the mycelia growth of *Botrytis cinecerea* which causes gray mold of straw berry at 125ppm to be 41 % and 31 % respectively. Huang *et al.* (2012) reported the inhibitory effects of *Allium tumberosum* on *F. oxysporum* Cubense. In PDA medium the crude extract of *A. tumberosum* at 0.5, 1.0 and 2.0 mL per petri-dish significantly inhibited the mycelia growth of *F. oxysporum* Cubense by 21 %, 43.5 % and 100 % respectively.

Gopi and Thangavelu (2014) found out the effective botanicals that will have significant effect on mycelia growth and spore germination of *F. oxysporu* Cubense. In their study, 33 botanical leaf extracts were screened which resulted in the identification of six different botanical leaf extracts (*Apinia galangal*, *Rhinacanthus nasutus*, *Hibiscus rosasinensis*, *A. cepa* L. × *A. sativum* L. (Zimmu), *O. tenuiflorum* and *Vitex* spp.) showing maximum effects in the inhibition of mycelia growth and spore germination of *F. oxysporum* Cubense pathogen of panama disease in banana

under *in vitro* conditions. However, among the six botanicals, *Zimmu* leaf extract alone exhibited 100 % inhibition of both mycelia growth and spore germination of *F. oxysporum* Cubense.

Fernando *et al.* (2013) studied the influence of plant extracts and essential oils against Panama disease (*F. oxysporum* cubense) in banana seedlings using the extracts and essential oils of *Cinnamomum zeylanicum* and *Syzygium aromaticum*. He discovered, they were effective at 500 ppm with direct effect against mycelia growth. The volatile essential oils of *C. zeylanicum* and *S. aromaticum* showed good effects in controlling the disease at both 1000 and 2000 ppm. The plant extracts of *C. zeylanicum* and *S. aromaticum* at 5 % concentration reduced mycelial growth to 40.37 % and 54.44 % respectively while the essential oils of *C. zeylanicum* and *S. aromaticum* also showed antagonistic effect on mycelia growth of *F. oxysporum* f. sp. Cubense at 250 ppm with a reduction of 78.70 % and 63.52 % respectively.

The floral parts of Nettle (*Urtica dioica* L.), Colocynthis (*Citrullus colocynthis* L. Schrad), Konar (*Z. spina-christi* L.), and Oleander (*Nerium oleander*) were screened *in vitro* against four important plant pathogenic fungi, viz; *A. alternate*, *F. oxysporum*, *F. solani* and *R. solani* using agar dilution bioassay. Extracts showed antifungal activity against all the tested fungi. Among the plants, Nettle and colocynthis were the most effective against *A. alternate* and *R. solani* while *oleanders* possess the best inhibition on *F. oxysporum* and *F. solani*. Konar was the most effective extract by reducing the growth of *R. solani* than other fungi (Hadizadeh and Kolani, 2009). Al-Rahma *et al.* (2013) reported the fungicidal activity of five methanolic plant extracts from *L. camara*, *Salvadora persica*, *Thymus vulgaris*, *Zingiber officinale* and *Z. spina-christi* against tomato phytopathogenic fungi, *F. oxysporum*, *Pythium aphanidermatum* and *R. solani*, the causative agents of tomato damping-off diseases. *T. vulgaris* extract was the most effective in suppressing the mycelia growth of phytopathogenic fungi followed by *Z. officinale* and *S. persica*. Satish *et al.* (2009) reported that

P. betle L. plant extracts was effective in controlling of *F. oxysporum* cubense. Hadi *et al.* (2013) investigated antifungal properties of some plant species and *Mentha piperita* extracts which all exhibited remarkable antifungal activity against *F. oxysporum*.

El-Mougy and Alhabeb (2009) reported the significant antifungal activity of ethanol and acetone extracts of leaves of *Piper betel*, *Carica papaya*, *Andrographis paniculata* and *L. inermis* against *F. oxysporum* the causal agent of *Fusarium* wilt in tomato. Caraway and peppermint crude extracts showed the complete growth inhibition of *S. rolfsii*. Moreira, (2008) reported no direct fungitoxicity against *C. lagenarium* isolated from water melon by ethanolic and methanolic extracts of *Cymbopogon nardus* and *Rosmarinus officinalis*.

Antifungal activity of aqueous and ethanolic extracts of *Tridax procumbens*, *Venonia amygdalina*, *Chromolaena odorata* and *Azadirachta indica* were determined *in vitro* against *A. niger*, *F. oxysporum*, *Rizospore stolonifer* and *Geotrichum candidum*, and were reported to all showed maximum efficiency in controlling the post harvest rot of tomato showing a broad range of fungitoxicity (Ijato *et al.*, 2011). Gatto *et al.* (2011) reported reduction in conidial germination and germ tube elongation of *Monilinia laxa*, *Penicillium digitatum*, *Penicillium italicum*, *A. niger* and *Botrytis cinerea* by *Sanguisorba minor* and *Orobancha crenata* extracts. Aman and Rai, (2015) screened synthetic fungicides and some botanicals for their efficacy against *Mycosphaerella musicola* causing yellow sigatoka in banana and confirm that, even though all fungicides inhibited the growth of the pathogen, mycelia inhibition concentration range varied contrastingly among them, F2 (Taqat) Captan 70 % + Hexaconazole 5 %) fungicides was more effective against *M. musicola*, with mycelia inhibition concentration range of 7.8 µg/mL, the methanolic extracts from *Orthosiphon diffuses* Benth (leaves) and *Redermacher axylocarpa* Roxb (Bark) completely inhibited the growth of the organism and maximally inhibited spore germination while the

methanolic extracts from *Garcinia cambogia* Gaertn inhibited ascospore germination efficiently but failed to exhibit any antifungal activity on actively growing fungal colonies.

The methanolic extracts from *O. diffuses* Benth, *G. cambogia* Gaertn and *R. xylocarpa* Roxb can be used in eco friendly management of sigatoka disease in banana plantations, as application of F2 can reduce the number of fungicides spraying cycles and the reduction in fungicide spraying cycles can reduce the overall consumption of fungicides, hence the combination of plant extracts (*O. diffuses* Benth, *G. cambogia* Gaertn and *R. xylocarpa* Roxb) with F2 will be more effective in integrated disease management strategy. Similarly, crude extracts of the leaf of *R. hastatus* exhibited moderate inhibitory activity against *Harpophora. Maydis* and *A. niger* and showed low activity against *F. solani*, *A. flavus* and *A. solani*. The crude extract of the leaf of *R. dentatus* showed moderate inhibitory activity against *F. solani* and showed low inhibitory activity against *A. flavus*, *A. niger* and *H. maydis*. The crude extract of *R. nepalensis* root exhibited highest activity against *A. niger* and show moderate activity against *A. flavus* and *A. solani* and no activity was noted against *F. solani* and *H. maydis*. The crude extract of *Polygonum persicaria* leaf showed highest activity against *A. flavus*, *H. maydis* and *A. solani* and low activity against *F. solani*. The crude extract of *Polygonum plebejum* (whole plant) exhibited moderate activity against *A. niger* and *H. maydis* and low activity was recorded against *F. solani*, *A. flavus* and *A. solani* (Farrukh *et al.*, 2010). Huang *et al.* (2012), studied the effect of Chinese leek (*Allium tuerosum*) on *Fusarium* wilt disease incidence and reported 58% reduction of incidence in the banana variety Baxi (AAA) and 79 % in the banana variety Guangfen No. 1 (ABB) under greenhouse conditions. In the work of Fernando *et al.* (2013), the extracts from *C. zeylanicum* and *S. aromticum* achieved best values in controlling *Fusarium* wilt disease of banana, were values obtained were equal to fungicide treatment used values.

The extracts and essential oils from *R. officinalis* and *C. nardus* were equal to fungicide in which they had no effects on the fungus (*in vitro*), and suggested that the result can represent something new on the treatment of the disease. Gopi and Thangavelu, (2014) reported in their work that *Zimmu* leaf extract at five different concentrations (5, 10, 25, 50 and 100) were used on banana plantlets inoculated with *F. oxysporum* f. sp. cubense to suppress *Fusarium* wilt disease. It was observed that at 50 and 100 % concentrations, the disease was completely suppressed and also increase the plant growth parameters such as plant height, girth, total number of leaves, leaf area, and total number of roots.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

This research was carried out in the laboratory and screen house respectively, in the Department of Plant Biology, Federal University of Technology Minna, Nigeria.

3.2 Collection of Plant Materials

Infected stalks of sugarcane and setts were collected from sugarcane farm centre at National Cereal Research Institute (NCRI) Baddegi and Wuya rake in Niger State. Fresh healthy leaves of christi thom (*Ziziphus spina-Christi*), neem (*Azadirachta indica*), henna (*Lawsonia inermis*), marhogany (*Khaya senegalensis*) and pawpaw (*Carica papaya*).were collected in sterile polythene bags from the mountain area at Egubagi villagi (table: 3.1). The plants were authenticated in the herbarium of the Department of Plant Biology, Federal University of Technology, Minna, Nigeria.

3.3 Experimental Design

A total of fifty one (51) plastic buckets (7 litre) were arranged in randomized complete block design (RCBD) with five treatments, one negative control (distilled water) and one positive control (clot) with three replicates each.

3.4 Preparation of Potato Dextrose Agar (PDA)

Two hundred grams (200 g) of peeled irish potato were washed and boiled for about 20minutes in 100ml of sterilize distilled water. The supernatant was drained in to conical flask (1000 mL) and made up to 1000 mL with sterile distilled water. Twenty grams (20 g) of agar agar powder and 20 g of glucose powder were weighed and added. The mouth of the conical flask was plugged with cotton wool wrapped in aluminium foil paper. The mixture was sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, the mixture was allowed to cool down for ten minutes, ten (10 mL) was poured into petri dishess and then 0.5 mL drop of 100 % concentration of chloramphenicol was added to prevent bacteria growth (Adebola *et al.*, 2019).

3.5 Isolation, Pusification and Identification of Pathogen from Infected Sugarcane

The infected sugarcane stalk was cut open into 1.0 - 1.5 cm segments using sterile blades with middle transition zone of healthy and infected portion. The samples were washed thoroughly under running tap water, surface sterilized using 0.5 % sodium hypochlorite for 30 to 40 seconds and washed five (5) times with sterile distilled water. The surface sterilized sample segments were inoculated on Petri dish containing Potato Dextrose Agar (PDA) at room temperature 28 ± 2 °C in an incubating chamber. Subcultures were done to obtain pure culture of the pathogen. The pathogen was identified using the cultural characteristics like colony colour and morphological characteristics such as the shape and size of macro and micro conidia under the microscope using

manuals of soil fungi (Gillman, 1957). Stock culture of the isolate was maintained in McCartney bottle slants and stored at 4 °C in refrigerator for subsequent use (Adebola and Amadi, 2010).

3.6 Preparation of Inoculum

Inoculum production was performed by inserting 5mm of culture medium with fungus in the centre of the Petri dishes containing Potato Dextrose Agar (PDA). The inoculated plate was transferred in to incubation chamber until the fungus filled the plate. After sporulation, conidia were collected and added to 10ml of sterile distilled water, rubbing a brush slightly over the colonies and subjected the suspension to constant agitation until the spores get liberate. This was used to determine the concentration of conidia suspension (Fernando, 2013). The macro and micro conidia were adjust to a concentration of 10^6 conidia /mL suspension (Ribeiro *et al.*, 2011)

3.7 Pathogenicity Test

To ascertain the pathogenicity of the organism, apparently healthy stalk of growing sugarcane (three stands) were planted inside seven (7) litre plastic buckets containing sterilized 6.9 kg of soil. The plants stalks were inoculated with 2 mL spore suspension of *C. falcatum* using a 2 mL syringe and needle, while the control pots were inoculated with sterile distilled water without conidia suspension and were observed for 30 days.

3.8 Preparation and Preservation of Plant Extracts

Plant samples collected were washed using tap water and disinfected with sodium hypochlorite (0.5 %) for 5 minutes, and rinsed thoroughly with sterile distilled water to remove the remaining sodium hypochlorite residue, and then dried with whatman No. 1 filter paper to remove excess moisture. They were shade dried under room temperature for two weeks. After drying, the leaves were grounded to powder using sterile mortar and pestle. Twenty gram (20 g) of each grounded

plant samples were weighed using electric weighing balance and poured into sterile No.1 Whatman filter paper, which was placed individually in the extracting flask of the soxhlet apparatus, after which 200 mL of ethanol was poured in to the round bottom flask of the Soxhlet setup, with the extracting chamber attached to the condenser and extracted for 24 hours at a temperature of 55 °C. More grounded plants were extracted until a substantial amount was obtained. The liquid extracts were concentrated in water bath and then preserved in airtight containers until needed for further analysis (Gnanasekara *et al.*, 2015).

Table. 3. 1. Scientific and Common names of the Plants Used for the Research

Plants (scientific names)	Common names (English)
<i>L. inermis</i>	Henna
<i>C. papaya</i>	Pawpaw
<i>K.senegalensis</i>	Marhogany
<i>Z. spina-christi</i>	Chisti thorn
<i>A.indica</i>	Neem

(Ziziphus, 1754: Orwa, 2009).

3.9 Qualitative Phytochemical Screening of the Plant Extracts.

The plant extracts were screened for biological active constituent using standard methods.

3.9.1. Test for alkaloids.

Zero point five gamms (0.5 g) of the five extracts were dissolved individually with 10 mL of dilute hydrochloric acids inside test tubes. They were filtered and filtrates were used to test for the presence of alkaloids. The filtrates were subjected to addition of Mayer's reagent and changes were

carefully observed. Formation of yellow creaming precipitate indicated the presence of alkaloids (Santhi and Sengottuvel, 2016).

3.9.2. Test for flavonoids.

About 0.5 g of plant extract from each of the extracts were heated with 10 ml of ethyl acetate in a test tube over a steam bath for 3 minutes. The mixture was filtered, 4 mL of the filtrates were shaken with 1mL of diluted ammonia solution and colour change was observed. The formation of bluish black colour reveals the presence of flavonoids (Akinyeye *et al.*, 2014).

3.9.3 Test for tannins

From each of the dried extracts, 0.5 g was taken, mixed with 20 mL of distilled water in test tubes and heated on a water bath. The mixtures were filtered and 0.1 % Ferric chloride (FeCl_3) solution was added to each extracts change was observed. Dark green or blue black colour reveals the presence of tanins (Sneh *et al.*, 2010).

3.9.4. Test for steroids

About 0.5 g from each of the plant extracts were mixed with two acetic anhydrous, additional 2 mL of chloroform and 3 mL of concentrated sulphuric acid (H_2SO_4) was carefully add and colour change was observed. Colour change from its original (violet) to blue or green indicated the presences of steroids (Santhi and Sengottuvel, 2016).

3.9.5. Test for terpenoids

Zero point five (0.5) g each from the extracts were dissolved in five (5)ml of distilled water. They were mixed with two (2) mL of chloroform and three (3) mL of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer and the appearance of reddish brown colour in the inner layer face or interface indicated the presence of terpenoids (Akinyeye *et al.*, 2014).

3.9.6 Test for anthraquinones

Five milliliters (5) mL of distilled water was used to dissolve 0.5 g from each of the extracts and was boiled with 10 % hydrogen chloride (HCl) for few minutes in a water bath. They were filtered and allowed to cool. Chloroform (CHCl₂) 10 mL was added to the filtrates, few drops of 10 % ammonia (NH₃) was added to the mixture heated and the resulting solutions were observed. Formation of pink colour shows the presence of anthroquinones (Krishnaiah *et al.*, 2009).

3.9.7 Test for phenols.

Ten (10) mL of distilled water were used to dissolve 0.01 g of each of the extracts. They were treated with addition of few drops of lead acetate solution. Noticeable yellow colour precipitates indicates the presence of phenols (Manickara and Verababu, 2014).

3.9.8 Test for saponins

Zero point five grams (0.5 g) of dried extracts from each plant were separately poured into test tubes containing five (5) mL of distilled water, shaken vigorously and observed. The appearance of creamy mix of small bubbles (frothing) showed that saponin is present (Sneh *et al.*, 2013).

3.9.9 Test for cardiac-glycosides

Five grams (5 g) from each of the plant extracts were separately mix with 2 mL of glacial acetic acid containing one drop of Ferric chloride (FeCl₃) solution, followed by the addition of 1 mL concentrated sulphuric acid. Brown ring formed at the interface indicates deoxysugar characteristics of cardenloides. A violet ring may appear beneath the Brown ring, while the acetic acid layer, formed a greenish ring gradually throughout the thin layer revealing the presence of cardiacglycosides (Akinyeye *et al.*, 2014).

3.10 *In vitro* Evaluation of the Five Plant Extracts on Mycelia Growth of *Colletotrichum falcatum*.

The plant extracts were evaluated for antifungal activities using the food poison techniques (Balouiri *et al.*, 2010). Five milliter (5) mL of the extract at different concentrations (75, 50 and 25 %) were thoroughly mixed with 15 mL of sterile potato dextrose agar (PDA) and 0.5ml of chloramphenicol contain in Petri dish with positive and negative control using 0.5 g/L and 0 % repectively. After solidification of the medium, seven days old isolate of *C. falcatum* were inoculated into the centre of the plates using 5 mm disc cork borer from their growing edges. The Petri dishes were incubated at 28±2 °C for 7 days. Three replicates for each treatment was made. Transparent meter rule (mm) was used to measure the radial mycelia growth of the pathogen and percentage inhibition was calculated after seven days using the formular (Adebola *et al.*, 2016).

$$\text{Growth inhibition (\%)} = \frac{\text{colony diameter of (control-treatment)}}{\text{Colony Diameter of control}} \times 100$$

3.11 *In vivo* Evaluation of Plant Extracts on Red Rot Disease of Sugarcane in the Screen House.

The experiment was conducted for a period of six (6) months in the screen house at Biological Garden, Department of Plant Biology Federal University of Technology, Minna, Nigeria. The treatments were evaluated on the sugarcane setts obtained from farmers on the field at Wuya Rake along Bida Mokwa road Niger State. A total of sixty (60) samples were collected and packaged inside newly bought sack and transported to the Screen House Federal University of Technology, Minna. They were planted into seven (7) litre plastic buckets containing 7 kg of sandy loam soil (cleared of unwanted plants debris and sterilized using autolave). Four weeks after germination of

the seedlings the plantlets were then inoculated using hypodermic syringe (Plate III) with 2 mL of the spore suspension at five (5) cm above the plants rhizomes. The potted plantlets were then treated with the plant extracts at three different concentrations (75, 50 and 25 %) by irrigating with 100mL of each plant extracts (Fernando *et al.*, 2013) Plate III. The control plants were irrigated with sterile distilled water (0 %) and commercial fungicide (Clot 0.5 g/L) as negative and positive controls respectively. Observation were taken after 23days of inoculation for a period of 6 months. Growth parameters such as plant height (using metre rule), girth (using tape), number of leaves and nodes (by physical counting) were measured and recorded while the severity of the vascular discolouration was also recorded using a scale of 1-6 (Gopi and Thangavelu, 2014).

$$\text{Percentage disease incidence} = \frac{\text{Number of infected leaves}}{\text{Total number of leaves}} \times 100$$

(Ajayi and Oyedele, 2016) .

Table 3.2 Disease Severity: Determined with a Visual Rating Scale of 0-5

Symptoms	Score
Reddening restrict to only point of inoculation.	0%
Reddening extend to another internodes.	1-10%
Red to brown colour extended more than two internodes.	11-25%
Red to brown colour more than 2 and 3.	26-50%
Red to brown colour more than 2 and 3 with alcoholic smelling	51-75%
Rotten of complete internal tissue of the cane with white mycelia.	75% & above

(Srinivasan and Bhat, 1961)

3.12 Data Analysis.

Data obtained from the antifungal activities of the extracts both *in vitro* and *in vivo* (field) were subjected to statistical analysis of variance (ANOVA) to determine the significant differences among means. Duncan multiple range test (DMRT) was used to separate the means, where there are significant differences. The analysis were carried out using the statistical package for social sciences, version 20, at 5 % level of significance.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

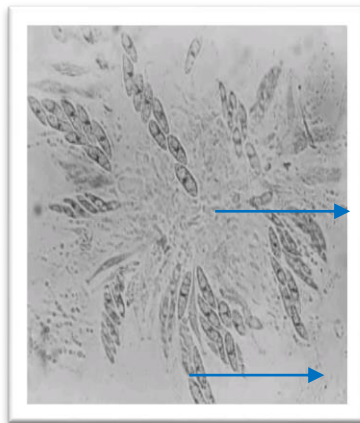
4.1 Results

4.1.1 Isolation and Identification of *Colletotrichum falcatum*

The colony turf of the isolate was light loose, cottony, and floccose, almost white in young cultures, becoming light ash with broad white margins in 7 to 14 days as shown in Plate 1. The conidia were curve or falcate shape with hyaline having two septate.



A



B



C

Plate 1: Morphology of *Colletotrichum falcatum*

A: Field Petri plate showing colony white cottony light ash Structure of *C. falcatum*.

B: photomicrograph of *C. falcatum* showing its macro and micro conidia *C. falcatum*.

C: photomicrograph of *C. falcatum* showing its mycelium bearing conidia. Source: (field photograph.)

4.1.2 Phytochemical screening of plant leaf extracts

The phytochemical analysis of the five plants extracts (Table 4.1), revealed the presence of alkaloids, flavonoids, tannins, terpenoids, cardiacglycosides and Saponins in leaf extracts of *A. indica*, while Phenols, steroids and Anthraquinones were absent. The leaf extracts of *Lawsonia inermis* contained alkaloids, terpenoids, saponnin, tannins, phenols, steroids, and anthroquinones but had no flavonoids and cardiacglycosides. *Khaya senegalensis* leaves extracts revealed the presence of alkaloids, flavonoids, phenols, and steroids while absence of terpenoids, saponins tannins, cardiacglycosides and anthroquinones were recorded. The leaves extracts from *Carica papaya* revealed that alkaloids, flavonoids, tannins, saponins, steroids phenols and steroids were present while cardiacglycosides and anthroquinones were not found. *Ziziphus spina- christi* leaves extracts contains the presence of alkaloids, flavonoids, tannins, saponnin, terpenoids, cardiacglycosides and anthroquinones, but phenol and steroids were recorded to be absent.

Table 4.1: Phytochemical Composition of *A. indica*, *L. inermis*, *K. senegalensis*, *C. papaya* and *Z. spina-christi* Plant Leaf Extracts.

Name of the plants	Phytochemicals									
	A	F	T	Sp	Tp	P	S	C	An	
<i>A. indica</i>	+	+	+	+	+	-	-	+	-	
<i>L. inermis</i>	+	-	+	+	+	+	+	-	+	
<i>K. senegalensis</i>	+	+	-	-	-	+	+	-	-	
<i>C. papaya</i>	+	+	+	+	+	+	+	-	-	
<i>Z. spina-christi</i>	+	+	+	+	+	-	-	+	+	

Key: += presence, - = absence.

A= Alkaloids, F= Flavonoids, T= Tannins, Sp= Saponins, Tp= Terpenoids, P= Phenols, S= Steroids, C= Cardiacglycosides and An= Anthraquinones.

4.1.3 Pathogenicity test

The results from the pathogenicity test (plate 2) showed that the tissue of the sugarcane that was inoculated appeared red in colour after fifteen (15) days of inoculation. The symptom thus observed was similar to the reddish colouration observed on the diseased sugarcane from which the test fungus was isolated.



Plate II: *in vitro* and *in vivo* pathogenicity test of *Colletotrichum falcatum*.

(source : field photograph).

4.1.4 Effects of ethanolic leaf extracts of *Azadirachta indica* on mycelia growth of *Colletotrichum falcatum*

The effects of ethanol leaf extracts of *A. indica* against *C. falcatum* was presented in Plate 3A and Figure. 4. 1. It shows that at day one (1), pots treated with different concentrations of plant extracts of *A. indica*, including the control pots had 0.00 % effects on the mycelial growth of the fungus. At day two (2), pots treated with 25 % and 75 % concentration of ethanolic extracts of *A. indica* inhibited the mycelia growth of *C. falcatum* to 0.9 mm and 0.5 mm respectively and there was significant difference ($p < 0.05$), while the control pot treated with sterile distilled water recorded 1.0mm. At day three (3), the concentrations of the leaf extracts at 75 % (1.2 ± 0.00), 25 % (1.6 ± 0.00) had significant difference ($p < 0.05$) and 0.5 g/L (0.0 ± 0.00) control (clot), 0 % (1.6 ± 0.00) control (sterile distilled water) significant difference ($p < 0.05$) with respect to their fungal mycelia growth. At day four (4), the concentration of 25 % (2.5 ± 0.00) with that of 0 % (2.7 ± 0.00) control had highest mycelia growth. At day five (5), there was significant difference ($p < 0.05$) between concentration of 50% (2.2 ± 0.00) and that of 0 % (3.0 ± 0.00) control (sterile distilled water). At day six (6), there was significant difference ($p < 0.05$) between the concentration of leaf extracts at 25 % (3.2 ± 0.00) and -ve 0 % (4.0 ± 0.00) control (sterile distilled water). At day seven (7), 0.5 g/L (0.0 ± 0.00) control (clot) had highest inhibitory effect with no fungal mycelia growth. But concentration of 25% (3.8 ± 0.00) and 0 % (4.35 ± 0.00) have significant difference ($p < 0.05$) compared with the other concentrations.

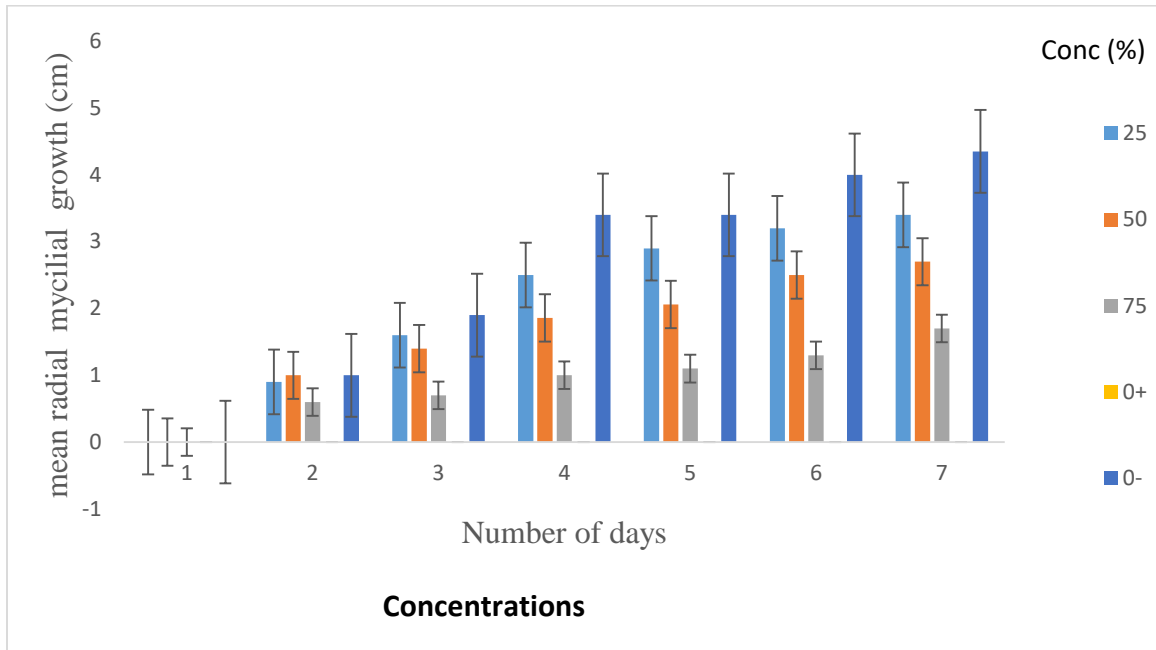


Fig. 4.1 Effect of Ethanolic Leaf Extract of *Azadiracta indica* at Varying Concentratio on Mycelial Growth of *Colletotrichum falcatum*.

4.1.5 Effects of ethanolic leaf extracts of *Lawsonia inermis* on mycelia growth of *Colletotrichum falcatum*

The effects of ethanolic leaf extracts of *Lawsonia inermis* against *C. falcatum* was presented in Plate 3E and Figure 4.2. It shows that at day one (1), all the different concentrations used had 0.00 % effect on the mycelial growth the fungus. At day two (2), there was significant difference ($p < 0.05$) between the treatment pots of 25 % (0.6 ± 0.00) concentration and that of 0 % (1.0 ± 0.00). At day three (3), there was no significant difference ($p < 0.05$) between the concentrations of 75 % (0.0 ± 0.00) pot and that of 0.5 g/L (0.0 ± 0.00), the 0 % (1.6 ± 0.00) however, had highest mycelia growth. At day four (4), there was significant difference ($p \leq 0.05$) between concentration of 75 % (0.0 ± 0.00) and 0 % (2.7 ± 0.00). At day five (5), the of leaf extracts with 50 % concentration (0.0 ± 0.00) and that of 0.5 g/L (0.0 ± 0.00) had no significant difference ($p < 0.05$). At day six (6), there was significant difference ($p < 0.05$) between concentrations of 75 % (0.0 ± 0.00) 0 % (4.0 ± 0.00). At day seven (7) there was no significant difference ($p < 0.05$) at 75 % (0.0 ± 0.00), 50 % (0.0 ± 0.00) and 0.5 g/L control (0.0 ± 0.00), however, there was significant difference ($p < 0.5$) between concentration of 25 % (1.5 ± 0.00) and 0 % (4.35 ± 0.00).

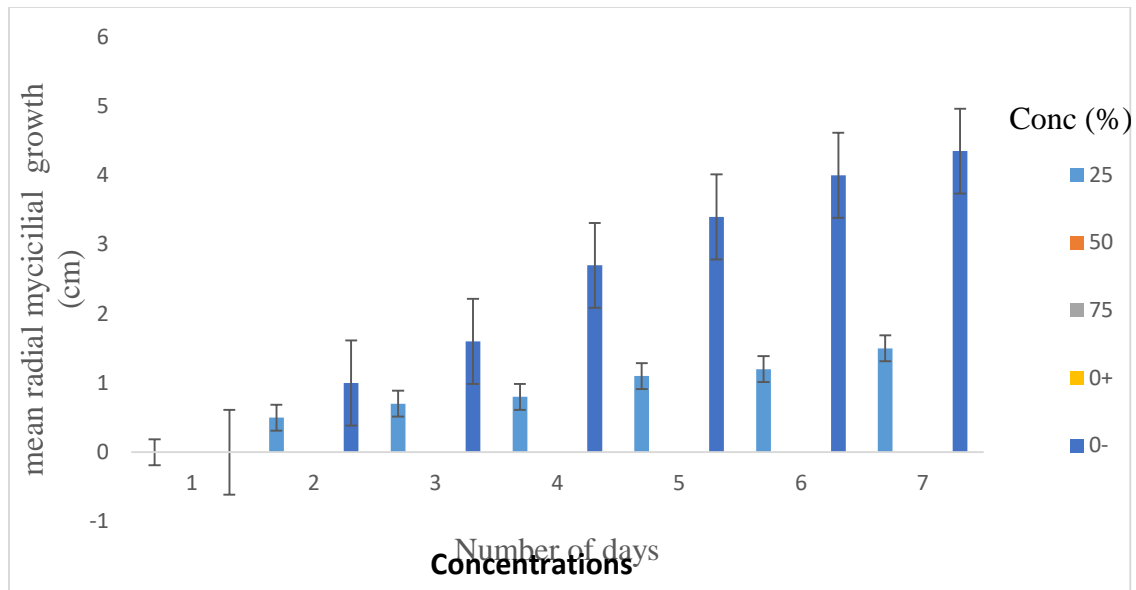


Fig. 4.2 Effect of Ethanolic Leaf Extract of *Lawsinia inermis* at Varying Concentration on Mycelial Growth of *Colletotrichum falcatum*.

4.1.6 Effect of ethanolic leaf extracts of *Carica papaya* on mycelia growth of *Collectotrichum falcatum*.

The result in Plate 3 and, Figure 4. 3 shows the effect of the ethanolic leaf extract of *Carica papaya* against *Colletotrichum falcatum*. At day one (1), It shows that all the different concentrations used had 0.00 % effect on the fungal mycelial growth of the fungus. At day two (2), there was significant difference ($p < 0.5$) between concentration of 75 % (0.0 ± 0.00) and 0 % (1.6 ± 0.00). At day three (3), there was significant difference ($p < 0.05$) between 75 % (0.6 ± 0.00) and 25 % (1.0 ± 0.00) concentrations, while 0% (1.6 ± 0.00) control (sterile distilled water) had highest mycelia growth. At day four (4), there was significant difference ($p \leq 0.05$) between concentration of 25 % (1.5 ± 0.00) and 0 % (2.7 ± 0.00). At day five (5), the concentrations of 25 % (2.0 ± 0.00) and 0 % (3.4 ± 0.00) showed significant difference ($p < 0.05$). Also at day six (6), the concentration of 25 % (2.13 ± 0.03) was significant difference ($p < 0.05$) from that of 0 % concentration (4.0 ± 0.00). At day seven (7), there was significant difference ($p \leq 0.05$) at 50% (1.8 ± 0.00) concentration and 25% concentration (2.24 ± 0.03). The concentration of 75% (1.0 ± 0.00) has significant difference ($p \leq 0.05$) with 0.5g/l (0.0 ± 0.00).

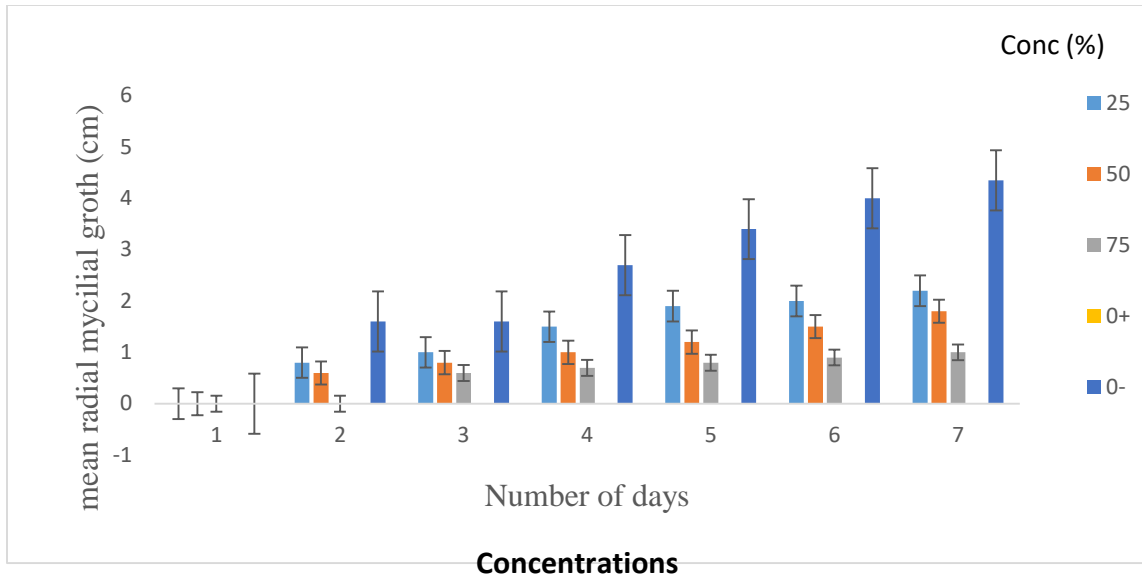


Fig. 4.3 Effect of Ethanolic Leaf Extract of *Carica Papaya* at Varying Concentration on Mycelial Growth of *Colletotrichum falcatum*.

4.1.7 Effect of ethanolic leaf extract of *Ziziphus spina-christi* on mycelia growth of *Colletotrichum falcatum*

The results in Plate 3C and Figure (4.4) revealed the inhibitory effect of the ethanolic leaf extract of *Ziziphus spina-christi* against *C. falcatu*. At day one (1), records shows that all the different concentrations used had 0.00 % effect on the mycelia growth of the fungus. Day two (2), the concentrations of 25 % (1.0 ± 0.00) and 0 % (1.03 ± 0.03) have no significant difference ($p < 0.05$). Also at day three (3), there was no significant difference ($p < 0.05$) at concentration of 25 % (1.4 ± 0.05) and 0 % (1.6 ± 0.00). At day four (4), there was significant difference ($p < 0.05$) at concentration of 25 % (1.9 ± 0.00) and 50 % (1.8 ± 0.03). At day five (5), the concentration of 75 % (1.9 ± 0.00) showed significant difference ($p < 0.05$) with that of 25 % concentration (2.3 ± 0.20), 0.5 g/L (0.0 ± 0.00) had highest inhibitory effect with no mycelial growth. At day six (6), the result revealed that there was significant difference ($p < 0.05$) between 25 % (2.7 ± 0.01) and 75 % (2.2 ± 0.01) concentrations. At day seven (7), the 0 % (4.35 ± 0.00) and 25 % (3.0 ± 0.00) concentration showed significant difference ($p < 0.05$).

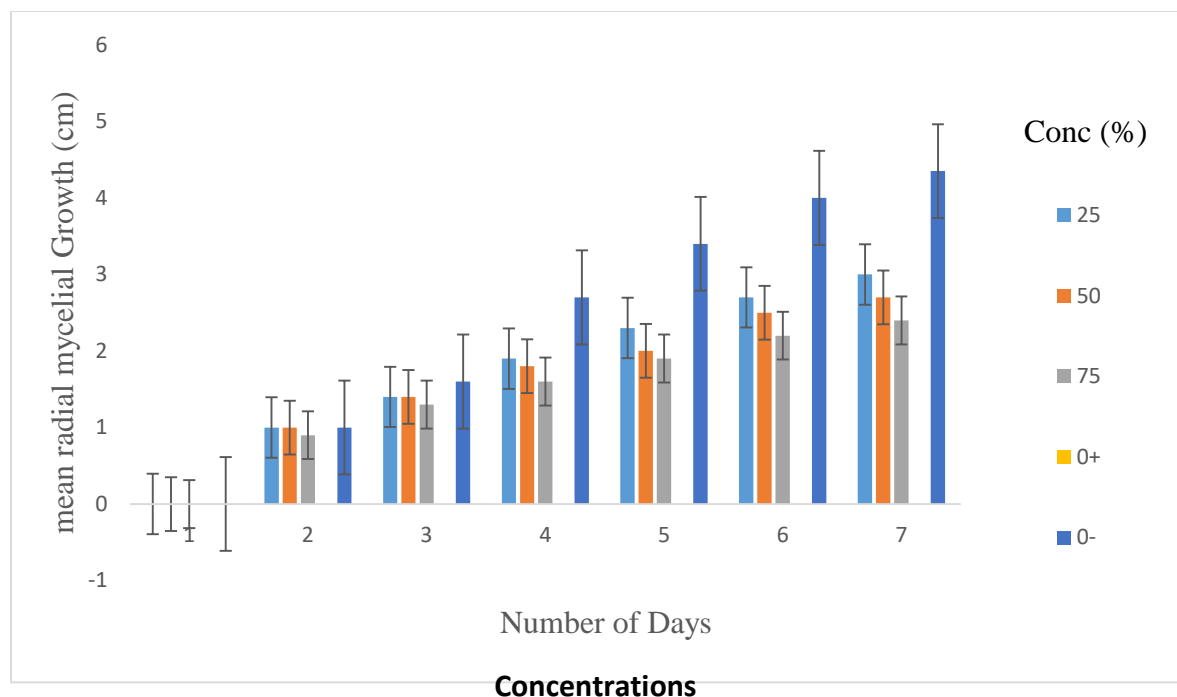


Fig. 4.4 Effect of Ethanolic Leaf Extract of *Ziziphirus spina-christi* at Varying Concentrations on Mycelial Growth of *Colletotrichum falcatum*.

4.1.8 Effect of ethanolic leaf extracts of *Khaya senegalensis* on mycelia growth of *Collectotrichum falcatum*

From the results in Plate 3B and Figure 4.5 it shows the inhibitory effects of the ethanolic leaf extracts of the *Khaya senegalensis* against *Colletotrichum falcatum*. At day one (1), record showed that all the concentrations used had 0.00 % effect on the mycelial growth of the fungus. At day two (2), there was significant difference ($p < 0.05$) on the fungal mycelia growth between the concentration of 25 % (0.7 ± 0.00) and 75 % (0.3 ± 0.00). At day three (3), there was significant difference ($p < 0.05$) on fungal mycelial growth between concentration of 25 % (1.6 ± 0.00) 0 % (1.9 ± 0.00), the 0.5 g/L (0.0 ± 0.00) control (clot) of highest inhibitory effect. At day four (4), the concentrations of 25 % (2.5 ± 0.00) and 0 % (2.4 ± 0.01) had no significant difference at ($p < 0.05$). Also at day five (5), there was significant difference at ($p < 0.05$) between the concentrations of 25 % (2.9 ± 0.01) and 0 % (3.4 ± 0.00). At day six (6), the concentrations of 50% (2.5 ± 0.05), 75 % (1.3 ± 0.00) and that of 25 % (3.2 ± 0.03) concentrations had significant difference at ($p \leq 0.05$). Day seven (7), 0.5g/l (0.0 ± 0.00). this showed significant difference at ($p < 0.05$) when compared with concentration of 25 % (3.4 ± 0.05).

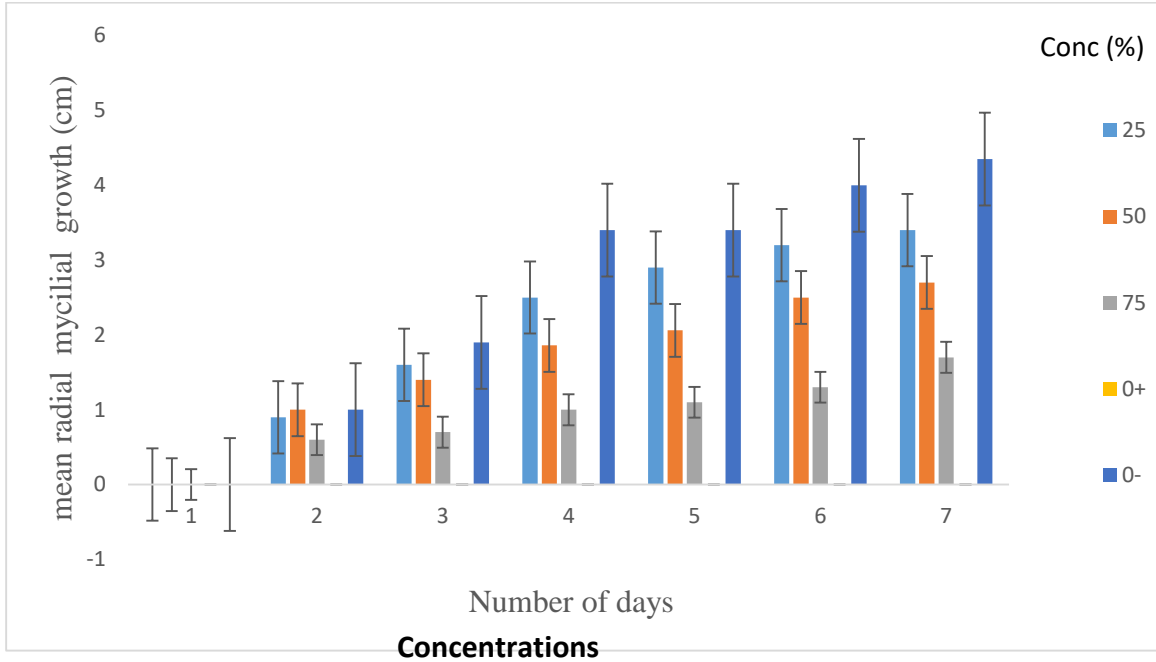


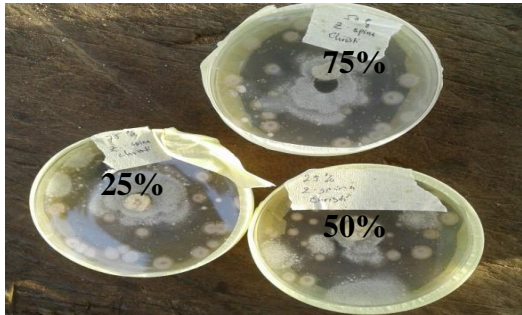
Fig. 4.5. Effect of Ethanolic Leaf Extract of *Khaya senegalensis* at Varying Concentrations on Mycelial Growth of *Colletotrichum falcatum*.



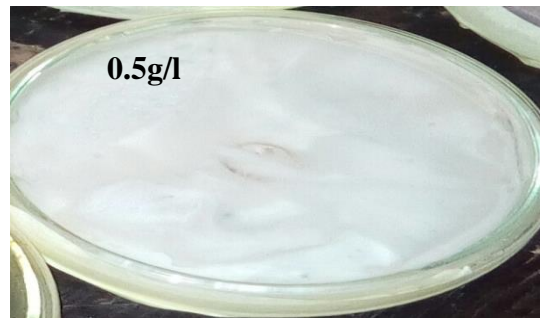
25% 50%

75%

A



B



C

D

Pate III: Inhibition effect of ethanol leaf extract of *K. senegalensis*, *A. indica*, *Z. spina-christi* and clot (chemical control)

A Inhibitory effect of the ethanol leaf extracts of *K. senegalensis* on *C. falcatum*.

B: Inhibitory effect of the ethanol leaf extracts of *A. indica* on *C. falcatum*.

C: Inhibitory effect of the ethanol leaf extracts of *Z. spina-Christi* on *C. falcatum*.

D: Inhibitory effect of the clot (positive control) on *C. falcatum*.

(Source: field photograph)



50%

25%

75%

0%

E

F



G

Plate IV: Inhibition effect of ethanol leaf extract *L. inermis*, *C. papaya* and sterile distilled water

A: Inhibitory effect of the ethanolic leaf extracts of *L. inermis* on *C. falcatum*.

B: Inhibitory effect of the water (negative control) on *C. falcatum*.

C: Inhibitory effect of the ethanolic leaf extracts of *C. papaya* on *C. falcatum*.

(source: field photograph)

4.1.9 *In vitro* Assessment of The Five (5) Plants Extracts on Mycelia Growth of *C. falcatum*

The percentage mycelial growth of *C. falcatum* at 7th day, inhibited by extracts of *A. indica*, *L.inermis*, *K. senegalensis*, *C. papaya* and *Z. spina-christi* is represented in table 4.2. The antifungal activity of the treatments on *C. falcatum* mycelial growth reduced with decrease in concentration of the extracts. The 75 % concentration of all the extracts tested showed that there was significant difference at (P <0.5) in the percentage growth inhibition, the extracts of *L. inermis*, *C. papaya*, *K. senegalensis*, in that order, have inhibition of 100, 78, 60.60 % respectively. The percentage growth inhibition at 50 % concentrations of the leave extracts of *Z. spina–christi* and *K. senegalensis* showed no significant difference (P<0.5), however, *A. indica* had 33% which observed to have significant different (P <0.05) from *L. inermis* 100 %, and *K. senegalensis* 40%. The percentage antifungal activity of extracts at 25% concentration showed significant difference (P<0.05). The extracts of *L. inermis*, *C. papaya*, *Z. spina–christi*, *K. senegalensis* and *A. indica*, in that order, have inhibition of 67, 48, 33, 25, 15.30 % respectively on the mycelial growth of *C. falcatum*. The positive control (clot) showed a maximum antifungal activity of 100 % inhibition, this is significantly different at (P<0.5) with the negative control (sterile distilled water).

Table 4.2 *In vitro* Assessment of *A. indica*, *Z. spina Christi*, *K. senegalensis*, *C. papaya* and *L. inermis* Plant Leaf Extracts on Pcentage Mycelia Growth Inhibition Seven Days After Inoculation with the Pathogen.

Treatment	CONTROLS
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Extract used	EXTRACT CONCENTRATION				
	SDTW	Clot	75 %	50 %	25 %
<i>A. indica</i>	0.00±0.00 ^{a*}	100±0.00 ^a	53.00±1.15 ^b	33.00±0.00 ^a	15.30±0.33 ^a
<i>Z. spina-christi</i>	0.00±0.00 ^a	100±0.00 ^a	45.00±1.00 ^a	40.00±1.15 ^b	33.00±0.00 ^c
<i>K. senegalensis</i>	0.00±0.00 ^a	100±0.00 ^a	60.60±0.00 ^c	40.00±1.15 ^b	25.00±0.00 ^b
<i>C. papaya</i>	0.00±0.00 ^a	100±0.00 ^a	78.00±0.00 ^d	60.00±0.00 ^c	48.00±0.00 ^d
<i>L. inermis</i>	0.00±0.00 ^a	100±0.00 ^a	100.00±0.00 ^e	100.00±0.00 ^d	67.00±0.00 ^e

Values are means of three replicates ± standard error*. Values with different superscript across the columns are significantly different (p<0.05).

Key: SDTW. S = sterile DT = destile W = water.

4.1.10 *In vivo* evaluation of *L. inermis* plant extracts used after six month of pathogen inoculation of treatment in the screen house

The results on the effects of the plant extracts on the growth parameters of the sugarcane plants were presented in table 4.3 The stalk girth of the plant treated with extract of *L. inermis* range

from (2.73–4.43 cm) due to effects of varying concentration). The controls 0.5g/l and 0 % were 4.30cm and 1.70 cm respectively. The plant height of the plants treated with the extracts of different concentration of *L. inermis*, was 41.83–64.60. The control treatments were 64.90 cm and 24.57cm long. The total number of cane nodes produced by the extracts of *L. inermis* was 3–6 while 0.5 g/L and 0% had 6 and 3 respectively. The length of internodes obtained with plant extracts ranged from 2.43-6.10 cm while 0.5 g /l and 0 % had 6.30 and 2.00 cm long respectively. Observation from the extract used showed that there was no significant different ($P<0.05$) in the cane girth of 50% and 75 % concentration while there was significant different ($P<0.05$) between 25 % concentration. Results showed that there was significant different ($P<0.05$) in cane height in extract of *L. inermis* at 75 % and 50 % concentrations. Result on the length of internodes show that there was significant difference in the extracts of 75 % and 25 % used.

Table. 4.3. Effect of *L.inermis* extracts on Qualitative Yield Parameters on Sugarcane Plant after six (6) Months of Inoculation with Pathogen

Concentrations	Cane girth(cm)	Cane height (cm)	Number of nodes	Lengths of internodes (cm)
75%	4.23±0.12 ^{c*}	64.60±0.21 ^c	6.00±0.00 ^b	6.10±0.27 ^b
50%	3.97±0.04 ^c	63.9±0.21 ^c	5.00±0.00 ^b	6.10±0.21 ^b
25%	2.73±0.06 ^b	41.83±3.06 ^b	3.00±0.00 ^a	2.43±0.23 ^a
0.5/l	4.30±0.12 ^c	64.90±0.58 ^c	6.00±0.00 ^b	6.30±0.00 ^b
0%	1.70±0.12 ^a	24.57±0.23 ^a	3.00±0.00 ^a	2.00±0.00 ^a

Values are means of three replicates ± standard error*. Values followed by the same superscript across the columns are significantly different ($p \leq 0.05$).

4.1.11 *In vivo* evaluation of *C. papaya* plant extracts after six month of pathogen inoculation of treatment in the screen house

The results on the effects of the plant extracts on the growth parameters of the sugarcane plants were presented in table 4.4. The stalk girth of the plant treated with extract of *C. papaya* range

from (1.97–4.0 cm) due to effects of varying concentration. The controls; 0.5g/l and 0 % were 4.30cm and 1.70 cm respectively. The plant height of the plants treated with the extracts of different concentration of *C. papaya* was (37.7–62.87 cm). The control treatments were 64.90 cm and 24.57cm long. The total number of cane nodes produced by the extracts of *C. papaya* was (4–5) while 0.5 g/L and 0% had 6 and 3 respectively. The length of internodes obtained with plant extract ranged from 2.33-6.10 cm while 0.5 g /L and 0 % had 6.30 and 2.00 cm long respectively. observation from the extract used showed that there was significant different ($P<0.05$) in the cane girth of all the plant extract concentrations. Results showed that there were significant different ($P<0.05$) in cane height in all extracts at different concentrations except 75 % with 0.5 5 g /L. Results on the the length of internodes show that there was significant difference in all the plant extract used.

Table. 4.4. Effect of *C. Papaya* Extracts on Qualitative Yield Parameters on Sugarcane Plant After six (6) Months of Inoculation with Pathogen

Concentrations	Cane girth(cm)	Cane height(cm)	Number of nodes	Length of internodes (cm)
75%	4.00±0.16 ^{c*}	62.87±0.24 ^d	5.00±0.00 ^c	6.10±0.00 ^c
50%	2.33±0.17 ^b	44.90±0.21 ^c	5.00±0.00 ^c	4.00±0.06 ^b
25%	1.97±0.04 ^a	37.70±0.21 ^b	4.00±0.00 ^b	2.33±0.28 ^a
0.5g/l	4.20±0.06 ^c	64.90±0.58 ^d	6.00±0.00 ^c	6.30±0.00 ^c
0%	1.70±0.12 ^a	24.57±0.23 ^a	3.00±0.00 ^a	2.00±0.00 ^a

Values are means of three replicates ± standard error*. Values followed by the same superscript across the columns are significantly different ($p \leq 0.05$).

4.1.12 *In vivo* evaluation of *K. senegalensi* plant extracts after six month of pathogen inoculation of treatment in the screen house

The results on the effects of the plant extracts on the growth parameters of the sugarcane plants were presented in table 4.5. The stalk girth of the plant treated with extract of *K. senegalensis* range from (2.03–4.03 cm) due to effects of varying concentration. The controls; 0.5g/l and 0 % were 4.30cm and 1.70 cm respectively. The plant height of the plants treated with the extracts of different concentration of *K. senegalensis* was (35.05–62.90 cm). The control treatments were 64.90 cm and 24.57cm long. The total number of cane nodes produced by the extracts of *K. senegalensis* was (3–5) while 0.5 g/L and 0% had 6 and 3 respectively. The length of internodes obtained with plant extracts ranged from (2.06-6.03cm) while 0.5 g /l and 0 % had 6.30 and 2.00 cm long respectively. Observation from the extract used showed that there was no significant different ($P<0.05$) in the cane girth of 50% and 75 % concentration while there was significant different ($P<0.05$) between these and 25 % concentration. Also it showed that there were significant different ($P<0.05$) in cane height in all extracts at different concentration. Results on the length of internodes showed that there was significant difference in all the extracts used.

Table. 4.5. Effect of *K. senegalensis* Extracts on Qualitative Yield Parameters on Sugarcane Plant after six (6) Months of Inoculation with Pathogen

Concetrations	Cane girth (cm)	Cane height (cm)	Number of nodes	Length of internode (cm)
75%	4.03±0.03 ^{c*}	62.90±0.10 ^d	5.00±0.00 ^b	6.03±0.03 ^d
50%	4.00±0.00 ^c	60.10±0.06 ^c	5.00±0.00 ^b	5.10±0.06 ^c
25%	2.03±0.03 ^b	35.04±0.03 ^b	3.00±0.00 ^a	2.06±0.06 ^b
0.5g/l	4.20±0.06 ^c	64.90±0.58 ^e	6.00±0.00 ^b	6.30±0.00 ^c
0%	1.70±0.12 ^a	24.57±0.23 ^a	3.00±0.00 ^a	2.00±0.00 ^a

Values are means of three replicates ± standard error*. Values followed by the same superscript across the columns are significantly different ($p \leq 0.05$).

4.1.13 *In vivo* evaluation of three plant extracts after six month of pathogen inoculation of treatment in the screen house

The results obtained in the Table (4.6) revealed that, at 75 % concentration of the extracts, there was promising reduction in the disease incidence of 6.94, 15.28 and 25.8 %, when treated with *L. inermis*, *C. papaya* and *K. senegalensis* respectively. The 0.5g /L concentration had 5.9 percent disease incidence, which is highly effective than 0% concentration with 40 % disease incidence. At the concentration of 50 %, it recorded that extracts of *L. inermis* had 14.67 % disease incidence which is slightly effective than *C. papaya* (20.64 %), and *K. senegalensis* was 29.17 %. At 25 % concentration, extracts of *L. inermis* showed 19.84 % disease incidence, while *C. papaya* and *K. senegalensis* showed 33.32 % and 35.09 % respectively. However, all the plants extracts recorded higher disease reduction than negative control with 40 %.

Table 4.6 Percentage Disease Incidence of Sugarcane Plants After six (6) Months of Inoculation with *C. falcatum* and Treatment with *L.inermis*, *C. papaya* and *K. senegalensis*.

Treatment (extracts)	Water control	Chemical control	75%	50%	25%
<i>L. inermis</i>	40.00±0.0 ^{a*}	5.90±0.00 ^a	6.94±1.39 ^a	14.67±3.5 ^a	19.84±4.14 ^a
<i>C. papaya</i>	40.00±0.0 ^a	5.90±0.00 ^a	15.28±3.68 ^{ab}	20.64±1.5 ^a	33.32±0.0 ^b
<i>K. senegalensis</i>	40.00±0.0 ^a	5.90±0.00 ^a	25.8±14.81 ^b	29.17±0.0 ^b	35.09±1.7 ^b

Values are means of three replicates ± standard error*. Values followed by the same superscript across the columns are significantly different (p≤0.05).

4.1.14 *In vivo* evaluation of three plant extracts after six month of pathogen inoculation of treatment in the tcreen house

The results on percentage disease severity were presented in table 4.7 and Plate 5 calculated using a scale of (0-5). The intensity of red rot pigment on the tissue of sugarcane plant treated with the three plant extracts. *L. inermis* extracts at the three concentrations 75, 50 and 25% was recorded

to suppress the disease with the disease score rate ranged from 1-2 %. *C. papaya* extracts with the concentrations of 75 %, 50 % and 25 % also had the disease score rate in the range of 1.57-3.0 %. Thus, *K. senegalensis* at the three concentrations 75, 50 and 25 % observed to have low reduction of disease with the disease score rate started from 1.95-4.0 %. The sugarcane plants treated with positive control 0.5 g/l (Clot) was observed to be recorded with a disease score rate of 1.0 % and the negative control 0 % (sterile distile water) treated plants had 5.0 % scored.

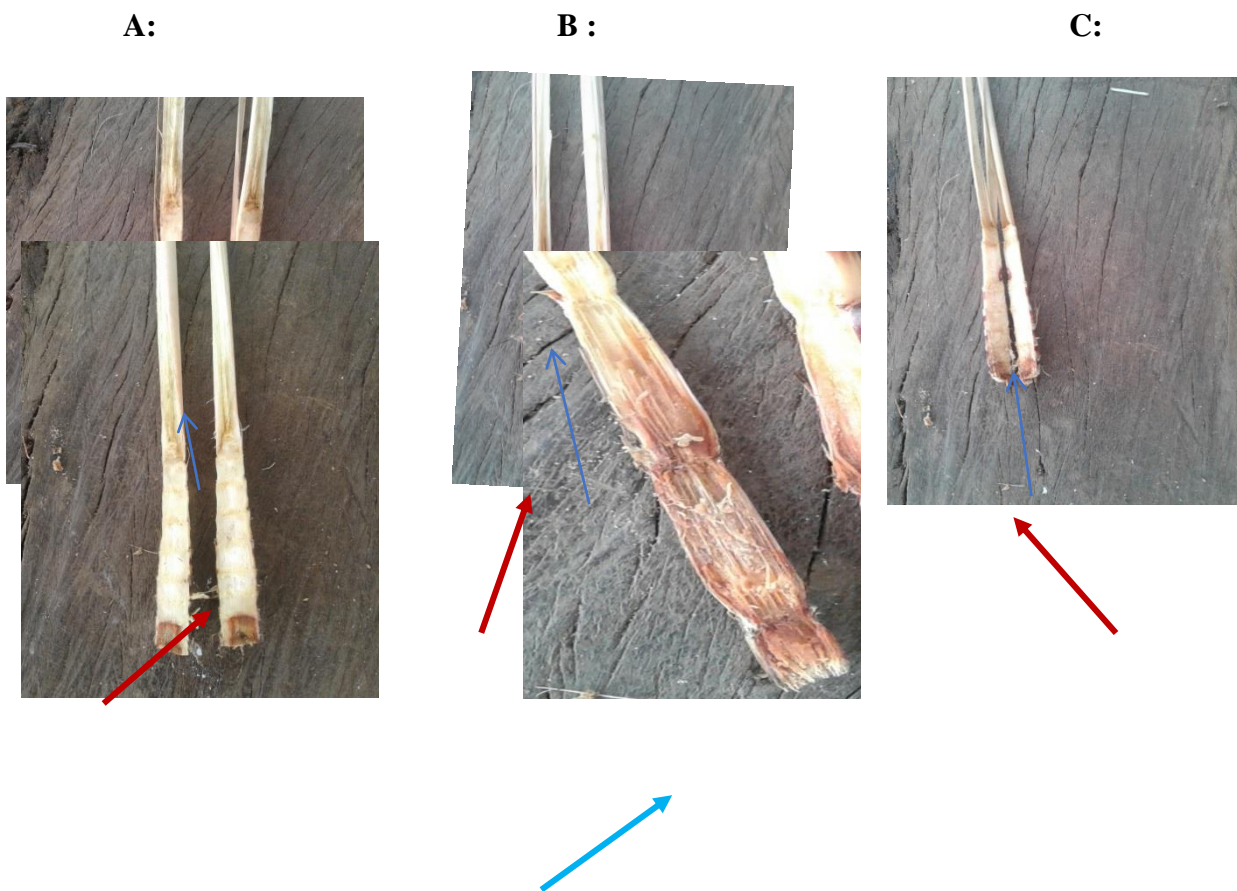
Table. 4.7 Disease Severity Score on Sugarcane Plants After Six (6) Months of Inoculation with *C. falcatum* and Treatment with *L. inermis*, *C. papaya* and *K. senegalensis*.

Disease Severity Score (0–5).

Treatment	Water	Chemical	75%	50%	25%
(extracts)	(control)	(control)			
L.inermis	5.00±0.00 ^{a*}	1.00±0.00 ^a	1.00±0.00 ^a	1.00±0.00 ^a	2.00±0.00 ^a

C. papaya	5.00±0.00 ^a	1.00±0.00 ^a	1.57±0.00 ^b	2.00±0.00 ^b	3.00±0.00 ^b
K. senegalensis	5.00±0.00 ^a	1.00±0.00 ^a	1.95±0.03 ^c	3.00±0.00 ^c	4.00±0.00 ^c

*Values are means of three replicates ± standard error**. Values followed by the same superscript across the columns are significantly different ($p \leq 0.05$).



Intensity of Red Rotten Tissue of Sugarcane Plants:

(a) Plant treated chemical (Clot) (b) plant treated with *K. senegalensis* (c) plant treated with *C. papaya*. (d) Plant treated with *L. inermis* (e) Plant treated with steriled distilled water.

Blue arrows: indicate points of rotten on sugarcane stalks and Red: arrow indicate rotten point with white mycelia on the sugarcane stalk. (field photograph)



4.2 Discussion

The morphological characteristics of *C. falcatum* observed in this study were similar to those reported by Vikash and Shukka. (2017) who reported that the colony colour of *C. falcatum* was appeared greyish white, white ashy and all conidia were falcate shape with hyaline. The results from pathogenicity test both in *in vitro* and *in vivo* revealed reddening of interner tissue after dissecting the cane stalk and wilther of the sugarcane leave planted starting from the tip of the leave, which is in conformity with the work of Sharma and Tamta, (2015) who reported after infection of *C. falcatum*, the spindle leaves display drying which wilther away at the tips along the margings and redining of the internal tissues which are usually elongated at right angles to the long axis of the stalk.

The presence of some biological active components such as alkaloids, flavonoids, tannins, saponnin, steroids, cardiacglycosides and anthraquinones detected in the leaf extracts of *A. indica* in this findings is in line with observations of Adebola *et al.* (2019) who reported the presence of tannins, flavonoids, saponnin, alkaloids, trepenes in the healthy leaves of the plant. The presence of phytochemical components reported to be present in the leaf extracts of *L. inermis* is similar to

previous findings of (Chukwu *et al.*, 2011; Wangini *et al.* 2014) who confirmed the presence of alkaloids, saponnins, tannins, glycosides and flavonoids in *L. inermis*.

Findings on the phytochemical constituent of the leaf extracts of *Z. spina-christi* observed in this study, were similar to those reported by Dangogo *et al.* (2012) and Haiti and Ahmed (2015) who also reported presence of alkaloids, tannins, saponnin, tannins, saponin, glycosides, steroids, flavonoids, and terpenoids in the leaf extracts of *Z. spina-christi*.

Bioactive compounds such as; cumarins, flavonoids, carbohydrates, steroids and terpenoids observed in the leaf extracts of *K. senegalensis* correlate with those reported by Elish *et al.* (2015). Also alkaloids, flavonoids, and steroids that were observed is in agreement with those reported by Kurta, *et al.* (2015).

The bioactive phytochemicals recorded in the leaves extracts of *C. papaya* in study is similar to those reported by (Eman *et al.*, 2014; Adebola *et al.*, 2018) who reported the presence of alkaloids, flavonoids, tannins, terpenoids, phenols in the leaves and seed extracts of *C. papaya*. However, the difference in phytochemicals recorded in this study as compared to those reported by other researchers, could be attributed to variation in environmental factors, type of solvent used and method of extraction (Eman *et al.* 2014).

Therefore, in respect to the five plant extracts evaluated in this study, it revealed the presence of some important bioactive compounds which are attributed to their antifungal activity. Some researchers also attributed antimicrobial activity of plant extracts to phenolic compound (Abu-Taleb *et al.*, 2011). Dangogo *et al.* (2012) also reported that, antimicrobial activity ranging from antifungi to antibacterial as a result of alkaloids, saponnin, and terpenoids that are found in the plant extracts made it possible to ascertain their antimicrobial potential on microorganism. Steroids

in plants have been also reported for their antimicrobial, cardiogenic and insecticidal properties (Aiyelagbe and Osamudiamen, 2009).

Interestingly, it was observed in this study that, an increase in concentration of all the plant extracts tested, resulted in an increase in their antifungal activity, this might be due to the increase in the quantity of the active secondary metabolites. Records on daily growth of *Colletotrichum falcatum* proved that the ethanolic leaf extract acted as an inhibitory constituent to the amended media. The inhibitory effects of the ethanolic leaf extracts of the plants may be due to the presence of some phytochemicals like tannins, saponins, flavonoids, alkaloids, phenols, anthraquinones and terpenoids that have antimicrobial properties. However, findings from this result revealed that, percentage inhibition on the tested organism decrease with a decrease in concentration. This opinion was shared by Adebola *et al.* (2016) who reported that extracts possessed significant antimicrobial activity in a concentration dependent manner. *L. inermis* leaves extracts exhibited the best antifungal activity, this view is in accordance with the works of Zarrin *et al.* (2013) and Khan and Nasreen, (2010) who tested several medicinally important plant and reported *L. inermis* exhibit best antifungal and antibacterial potency.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The findings of this research study conclude that the phytochemical constituents: alkaloids, flavonoids, tannins, saponins, terpenoids, phenols, steroids, cardiacglycosides and anthraquinones were present in the ethanolic leaf extracts of *A. indica*, *L. inermis*, *C. papaya*, *K. senegalensis* and *Z. spina-christi*. It is also concluded that the efficacy of the ethanolic leaf extracts of these plants increased with increase in concentration. The extracts of *L.inermis* demonstrated the best inhibitory effect on the fungus during *in vivo* evaluation and improved the growth parameters of the sugarcane plant. Further more, it is also concluded that the leaf extracts have differential effect on plant growth parameter and fungal radial growth.

5.2 Recommendations

From the findings of current research work, the following are recommended.

- i. Further research work should be carried out using Gas Chromatographyl Mass Spectroscopy (GCMS) and Nuclear Magnetic Resonance (NMR) to find out active ingredients responsible for clearing the growth of fungi and improving the growth parameter of crops.
- ii. It is also recommmed that the active ingredients responsible for enhancement of growth parameter can be used as bio control.

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APPENDICE A

Percentage disease incidence of sugarcane plants after six (6) months of inoculation with *C. falcatum* and treatment with *L.inermis*, *C. papaya* and *K. senegalensis*

		Sum of Squares	df	Mean of Square	F	Sig.
0% (water)	Between Groups	0.000	2	0.000		
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
0.5%g/l (clot)	Between Groups	0.000	2	0.000		
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
75%	Between Groups	536.543	2	268.271	6.94	.027
	Within Groups	231.651	6	38.609		
	Total	768.194	8			
50%	Between Groups	318.805	2	159.403	10.6	.011
	Within Groups	89.772	6	14.962		
	Total	408.577	8			

		Sum of Squares	df	Mean of Square	F	Sig.
GIRT	Between Groups	14.965	4	3.741	116.650	.000
	Within Groups	.321	10	.032		
	Total	15.286	14			
HEIGHT	Between Groups	3900.487	4	975.122	165.552	.000
	Within Groups	58.901	10	5.890		
	Total	3959.388	14			
NODES	Between Groups	28.267	4	7.067	15.143	.000
	Within Groups	4.667	10	.467		
	Total	32.933	14			
INTERNODES	Between Groups	47.979	4	11.995	116.447	.000
	Within Groups	1.030	10	.103		
	Total	49.009	14			
25%	Between Groups	417.292	2	208.646	10.3	.011
	Within Groups	121.421	6	20.237		
	Total	538.713	8			

APPENDICE B

Effects of *C.papaya* extracts on qualitative yield parameters on sugarcane plant after six (6) months inoculation with pathogen

APPENDICE C

**Effects of *K. senegalensis* extracts on qualitative yield parameters sugarcane plant
 afters six (6) months inoculation with pathogen**

		Sum of Squares	df	Mean of Square	F	Sig.
GIRT	Between Groups	17.836	4	4.459	393.441	.000
	Within Groups	.113	10	.011		
	Total	17.949	14			
HEIGHT	Between Groups	3998.605	4	999.651	4143.231	.000
	Within Groups	2.413	10	.241		
	Total	4001.018	14			
NODES	Between Groups	20.267	4	5.067	76.000	.000
	Within Groups	.667	10	.067		
	Total	20.933	14			
INTERNODES	Between Groups	28.303	4	7.076	1516.214	.000
	Within Groups	.047	10	.005		
	Total	28.349	14			

APPENDICE D

Disease severity score on sugarcane plants after six (6) months of inoculation with *C. falcatum* and treatment with *L. inermis*, *C. papaya* and *K. senegalensis*

Disease severity score (0-5)

		Sum of Squares	df	Mean of Square	F	Sig.
0%(water)	Between Groups	0.000	2	0.000	297.063	.000
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
0.5g/l	Between Groups	0.000	2	0.000		
	Within Groups	0.000	6	0.000		
	Total	0.000	8			
75%	Between Groups	1.373	2	.687		
	Within Groups	.014	6	.002		
	Total	1.387	8			
50%	Between Groups	6.000	2	3.000		
	Within Groups	0.000	6	0.000		
	Total	6.000	8			
25%	Between Groups	6.000	2	3.000		
	Within Groups	0.000	6	0.000		
	Total	6.000	8			