EFFECTS OF SOME PLANT EXTRACTS AGAINST *Pythium myriotylum* CAUSAL AGENT OF POST EMERGENCE DAMPING-OFF DISEASE IN *Amaranthus hybridus*

BY

MOHAMMED, Habib Baba
MTECH/SLS/2018/8260

DEPARTMENT OF PLANT BIOLOGY
FEDERAL UNIVERSITY OF TECHNOLOGY
MINNA

FEBRUARY, 2022

EFFECTS OF SOME PLANT EXTRACTS AGAINST *Pythium myriotylum* CAUSAL AGENT OF POST EMERGENCE DAMPING-OFF DISEASE IN *Amaranthus hybridus*

BY

MOHAMMED, Habib Baba
MTECH/SLS/2018/8260

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF TECHNOLOGY (M. TECH) IN BOTANY (MYCOLOGY)

FEBRUARY, 2022

ABSTRACT

The use of plant botanicals have gained a lot of attention in recent times, owing to the health and economic implications associated with their uses. *Amaranthus hybridus* has numerous nutritional values but it is affected largely by post emergence damping off disease. This research investigated the efficacy of the leaf extracts of three plant Neem (*Azadirachta indica*), Mango (*Mangifera indica*) and Mahogany (*Khaya senegalensis*) against *Pythium myriotylum* the causative pathogen of post emergence damping off disease in *Amaranthus hybridus*. The plant samples were collected, dried and extraction was done using the soxhlet apparatus. Qualitative and quantitative phytochemical screening was done using standard procedures. The causative pathogen (*Pythium myriotylum*) was isolated from infected A. *hybridus* leaves and the rhizosphere on PDA medium. Agar well diffusion technique was used to test for antagonism using three extract concentrations at 500, 800 and 1000 mg/mL. The control was without extract and three replicates of each were made. Incubation was done at 28±2 ^{o}C for 7 days. The radial growth of *P. myriotylum* at day 7 from Mahogany extract at 500, 800 and 1000 mg/ml concentrations were 4.00, 3.99 and 3.99 cm respectively, while values for Neem extracts were 3.98, 3.86 and 3.55cm and Mango extract were 4.00, 3.12 and 2.75 cm. The results obtained showed that the plant extracts have positive potentials towards inhibiting the pathogen (P. *myriotylum*) with Mango extract having the highest inhibition potential followed by Neem extract and Mahogany extract recorded the lowest. The percentage inhibition at 500, 800 and 1000 mg/mL from Mahogany extract across the concentrations were 0.00, 0.25 and 0.25 % respectively. While Neem were 0.5, 3.5 and 11.25 % and the percentage inhibition from Mango extract were 0.00, 22.00 and 31.25 %. The study shows that mango extract had the highest inhibitory potential against the pathogen with 31.25 % at 1000 mg/ml concentration, while Neem extract followed with 11.25 % and Mahogany extract had the lowest inhibition with 0.25 % at 1000mg/ml. The results obtained from the *invivo* study showed that A *hybridus* seedlings that were infected with the pathogen developed resistance to the pathogen across all concentration gradients of the plant extracts except from Mahogany which could only suppress the disease at the highest concentration of 1000 mg/ml. Therefore, this study supports the use of these botanicals in the control of plant damping off disease of A. *hybridus* as it has proven to be effective.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the Study

Amarathus hybridus L., popularly called *amaranth* or pigweed is an annual herbaceous plant of 1.6 feet high. The leaves are alternate petiole, 3 - 6 inches long, dull green and with rough hairy ovate or rhombic and wavy margins. The flowers are small, with greenish or red terminal panicles. Taproot is long flashy red or pink. The seeds are long lenticellular in shape, with each seed weighing 0.6 - 1.2 g (Oluwole *et al.*, 2013)

Amarathus hybridus is an annual herbaceous plant belonging to the family *amaranthaceae* with green or red leaves branches stalks bearing small seeds variable in colour from cream to gold and pink to black. There are about 60 species of *Amaranthus*, however only a limited number are of the cultivated type, while most are considered weedy species and hence, rarely preserved. Many amaranth species are collected from the wild for subsistence, while only few are cultivated or occur as protected weeds in backyards and home gardens (Oluwole *et al.*, 2013).

Most developing countries depend on starch-based foods as the main staple food for supply off both energy and protein. This account in part for protein deficiency which prevails among the populace as recognized by food and agricultural organization (Ladeji *et al.*, 2019). In Nigeria as in most other tropical countries of Africa, where the daily diet is dominated by starch staple foods vegetables are the cheapest and most readily available source of important proteins, vitamin, minerals and essential amino acids (Okafor, 2020).

Damping off is a horticultural disease caused by pathogens that kill or weaken seeds or seedling before or after germination. There are various symptoms association with damping off, these reflect the variety of different pathogenic organisms which can cause the condition, however all symptoms result in the death of at least seedlings in any given population. Fungi species implicated are *Pythium, Sphanomyces* and *Fusarium*. (Abang *et al.,* 2012).

Chemicals are used to control the diseases but the use of chemicals have been discouraged owing to the toxic effect it has on humans and the ecosystem (Walker and Lynch, 2007). Hence, other measures or biological control such as the use of plant extracts which are non-toxic, eco-friendly and easy to obtain (Gurjar *et al.*, 2012).

Hence the effect of *Azadirachta indica, Khaya senegalensis* and *Carica papaya* leaf extracts were examined for their viability in the control of *Pythium myriotylium* damping off disease in *Amaranthus hybridus.*

1.2 Statement of Research Problems

Human beings have been using plants ever since the beginning of the civilization and out of the estimated total of 30,000 plants that have been found to be edible in nature, approximately 7,000 plants have been cultivated by mankind at one point or the other out of which only 158 plants are widely used for food (Gupta *et al.*, 2008).

Damping off in *A. hybridus* is a condition caused by a number of different pathogens that kill or weaken seedlings before or after germination. Leading to loss of most or sometimes all the seedling in a population (Abang *et al.*, 2012). Numerous methods are used to control the damping off disease in *Amaranths* such as the use of chemicals, but due to the health and cost implication on the use of chemicals, biological controls are employed (Sengar *et al.*, 2009).

1.3 Aim and Objectives of the Study

The aim of this study is to evaluate the effects *Azadirachta indica, Carica papaya* and *Khaya senegalensis* leaf extracts on the control of *Pyium myriotylium* damping off disease in *Amaranthus hybridus*.

The specific objectives are to:

i. determine the Phytochemical constituents of *Azadirachta indica, Mangifera indica* and *Khaya senegalensis* leaf extracts.
ii. isolate and identify the fungi species causing damping off disease in *A hybridus.*
iii. control damping off disease using the selected plant extracts.

1.4 Justification for the Study

Amaranthus hybridus has various nutritive value which are essential for human consumption. Botanicals are increasingly gaining more significance as they are use in the control of fungi pathogens. The phytochemicals act on the pathogen or induce resistance in plants (Yulier *et al.,* 2015). They are also less expensive than chemicals readily available and friendly to the environment, they play significant roles in disease management including Amaranth damping-off (Hafiz *et al.,* 2016). Plant extracts have unveiled a new scope for bio-control of plant diseases and the secondary metabolites such as phenols, alkaloids and terpenoids in *Khaya senegalenss, Azadirachta indica* and *Carica papaya* which have been used by various scientists enacted the curiosity for this work.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Damping-Off Disease

Soilborne plant pathogens causing wilts, root and crown rots, and damping-off are major yield-limiting factors in the production of fiber, food and ornamental crops. Damping-off diseases are found worldwide and can be caused by several species of fungi under various weather conditions. The name damping-off is its standard use in the literature usually refers to the disintegration of stem and root tissues at and below the soil line. The plant tissues become water-soaked and mushy, and the seedling wilts and falls over. Damping-off is caused due to the attack of seedlings by soil-inhabiting fungi usually just at the soil level on to hypocotyls or upper taproot. This causes partial or complete rot and the seedlings suddenly topple over in a characteristic manner (Moorman, 2019).

The damping-off has been known for a long time in both Europe and America (Tomioka *et al*., 2018). By the beginning of the twentieth century in the United States, as early as 1901 to 1905, the damping-off disease was found in forest nursery seedbeds and began to receive attention (Smith *et al*., 2019). This disease is encountered in seedbeds where plants are propagated to be transplanted later in the fields. However, greenhouses and nursery seedbeds are ideal places for outbreak of damping-off, if moisture and temperature conditions are favorable (Bahramisharif *et al*., 2018).

Damping-off diseases, however, can have several phases, the fungi that cause these diseases can attack the seed or the seedling below the soil line before it emerges, causing a pre-emergence damping-off where seeds become soft and mushy, turn dark

brown and germinating seedlings shrivel, and may darken. Pre-emergence damping-off disease is difficult to be diagnosed because the seeds are not visible; consequently, the losses are often attributed to poor seed quality or seed maggots rather than to the presence of a disease. On the other hand, post-emergence damping-off causes death of seedlings after emergence or transplanting at the soil line where stem tissue near the soil line is weakened and decayed, usually causing plants to topple and die. When only roots are decayed, plants may continue growing but remain stunted, wilt, and eventually die. As seedlings get older, they become more resistant to damping-off pathogens (Tomioka *et al.*, 2018). Most pathogens that cause damping-off diseases are responsible for diseases as the plant grows to maturity. Root rot, rown rot, stem lesions, basal rot, crater rot, bottom rot, and stem girdling diseases may all be associated with damping-off pathogens attacking mature plants.

Damping-off has been found to affect up to 80 % (in some cases even 100 %) of seedlings, resulting in poor seedling stands in nurseries of various crops (Agrios, 2019). Almost any kind of plant may be attacked by damping-off fungi while in the young, tender, succulent stage of development. The incidence of disease is dependent more upon the conditions under which the seedlings are grown than the particular species of plant concerned (Bahramisharif *et al*., 2019).

The two fungi that are most often associated with damping-off are *Rhizoctonia solani* and *Pythium species*. *Rhizoctonia solani* is found in most agricultural soils and survives between crops on plant residues and as microsclerotia. This pathogen usually attacks seedlings at or near the soil surface. Initial symptoms are stem lesions that are brick red to brown and sunken. If the disease progresses, the stem may become girdled. Stem canker, soreshin, wirestem, and damping-off are names associated with seedling and post emergent diseases caused by *R. solani.*

Pythium on the other hand is a cosmopolitan and biologically diverse genus. Most species reside in soil inhabitants and are saprobes or facultative or opportunistic plant pathogens, although some are aquatic inhabitants. Damping-off diseases caused by *Pythium* species usually begin as root rot. This group of fungi survives as oospores in the soil that germinate to attack root tips and root hairs, causing a progressive deterioration of the root. The seedling may wilt or rot in the ground. *Pythium* species are often responsible for pre emergence damping-off (Agrios, 2019). The environmental conditions that favor damping-off vary according to the pathogen. Pythium spp. tend to be most active during the period when soil moisture is plentiful (Yang, 2019). Research has reported that although damping off disease is usually caused by fungi or oomycetes, stresses such as high surface soil temperatures and chemicals can also cause damping-off symptoms. Other fungi that can be associated with seedling or transplant damping-off are *Botrytis cinerea, Sclerotinia sclerotiorum, S. minor, Alternaria* species, *Phytophthora* species, *Fusarium* species, and *Thielaviopsis basicola*. Damping-off disease may affect 5% to 80% of the seedlings, thereby inducing heavy economic consequences for farmers. Much research efforts have been made in recent years to develop bio-control solutions for damping-off, which have interesting future perspectives.

2.1.1 Economic importance of damping-off disease

Damping-off can be a devastating disease and cause huge economic loss. Damping-off mainly affects plants prior to seed germination and throughout the seedling stage (Agrios, 2019). Crop losses due to damping-off disease vary from place to place. Moderate to severe incidences, leading to crop loss of more than 50 % to 80 %, have been reported because of this disease (Lamichhane *et al*., 2017). The amount of crop loss depends on the stage of crop growth at which the infection starts. If it occurs early,

total crop loss of the affected clump will result into huge loss, whereas the loss is partial if the plant is affected at a later stage (Agrios, 2019). *Pythium* spp. also cause catastrophic loss in storage. Crop losses inflicted by soil-borne pathogens continue to increase and become a limiting factor in stabilizing and maximizing crop yields on a worldwide basis (Kennelly, 2019).

A **B**

Plate I; Damping-off of seed and B; Damping-off of leaves caused by Pythium (Source: Martin and Loper, 2019).

2.1.2 Environmental conditions of disease development

The risk of *Pythium* blight is high during humid weather when day temperatures are 30 ^{o}C to 35 ^{o}C and a consistent decrease overnight to 20 ^{o}C at least (Kennelly, 2019). The disease is most common when soils are saturated with water, due to excessive rainfall or irrigation. Also, long dew periods, high relative humidity, and lush and dense turfgrass growth favors the disease development. Areas of low-altitude, poor airflow and poor drainage are particularly vulnerable (Mundel *et al*., 2018). The soil pH influences some phases of life-cycle of the *Pythium* species, such as the formation of oospores and

sporangia. *Pythium* species prefer to grow in alkaline soils (above pH 8 - 9) (Lumsden *et al*., 2017). While the soil pH between 5.2 and 5.7 (moderately acidic) prevents damping-off problems. Aluminum sulfate drenches, sulfur (200 to 500 pounds/acre), and acid peat applications can be used to maintain the soil pH.

Pythium species differ in soil temperature requirements. Some *Pythium* species prefer low soil temperature (5 to 10 ^{o}C), such as *P. ultimum* and *P. irregulare*, while others thrive in warmer temperatures (25 to 30 ^{o}C), such as *P. aphanidermatum*. Spore germination and germ tube growth are influenced by soil temperature, with greater zoospore discharge from sporangia at lower soil temperature, while oospore germination and germ tube growth occur at relatively higher temperatures (Mundel *et al*, 2018; Agrios, 2019).

2.1.3 Symptoms of damping-off

Based on the time of appearance, the disease symptoms can be classified into two phases. The first is pre-emergence, in which sprouting seeds decay in soil and young seedlings rot before emergence. The second is post-emergence phase, which is characterized by the infection of the juvenile tissues of the collar at the ground level (Bacharis *et al*., 2020). Damping-off symptoms are most commonly seen as poor stand establishment in seeded areas, often giving them a ‘patchy’ look, since the plants are highly vulnerable after germination when a little damage can kill the plant (Tomioka *et al*., 2018). Typical symptoms of damping-off are rotting stems at or near the soil; line and root decay (post-emerging damping off). Affected areas in the seedbed are usually a 30cm or more in diameter with shriveled brown, collapsed or stunted seedlings (Olson *et al*., 2019). *Pythium* attacks often at root tips (Moorman, 2019) while moldy fungal growth may be observed on affected plants at the soil line. Also, germinating seeds can

be attacked by these fungi before they emerge from the soil (pre-emerging damping off), resulting in poor stands. Usually, affected plants occur in patches in nursery beds or in the fields at low elevations (Olson *et al*., 2019).

Seedlings that survive from infected nurseries often show the so-called “wire stem” or “stem girdling” syndrome (i.e. a blackish sunken lesion girdles the base of the stem, where the stem becomes thin and hard, especially at soil level, and may bend or twist) resulting in reduced vigor (Lamichhane *et al*., 2017). If the infection occurs before the emergence, the germinating plants become soft, brown, and decompose. While, if the infection happened after emergence, water-soaked lesions form about one cm above or below the soil line (Alcala *et al*., 2019).

The stem will be softened and cannot support the seedling, which collapses and die. Stunting of plants due to root-rot may also occur. In this case, the root system rots become brown and develops few secondary roots (Kennelly, 2019). In flat fields, affected plants are generally found in scattered parts (Horst, 2018). Infection by the pathogen may occur much later after emergence; in this case, the infection usually is not lethal but plant growth and yield may be reduced (Moorman, 2019). Severe infection in the root region may cause wilting in warm or windy weather. Also, extensive root rotting induces symptoms of nutritional deficiencies in the plant, since nutrients cannot move sufficiently from the soil up through the plant. However, pathogen isolation and identification are needed to confirm diagnosis, because several pathogens can cause similar symptoms, (Horst, 2018; Olson *et al*., 2019).

The fungus attacks the seedlings at hypocotyls or taproot and when the tissue is susceptible and contributes to the initiation of a thin cell wall during rapid growth (Scheuerell *et al*., 2018). The disease symptoms can be observed from seeding until four

to six weeks post-sowing (Horst 2018; Olson *et al*., 2019). The only evidence of pre-emergence damping-off in nursery beds is that the germinating seedlings are sparse and patchy (Agrios, 2019). This phase is difficult to detect, but sometimes may be diagnosed by digging up seeds that have not emerged and checking to see whether seeds or germinated seeds are decayed or withered. Post-emergence damping-off, which occurs at the cotyledon stage, causes wither and collapse of the seedlings. When the succulent root-collar tissue or the roots is penetrated by the pathogen, the disease is defined as soil-infection damping-off. While, when the fungal invasion occurs at a higher level on the stem or cotyledons, it is called top infection damping-off (Moorman, 2019).

There are multiple cases that have similar symptoms to damping-off, where sometimes the seed may not germinate if it is old or if conditions have not been favorable for germination. Also, if tender young seedlings are over fertilized, roots appear shriveled and desiccated, and the plants die due to the high salts. In addition, hot water, heat stress, lack of water and chemical sprays can also prompt death to the tender young seedlings (Daughtery *et al*., 2019).

2.2 Pythium Species: Causal Agent of Damping-Off and Root Rot

The genus Pythium is a part of the Peronosporales order of the class Oomycetes or the phylum Oomycota, a member of the kingdom Chromista (Stramenopiles) (Kirk *et al*., 2018). The Pythium spp. are no longer considered to be true fungi; instead, they were found to be more closely related to algae (Bauldaf *et al*., 2020). The class Oomycetes differ from true fungi in that they have coenocytic hyphae, a diploid vegetative stage, cell wall content of glucan and cellulose, biflagellate zoospores with tinsel, and whiplash flagella. The Pythium genus contains species that are pathogenic to plants, animals, algae and other fungi. More than 330 species of Pythium have been identified

to date of which many are pathogenic to plants. These pseudo-fungi cause many plant diseases, including damping-off, root rot, collar rot, soft rot, and stem rot in different production systems such as nurseries, greenhouses, and agricultural fields (Bauldaf *et al*., 2020).

2.2.1 Host range and distribution

Pythium has been found in Australia, Canada, Brazil, China, Korea, Japan, South Africa and many other countries in the world including most states within the United States of America. It causes *Pythium* blight of turfgrass, inflicting very serious damage in golf courses (Allen *et al*., 2019). It can also infect crops like cabbage, broccoli, cucumber, carrot, melon, cotton and wheat (El-Mohamedy, 2017). Surveys of the diversity of *Pythium* species in agricultural soils have demonstrated that it can be common to isolate more than one species in a field, including both pathogenic and non-pathogenic species. However, several pathogenic *Pythium* species can be associated with damping-off in a particular field, making management of the disease more complicated (Broders *et al*., 2019).

2.2.2 *Pythium myriotylum*

Pythium myriotylum is one of the most common species found in greenhouse production systems due to its broad host range. *Pythium myriotylum* isolated from root systems of bell peppers was also pathogenic to tomato, causing severe weight loss (Chellemi *et al*., 2000). Four *Pythium* species were isolated from diseased hydroponic tomato roots, one of which was *P. myriotylum* (Jenkins and Averre, 2018). *P. myriotylum*, which was first isolated from tomato, is very similar to *P. zingiberis* in terms of morphology and some gene sequences (Lévesque and De Cock, 2019). It is however known to have a worldwide distribution and attacks numerous hosts. Stirling *et al.* (2019) observed that

disease severity caused by *P. myritotylum* on vegetables was more strongly influenced by temperature rather than soil moisture. The pathogen was also very aggressive on vegetables showing symptoms on above ground parts of plants within a week of inoculation (Wang and Chang, 2018).

2.2.3 Integrated disease management

Utilization of a range of control strategies such as biological, cultural, and chemical is needed to keep the disease below the economic thresholds of a sustainable agro-ecosystem. Integrated disease management has been reported to be quite effective for control of soil-borne plant pathogens (Castillo, 2019). Preventing the disease is considered one of the most effective control strategies, as there is no cure for damping off once it occurs. Even though some fungicides can control this pathogen, nowadays researchers are more interested in biological control agents and their antifungal metabolites due to the development of resistance in the pathogen. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and ecofriendly method for plant disease management (Rajendraprasad *et al*., 2017).

2.2.3.1 Prevention and cultural management

Economical control of plant disease is rarely achieved by a single procedure. It must be supported by the use of planting material that is free of the pathogen, tillage methods that are unfavorable to the pathogen but favorable to the host, the use of antagonists, sanitation, use of fungicides, and especially by host plant resistance. Such integrated control is based on the fact that different methods work best at different times and places or under different conditions, each compensating to some extent for the deficiencies of the others (Kumar *et al*., 2017). Hence, it is recommended to adopt

integrated disease management. Accordingly, mentioned below are several general methods used in preventing damping off:

a. Proper soil preparation and management to provide good soil drainage, structure, aeration, water-holding capacity and plant nutrition by including proper amounts of fertilizer and lime according to the soil test report (Bradley, 2018).
b. Proper soil treatment with heat or chemicals to reduce the level of fungi that cause damping- off (Milijasevic *et al*. 2017).
c. Proper seeding rates to avoid thick plant stands, poor air movement and low light intensity (Horst, 2018).
d. Proper planting depth and soil temperature to assure rapid seeding emergence and growth (Moorman, 2019).
e. The growth medium should be free of chemicals and other toxic residues. Using plant containers with one or more openings in the bottom to provide proper drainage. Avoid excess watering. Placing the plants in the best site possible for maximum growth (Moorman, 2019).
f. Use a sterile potting mix, rather than soil from the farm. Outdoor soil can harbor fungus spores.
g. Avoid watering before taking cuttings or otherwise handling plants. The chances of transmitting pathogens are reduced when only dry plants are handled. Cuttings should be made as far from the soil surface as practical to minimize contamination.
h. Giving seedlings plenty of heat and light, so they germinate and grow quickly. Damping-off only affects seedlings. If the seeds can get them past the seedling stage, they're safe (Bradley, 2018).

i. Avoid spreading soil from infested areas. Carefully dig up diseased plants, removing them from growing areas, and place them in a covered trash container (Milijasevic *et al*., 2017).

2.2.3.2 Chemical control

Once pathogens become established on crops especially perennials and reach damaging levels, chemical control is needed. Pests and pathogens may be killed by exposing them to toxic substances. Toxic chemicals lethal to all forms are used in agriculture only as sterilant for the eradication of pests and pathogens from soil. Chemical control has to be followed with each outbreak. It is however, very quick in action and still the method by which best results were obtained for mortality of disease outbreaks (Hill and Waller, 2019).

2.2.3.3 Biological control

In an attempt to reduce the use of pesticides, there is an increasing interest in introducing biological agents and putting to use plant compounds as natural commercial products for managing soil-borne pathogens (Cook, 2018). Biological control using beneficial microorganisms has been considered a viable alternative strategy for the replacement of chemical methods. The most common antagonistic agents that have been used to control damping-off include bacteria such as *Pseudomonas, Bacillus* and *Burkholderia* isolates (Ramarathnam *et al*., 2019; Sallam *et al*., 2019), whose presence in composted materials is very significant. Their involvement both in the compost suppressive abilities and plant growth promotion is widely known (Haruta *et al*., 2018).

2.2.3.4 Soil sterilization

Soil solarization is a non-chemical approach for soil disinfestation and one of the most promising methods to control soil-borne pathogens. It utilizes solar radiation and a thin film of transparent mulch, usually of polyethylene, to heat the soil to a range of 38 to 50 °C to a depth of about 10 to 20 cm after sufficient irrigation (Gamliel and Katan, 2017) for soil pasteurization. It is also a method for controlling ,mesophilic organisms, which include most plant pathogens (such as soil -borne fungi) and other pests, without destroying the beneficial bacteria and mycorrhizal fungi (Minuto *et al*., 1995).

2.2.4 Virulence mechanism of *Pythium* spp. and challenges in resistant breeding against *Pythium* spp.

Disease development by Pythium spp. involves an extensive repertoire of carbohydrate active enzymes (CAZymes), including glycoside hydrolases, polysaccharide lyases, carbohydrate esterases, proteases, etc., which help in plant cell wall penetration and further colonization. Adhikari *et al*. (2018), in their study of the *P. ultimum* genome, observed high sequence similarity and synteny with another phytopathogenic oomycete, Phytophthora, sharing genes and encoding enzymes involved in the metabolism of carbohydrates. However, the absence or underrepresentation of many notable genes in *Pythium* encoding for cutinases, xylanases, pectinases, etc., that are present in the Phytophthora genome suggests some difference in the methods of virulence between these oomycetes (Adhikari *et al*., 2018).

2.3 *Amaranthus hybridus*

Most developing countries depend on starch-based foods as the main staple food for the supply of both energy and protein. This accounts in part for protein deficiency which

prevails among the populace as recognized by Food and Agricultural Organization (Ladeji *et al*., 2019). In Nigeria, as in most other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acid (Okafor, 2020). Many of the local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials.

Vegetable is defined as the edible part of a plant, especially leafy or fleshy type that is usually consumed as side dishes, food supplement, herbal medicine and soup condiments. although, people generally believe that vegetable refers to the green leaves of some plants, but in the actual sense so many parts of a plant can be called or consumed as vegetable. Vegetables are therefore, categorized based on the part of plant consumed; they include flower bud, seeds (sweet corn (maize), peas, beans, corn), leaves (spinach, lettuce, garlic chives), stem (stems of leaves), stem shoots (ginger), tubers (potatoes, yams), roots (carrots) and bulbs (onions) as well as fruits in the botanical sense, but used as vegetables; tomatoes, cucumbers, pumpkins, peppers, okra (Munro *et al*., 2018).

In general, and regardless of the variation in reported data, one can conclude that the leafy vegetables are rich in good quality proteins, essential amino acids and minerals, and the seeds in fats. Among the plants, vegetables are excellent sources of essential nutrients and contribute to the recommended dietary allowance (RDA) of individuals. Minerals are very important and essential ingredients of diet required for normal metabolic activities of body tissues (Turan *et al*., 2018).

The *Amaranthus hybridus*, commonly called pigweed, is specie of an annual flowering plant. It is locally called “Alefo” in native Hausa language and “Efo tete” in Yoruba. It

is specie commonly found over the Southwestern and North central part of Nigeria. It grows from a short taproot, which could be up to 25 cm in height and grows in many different places, including disturbed habitat. It is a highly nutritious food. The leaves, shoots and tender stems are eaten as a potherb in sauces or soups, cooked with other vegetables, with a main dish or by itself. The seed or grain is also edible (Acar *et al*., 2020).

2.3.1 Taxonomic tree

Domain: *Eukaryota*

Kingdom: *Plantae*

Phylum: *Spermatophyta*

Subphylum: *Angiospermae*

Class: *Dicotyledonae*

Order: *Caryophyllales*

Family: *Amaranthaceae*

Genus: *Amaranthus*

Species: *Amaranthus hybridus.*

2.3.2 Origin and distribution

Amaranth originated in America and is one of the oldest food crops in the world, with evidence of its cultivation reaching back as far as 6700 BC. The genus Amaranth consists of nearly 60 species, several of which are cultivated as leaf vegetables, grains

or ornamental plants, while others are weeds. Grain amaranth species have been important in different parts of the world and at various times during the past few thousand years. At present, amaranth is extensively grown as a green, leafy vegetable in many temperate and tropical regions. The largest area ever grown was during the height of the Aztec civilization in Mexico during the 1400s. After the arrival of the Spanish conquistadors in Mexico in the early 1500s, amaranth almost disappeared in the Americas as a crop until research began on it in the US in the 1970s. Meanwhile, amaranth had spread around the world and had become established for food use (the grain or leaves) in places such as Africa, India and Nepal (Okafor, 2020). During the past two centuries, grain amaranth has been grown in scattered locations, including Mexico, Central America, India and Nepal. Although it is also used as a grain, Amaranth is more widely used as a pot herb and in some instances supplies a substantial part of the protein, minerals and vitamins in the diet. This makes it an easy crop to cultivate and domesticate (Okafor, 2020).

A **B**

Plate II: *A. Amaranthus hybridus* plant, B: *Amaranthus hybridus* leaves

2.3.3 Description of the plant

Mature plant: Amaranth species are erect or spreading annuals with a rough or prickly appearance. Grain amaranths vary in flower, leaf and stem colour, but maroon or crimson colouring is common in all three plant parts. Some varieties have green flowers and some are more golden. Some of the deep crimson varieties can be very striking when in full bloom. The height of the plant varies between 0.3 m and 2 m, depending on the species, growth habitat and environment.

Stems: Stems are usually longitudinally grooved. Grain amaranth plants are about 1,524 m to 2,134 m tall when mature and are dicots (broadleaf) plants with thick, tough stems similar to those of sunflowers (Mugerwa and Bwabye, 2019).

Leaves: The leaves are variable in size, green or purple, with slender stalks. These are alternate, usually simple, with entire margins and distinct markings, depending on species.

Flowers: Tiny green flowers are borne in dense, elongated clusters, usually on the tips of the branches. They are borne in spikes or plumes and are white, green, pink or purplish in colour.

Seed: The small seeds are usually shiny black in colour, in contrast to those of grain types which are cream-coloured. There are up to 3000 seeds per gram. The tiny, lens shaped seeds are usually pale in colour.

2.3.4 Disease control

No significant disease problems have been conclusively identified for grain amaranth. One possible problem is a damping-off fungus, which can kill seedlings. Therefore, use

disease-free seeds and avoid both overwatering and dense planting. Leaf amaranth suffers damage from the armyworm and the curly top virus disease, which is transmitted by the beet leafhoppers (*Circulifer femellus*).

2.3.5 Economic importance of *Amaranthus hypochondriacus*

A. hybridus has been reported to be a highly nutritious herbage and a potentially valuable forage crop (Mugerwa and Bwabye, 2019). The grain has some protein (12 % to 17 %) and is high in lysine, an amino acid that is low in other grain crops. The grain is high in fibre and low in saturated fats, factors which contribute to its use by the health food market. It is an exceptionally rich source of calcium, iron and vitamin C, a very rich source of potassium, vitamin A and riboflavin, a rich source of niacin and an above-average source of protein. Amaranth contribute to the nutritional well-being of rural people by providing the essential nutrients required for body growth and development and for prevention of diseases associated with nutritional disorders such as blindness due to vitamin A deficiency.

In Malawi, leaves of Amaranth are eaten as spinach or green vegetables. The leaves boiled and mixed with groundnuts sauce are eaten as salad. The grain of *Amaranthus hypochondriacus* is pound and bottled and sold as immune boosters in different pharmacies and supermarkets in Malawi. Leaves and young seedlings are cooked like spinach and added to soups, etc. Leaves have a mild flavour. Seeds can be used raw or cooked as a cereal substitute. An astringent medical tea can be made from the leaves. Hauptli and Jain (2020) suggested that it could be used as breeding material for recombining desirable yield characteristics in the cultivated grain amaranths. The processed grain is a potentially valuable energy enhancement for broiler diets and can

be integrated at levels up to 400 g per kg without adverse effects (Ravindran *et al*., 2019)

2.4 Phytochemistry of *Amaranthus hypochondriacus*

It will be recalled that in the food chain, plants are referred to as the producers because they had the ability to trap energy from sunlight, harness and assemble some basic units which they transform through some chemical process into complex high energy-yielding compounds that are readily available to organisms. Their generosity became overwhelmingly and practically complex to comprehend at a glance. A field has to emerge – "phytochemistry." Phytochemistry is the study of chemicals produced by plants, particularly the secondary metabolites. It takes into account their structural compositions, the biosynthetic pathways, functions, and mechanisms of actions in the living system.

The study of phytochemicals has been instrumental in the discovery of new plant natural products, which are of commercial values in various industries such as the traditional and complementary medicine systems, pharmaceutical industries, nutraceuticals, and dietary supplement industries. Owing to the consistent threat of microorganisms, environmental hazards to public health, the significance of phytochemistry in the medical and pharmaceutical industries for the quest for the discovery of new drugs has overshadowed their essence in other industries (Chukwuebuka *et al*., 2020).

2.4.1 Phytochemicals of *Amaranthus hypochondriacus*

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They

protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals. Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics and About 150 phytochemicals have been studied in detail (American Cancer Society, 2020).

2.4.2 Classification of phytochemicals

The exact classification of phytochemicals could have not been performed so far, because of the wide variety of them. In resent year, Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, amino acids, proteins, purines and pyrimidines of nucleic acids, chlorophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids and glucosides (Hahn, 2019). Literature survey indicate that phenolics are the most numerous and structurally diverse plant phytocontituents.

2.4.2.1 Phenolics contents

Phenolic phytochemicals are the largest category of phytochemicals and the most widely distributed in the plant kingdom. The three most important groups of dietary phenolics are flavonoids, phenolic acids, and polyphenols. Phenolic are hydroxyl group (-OH) containing class of chemical compounds where the (-OH) bonded directly to an aromatic hydrocarbon group. Phenol (C_6H_5OH) is considered the simplest class of this

group of natural compounds. Phenolic compounds are a large and complex group of chemical constituents found in plants (Walton *et al*., 2020).

2.4.2.2 Activity of phenolic compounds

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. Phenolic acid compounds and functions have been the subject of a great number of agricultural, biological, chemical and medical studies. Phenolic compounds in many plants are polymerized into larger molecules such as the proanthocyanidins (PA; condensed tannins) and lignins. Moreover, phenolic acids may arise in food plants as glycosides or esters with other natural compounds such as sterols, alcohols, glucosides and hydroxy fatty acids. Varied biological activities of phenolic acids were reported. Increases bile secretion, reduces blood cholesterol and lipid levels and antimicrobial activity against some strains of bacteria such as *staphylococcus aureus* are some of biological activities of phenolic acids. Phenolics acid possesses diverse biological activities, for instance, antiulcer, anti-inflammatory, antioxidant, cytotoxic and antitumor, antispasmodic, and antidepressant activities (Ghasemzadeh *et al*., 2019).

2.4.2.3 Flavonoids contents

Flavonoids are polyphenolic compounds that are ubiquitous in nature. More than 4,000 flavonoids have been recognized, many of which occur in vegetables, fruits and beverages like tea, coffee and fruit drinks (Pridham, 2020). The flavonoids appear to have played a major role in successful medical treatments of ancient times, and their use has persisted up to now. Flavonoids are ubiquitous among vascular plants and occur as aglycones, glucosides and methylated derivatives.

2.4.2.4 Activity of flavonoids

Flavonoids have gained recent attention because of their broad biological and pharmacological activities in these order Flavonoids have been reported to exert multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory as well as antitumor activities but the best-described property of almost every group of flavonoids is their capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species (Pridham, 2020).

The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The position of hydroxyl groups and other features in the chemical structure of flavonoids are important for their antioxidant and free radical scavenging activities. On the other hand, flavonoids such as luteolin and cathechins, are better antioxidants than the nutrients antioxidants such as vitamin C, vitamin E and β-carotene (Pridham, 2020).

2.4.2.5 Tannin contents

From a chemical point of view, it is difficult to define tannins since the term encompasses some very diverse oligomers and polymers. It might be said that the tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins (mainly), polysaccharides (cellulose, hemicellulose, pectin, etc.), alkaloids, nucleic acids and minerals, etc (Schofield *et al*., 2019).

2.4.2.6 Activity of tannins

In medicine, especially in Asian (Japanese and Chinese) natural healing, the tannin-containing plant extracts are used as astringents, against diarrhoea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, antiseptic, antioxidant and

haemostatic pharmaceuticals. Tannins are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and in the production of inks (iron gallate ink). In the food industry tannins are used to clarify wine, beer, and fruit juices. Other industrial uses of tannins include textile dyes, as antioxidants in the fruit juice, beer, and wine industries, and as coagulants in rubber Production. Recently the tannins have attracted scientific interest, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers. The search for new lead compounds for the development of novel pharmaceuticals has become increasingly important, especially as the biological action of tannin-containing plant extracts has been well documented (Palavy and Priscilla, 2019; Mueller-Harvey, 2019).

2.4.2.7 Alkaloids contents

Alkaloids are natural product that contains heterocyclic nitrogen atoms, are basic in character. The name of alkaloids derives from the “alkaline” and it was used to describe any nitrogen-containing base (Mueller-Harvey, 2019). Alkaloids are naturally synthesis by a large number of organisms, including animals, plants, bacteria and fungi. Some of the first natural products to be isolated from medicinal plants were alkaloids when they were obtained from plants materials in the early years of 19th century, it was found that they were nitrogen containing bases which formed salts with acid.

2.4.2.8 Activity of alkaloids

Alkaloids are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores (feeding deterrents) and also against other plants by means of allopathically active chemicals. The use of alkaloids containing plants as dyes, spices, drugs or poisons can be traced back almost to the beginning of civilization. Alkaloids

have many pharmacological activities including antihypertensive effects (many indole alkaloids), antiarrhythmic effect (quinidine, spareien), antimalarial activity (quinine), and anticancer actions (dimeric indoles, vincristine, vinblastine). These are just a few examples illustrating the great economic importance of this group of plant constituents. Some alkaloids have stimulant property as caffeine and nicotine, morphine is used as the analgesic and quinine as the antimalarial drug (Rao *et al*., 2019).

2.4.2.9 Terpenoids contents

The terpenoids are a class of natural products, which have been derived from five-carbon isoprene nits. Most of the terpenoids have multi cyclic structures that differ from one another by their functional groups and basic carbon skeletons. These types of natural lipids can be found in every class of living things, and therefore considered as the largest group of natural products. Many of the terpenoids are commercially interesting because of their use as flavours and fragrances in foods and cosmetics examples menthol and sclareol or because they are important for the quality of agricultural products, such as the flavour of fruits and the fragrance of flowers like linalool (Harborne and Tomas-Barberan, 2019).

2.4.2.10 Activity of Terpenes

Among plant secondary metabolites terpenoids are a structurally most diverse group; they function as phytoalexins in plant direct defense, or as signals in indirect defense responses which involves herbivores and their natural enemies. Many plants produce volatile terpenes in order to attract specific insects for pollination or otherwise to expel certain animals using these plants as food. Less volatile but strongly bitter-tasting or toxic terpenes also protect some plants from being eaten by animals (antifeedants) (Degenhardt *et al*., 2020). Last, but not least, terpenes play an important role as signal

compounds and growth regulators (phytohormones) of plants, as shown by preliminary investigations. In addition, terpenoids can have medicinal properties such as anticarcinogenic (e.g. perilla alcohol), antimalarial (e.g. artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g. glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artimisinin and the diterpenoid anticancer drug taxol (Langenheim, 2019).

2.4.2.11 Saponin contents

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. They form a stable foam in aqueous solutions such as soap, hence the name "saponin". Chemically, saponins as a group include compounds that are glycosylated steroids, triterpenoids, and steroid alkaloids. Two main types of steroid aglycones are known, spirostan and furostan derivatives. The main triterpene aglycone is a derivative of oleanane. The carbohydrate part consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid glycosidically linked to a sapogenin (aglycone).

2.4.2.12 Activity of saponins

The physiological role of saponins in plants is not yet fully understood. While there is a number of a publication describing their identification in plants, and their multiple effects in animal cells and on fungi and bacteria, only a few have addressed their function in plant cells. Many saponins are known to be antimicrobial, to inhibit mould, and to protect plants from insect attack. Saponins may be considered a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants (Dudareva *et al*., 2019). Saponin mixtures present in plants and plant products possess diverse biological

effects when present in the animal body. Extensive research has been carried out into the membrane-permeabilizing, immunostimulant, hypocholesterolaemic and anticarcinogenic properties of saponins and they have also been found to significantly affect growth, feed intake and reproduction in animals (Lacaille-Dubois and Wagner, 2020).

2.5 Neem (*Azadirachta indica*)

Neem (*Azadirachta indica*) belonging to meliaceae family is one of the most suitable and valuable tree species found in India. Neem (*Azadirachta indica*) commonly called 'India Lilac' or 'Margosa' and Dogon yaro in Hausa, belongs to the subfamily Meloideae and tribe Melieae (Tomoko *et al*., 2018).

It can grow on wide range of soils up to pH 10 which makes it one of the most versatile and important trees in Indian sub-continent. Due to its multifarious uses, it has been cultivated by Indian farmers since vedic period and it has now become part of Indian culture. In India, it occurs throughout the country and can grow well in every agro-climatic zones except in high and cold regions and dam sites. In fact, in India, *Neem* trees are often found growing scattered in the farmers' fields and on the boundaries of fields without affecting the crops. Farmers practice this system just to meet the local demand for timber, fodder, fuelwood and also for various medicinal properties. Due to its deep tap root system, it does not compete with annual crops for scarce soil moisture. *Neem* tree can be labelled as wonder tree for its multipurpose uses in real sense (Tomoko *et al*., 2018). This has been used as a medicinal plant for long time and provides almost all the requirements of rural areas - be the timber, fuelwood, fodder, oil, fertilizers, pest repellent or the ubiquitous 'datun'. Today, it has been recognized as the most potential tree of India due to its evergreen nature (deciduous in drier areas) and

ability to grow in even the most arid and nutrient deficient soils as well as for its many commercially exploitable by-products and environmentally beneficial characteristics (it has therefore been labelled as tree of the future). If plantation of this tree has to be taken up on large scale, it has to be integrated as an important component of agriculture under various agro-forestry systems. It has been estimated that India's *Neem* bear about 3.5 million tonnes of Kernels every year. From this about 7 lakh tonnes of oil might be recovered (Tomoko *et al*., 2018).

2.5.1 Description of plant

A large evergreen tree, 12 to 18 meters in height and 1.8 to 2.4 meter in girth with a straight bole and long spreading branches forming a broad crown as much as 20 meters across, commonly found throughout greater parts of India. Bark Grey or dark reddish brown with numerous and scattered tubercles (Tomoko *et al*., 2018). The bark exudes a gum known as East India gum. Leaves alternate 20 - 30 cm long, leaflets 8 - 19 alternate or opposite ovate glossy, bluntly serrate. Flowers: white or pale yellow, small, scented, numerous on long axillary panicles, have a honey like scent and attract many bees. Fruit: Fruit is a ovoid bluntly pointed, smooth drupe green when young and turns yellow with a very thin epicarp, mesocarp with scanty pulp and a hard bony endocarp, enclosing one seed. The timber is relatively heavy with a specific gravity varying from 0.56 to 0.85 (average 0.68) when freshly cut, it has a strong smell. The flowering season of neem varies from place to place. Generally, it flowers from January to May and the ripening time of fruits is from May to August. The fruit pulp is edible (Tomoko *et al*., 2018).

2.5.2 Ethno- and medicinal use

Neem bark is used in villages for rope making. Neem oil is used in soap manufacture. Neem has proved effective against certain fungi that infect humans. Neem extracts are used as insecticides, pesticides and fungicides. Neem oil has antibacterial, antiviral properties and used in skin and dental problems. *Neem* products are being used for malaria, fever, pain and also as contraceptive; neem oil is a powerful spermicide and can therefore be used as an inexpensive birth control method (Tomoko *et al*., 2018). A neem oil-based product, Sensal, is being marketed in India as an intravaginal contraceptive. Leaf teas are used to treat malaria. People use the twigs as toothbrushes, and dentists find twigs effective in preventing periodontal disease. Neem oil has been used traditionally as a topical treatment for skin symptoms in both humans and livestock, but it should not be ingested orally (Tomoko *et al*., 2018).

Neem is also being used in cosmetics, lubricants and fertilisers. In a laboratory study, neem preparations showed toxicity to cultures of 14 common fungi. The tree has suppressed several species of pathogenic bacteria, including *Salmonella typhosa* and *Staphylococus aureus*. Various parts of *A. indica* have anthelmintic, antiperiodic, antiseptic, diuretic and purgative actions, and are also used to treat boils, pimples, eye diseases, hepatitis, leprosy, rheumatism, scrofula, ringworm and ulcers. Neem extracts are rich in antimicrobial compounds as some studies have clearly shown that neem extracts can be potentially useful to control some foodborne pathogens and other spoilage organisms (Mahfuzul *et al*., 2019).

2.5.3 Chief phytochemical constituents in neem methanolic leaves extract

Neem Leaves mainly yield quercetin (flavonoid) and nimbosterol (ß- sitosterol) as well as number of liminoids (nimbin and its derivatives). Quercetin (a polyphenolic

flavonoid) is known to have antibacterial and antifungal properties. This may perhaps account for the curative properties of leaves for sores and scabies. Limonoids like nimocinolide and isonimocinolide affect fecundity in house flies (*Musca domestica*) at a dose ranging between 100 and 500 ppm. They also show mutagenic properties in mosquitoes (*Aedes aegypti*) producing intermediates. Fresh matured leaves yield an odorous viscous essential oil, which exhibits antifungal activity against fungi (*Trichophyton mentagrophytes*) in vitro. White crystalline flakes obtained from petroleum ether extract of leaves consisting of a mixture of C - 14, C - 24, C - 31 alkanes were found to exceed or equal the lavicidal activity of pyrethrum extract. The principal constituents of neem leaves include protein (7.1 %), carbohydrates (22.9 %), minerals, calcium, phosphorus, vitamin C, carotene etc. But they also contain glutamic acid, tyrosine, aspartic acid, alanine, praline, glutamine and cystine like amino acids, and several fatty acids (dodecanoic, tetradecanoic, elcosanic, etc.) (Mahfuzul *et al*., 2019).

2.6 Mahogany (*Khaya senegalensis*)

Khaya senegalensis is a tree in the family Meliaceae is a native of West Africa (Senegal) and extends to Sudan and Uganda. The tree is commonly called the dry zone mahogany, and it is widely distributed in the Savannah regions (Makut *et al.,* 2007). In Nigeria, the tree is called with several local names in different parts of the country; ‘Madachi’ in Hausa, ‘Dalehi-Kahi’ in Fulfulde, ‘Oganwa’ in Yoruba and ‘Ono’ in Igbo languages (Makut and Ekeleme, 2018). The plant is easily recognized by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules.

2.6.1 Botany of mahogany

In its natural habitat, the plant is a medium to large sized tree that grows up to 30 m high and 3 m girth, with dense crown and short bole covered with dark drey scaly bark. Slash dark pink, bark bitter yielding gum when wounded. Leaves with 3 - 4 (exceptionally 5) pairs of leaflets, 5 – 10 cm long by 2.5 - 5 cm broad, more or less elliptic, round, obtuse or shortly acuminate at apex; stalks of leaflets 4 mm long. It has attracted world-wide attention for its high-quality timber production and its high usage in the tropics (Arnold *et al.*, 2004). Fruit is 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.

2.6.2 Medicinal uses of mahogany

In northern Nigeria, the Hausa and the Fulani tribes utilize *K. senegalensis* traditionally as a remedy for several human and animal ailments. It is used extensively as a bitter tonic for the treatment of a variety of pro-inflammatory disease. The plant is also commonly used in African traditional medicine for pain and Inflammation (Oguntibeju, 2018). The stem-back and leaves of *K. senegalensis* have been used in Adamawa State in forms of decoction and concoctions for the cure of mucous diarrhea, syphilis, pyrexia, malarial fever, dysentery and wound infections.

The very bitter bark of *K. senegalensis* has a considerable reputation in its natural range as a fever remedy. It is also used as a laxative, vermifuge, taenicide, depurative and for treating syphilis. The bark extract is used for treating jaundice, dermatoses, scorpion bite, allergies, infection of the gums, hookworm, bleeding wounds (disinfectant). The crushed bark and seeds are regarded as an emmenagogue. The seeds and leaves are used

for treating fevers and headache. The roots are used as a treatment against sterility, for the treatment of mental illness, against syphilis, leprosy and as an aphrodisiac (Eid *et al*., 2018; Naveen *et al*., 2019).

2.7 Mango (*Mangifera indica* L)

Mango (*Mangifera indica* L.) ascribed to the family Anacardiaceae has been adjudged as the vital traditionally significant and one of the most economically important tropical fruit crops globally (Barreto *et al*., 2018). Mango is an evergreen tree with a lot of traditional medicinal resources apart from its very famous fruits. Mangoes are native to the South and Southeast Asia, and in 2018, the global production of mangoes (the report includes guavas and mangosteens) was 55.4 million tonnes. The largest mango producing countries are India, China, Thailand, Indonesia, Pakistan, Mexico, Brazil, Bangladesh, Nigeria, and the Philippines. Apart from its economically important portion (fruit), large amounts of crop residues such as leaves, flowers, stem, and bark are generated during pruning, which causes complications of disposal to the farmers. Mango leaves (MLs) are the potential source of minerals, viz. nitrogen, potassium, phosphorus, iron, sodium, calcium, magnesium, and vitamins, viz. A, B, E, and C. A major bio-macromolecule present in mango leaves is protein. MLs can be utilized as an alternative source of livestock feeding in developing countries for alleviating the food shortage for livestock.

2.7.1 Botanical characterization

The mango tree is an evergreen, fast-growing, and long-lived orchard. It is very vigorous with a large canopy and an almost circular projection. The leaves are perennial, deep green, pointed, and shiny, while the inflorescence occurs in panicles consisting of about 3000 whitish-red or yellowish–green flowers. In tropical regions, the

trees can reach up to 30–40 m in height, while in subtropical areas the growth rate is consistently reduced. The mango fruit has hundreds of varieties, each having its own characteristic taste, shape, and size. Each fruit is 5 – 15 cm long and 4 – 10 cm in diameter. Usually its weight ranges from 150 g to around 750 g, reaching approximately 390 g in Sicilian mango fruit (Farina *et al*., 2018). The outer peel (exocarp) is smooth and is green in unripe mango, but it turns golden yellow, crimson red, yellow, or orange-red in ripe fruits, depending upon the cultivar type. The endocarp is a large ovoid-oblong core that contains a single seed. The pulp (mesocarp) is orange-yellow in colour, well endowed with numerous soft fibrils. Its flavor is pleasant and rich, and its taste is sweet with mild tartness. Mango is consumed fresh or is processed for chutney, pickles, curries, dried products, puree, nectar, and canned or frozen slices that are popular worldwide.

2.7.2 Medicinal use of mango

Extracts of the MLs have been utilized for traditional medicines to cure diabetes, bronchitis, diarrhea, asthma, kidney, scabies, respiratory problems, syphilis, and urinary disorders (Kulkarni *et al*., 2020; Kumar *et al*., 2021). The most active biological constituent of MLs is mangiferin, followed by phenolic acids, benzophenones, and other antioxidants such as flavonoids, carotenoids, quercetin, isoquercetin, ascorbic acid, and tocopherols. Mangiferin is the main contributor of most of the biological activities of MLs extract. MLs have a great scope of valorization as they are recognized to possess varied phytochemical, biological, and pharmacological properties, viz. anti-microbial, antioxidant, anti-diabetic, anti-tumour, and immunomodulatory effects. ML oil (MLO) contains monoterpenes, sesquiterpenes, minor quantities of other analogues, and trace amounts of non-terpenoid hydrocarbons and oxygenated hydrocarbons (Batool *et al*., 2019).

2.7.3 Antimicrobial activities

Distinct morphological parts of the mango plant like leaves, stem, kernel, seeds, and bark have been manifested to show antimicrobial activities against microbes like *Staphylococcus sp., Bacillus subtilis, Escherichia coli, Candida albicans, Proteus vulgaris, Pseudomonas fluorescens, Shigella flexneri, Klebsiella pneumoniae*, and *Salmonella typhi*. MLs extract is the most studied part for antibacterial effects. Bharti (2018) observed that hexane and hexane/ethyl acetate extracts of MLs exhibit favorable antibacterial effects against Mycobacterium tuberculosis and Enterobacter aerogenes. Antimicrobial investigation of the essential oils extracted from leaves of five Egyptian mango cultivars to be used as preservatives materials has been demonstrated by Kumar *et al*. (2021) against *Staphylococcus sp., Escherichia coli, Pseudomonas aeruginosa, Aspergillus* and *Salmonella typhi*. The major phytochemicals responsible for the antimicrobial activity in mango leaves include phenolics, alkaloids, saponins, glycosides, terpenes, and tannins (Bharti, 2018).

These compounds are identified to be synthesized immediately after a fungal attack, and a concentration of 1000 ppm has curtailed the growth of target fungal species like *Aspergillus* and *Alternaria* from 56 - 97 %. A myriad of terpenes identified from MLs extract exhibit bacteriostatic and bactericidal effects against different pathogens. HPLC analysis of leaf extract to identify hydrolyzable tannins revealed the presence of gallatotannins. Antimicrobial properties of gallatotannins have been associated with their ability to hinder the enzymes of the pathogen, disintegrate lipid bilayer membranes, and promote chelation of metal ions. The aforementioned phytocompounds purified from ML extract can be directly used as food additives to enhance the shelf-life of foods, and as an alternative for the synthetic antimicrobial agents, owing to their broad biological and pharmacological activities (Kumar *et al*., 2021).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experimental Site

The *in vitro* and *in vivo* studies of this research was carried out in the laboratory and screen house respectively, in the Department of Plant Biology, Federal University of Technology Minna, Nigeria.

3.1.1 Soil sampling

Soil samples were collected from the fields at the soil cores/transect to a depth of 15 cm using a soil probe with a diameter of 2.5 cm. The soil cores for each transect were then pooled into a polyethylene bag. At the time of sampling, the soil samples were placed in a cooler with ice and transferred to a cold storage refrigerator (4±2 °C) at the Microbiology Department, Federal University of Technology, Minna.

3.1.2 Collection of plant materials

The seeds and infected leaves of *A. hybridus* were collected alongside fresh and healthy leaves of Neem (*Azadirachta indica*), Mahogany (*Khaya senegalensis*) and Mango (*Mangifera indica L*) were collected and the plants were authenticated in the herbarium of the Department of Plant Biology, Federal University of Technology, Minna, Nigeria

3.2 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 rinse 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 (Gnannasekaran *et al.,* 2015). The percentage yield of the plant extracts were calculated using the formula represented below;

$$\% \text{ Yield} = \frac{the\ weight\ of\ the\ extracts}{the\ weight\ of\ the\ plant\ samples\ extracted} \times 100$$

3.3 Qualitative Phytochemical Screening of the Methanol Extracts

3.3.1 Test for alkaloids.

A 0.5 g of the five extracts were dissolved individually with 10ml 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).

3.3.2 Test for flavonoids

Portions 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 shake 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).

3.3.3 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 Tannins (Sneh *et al*., 2013).

3.3.4 Test for phenols.

About ten (10) mL of distilled 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 indicate the presence of phenols (Manickara and Veerababu, 2014).

3.3.5 Test for saponins

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

3.3.6 Test for steroids

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

3.3.7 Test for terpenoids

About 0.5 g each from the extracts were dissolve in five (5) mL of distilled water. They were mixed with two (2) mL of chloroform and three (3) mL of concentrated surfuric 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).

3.3.8 Test for anthraquinones

Five (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 ($CHCL_2$) oabout 10ml was added to the filtrates, few drops of 10% ammonia (HH_3) 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).

3.3.9 Test for cardiac-glycosides

Five (5) g from each of the plant extracts were separately mix with (2) ml of glacial acetic acid containing one drop of Ferric chloride ($Fecl_3$) solution, followed by the addition of 1ml 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).

3.4 Quantitative Phytochemical Screening of the Methanolic Extract of Plant Sample

According to method described by Oloyede *et al.* (2010), were estimated. The total phenolic content was calculated by means of the method of Singleton *et al.* (1999) and the content of the flavonoids by the aluminum chloride colorimetric method of Chan *et al.* (2019).

3.4.1 Total flavonoid determination

Zero-point five milliliter (0.5 mL) of the plant extract and the methanol of 1.5 mL were mixed, 0.1 mL of 10 per cent aluminum chloride and same volume of 1 M sodium acetate with 2.7 mL distilled water was 30 minutes at room temperature. Spectrophotometer was utilized at 415 nm to determine the concentration of flavonoid.

3.4.2 Determination of total phenol

The total phenol content of the crude extracts was determined using the method described by Singleton *et al.* (1999). The raw extract (0.5 mL) was oxidized in a range of 2.5 mL of Ciocalteau's folin reagent of 10 % and sodium carbonate neutralized in a range of 2 mL of 7.5 %. The reaction mixture has been incubated at 45 °C for 40 minutes, with a spectrophotometer measuring the absorption at 765 nm.

3.4.3 Alkaloids determination

The crude extract (0.5 g) was mixed with a 1:1 ratio of 20 ml of 96 % ethanol and 20 mL of 20 % H_2SO_4. A milliliter of filtrate was put in 5ml of 60 percent of H_2SO_4. After 5 minutes of mixing, 0.5 % formaldehyde solution was added to 5ml and allowed to sit for 3 hours. The reading was recorded at a wavelength of 565 nm.

3.4.4 Saponins determination

A total of 0.5 grams (0.5 g), 20 mL of 1M HCl and boiled for 4 hours of raw extract was added. 50 mL petroleum ethers for the ether layer were added and evaporated to dryness after cooling and filtering. The residue has been treated with five ml of acetone and ethanol, six ml of ferrous sulfate reagent and two ml of Conc. H_2SO_4. The residue has been treated. The absorption was measured at 490 nm after the mixture had been homogenized and allowed to lie for 10 minutes (Oloyede *et al*., 2010).

3.4.5 Tannin determination

Zero-point two grams of extract (0.2 g) were weighed into a 50 ml beaker, which was then filled with 20 ml of 50 % methanol, wrapped in parafilm, and heated to 800 °C for one hour. The contents of the mixture were transferred to a 100 mL volumetric flask after vigorous shaking. 20 milliliters water, 2.5 milliliters Folin-Denis reagent, and ten milliliters 17 percent Na_2CO3 were thoroughly combined. The mixture was permitted to settle for 20 minutes. The end of the 12.5 - 100 g/ml range was selected to search for bluish green colouration. The absorption of tannins, acids and the samples were evaluated using a spectrophotometer at 760 nm following color development.

3.4.6 Glycoside determination

The method of El-Olemy *et al.* (1994) was used. 1 g of the fin powder was soaked in 10ml of 70 % alcohol for 2 hours and then filtered. The extract obtained was then purified using lead acetate and Na2HPO4 solution before the addition of freshly prepared bullet`s reagent (containing 95ml aqueous picric acid + 5 mL of 10 % aqueous NaOH). The difference between the intensity of colors of the experimental and blank (distilled water and Buljet`s reagent sample gives the absorbance and is proportional to the concentration of the glycoside.

3.4.7 Terpenoids content determination

The extract (1 g) will be marcarated with 50 mL of ethanol and filtered. To the filtrate (2.5 ml), 2.5 ml of 5 % aqueous phosphomolybdic acid solution will be added and 2.5 ml of concentrated H2SO4 will be gradually added and mixed. The mixture will be left to stand for 30 min and then made up to 12.5 mL with ethanol. The absorbance will be taken at 700 nm.

3.4.8 Steroids content determination

The extract (1 g) will be marcarated with 20 ml of ethanol and filterd. To the filterate (2 ml), 2 mL of chromagen solution will be added and the solution left to stand for 30 min. The absorbance will be read at 550 nm.

3.4.9 Anthraquinones content determination

For estimating the Anthraquinones content, take 1ml of sample solution and add 1 ml of 5 % phenol and then add 5 ml of concentrated sulphuric acid mix well and leave for 10 minutes. Measure the absorbance at 488 nm against blank. Then compare it with

standard solution of glucose. To prepare blank, 1 mL of distilled water added to1 mL of 5 % phenol followed by 5 mL of concentrated sulphuric acid.

3.5 Preparation of Potato Dextrose Agar

Two hundred grams (200 g) of peeled irish potato was washed and boiled for about 20 minutes in 100 mL of sterilized distilled water. The supernatant was drained into a conical flask (100 mL) and made up to 1000 mL with sterile distilled water. Twenty grams of agar agar powder and 20 g of glucose powder was weighed and added. The mouth of the conical flask was plugged with cotton wool wrapped in aluminum foil paper. The mixture was sterilized in a autoclave at 121 ^{o}C for 15 minutes after sterilization the mixture was then be allowed to cool down for ten minute, poured into petri dish and then 0.5 mL drop of 100 L concentration of chloramphenicol was added to prevent bacteria growth (Adebola *et al.,* 2014)

3.5.1 Isolation of *Pythium* myriotylum

The infected A. *hybridus* samples were taken to the Department of Biological Science laboratory (FUT) Minna, Nigeria for analysis. The leaves were washed thoroughly under running tap water, then surface sterilized with 70 % sodium hypochlorite solution for 30 to 40 seconds and washed 5 times with sterilized distilled water. Two discs taken from the periphery of necrotic region were placed on Potato Dextrose Agar (PDA), to which streptomycin (1 mlL^{-1}) was added and incubated at 22±2 ^{o}C for 3 days. A Single conidium was picked up with a sterile needle under microscopic observation, transferred individually to PDA plates and incubated at ambient temperature (Subramanian *et al.,* 2013). The monoculture was prepared and stored on PDA slants at 4 ^{o}C. Subculture was made at regular intervals. The fungal isolate was identified using microscopic examination of sporangia, oospores, antheridia, and oogonia characteristic of *Pythium*

spp and also using the fungal family of the world mycological monograph (Adebola and Amadi, 2012).

The method used for baiting *Pythium* species from the soil was also employed as described by Watanabe *et al*. (2018). A 20 g soil sample from each field was placed in a 15 cm diameter Petri plate and moistened to field capacity with 3 to 5 mL tap water. Plates were incubated at room temperature (22 ± 2 ^{o}C) on a laboratory bench for 24 to 48 h. Distilled water (10 ml) was added to each plate, and infected A. *hybridus* leaves., cut to about 2.5 cm long and sterilized by autoclaving at 1.1 kg/cm^{2} and 121 ^{o}C) for 20 min, were floated on the water in each plate. After 24 to 36 h, the leaves were removed, blotted dry on sterilized paper towel, and plated on a *Pythium* selective agar medium (PSM) (Alcala *et al.,* 2016). After another 24 to 36 h, emerging hyphal tips from the leaves were transferred onto plates of water agar (WA). Hyphal growth patterns on WA were observed microscopically to select and verify isolation of *Pythium* species. A pure culture of each isolate of *Pythium* was maintained on WA and stored at 4 °C. For long term storage, a WA block (1 cm^{3}) colonized by the *Pythium* isolate was placed in a glass vial containing sterilized water and four sterilized *A. hybridus* seeds (HBD International Inc., Brentwood, TN), and stored at room temperature.

3.6 Preparation of Inoculum

Inoculum production was performed by inserting little discs 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 10 mL of sterile distilled water, rubbing a brush slightly over the colonies and subjected the suspension to constant agitation untill the spores get liberate. This was used to determine the

concentration of conidia suspension (Fernanado *et al*., 2013). The macro and micro conidia were adjusted to a concentration of 10^6 conidia /ml suspension (Ribeiro *et al*., 2011).

3.7 In Vitro Antifungal Activities of the Three Plant Extracts on Mycelia Growth of *Pythium myriotylum*

The plant extracts were evaluated for antifungal activities using food poison techniques (Gnannasekaran *et al.,* 2015) five (5 ml) of the extracts at different concentrations (500, 800 and 1000 mg/mL) was thoroughly mixed with 15 ml of sterile potato dextrose agar (P. D. A.) and 0.5 mL of chloramphenicol contained in the petri-dish with control respectively. After gelling of the medium, seven days old of P. *myriotylum* was inoculated into the centre of the plate using 5mm disk cork borer from their growing edges. The petri-dishes was incubated at 28±2 ^{o}C) for 7 days. On each day, the diameter of the extension was measured using a meter rule.

The percentage (%) inhibition was calculated using the formula below;

$$\% \text{ inhibition} = \frac{R1-R2}{R1} \times 100$$

Where R1 is growth in the control and R2 is the growth in the treatment.

3.8 *In-Vivo* Experimental Design

A total of thirty (30) planting container in the screen house were arranged in randomized complete block design (RCBD. The planting containers were divided into four (4) groups, by pathogen treated groups with the 3 plant extracts and the pathogen untreated group which served and based on the plant extracts concentration treatment (with or without). There was a total of 3 replicates for each extract.

3.8.1 Seed and soil treatment

Two hundred (200) seeds were soaked into 10mls of each concentration of the plant extract (500, 800 and 1000 mg/ml) respectively for 20 minutes. The soil was then drenched with 10 mLs of each concentration of the extract before spreading (dispersing) the seeds, the seeds were then dispersed into the planting poly-pot 20 cm wide in diameter, containing 4.5 kg of sterilized soil. Two day after seedling germination, they were sprayed with five (5 mL) of each plant extract with respect to concentration (500, 800 and 1000 mg/mL respectively). Three (3) days later, after which the seedlings must have imbibed the plant extracts, they were then sprayed with five (5 mL) of the fungal isolate and observed for seven days. The control pots were not sprayed with the extracts.

3.9 Data Collection

3.9.1 Statistical analysis

The data were statistically evaluated using SPSS 21.0 and an ANOVA one-way test, followed by a post-hoc DUNCAN-ALPHA test to determine its importance. Statistically significant P values less than 0.05, but not more than 0.05 are considered statistically significant ($p \leq 0.05$). The data were shown as a medium of a. ± 00 SEM defect.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Extract yields

The percentage yields of methanol crude extracts of *Mangifera indica* L, *Azadirachta indica* and *Khaya senegalensis* samples was found to be 22.18 %, 20.55 % and 24.94 % respectively as illustrated in (Table 4.1)

Table 4.1: Percentage yields of plant sample crude methanolic extract

S/N	Plant Samples	Methanol Extract Yield (%)
1	Mango (*Mangifera indica* L) leaves	22.18
2	Neem (*Azadirachta indica*) leaves	20.55
3	Mahogany (*Khaya senegalensis*) leaves	24.94

4.1.1.2 Preliminary phytochemical composition of plant leaf extract

Saponins, alkaloids, tannins, phenols, tannins and terpenoids were found to be present in the methanol extracts of the plant samples, while glycosides, steroids and anthraquinones were found to be mostly absent (Table 4.2).

Table 4.2: Qualitative phytochemical constituents of plant extracts.

S/N	Phytochemicals	Mango Extract	Neem Extract	Mahogany Extract
1	Alkaloids	+	+	++
2	Total phenols	+++	++	+
3	Saponins	++	+	+
4	Tannins	+++	+++	++
5	Flavonoids	++	++	+
6	Glycosides	-	+	-
7	Steroids	+	-	-
8	Terpenoids	++	++	+
9	Anthraquinones	-	-	-

Present; **+;** Absent **-** .

4.1.1.3 Quantitative phytochemical constituents of plant extracts

Total phenol (1388.40 ± 46.47^{h} mg/g) and Flavonoids (810.10 ± 29.94^{g} mg/g) were the most abundant in the mahogany extracts, while the least concentration of phytochemical was steroids (0.26 ± 1.23^{ab} mg/g) and glycoside found in mango extract (0.16 ± 0.53^{a} mg/g), and (0.16 ± 0.53^{a} mg/g) in Mahogany extract. Other phytochemicals were found to be significantly present in the plant methanol extracts (Table 4.3).

Table 4.3: Phytochemical composition of methanolic extract of plant samples.

		Quantity (mg/100g)		
S/N	**Phytochemicals**	**Mahogany Extract**	**Neem Extract**	**Mango Extract**
1	Alkaloids	9.66 ± 0.20 [b]	169.80 ± 0.64 [g]	165.17 ± 8.10 [f]
2	Total phenols	75.23 ± 0.22 [f]	118.51 ± 0.58 [f]	1388.40 ± 46.47 [h]
3	Saponins	8.48 ± 0.13 [b]	40.20 ± 0.58 [c]	4.02 ± 0.67 [c]
4	Tannins	42.10 ± 0.20 [d]	86.80 ± 0.58 [e]	22.72 ± 1.05 [d]
5	Flavonoids	68.6 ± 0.20 [e]	68.61 ± 0.58 [d]	810.10 ± 29.94 [g]
6	Glycosides	0.16±0.53 [a]	1.56 ± 6.31 [b]	0.92±66.1 [a]
7	Steroids	0.26±1.23 [ab]	0.45 ± 0.10 [ab]	3.21 ± 0.41 [bc]
8	Terpenoids	11.24 ± 0.10 [c]	62.9 ± 0.20 [d]	96.33 ± 9.81 [e]
9	Anthraquinones	0.84 ± 0.11 [ab]	0.84 ± 0.11 [ab]	0.26±0.35 [a]

Values are in ± mean S.E. (*S.E = Standard error of Mean*).Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level ($P < 0.05$).

4.1.2 Morphological identification of *Pythium myriotylum*.

All cultures grew well on PDA and formed cottony colonies within a week at 25 °C. Hyphae were hyaline, aseptae and up to 8 μm wide when viewed under the microscope as shown in Plate III. Finger-like appressoria formed abundantly in singles or clusters in water cultures. Sporangia were filamentous inflated and up to 12 μm wide. All isolates were homothallic; sexual structures formed abundantly on agar and water cultures. *Pythium myriotylum* produced the smallest ones on average (30.01 μm diam). Based on the classical definition, oospores an average of about (26.06 μm diam) were mostly aplerotic, but occasionally, plerotic oospores were also observed.

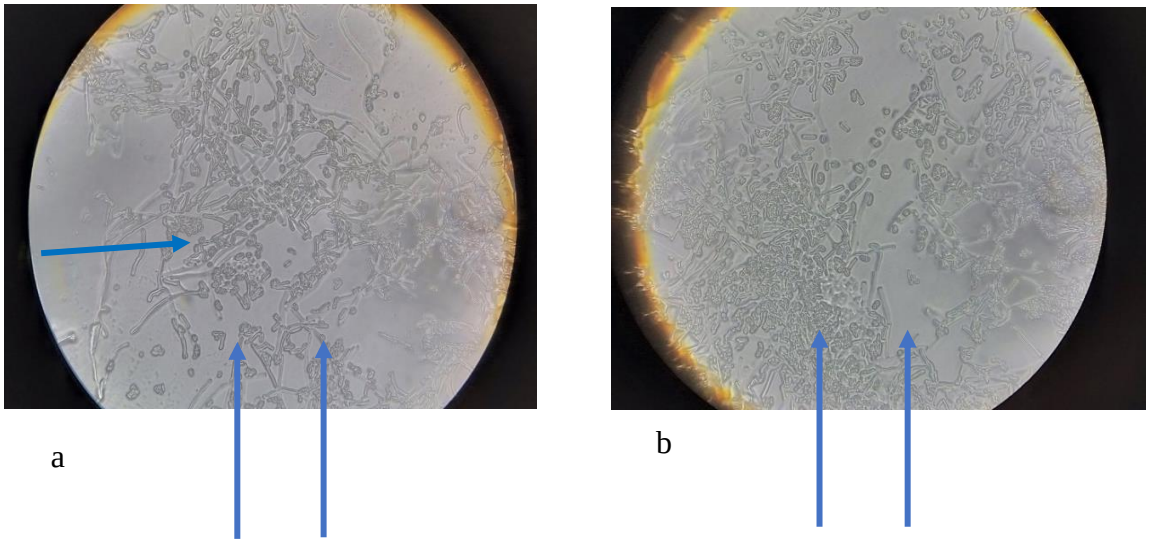

Plate III: ***Macro and micro identification of the hyphae and sporangia of Pythium myriotylum.*** **a. Clustered appressoria under microscope b. Filamentous sporangia under microscope.**

4.1.3 In vivo antifungal activities of the three plant extracts

4.1.3.1 Ratio of growth diameter

(Table 4.5 – 4.7) shows the ratio of growth in diameter of *Pythium myriotylum* on PDA containing the methanolic extract of Mango, Mahogany and Neem leaves which was determined based on comparison with growth diameter on control PDA plate. After day 3, the 500 mg/ml of the methanolic extracts of mahogany shows the highest radial growth of Pythium (1.86 cm) as compared with other concentration of the extracts and with the control groups (1.88 cm). From the table after day 7, 1000 mg/ml of the plant extract still shows a little zone of radial inhibition in growth (3.99 cm) as compared with the control group, which was already (4.00 cm).

Table 4.5: Mahogany extract Concentration (mg/ml)

Days	500	800	1000	Control
Day 1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 2	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
Day 3	1.86±0.005[b]	1.63±0.010[b]	1.53±0.005[b]	1.88±0.015[b]
Day 4	2.84±0.015[c]	2.79±0.010[c]	2.64±0.015[c]	2.90±0.010[c]
Day 5	3.86±0.010 [d]	3.77±0.010[d]	3.55±0.010[d]	3.94±0.005[d]
Day 6	3.97±0.015[e]	3.95±0.010[e]	3.87±0.005[e]	3.99±0.015[e]
Day 7	4.0±0.020[f]	3.99±0.020[f]	3.99±0.010[f]	4.00±0.00[f]

Values are in ± mean S.E. (*S.E = Standard error of Mean*)Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level ($P < 0.05$).

4.1.3.2 Radial growth of Pythium myriotylum measured in cm.

In this table below, the results show that After day 3, the 500 mg/ml of the methanolic extracts of neem leaves shows the highest radial growth of Pythium (1.84 cm) as compared with other concentration of the extracts and the control groups (1.88 cm). From the table after day 7, 1000 mg/ml of the plant extract still shows a little zone of radial inhibition in growth (3.55 cm) as compared with the control group which was already (4.00 cm).

In this table below, the results show that After day 3, the 500 mg/ml of the methanolic extracts of mango leaves shows the highest radial growth of Pythium (1.74 mm) as compared with other concentration of the extracts and the control groups (1.88 mm). From the table after day 7, 1000 mg/ml of the plant extract still shows a little zone of

radial inhibition in growth (3.55 mm) as compared with the control group which was already (4.00 mm).

Table 4.6: Neem extract Concentration (mg/ml)

Days	500	800	1000	Control
Day 1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 2	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
Day 3	1.84±0.005[b]	1.45±0.010[b]	1.23±0.005[b]	1.88±0.015[b]
Day 4	2.79±0.015[c]	2.58±0.010[c]	2.13±0.015[c]	2.90±0.010[c]
Day 5	3.64±0.010 [d]	3.17±0.010[d]	2.55±0.010[d]	3.94±0.005[d]
Day 6	3.92±0.015[e]	3.54±0.010[e]	3.05±0.005[e]	3.99±0.015[e]
Day 7	3.98±0.020[f]	3.86±0.020[f]	3.55±0.010[f]	4.00±0.00[f]

Values are in ± mean S.E. (*S.E = Standard error of Mean*) Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level (P < 0.05).

Table 4.7: Mango extract Concentration (mg/ml)

Days	500	800	1000	Control
Day 1	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Day 2	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]	0.00±0.00 [a]
Day 3	1.74±0.005[b]	1.23±0.010[b]	0.94±0.005[b]	1.88±0.015[b]
Day 4	2.64±0.015[c]	2.07±0.010[c]	1.75±0.015[c]	2.90±0.010[c]
Day 5	3.56±0.010 [d]	2.87±0.010[d]	2.14±0.010[d]	3.94±0.005[d]
Day 6	3.84±0.015[e]	3.03±0.010[e]	2.52±0.005[e]	3.99±0.015[e]
Day 7	4.0±0.020[f]	3.12±0.020[f]	2.75±0.010[f]	4.00±0.00[f]

Values are in ± mean S.E. (*S.E = Standard error of Mean*)Values between experimental treatments Within Groups bearing the same superscript are not significantly different at the 5 % level (P < 0.05).

4.1.3.3 Percentage inhibition of the plant extracts concentrations.

The results of this study as shown in (Table 4.8 – 4.10) expresses that at different methanolic plant extracts concentration, the extracts exhibited high potency in inhibiting mycelial growth of *Pythium myriotylum*. The result below shows that there was a significant decrease in the % inhibition of the fungi by the extracts as the days proceeds, at Day 3, 100 mg/ml of the mahogany extract shows the highest % inhibition of 18.61 %, followed by 800 mg/ml (13.29 %) and the least was 500 mg/ml (1.06 %)

Table 4.8: Mahogany extracts Concentrations (mg/ml)

Days	**500**	**800**	**1000**
Day 1	0.00	0.00	0.00
Day 2	0.00	0.00	0.00
Day 3	1.06	13.29	18.61
Day 4	2.07	3.79	8.96
Day 5	2.03	4.32	9.90
Day 6	0.50	1.00	3.00
Day 7	0.0	0.25	0.33

4.1.3.4 Effects of neem extract concentrations on the growth and resistance of Pythium myriotylum.

The table below showing the results of the % inhibition revealed that there was a significant decrease in the % inhibition of the fungi by the extracts as the days proceeds, at Day 3, 100 mg/ml of the neem leaves extract shows the highest % inhibition of 34.57 %, followed by 800 mg/ml (22.87 %) and the least was 500 mg/ml (2.13 %)

Table 4.9: Neem extract Concentrations (mg/ml)

Days	**500**	**800**	**1000**
Day 1	0.00	0.00	0.00
Day 2	0.00	0.00	0.00
Day 3	2.13	22.87	34.57
Day 4	3.79	11.03	26.55
Day 5	7.61	19.54	35.28
Day 6	1.75	11.28	23.56
Day 7	0.50	3.50	11.25

4.1.3.5 Effects of mango extract concentrations on the growth and resistance of Pythium myriotylum.

The result below shows that there was a significant decrease in the % inhibition of the fungi by the extracts as the number of days increases as shown below, at Day 3, when the concentration of the mango leaves extract was 100 mg/ml, the highest % inhibition of 50 % was recorded, followed by 800 mg/ml (34.57 %) and the least was 500 mg/ml (7.45 %).

Table 4.10: Mango extract Concentrations (mg/ml)

Days	500	800	1000
Day 1	0.00	0.00	0.00
Day 2	0.00	0.00	0.00
Day 3	7.45	34.57	50.00
Day 4	8.96	28.62	39.66
Day 5	9.65	27.16	45.68
Day 6	3.76	24.06	36.84
Day 7	0.00	22.00	31.25

4.2 Discussion

4.2.1 Phytochemical contents of the three plant extracts

The phytochemical contents of the three extracts from this study indicates phyto - constituents were found in the crude methanol extract of *Mangifera indica* L, *Azadirachta indica* and *Khaya senegalensis* extracts. Some of the phytoconstituents present significantly include alkaloids, saponins, tannins, phenols and flavonoids while Glycosides, Steroids, Terpenoids and Anthraquinones are found in trace amount this findings is in agreement with the work of Gnannasekaran *et al.* (2015) who reported similar results, also Imam *et al.* (2019) who reported high content of phytochemicals in *Azadirachta indica*.

The Quantitative analysis shows Total phenols (1388.40 mg/100g) to be the highest and Steroid (3.21 mg/100g) as least concentration of phytochemical as both found in Mango (*Mangifera indica* L) extract which is line with previous findings by Ntengna *et al.* (2019), who discovered the presence of the phyto-constituents in methanol mango leaves. The presence of this phytochemical might explain the antifungal effects of the extract against *Pythium myriotylum*. Phytochemicals are responsible for the majority of plant extract biological activity. The existence of these metabolites undeniably indicates the potential medical benefit of the plant extract, since it can be used bioremediation. The presence of phenol, flavonoids and tannins in these plants, as the findings demonstrate, shows that it has the capacity to act as an antifungal agent to help rehabilitate and control the spread of infection caused by this microorganism (Imam *et al.,* 2019). The existence of these metabolites undeniably indicates the potential medical benefit of the plant extracts, since it can be used to produce medicines. Anti-fungal properties have been proven for tannins and saponins, making them potentially

beneficial for fungal treatment. The presence of flavonoids and alkaloids in this plant, as the findings demonstrate, shows that it has the capacity to act as fungal deterring agent to help regulate and rehabilitate stem cells for repair caused by fungal damage in plants (Imam *et al.* 2019).

4.2.2 In vivo antifungal activities of the three plant extracts

Stem diameter and Shoot height of *Amaranthus hybridus* seedlings grown in medium biotreated with Mango (*Mangifera indica* L) and Neem extract from this study were significantly increased as compared to all other treatments this results in line with previous of Imam *et al.* (2019) who reported similar findings also the work of Mahfuzul *et al*. (2019). There was also a decrease in the observed disease index with increase in the extract concentration of the plant samples as reported by Smith *et al*. (2019).

4.2.3 In vitro antifungal activities of the three plant extracts

Radial growth of *Pythium myriotylum* assay using *Mangifera indica* L, *Azadirachta indica* and *Khaya senegalensis* methanolic plant extracts from this study reveal that every extract tested decreased mycelial growth of *Pythium myriotylum* by some degree this findings is in agreements with Smith *et al*. (2019) who both reported similar findings and attributes changes to the differing abilities of fungal mycelia to absorb plant extracts.

The percentage (%) inhibition of the extracts on the growth of the fungal strains (*Pythium myriotylum*) on PDA containing plant extracts which were determined based on growth diameter on the control plate. The results of the percentage inhibition also prove the efficacy of the methanolic extract of mango over other plant extracts used in this study. The result shows that there was a significant decrease in the % inhibition of

the fungi by the extracts as the days proceeds the finding corroborates with Gnannasekaran *et al*. (2015) who highlighted that the Resistance of fungi growth to methanolic plant extract concentrations.

Results from this research are strong indicators of the capabilities of Mango (*Mangifera indica*) as most preferable as well as Neem (*Azadirachta indica*) based products as biological controls for soilborne pathogenic fungi. The Development of these products requires a balance between the antifungal activity and phytotoxicity effects of the plant extracts and the phytochemical constituents. Mango (*Mangifera indica*) appeared superior in the suppression of damping-off in *Amaranthus hybridus* seedlings. High concentrations of the plant samples did not appear to limit germination of *Amaranthus hybridus* seedlings as documented in other studies (Al-Rahman *et al.,* 2013). Also, Al-Rahman *et al*. (2013) reported the fungicidal activity of five methanolic plant extracts from *Lantana camara, Salvadora persica, Thymus vulgaris, Zingiber officinale and Ziziphus spina-christi* against tomato phytopathogenic fungi, *Fusarium oxysporum, Pythium aphanidermatum* and *Rhizoctonia solani,* the causative agents of tomato damping-off diseases.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATION AND CONTRIBUTION OF RESEARCH TO KNOWLEDGE

5.1 Conclusion

In conclusion, the plants extract shows differences in their efficacy to protect the seedling from the manifestation of the infection with *Pythium*. It was deduced that the mango leaf extract proves to be the most efficient against damping-off disease caused by *Pythium myriotylum.*

Plant extracts could have different effects on soil microflora which could influence the microbial balance of soil which play vital role in soil fertility and crop yield. The present study revealed that there is a negative growth effect of the plant extracts on soil-borne mycoflora (*Pythium myriotylum*). These mycoflora which are adversely affected by these phytochemicals present in the plant samples are beneficial to the crop by the way of degrading the organic materials in the soil thereby making nutrients available to the crop and protection against infections. Based on the results obtained from this study, it is obvious that when plant extracts are applied, the phytochemicals exert certain effects on the target organisms soil mycoflora particularly *Pythium myriotylum.*

This study indicates that treatment of soil with methanolic plant extracts especially that of Mango leaves as shown in the analysis inhibits and degrades fungus particularly *Pythium myriotylum* would be useful in some areas where this fungal infection is extensively prevalent.

5.2 Recommendation

All experiments in this study used the crude methanolic plant extracts as bioremediation of *Pythium myriotylum* post emergence seedling damping off disease. Fractions of these plant extract might be more suitable so as to determine if the rate of infection will be reduced or if the extracts are mixed (in synergy) to maximize their pathogen control and also toxicity studies of the effect of these plant extracts will also be important so as to minimize the effect of phytotoxicity in the treated plant for its safety for human consumption.

5.3 Contribution of Research to Knowledge

This thesis established three concentration gradients of 500, 800 and 1000 mg/mL against post-emergence damping-off disease (caused by *Pythium myriotylum*) in *Amaranthus hybridus*. The inhibition potential showed by Mahogany across the concentrations 0.00, 0.25 and 0.25% respectively while Neem had 0.5, 3.5, 11.5 % respectively, and Mango recorded 0.00, 22.00 and 31.25 % respectively. Therefore Mango leaf extracts exhibited the highest inhibitory potential in suppressing *Pythium myriotylum* post-emergence damping-off disease. It additionally revealed that *A. hybridus* seedlings treated with the extracts developed 100 % resistance to the pathogen across all concentrations gradients.

REFERENCES

Abang, M. M., Kihurani, A. W. & Srinivasan, R. (2012). Managing diseases and pests of indigenous vegetables for GAP compliance in sub-Saharan Africa. *Scripta Horticulturae*, 15, 191-232.

Acar N, P., Vohra, R., Becker, G. D., Hanners, M. D. & Saunders, R.M. (2020). Nutritional evaluation of grain amaranth for growing chickens. *Poultry-Science*, 67, (8), 1166-1173.

Adebola, M. O. & Amadi, J. E. (2012). Studies on *Penicillium digitatum*, *Botryodiploidia theobromae*, *Alternaria tenuis* and *Trichoderma harzianum* for bicontrol of *Phytophthora palmivora* cocoa black pod disease pathogen. *American-Eurasian Journal of Agronomy* 5 (2), 30-34

Adebola, M. O., Dadi-Mamud, N. J. & Yakima, M. K. (2014). Effect of packaging materials on shelf-life stability of Gari brought from markets in Lapai, Niger state, Nigeria. *Journal of Applied & Environmental Microbiology*, 2, (1), 12-1.

Adhikari, B. N., Hamilton, J. P., Zerillo, M. M., Tisserat, N., Lévesque, C. A. & Buell, C. R. (2018). Comparative genomics reveals insight into virulence strategies of plant pathogenic oomycetes. *PLoS ONE*, 12, 8-14.

Agrios, G. N. (2019). Plant Pathology. Fifth edition. *Elsevier Academic press, London U.K*, 922.

Akinyeye, A. J., Solace, E. O. & Adebiyi, I. O. (2014). Phytochemical and antimicrobial evaluation of leaf and seed of moringa olifer a extracts. *International Journal of Research in Medicine and Health Science*, 4, 656-660.

Alcala, A. V. C., Paulitz, T. C., Schroeder, K. L., Porter, L. D., Derie, M. L., & du Toit, L. J. (2016). Pythium species associated with damping-off of pea in certified organic fields in the Columbia Basin of central Washington. *Plant disease*, 100, (5), 916-925.

Alcala, A. V. C., Paulitz, T. C. & Schroeder, K. L. (2019). *Pythium* species associated with damping-off of pea in certified organic fields in the Columbia basin of Central Washington. *Plant Diseases*, 100, 916 -925

Allen, T. W., Martinez, A. & Burpee, L. L. (2019). *Pythium* blight of turfgrass. Retrieved on 21st November 2020 from http://www.apsnet .org/edcenter /intropp /lessons/fungi/Oomycetes/Pages/PythiumBlight.aspx.

Al-Rahman, A. N, Mostafa, A. A., Abdel-Megeed, A., Yakout, S. M. & Hussein, S. A. (2013). Fungicidal Activities of Certain Methanolic Plant Extracts Against Tomato Phytopathogenic Fungi. *African Journal of Microbiology Research*, 7, (6), 5 17 -524.

American Cancer Society (2020). Phytochemicals. Retrieved on 21st November 2020 from http://www.cancer.org/eprise/main/docroot/ETO/content/ETO_5_3X_Phytochemicals,

Arnold, R., Reilly, D, Dickinson, G. & Jovanovic, T. (2004). Determining the climatic suitability of *K. senegalensis* for plantations in Australia. Working Paper presented at a Workshop on "Prospects for high-value hardwood timber plantations in the 'dry' tropics of northern Australia. *Mareeba, Queensland*, 19-21.

Bacharis, C., Gouziotis, A. & Kalogeropoulou, P. (2020). Characterization of *Rhizoctonia* spp. isolates associated with damping-off disease in cotton and tobacco seedlings in Greece. *Plant Diseases*, 94, 1314–1322.

Bahramisharif, A., Lamprecht, S. C., Calitz, F. & Mcloed, A. (2018). Suppression of *Pythium* and *Phytophthora* damping-off of rooibos by compost and a combination of compost and non-pathogenic *Pythium* taxa. *Plant Disease*, 97, 1605–1610.

Bahramisharif, A., Lamprecht, S. C., Spies, C. F. J., Botha, W. J., Calitz, F. J. & McLoed, A. (2019). *Pythium* spp. associated with rooibos seedlings, and their pathogenicity toward rooibos, lupin, and oat. *Plant Disease*, 98, 223–232.

Barreto, J. C., Trevisan, M. T. S., Hull, W. E., Erben, G., De Brito, E. S., Pfundstein, B., Würtele, G., Spiegelhalder, B. & Owen, R. W. (2018). Characterization and Quantitation of Polyphenolic Compounds in Bark, Kernel, Leaves, and Peel of Mango (Mangifera indica L.). *Journal of Agriculture and Food Chemistry*, 56, 5599–5610.

Batool, N., Ilyas, N., Shabir, S., Saeed, M. & Mazhar, R. (2019). Mini-Review of therapeutic potential of Mangifera indica L. *Pakistan Journal of Pharmaceutical Science*, 31, 1441–1448.

Bauldaf, S. L., Roger, A. J., Wenk-Siefert, I., & Doolittle, W. F. (2020). A kingdom level phylogeny of Eukaryotes based on combined protein data. *Science*, 290, 972-977.

Bharti, R. P. (2018). Studies on antimicrobial activity and phytochemical profile of Mangifera indica leaf extract. *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 7, 74–78.

Bradley, C. A. (2018). Effect of fungicide seed treatments on stand establishment, seedling disease and yield of soybean in North Dakota. *Plant Disease*, 92, 120-125.

Broders, K. D., Wallhead, M. W., Austin, G. D., Lipps, P. E., Paul, P. A., Mullen, R. W. & Dorrance, A. E. (2019). Association of soil chemical and physical properties with *Pythium* species diversity, community composition, and disease incidence. *Phytopathology*, 99, 957- 967.

Castillo, J. V. (2019). Inoculating composted pine bark with beneficial organisms to make a disease suppressive compost for container production in Mexican forest nurseries. *Native Plants Journal,* 5, 181-185.

Chan, R., Suharti, S., Murniati, Sumarhani & Pradjadinata, S. (2019). Teknik pemupukan tanaman mahoni (*Swietenia macrophylla* King). *Indonesian Journal of Forest Research and Nature Conservation,* 16, (2), 11-19.

Chellemi, D. O., Mitchell, D. J., Kannwischer-Mitchell, M. E., Rayside, P. A., & Rosskopf, E. N. (2000). Pythium spp. associated with bell pepper production in Florida. *Plant disease*, 84(12), 1271-1274.

Chukwuebuka, E., Jonathan, C., Ifemeje1, T. L., Kryeziu, M. M., Hameed, S. G. M., Narasimha, R., Laurence, J., Francis J. G. & Habibu, T. (2020). Introduction to phytochemistry, *Apple Academic Press and Taylor & Francis Group*, 1, 4-8.

Cook, R. J. (2018). Making greater use of introduced microorganisms for biological control of plant pathogens. *Annals Review of Phytopathology*, 31, (1), 53-80.

Daughtery, M. L., Wick, R. L. & Peterson, J. L. (2019). Compendium of Flowering Potted Plant Diseases. *American Phytopathological Society,* 9(14), 90-100.

Degenhardt, J., Gershenzon, J., Baldwin, I. T. & Kessler A. (2020). Attracting friends to feast on foes: Engineering terpene emission to make crop plants more attractive to herbivore enemies. *Current Opinion Biotechnology*, 14, 169–176.

Dudareva, N., Pichersky, E. & Gershenzon, J. (2019). Biochemistry of plant volatiles. *Plant Physiology*, 135, 1893–1902.

Eid, A. M., Marzugi, N. & Ali-El Enshasy. (2018). Review on the phytopharmacological effect of Swietenia macrophylla. *International Journal of Pharmacology and Pharmaceutics Science*, 5, 3, 47-53.

El-Mohamedy, A. (2017). Biological control of *Pythium* root rot of broccoli plants under greenhouse conditions. *Journal of Agricultural Technology*, 8, 1017-1028.

El-Olemy, M. M., Farid, J. A. & Abdel-Fattah, A. A. (1994). Ethanol Extract of *P. stratiotes; NISEB Journal* 1(1), 51-59.

Farina, V., Corona, O., Mineo, V. & D'Asaro, A. (2018). Barone, F. Qualitative characteristics of Mango fruits (*Mangifera indica* L.), which have undergone preservation (Italian). *Italian Horticultural Society,* 12, 70–73.

Fernanado, P. M., Larissa, C. F., Jhonata, L. S., Leandro, P. P. & Paulo, E. S. (2013). Influence of plant extracts and essential oils against panama disease (*Fusarium oxysprum f. sp. cubense*) in banana seedlings. *Journal of Agricultural Science,* 5 (4), 1915-9752.

Gamliel, A. & Katan, J. (2017). Soil Solarization: Theory and Practice. *American Phytopathological Society, St.Paul. Forest Service, Washington, DC,* 171. Retrieved on 20th November, 2021 from https://www.mdpi.com/2311-7524/8/6/517.

Ghasemzadeh, A., Jaafar, H. Z. E. & Rahmat, A. (2019). Antioxidant activities, total Phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules,* 15, 4324-4333.

Gnanasekaran, P., Mohamed, S. S., Panneerselvam, A. & Umamagheswari, K. (2015) In vitro Biological Control of *Fusarium o xysporum* f. sp. Cubense by using Some Indian Medicinal Plants. *International Journal for Current Research and Academic Reviews,* 3, 11, 107-116.

Gupta, C., Garga, A. P., Unocal, R. C. & Kumar, A. (2008).Antimicrobial activity of some herbal oils against common food borne pathogens. *Africa Journal of Microbiology Research,* 2, 254-261.

Gurjar, M. S., Ali, S., Akhtar, M. & Singh, K. S. (2012). Efficacy of plant extracts in plant disease management. Central Potato Research Station, Shillong, India. *Indian Journal of Agricultural Sciences*, 3 (3), 423-433.

Hafiz, T. A., Shabbaz, T. S., Amer, H. & Sohail, A. (2016). Laboratory evaluation of fungicide and plant extracts against different strains of *Colletotrichum falcatum* the cause of red rot of sugarcane. *Journal of Agricultural Science*. 53, (1), 181-2016.

Hahn, N. I. (2019). Is Phytoestrogens Nature's Cure for What Ails Us? A Look at the Research. *Journal of the American Dietetic Association*, 98, 974- 976.

Harborne, J. B & Tomas-Barberan F. A. (2019). Ecological Chemistry and Biochemistry of Plant Terpenoids, *Clarendon, Oxford*. Retrieved on 21st December, 2022 from https://catalogue.nla.gov.au/Record/2609873.

Haruta, S., Nakayama, T., Nakamura, K., Hemmi, H., Ishii, M., Igarashi, Y. &Nishini T. (2018). Microbial diversity in biodegradation and reutilization processes of garbage. *Journal of Bioscience and Bioengineering*. 99 (1), 1–11.

Hauptli, H. & Jain, S. K. (2020). Biosystematics and agronomic potential of some weedy and cultivated amaranths. *Theories of Applied Genetics*, 52, 177-185.

Hill, D. S. & Waller, J. M. (2019). Integrated control, in Pests and Diseases of Tropical Crops Volume I. *Principles and Methods of Control*, 175, 64-65.

Horst, R.K. (2018). Damping-off. *Westcott plant disease handbook. Springer Netherlands, ordrecht,* 177-186.

Imam, H., Riaz, Z., Azhar, M., Sofi, G., & Hussain, A. (2019). Sweet flag (Acorus calamus Linn.): An incredible medicinal herb. *International Journal of Green Pharmacy*, 7(4) 111-119.

Jenkins, S. F. Jr. & Averre, C. W. (2018). Root diseases of vegetables in hydroponic culture biological component: nursery pests and mycorrhizae. *Agriculture handbook*, 5, 674-680.

Kennelly, M. (2019). *Pythium* Blight of Turfgrass. *Plant Pathology*. 150-159.

Kirk, P. M., Cannon, P. F., Minter, D. W. & Stalpers, J. A. (2018). Ainsworth and Bisby's Dictionary of the Fungi, 10th Edition. *Center for Agriculture and Bioscience International, Wallingford, UK.*

Krishnaiah, D., Sarbatly, R. & Bono, A. (2009). Phytochemical antioxidants for health and medicine. A move towards nature. *Biotechnology Mol. Biology. Review.* 1(4), 097-104.

Kulkarni, V. M. & Rathod, V. K. (2020). Extraction of mangiferin from Mangifera indica leaves using three phase partitioning coupled with ultrasound. *Industrial Crop Production*, 52, 292–297.

Kumar, M., Saurabh, V., Tomar, M., Hasan, M., Changan, S., Sasi, M. & Mekhemar, M. (2021). Mango (*Mangifera indica* L.) leaves: Nutritional composition, phytochemical profile, and health-promoting bioactivities. *Antioxidants*, 10(2), 299-309.

Kumar, V., Gupta P. & Dwivedi, S. (2017). Bio-efficacy of fly-ash based *Trichoderma* formulations against damping-off and root-rot diseases in tomato (*Lycopersicum esculentum*). *Indian Phytopathology*, 65, (4), 404-409.

Lacaille-Dubois, M. A. & Wagner, H. (2020). Bioactive saponins from plants: An update. In Studies in Natural Products Chemistry. *Atta-Ur-Rahman, ed. Elsevier Science Amsterdam*, 21, 633-687.

Ladeji, O, Okoye Z. S. & Ojobe, T. (2019). Chemical evaluation of the nutritive value of leaf of fluted pumpkin (*Telferia occidentalis*). *Food Chemistry*, 53, 353-355.

Lamichhane, J. R., Dürr, C., Schwanck, A. A., Robin, M. H., Sarthou, J. P., Cellier, V., Messéan, A. & Aubertot, J. N. (2017). Integrated management of damping-off diseases. A review. *Agronomy Sustenance and Development*, 37, 10-20.

Langenheim, J. H. (2019). Higher plant terpenoids A phytocentric overview of their ecological roles. *Journal of Chemical Ecology*, 20, 1223- 1280.

Lévesque, C. A. & De Cock, A. W. (2019). Molecular phylogeny and taxonomy of the genus *Pythium*. *Mycology Research*, 108, 1363-1383.

Lumsden, R. D., Ayers, W. A., Adams, P. B., Dow, R. L., Lewis, J. A., Papavizas, G. C. & Kantzes, J. G. (2017). Ecology and epidemiology of *Pythium* species in field soil. *Phytopathology*, 66, 1203-1209.

Mahfuzul, H. M. D, Bari, M. L, Inatsu, Y, Juneja, V. K. & Kawamoto, S. (2019). Antibacterial activity of guava (*Psidium guajava* L.) and neem (*Azadirachta indica* A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathogens and Disease*, 4, 481‑8.

Makut, M. D., Gyar, S. D, Pennap G. R. I. & Anthony, P. (2007). Phytochemical screening and antimicrobial activity of the ethanolic and methanolic extracts of the leaf and bark of *Khaya senegalensis*. *Africa Journal of Biotechnology*, (9), 1216-1219.

Makut, M. D., & Ekeleme, I. K. (2018). Citric Acid Production by Aspergillus niger and Trichoderma viride Using Hydrolysed Potato Peels Substrate. *Asian Journal of Advances in Agricultural Research*, 1-7.

Manickara, M. & Veerababu, R. M. (2014). Petrochemical, FI-IR antibacteria activity of whole plant extracts of *Aerva lanata* (L) just. Ex schult. *Journal of Medicine and Plant Studies*, 2(3), 51-57.

Martin, F. N. & Loper, J. E. (2019). Soilborne Plant Diseases Caused by Pythium spp.: Ecology, Epidemiology, and Prospects for Biological Control. *Critical Revised Plant Science*, 18, 111–181.

Milijasevic, S., Rekanovic, E., Hrustic, J., Grahovac, M. & Tanovic, B. (2017). Methods for management of soilborne plant pathogens, *Pesticide Phytomedicne*, 32, (1), 9–24

Minuto, A., Migheli, Q. & Garibaldi, A. (1995). Integrated control of soil-borne plant pathogens by solar heating and antagonistic microorganisms. *International society for Horticulture Science*, 382, 138-144DOI:10.17660/ActaHortic.1995. 382.14.

Moorman, G. W. (2019). Damping off. Pennsylvania State University Cooperative Extension. *Plant Disease Fact sheet*, 2, 222-230

Mueller-Harvey I. (2019). Tannins their Nature and Biological significance. In: Secondary plants products. Antinutritional and beneficial actions in animal feeding Caygill J. C and Mueller-Harvey I, eds. *Nottingham University Press* (UK), 17-70.

Mugerwa, J. S. & Bwabye, R. (2019). Yield, Composition and Vitro Digestibility of *Amaranthus hybridus* subspecies incurvatus. *Tropical Grasslands*, 8, 49-53.

Mundel, H. H., Huang, H. C., Kozub, G. C. & Barr, D. J. S. (2018). Effect of Soil Moisture and Temperature on Seedling Emergence and Incidence of *Pythium* Damping-off in safflower. *Carthamus tinctorius* L. *Canadian Journal of Plant Science*, 75, 505–509

Munro, A., Derek, B. & Small, E. (2018). Vegetables of Canada. National Research Council Canada, 464-470. ISBN 0-660-16708-5.Retrieved on 26th November 2021 from https://libcat.aun.edu.ng/cgi-bin/koha/opac-detail.pl?biblionumber=9453

Naveen Y.P., Divya Rupini G., Ahmed F. & Urooj A. (2019). Pharmacological effects and active phytoconstituents of *Swietenia mahagoni*: a review. *Journal of Integrated Medcine*, 12(2), 86-93.

Ntengna, Y. F., Tchameni, N. S., Fokom, R., Sameza, M. L., Minyaka, E., Ngonkeu, M. E. L., & Nwaga, D. (2019). Effects of Arbuscular Mycorrhiza fungi on Stimulation of Nutrient Content and Induction of Biochemical Defense Response in *Xanthosoma sagittifolium* Plants against Root Rot Disease caused by *Pythium myriotylum*. *International Journal of Advance Agricultural Research*, 7, 98, 107.

Oguntibeju, O. O. (2018). Medicinal Plants with Anti-Inflammatory Activities from selected countries and regions of Africa. *Journal of inflammation research*, 11, 307-312.

Okafor, J. C. (2020). Horticultural Promising Indigenous wild Plant Species of the Nigerian Forest Zone. *Acta Horticulture*, 123, 165 – 176.

Oloyede, G. K., Onocha, P. A., Soyinka, J., Oguntokun, O., & Thonda, E. (2010). Phytochemical Screening, Antimicrobial and Antioxidant Activities of Four Nigerian Medicinal Plants. *Annals of Biological Research*, 1(2), 114-120.

Olson, J. D., Damicone, J. P. & Kahn, B.A. (2019). Identification and characterization of isolate of *Pythium* and *Phytophthora* spp. from snap beans with cottony leak. *Plant Disease*, 100, 1446–1453.

Oluwole, S. O., Olubunmi, M. S. C., Kafeelah, A. Y., Olusegun, O. F., & Ayobami, O. O. (2013). Determination of heavy metal contaminants in leafy vegetables cultivated by the road side. *International Journal of England Research Development*, 7, (3), 1-5.

Palavy, K. & Priscilla M. D. (2019). Standardization of selected indian medicinal herbal raw material. *Journal Pharmaceutical sciences*, 68, 506- 509.

Pridham, J. B. (2020) In Phenolics in plants in health and disease, *Pergamon Press, New York*, 34-35. Retrieved on 12th December 2020 from https://www.cabdirect.org/cabdirect/abstract/19611101181.

Rajendraprasad, M, Vidyasagar M. B., Uma Devi, G. & Koteswar, S. R. (2017). Biological control of tomato damping-off caused by *Pythium debaryanum. International Journal of Chemical Studies*, 5, (5), 447-452.

Ramarathnam, R., Fernando, W. D. & de Kievit, T. (2019). The role of antibiosis and induced systemic resistance, mediated by strains of *Pseudomonas chlororaphis*, *Bacillus cereus* and *B. amyloliquefaciens*, in controlling blackleg disease of canola. *Biology Control*, 56, 225–235.

Rao, R. V. K., Ali, N. & Reddy, M. N. (2019). Occurrence of both sapogenins and alkaloid lycorine in *Curculigo orchioides*. *Indian Journal of Pharmacy Science*, 40, (3), 104-105.

Ravindran,. R. J. Hood, C. R. Gill, W.L. Kneale, O. & Bryden T. (2019). Nutritional evaluation of grain amaranth (*Amaranthus hypochondriacus*) in broiler diets. *Animal Feed Science and Technology*, 63, 1(4), 323-331.

Ribeiro, L., Amoriu, E. P., Cordeiro, Z. M. J., Silva, D. O. S. & Dita, M. A. (2011). Discrimination of genotypes for *Fusarium* wilt resistance in the green house. *Acta Horticulturae*, 897, 381-385.

Sallam, N. A., Riad, S. N., Mohamed, M. S. & El-Eslam, A. S. (2019). Formulations of *Bacillus* spp and *Pseudomonas fluorescens* for bio control of cantaloupe root caused by *Fusarium solani*. *Journal of Plant Protection Research*, 53, (3), 295–300.

Santhi, K. & Sengottuvel, R. (2016). Qualitative and quatitative phytochemical analysis of *Moringa concanesis* nimmo. I*nternational Journal of Microbiology and Applied Science.* 5, (1), 633-640.

Scheuerell, S. J., Sullivan, D. M. & Mahaffee, W. F. (2018). Suppression of seedling Damping-off caused by *Pythium ultimum*, *P. irregulare*, and *Rhizoctonia solani* in container media amended with a diverse range of pacific Northwest compost sources. *Phytopathology*, 95, 306-315.

Schofield, P, Mbugua, D. M. & Pell A. N. (2019). Analysis of condensed tannins: a review. *Animal Feed Science Technology*, 91, 21-40.

Sengar, A., Kumar, B., Pavavi, M. & Gosal, S. (2009). In Vitro selection at cellular level for red rot resistance in sugarcane (*Saccharum* spp.) *Plant Growth Regulation*, 58, 201-209.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, 299, 152-178.

Smith, M., Le, D. P., Hudler, G. W. & Aitken, E. (2019). *Pythium* Soft rot of ginger: detection and identification of the causal pathogens, and their control. *Crop Protection*, 65, 153–167.

Sneh, V., Tundra, M. T., Revathy, K., Suthindhiran, K. & Jayasri, M. A. (2013). Phytochemical and pharmacological evaluation of selected plants. *American Journal of Biochemistry and Biotechnology*. 9, (3), 291-299.

Stirling, G.R., Turaganivalu, U., Stirling, A.M., Lomavatu, M.F. & Smith, M.K. (2019). Rhizome rot of Ginger (*Zingiber officinale*) caused by *Pythium myriotylum* in Fiji and Australia. *Australia Plant Pathology*, 38, 453-460.

Subramanian, R., Subbramaniyan, P., & Raj, V. (2013). Antioxidant activity of the stem bark of *Shorea roxburghii* and its silver reducing Power. *Springer Plus*, 2(1), 1-11.

Tomioka, K., Takehara, T., Osaki, H., Sekiguchi, H., Nomiyama, K. & Kageyama, K. (2018). Damping-off of soybean caused by *Pythium myriotylum* in Japan. *Journal of General Plant Pathology*, 79, 162–164.

Tomoko, N., Takashi, A., Hiromu, T., Yuka, I., Hiroko, M. & Munekazu, I. (2018). Antibacterial activity of extracts preparated from tropical and subtropical plants on methicillin-resistant *Staphylococcus aureus*. *Journal of Health Science.* 48, 273-276.

Turan, M. S. Kordali, H. Zengin, A. Dursun, & Y. Sezen, (2018). Macro- and micro-mineral content of some wild edible leaves consumed in Eastern Anatolia. *Acta Agricultural Scandia Plant and Soil Science*, 53, 129 – 137.

Walker, K., & Lynch, M. (2007). Contributions of anopheles larval control to malaria suppression in tropical Africa: review of achievements and potential. *Medical and veterinary entomology*, 21, (1), 2-21.

Walton, N. J, Mayer, M. J. & Narbad, A. (2020). Molecules of interest: vanillin. *Phytochemistry*, 63, 505-515.

Wang, P. H. & Chang, C. W. (2018). Detection of the low germination rate resting oospores of *Pythium myriotylum* from soil by PCR. *Letters of Applied Microbiology*, 36, 157-161.

Watanabe, H., Kageyama, K., Taguchi, Y., Horinouchi, H. & Hyakumachi, M. (2018). Bait method to detect *Pythium* species that grow at high temperatures in hydroponic solutions. *Journal of Genetics and Plant Pathology*, 74, 417-424

Yang, X. B. (2019) Identification of soybean seedling diseases. *Integrated Crop Management*, 486, (10),79–80

Yulier, A., Nion, Y.U. & Toyota, K. (2015). Recent trends in control methods for bacterial with disease caused by *Ralstonia solanaceaerum*. *Microbes and Environments*, 30, (1), 1-11.