CHEMICAL ECOLOGY AND MANAGEMENT OF *Phytolyma fusca* WALKER (HEMIPTERA: PSYLLIDAE) USING ORGANIC AMENDMENTS ON *Milicia excelsa* C.C. BERG

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CERTIFICATION

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DEDICATION

This work is dedicated to the Most Merciful and Gracious GOD, the Creator of Heaven and Earth and the giver of life, and all foresters and entomologists for their tireless efforts in creating a sustainable environment. I give a profound gratitude to Jehovah who Bestowed me with good health and wellness to pursue and complete this higher degree programme amidst turbulence and tribulation.

May Honour and praises be unto Him for his goodness, wisdom, knowledge, understanding and mercy upon me.

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ABSTRACT

Milicia excelsa (Me) is an important timber tree whose establishment has been constrained by *Phytolyma fusca* (Pf) attack. A commonly used method of Pf control with systemic pesticides has been unsuccessful but cultural management with organic amendment could be feasible. Information on the chemical interactions between Pf and Me, and effectiveness of organic amendments for the management of Pf is scanty. Therefore, phytochemical constituents, reactive oxygen species of Me seedlings attacked by Pf and effect of soil amendments on its growth and gall formation were investigated.

Seeds of Me (n=272) collected from a mother tree were germinated and raised in a screen cage, out of which 20-seedlings were collected and infested with Pf following standard procedure. Galled Leaves (GL) and Healthy Leaves (HL) were analysed at 32- and 64-weeks for phytochemical contents using Gas Chromatography-Mass Spectrometry. Third-leaf from the apex of healthy seedlings (n=12), aged 32-weeks were mechanically wounded using a sterile needle perforator, excised after 6 hours; and analysed using standard staining technique for Superoxide Anion (SA) and Hydrogen Peroxide (HP). Soil was amended with Poultry droppings (Pd), Cattle dung (Cd) and Pig faeces (Pgf) at ratio 2:1. Sixty replicates of healthy seedlings (n=240), aged 24-weeks were randomly transplanted and immediately exposed to Pf, while seedlings on untreated soil served as control. Seedling survival (SS),%, Seedling height (SH),cm, Collar diameter (CoD),cm and Number of galls (NoG) were assessed fortnightly for 22 weeks. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Six phytochemicals; tannins, flavonoids, alkaloids, saponins, steroids and terpenoids were identified in GL and HL at 32- and 64-weeks, respectively. Alkaloids were higher in GL (2.44±0.02%) and lower in HL (2.12±0.03%) at week-32. Saponins, terpenoids, tannins and flavonoids decreased from 0.09±2.08 mg/g, 0.55±0.01 mg/g, 0.44±0.45 mg/g, 0.49±0.08 mg/g in HL to 0.01±0.01 mg/g, 0.16±0.07 mg/g, 0.23±0.06 mg/g, 0.23±0.06 mg/g in GL at week-32, respectively. At week-64, saponins and alkaloids were higher in GL (0.25±0.16 mg/g and 6.30±0.14%) and lower in HL (0.17±2.08 mg/g and 3.78±0.13%), respectively. Terpenoids, tannins and flavonoids were higher in HL (0.70±0.31 mg/g 0.84±0.85 mg/g and 0.73±0.39 mg/g) and lower in GL (0.54±0.43 mg/g, 0.57±0.65 mg/g and 0.50±0.19 mg/g), respectively. Numbers of Heterocyclic Compounds (NHC) in the GL (18) was lower in HL (20) at week-32. Also, NHC in GL (15) reduced by 45% and HL (18) by 47% at week-64, respectively. Terpenoids present in HL were absent in GL at week-64. Brown colouration on wounded leaf-tissues indicated the presence of HP while SA was absent in both leaf treatments. The SS was least in Pd (50.0%) and highest in control (80.0%). The SH and CoD significantly increased from 23.4±0.82 to 46.21±2.59; and 0.46±0.02 to 0.77±0.03, in control and PD seedlings, respectively. The NoG was lowest in CD (0.75±0.06) and highest in control (1.25±0.09).

Heterocyclic compounds in galled leaves reduced with persistent attack, while *Phytolyma fusca* activity decreased the amount of terpenoids in healthy leaves. Mechanical wounding induced hydrogen peroxide production. Soil amendment with poultry droppings enhanced growth of *Milicia excelsa* seedlings.

Keywords: *Milicia excelsa*, Plant secondary metabolites, Terpenoids, Reactive oxygen species.

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CHAPTER ONE
INTRODUCTION

Insect-Plant interactions are a complex systems, subject to continuous change. These interactions are mediated by chemical and biophysical barriers on plants that induce the production of defensive proteins, the release of volatile compounds which entice predators, production of metabolites, trichomes and spikes (Birkett *et al*., 2000; Baldwin, 2001; Fordyce and Agrawal, 2001; Haruta *et al*., 2001; Kliebenstein *et al*., 2001). The tissues of plants can adequately respond to biotic stress by forming galls in some cases, alteration in biochemical constituents, and their biophysical components such as trichome density and leaf texture (Oliveira and Isaias, 2010). The galling response from plant tissues is one of the major biotic stressors (Mani, 1964; Shorthouse and Rohfritsch, 1992). The formation of gall in plants and its growth comprises re-differentiation of cells which usually metamorphosis into the plant producing new and different internal organs (Oliveira and Isaias, 2010).

Gall forming insects have been reported to use phytochemical compounds signals like those normally used in the metabolic processes in plants for gall formation (Abrahamson and Weiss, 1997; Detoni *et al*., 2011). This interaction has made some plants evolve, strategic means by which they can cope (Ballhorn *et al*., 2009). Unfortunately, empirical studies on the ecology of chemical mediated interfaces between plants and insects have been guided by theoretical advancement over a long time, which are often time-constrained by inadequate data (Arnason *et al*., 2004).

In plant-insect interaction, the plant chemical defence system could either be produced or induced by the inducer. This often time assists in the protection of the plant against stress. An example is the prevention of herbivory by an insect in plants due to the possession of a diverse array of phytochemicals that serve as toxin and repellents. These chemical compounds, produced through the plant's metabolic processes, play a significant function in the plant, especially in their growth and defence from attacks. The involvement

of primary metabolites in plant growth and development is very sacrosanct, while the secondary metabolite is sketchy but could act as plants defence compounds (Jander *et al*., 2015).

Insect attack on plants is generally associated with wounding, which induces modifications in the plant's response (Kessler and Baldwin, 2002). When this attack leads to galling, these galls are often adaptive for the insect (Matsukura *et al*., 2012). This is because wounding causes hypersensitive reactions such as the accumulation of reactive oxygen species molecules (Van Breusegem *et al*., 2001). The role of these molecules in controlling important processes in the tissue of plants and its stress reaction is sacrosanct (Moloi and van der Westhuizen, 2006).

The interaction that causes gall formation in *Milicia excelsa* (*M. excelsa*) by *Phytolyma fusca* (*P. fusca*) has become a major field of interest in the management of this important tropical tree species. *Milicia excelsa*, also known and called Iroko or/and African teak in the International timber market, is a deciduous, valuable hardwood tree species that is widespread in tropical Africa forests (Ouinsavi *et al*., 2005; Oteng-Amoako, 2006; Ofori and Cobbinah, 2007; Omolokun and Oladele, 2010). The wood of *M. excelsa* is useful for decoration, furniture and construction work, and it is very hard and can withstand fungal and termite attacks (Hawthorne, 1995). The exceptionally durable wood is used for construction, furniture, cabinetwork, panelling, frames and floors, production of fuelwood, and other uses such as mulch, shade, and windbreak.

Regeneration of *M. excelsa* has been seriously affected by *P. fusca*, an important and common insect pest across its area of endemism (Ugwu and Omoloye, 2014; Ugwu *et al*., 2019). In Nigeria, the *M. excelsa* tree is of great economic value used in furniture and other wood and spiritual purposes, but it is seriously threatened (Olajuyigbe *et al*., 2015). Furthermore, its regeneration is critically constrained by the Iroko gall midge, which attacks young seedlings causing death.

Phytolyma fusca attack the shoot of young seedlings and, in some cases, saplings of *M. excelsa*, which invariably lead to the formation of galls on the leaf and apical meristem. The psyllid, *P. fusca*, attacks when the female lays its eggs on the leaves and buds. The nymph gets its nutriment inside the gall chamber by breaking down the internal

cells of the plants. This invariably leads to the swelling of parenchyma cells in the leaf tissue (Wagner *et al*., 2008). After growing to the stage of maturity, the nymph exits the gall chamber; the gall then becomes exposed to a pathogen which usually leads to disease outbreak such as the dieback disease that causes the plant to lose vigour and, in some cases leading to the death of young seedlings (Djagbletey *et al*., 2011). This attack automatically initiates galling at the regions of induction, and gall has been ruptured after 14 days to release matured insects, subsequently causing young plant death (Nichols *et al*., 1999; Agyeman *et al*., 2009; Ugwu and Omoloye, 2015).

Galls caused by the insect are uncharacteristic growths that look very different from other and normal cells and tissues. The link between the host plant and gall-forming insects has been a subject of continued dispute for decades (Mani, 1964; Krishnamurthy, 1984). Galls caused by gall formers have been reported to be mummified with some specific fungi (Batra and Lichtwardt, 1963). A report on the existing relationship between plant and insect concerning fungi has made the whole theory complex.

Factors that serve as constraints to the propagation of *M. excelsa* include slow growth, bush burning, people's perception, unavailability of viable seeds and insect pests (Ofori and Cobbinah, 2007; Olajuyigbe *et al*., 2015; Ugwu and Omoloye, 2015; Ugwu *et al*., 2019). These constraints have made regeneration of *M. excelsa* difficult. The pestiferous damage done by *P. fusca* is the most imperative restraining cause to establishing the *M. excelsa* tree under plantations condition in Nigeria.

Several pest control methods have been evaluated for *P. fusca* on *M. excelsa*. This includes the use of chemicals, plants extract, physical, mechanical, and cultural barriers, and adopting some integrated pest management strategies (Ofori and Cobibinah, 2007; Ugwu and Omoloye, 2015; Olajuyigbe *et al*., 2015).

Insect growth and population increase have been related to the chemistry of the soil on which the plant is grown (Sinha *et al*., 2018). The promotion of rapid and healthy plant growth and yield increment can be attributed to effective fertilisation. Promoters of the use of organic matter in plant production believe that plants enriched solely with nutrients from organic matters or components are more resistant to insect pest attacks.

Similarly, the plant's resistance to pests is strictly hinged on the different characteristics of soils at its best (Sinha *et al*., 2018).

Plants growing on well-nourished soil are believed to grow better and healthier than plants grown on nutrient-deficient soil, especially in their ability to compensate for pest damage (Teetes, 1980; Listinger, 1993). Therefore, plant nutrition management is an important system deployed in plant production to attain high yield and stimulate plant responses to insect pests and diseases (Chau and Heong, 2005).

The system of plant nutrition management is a major contributor to improved plant output, and this contributor stimulates the plant growth population of the insect pest. This is often subjected to the kind of fertiliser applied and the insect pest. Nevertheless, when plant nutrition management is not properly done, it could lead to pest problems (Jahn, 2004). The plant grown under organic materials are said to exhibit great tolerance or/, and resistance against insect pest, whereas those planted under poor soil nutriment tends to have weak resistance and suffers during insect pest attack (Magdoff and Van, 2000). The availability of required and sufficient mineral nutrients in the soil directly impact the extent of damage received from insect pest as well as the ability of the plant to withstand and recover after being attacked. Historically, organic soil amendments have been a major source of plant input to boost soil productivity and improve the nutrient status of soil (Beckman, 1973). Generally, aside from potassium, phosphorus, magnesium, and some other macronutrients, nitrogen is the major minerals element in organic materials, and these elements are essential for plant growth and development. Attempts by insect scientists to assess plant growth response to organic amendment and insect pest populations are still under experimental trials. However, few results showed that soil amendments using manure diminishes insect pest population (Culliney and Pimentel, 1986; Eigenbrode and Pimentel, 1988; Listinger, 1993; Fallahpour *et al*., 2015), contrary results reported that soil amendments with manure increased the population of insect pest (Costello, 1994; Costello and Altieri, 1995).

The chemical interactions that take place due to the attack by *P. fusca* attack on the *M. excelsa* plant have not been elucidated. Reports suggest that secretions injected during insect oviposition or feeding by the insect larva could trigger gall development

(Shorthouse and Rohfritsch, 1992; Higton and Mabberly, 1994; Yamaguchi *et al*., 2012). The dearth of information on the reaction during gall formation created puzzles on the experimental pathway of the organic chemical processes.

Because of the great and important value of this tropical rainforest tree species and the grave consequences of losing it to unregulated logging activities, it has become the focus of increasing public attention, such as the advocates for the cultivation of indigenous tree species. It, therefore, becomes imperative that efforts directed at replenishing these depleted forests species would be a giant stride at restoring these important indigenous tropical tree species.

Previous studies on the psyllid have focused on its biology and ecology and natural regeneration and management using different strategies such as assessment for resistant lines (Cobbinah, 1990; Cobbinah and Wagner, 1995; Nichols *et al*., 1998; Ugwu and Omoloye, 2015). This study aims to fill the information gap on the chemical interaction between *M. excelsa* and *P. fusca* vis-à-vis the role of phytochemical constituents and the hypersensitivity reaction in the plant. This is required to develop appropriate management tactics for restocking M. excelsa in the Nigeria tropical rainforest.

Main objective: The main objective of this research work is to characterise the phytochemical compounds and reactive oxygen species in the leaves of *M. excelsa* seedlings attacked by *P. fusca* to identify the biochemical pathways in the insect-plant interaction.

Specific objectives: The study-specific objectives were to:

i. determine phytochemical constituents in *Milicia excelsa* gall due to attack by *Phytolyma fusca*

ii. characterise and quantify Reactive Oxygen Species of galled *Milicia excelsa* mediate the constitutive defence system against *Phytolyma fusca*

iii. identify fungi associated with *Phytolyma fusca* gall formation on *Milicia excelsa*

iv. assess the morphological response of *Milicia excelsa* to *Phytolyma fusca* attack

v. evaluate the effects of poultry droppings, cattle dung and pig faeces on growth and gall formation of *Milicia excelsa* seedlings during *Phytolyma fusca* attack.

CHAPTER TWO
LITERATURE REVIEW

2.1 Origin and Distribution of *Milicia excelsa*

Milicia comprises two species; *Milicia excelsa* and *Milicia regia*, which belong to the family Moraceae. They are indigenous to tropical Africa and are commercially known as Iroko in Nigeria. The trees of *M. excelsa* are dioecious and grow tall to 50 m and diameter of 3 m. The colour of the tree bark is dark grey with a thick texture. It exudes milky-like substances when cut. The tree branches are usually horizontal and appear in an umbrella shape. The leaf structure is ovate with a toothed edge measuring between 5 cm to 10 cm. The colour of the leaf is green but turn yellow as it gets old and matures (Ofori, 2007).

The features of the male and female trees are very distinct. The female, during flowering, develop flower spikes that are green in colour, while the male trees produce whitish catkins. Female trees produce erect flower spikes, and flowers found on separate (male and female) have been attributed to seasonal variations. Flowers appear a few weeks after shedding or with the new leaves. *Milicia excelsa* annually during dry seasons shed its leaves for a short period, and propagation of the plant can be done using viable seeds and young stem cuttings (Ofori, 2007).

2.2 Botanical and Ecological Characteristics of *Milicia excelsa*

Milicia excelsa naturally originated from Guinea Bissau, and it is ecologically distributed through Mozambique before spreading to other parts of Africa, including Nigeria (White, 1966). The main habitats of the tree are deciduous, semi-deciduous, primary or secondary forest, as well as in other types of forest landscapes and cultivated areas (Nichols *et al*., 1998). *Milicia excelsa* grows best in areas with temperature and rainfall amounts ranging from 25°C – 35°C and 1150 mm – 1900 mm annually, respectively. *Milicia excelsa* grows on various soil but has high demands concerning soil fertility, making it an excellent pointer to well-nourished soil suitable for other agronomic

purposes (Taylor, 1960). It grows best in sandy-loamy soils, does not tolerate high precipitate soil, and can withstand a mild drought for up to 6 months (Ofori *et al*., 2003).

2.3 Utilisation of *Milicia excelsa*

The wood of *M. excelsa* is highly valued, and it commands great demand and price in the timber market. It is used for various wood construction work and all manners of woodwork purposes and high-density boards for decoration and sliced veneer (Ofori, 2007). The bark of the tree is a vital substrate used in dyeing. It is prominent in marking boundaries between farms or villages (Bolza and Keating, 1972). *Milicia excelsa* root parts are used in the preparation of decoction in some parts of Africa for the treatment of female sterility and to also stimulate sexual desire (Neuwinger, 2000). Herbal formulation from the stem-bark of mature trees is administered to treat cough, asthma, heart trouble and other primary health challenges (Padayachee and Odhav, 2001). This herb is also taken against other health challenges such as tumours and obstructions of the throat (Olajide *et al*., 2005). Preparation from the leaves is eaten to treat insanity gallstones and is externally applied to treat snakebites and fever. In West African countries, 'Iroko' trees are highly adorned and critically conserved near the house and in places where it is being cultivated. The leaves of the tree are used to prepare charms by voodoo, and the tree is vital to the customs and traditions of most African countries (Ofori, 2007).

In southwestern Nigeria, *M. excelsa* is an economic tree of great benefits. Benefits derived from the tree includes financial, medicinal, security, worship and environmental (Ugwu and Omoloye, 2015). The financial benefits derived are from the timber sale in local and international timber markets.

2.4 Constraints to Production of *Milicia excelsa*

Milicia excelsa, otherwise known as Iroko, is propagated mainly through seeds and vegetative methods (Ofori, 1994). Mature fruits are usually collected after they are dropped on the ground, and the seeds are removed before the fermentation process sets in on the fruit (Ofori, 2007). Seeds can be extracted from the fruit by squeezing after being soaked for days in water, and they are best sown immediately or after a few days (Ofori *et al*., 1996). After seeds extraction, sowing is done in germination box, and sprouted

seedlings are transplanted in singles to plant pot, usually polythene bags filled with soil three weeks after germination. Generally, seedlings are transplanted to the field about four months after sowing. In addition, *M. excelsa* can be propagated through vegetative methods such as layering grafting using stem and root cuttings (Ofori *et al*., 1996).

Milicia excelsa, in its area of endemism, are scarce and becoming sparsely distributed due to the unsustainable exploitation of the timber from the natural forest and insect pest problems (Ofori and Cobbinah, 2007; Ugwu and Omoloye, 2015). Slow growth, bush burning, belief and scarcity of viable seeds are some of the other foremost hindrances to the production of Iroko trees (Ugwu and Omoloye, 2015). There is a general traditional belief that the Iroko tree is sacred and should be worshipped in southwest Nigeria. It is also believed to possess spiritual power and that its cultivation should be restricted (Ugwu and Omoloye, 2015). In some farmland areas in southwestern Nigeria, farmers do not nurture it due to the general belief that tree is associated with certain spirits (Ugwu and Omoloye, 2015). However, it is expected that *M. excelsa* is important in the mythology and tradition of the societies it grows (Neuwinger, 2000). Nevertheless, there has been some renewed interest in some parts of Nigeria on the need for propagation and conservation of the species because of its socio-economic importance (Ugwu and Omoloye, 2015).

Microbes, insects, birds, rodents have been reported to attack the fresh fruits and seeds and stored seeds of *M. excelsa*. These attacks usually lead to the loss of fruits, seeds, and seed viability, especially when the embryo of the seeds is consumed (Apetorgbor *et al*., 2001). Insects and microbes have been recognised as significant pests that cause setbacks in establishing *M. excelsa* seedlings under plantations condition (Wagner *et al*., 2008). The insect attack by defoliating the leaves sucking saps, thereby disrupting the plant's physiological process, leading to the death of the entire tree (Ofori, 2007). Some insects, such as the hemipterans and dipterans, are disease vectors that transmit pathogens to plants during and after an attack. They weaken the tree by sucking the sap resulting in damage or death (Apertorgbor *et al*., 2001).

Generally, the loss of fauna biodiversity such as *M. excelsa* into extinction is alarming, and this could spell doom to them in the future (Pimm *et al*., 1995; Ricketts *et al*., 2005). Because of climate change globally, it is imperative to pinpoint extinction to

reverse the trend through appropriate risk driver diagnosis (Darrah *et al*., 2017). According to Kakpo *et al*. (2019), modelling the *M. excelsa* niche shows the species marginalisation in its areas of endemism. This can best be described by the significant variations projected for rainfall and temperature. According to Daïnou *et al*. (2012), the *M. excelsa* plant is best adapted to the temperature and rainfall ranging between 25 °C and 35 °C and 1150 mm and 1900 mm, annually, respectively. Due to climate change, variations in temperature and rainfall significantly affect the production of suitable habitats for *M. excelsa* biological diversity and distribution (Busby *et al*., 2010).

2.5 Current status of *Milicia excelsa* in Nigerian Forests

Anthropogenic pressure on account of industrialisation, urbanisation and agriculture in the Nigerian forest heightened tremendously. Forest reserves are currently farmed and, in some cases, transformed into single-dominant plantations of exotic tree species. *Milicia excelsa* seedlings were, for the first time in Nigeria, systematically planted in Mamu and Olokemeji forest reserves in 1907 (Kennedy, 1933). After that, in 1908, 16,700 seedlings were planted, and in the year 1909, twenty-five acres were planted, while 15,000 seedlings were planted along the railway line between Olokemeji and Eruwa Road stations. In 1910, a sample plot consisting of 2,500 *M. excelsa* seedlings was planted in Mamu, while the Railway plantations at Olokemeji and Eruwa Road were extended by 12 acres, containing about 8,000 plants (Kennedy, 1933).

Milicia excelsa, according to IUCN (2010), is a near-threatened species. Therefore, the tree is usually not cultivated in most parts of Nigeria. However, Onefeli and Agwu (2015) reported that *M. excelsa* could be used as a candidate plant for mix-cropping because of the tree association with other tree species, which are of great economic importance to users who depend on it for livelihood and sustenance. A survey in Ibadan revealed 62 stands of *M. excelsa*, and this was a tremendous drift from the population that existed several decades ago (Onefeli and Agwu, 2015). In the last five decades, Nigeria has consistently experienced increased urbanisation. Anthropogenic activities on land mainly cause the alteration of ecosystem structure and function and plant availability.

Milicia excelsa, 'Iroko' trees, have become sparsely distributed in Nigeria (Babalola *et al*., 2012; Ugwu and Omoloye, 2015). Apart from those situated in restricted

and protected areas, the tree population is faced with the threat of being fell at any point in time. Babalola *et al*. (2012) revealed that protected areas and institutions vicinities harboured most *M. excelsa* trees in a metropolitan area. The protective status conferred on the tree species made it illegal to fall in these areas. Most of the trees are situated in protected areas where complicated hauling operations. Some of the stands were also close to residential buildings, and harvesting operations could cause severe infrastructural damage. The situation is similar all over southwestern Nigeria, with the population of M. excelsa being deficient and sparsely distributed. The population of *M. excelsa* trees in Ekiti State is very high compared to other states in South-West Nigeria. (Ugwu and Omoloye, 2015).

2.6 Taxonomy of *Phytolyma fusca* and its damage to *Milicia excelsa*

Class – Insecta

Order – Hemiptera

Superfamily -Psylloidea

Family – Homotomidae

Genus – *Phytolyma*

Species – *Phytolyma fusca*

The genus Phytolyma is found throughout the ecological range of *M. excelsa*. It consists of four species, *Phytolyma lata* Walker, *Phytolyma tuberculata* Alibert, *Phytolyma fusca* Walker and *Phytolyma minuta* Hollis (Ofori, 2007; Ugwu *et al*., 2019). Phytolyma species in Nigeria are closely related to *Craspedolepta canadensis*, a psyllid found in North America. However, a taxonomic review of the insect recently showed that *P. fusca* (Alibert) is predominant around Milicia species and is widely distributed in Nigeria (Ugwu *et al*., 2019). It is also believed that *P. lata* is very common in tropical rainforest areas where its prominent host is *M. excelsa* (Wagner *et al*., 2008). The life-cycle of the insect is an incomplete metamorphosis. The female oviposits on the host plant leaf (Plate 2.1). The emergence of the nymph takes place after about eight days of incubation. The young first instar nymph penetrates and burrows through the internal structures of the leaf, causing damages. Gall formation is noticed in 1 to 2 days after this

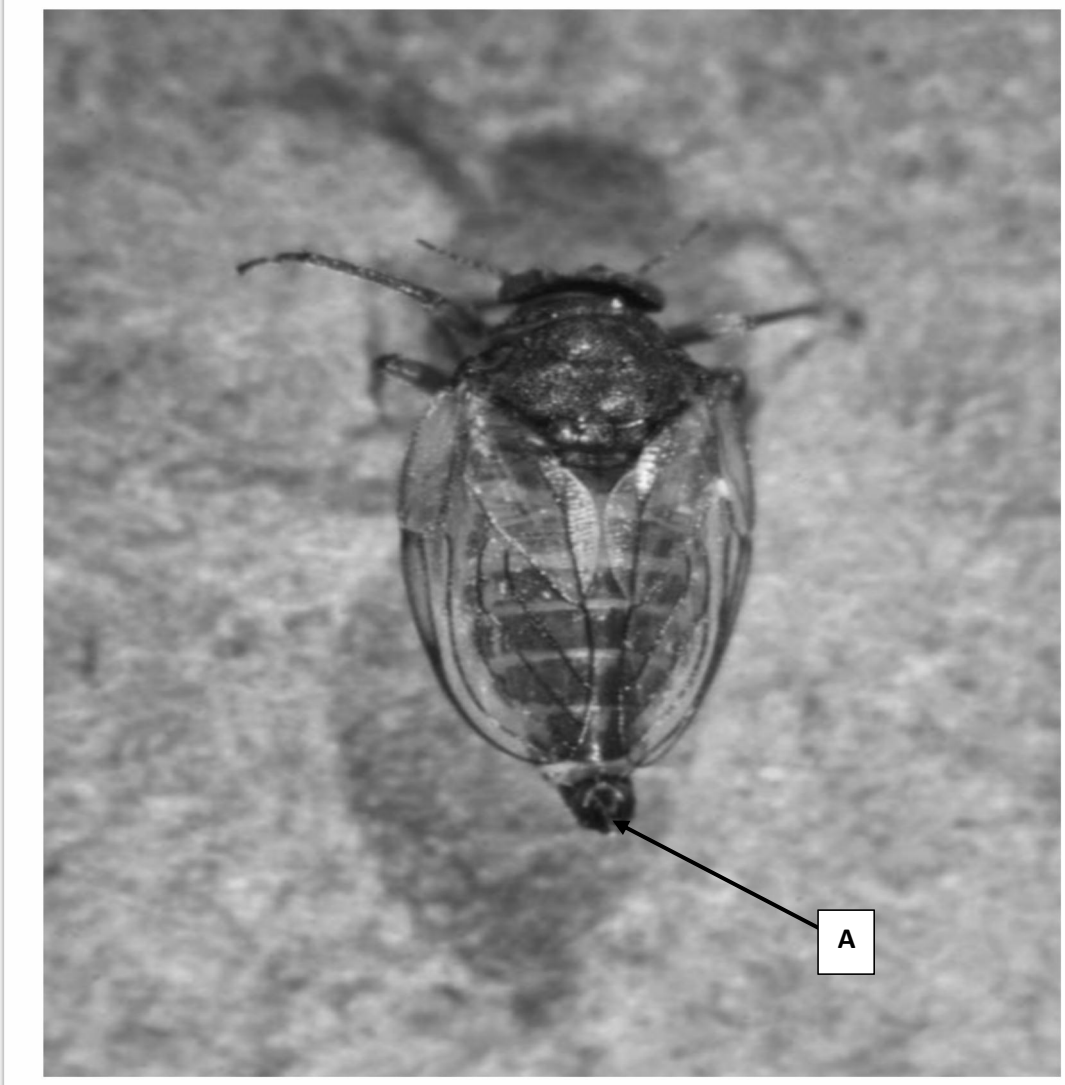

Plate 2.1: Adult female *Phytolyma spp* showing its ovipositor (A) (Wagner *et al*., 2008)

attack. The gall completely encloses the nymph, and the young nymph gets nutrients inside the gall. After some days, the immature insect finds its way out of the gall through the slit opening created during penetration (Wagner *et al*., 1991; Ugwu and Omoloye, 2014).

After the gall rupture, decay sets in rapidly and this make the infested plant parts become a putrefying mass which invariably affects the physiological process and functions of the tissue. After the sustained attack, seedling growth is retarded and sometimes stunted and bushy and may eventually die. This pestiferous attack has led to propagation and production failures under plantation conditions (Wagner *et al*., 2008; Olajuyigbe *et al*., 2015).

2.7 Chemical Ecology of insect-plant interaction

The interaction between plant and insect comprises studies investigating plant volatiles, pheromones, hydrocarbons in insect cuticles and numerous compounds that mediate in their interactions (Hay, 2014; Jeffrey *et al*., 2018). Plants and insects are connected biochemically, which is prominent in food chains. The sensibility for an organism to locate food, host, and mediate interaction and repel them, which are constituent defence phytochemicals required to elicit behavioural and physiological responses (Nordlund, 1981). In addition, plants produce semiochemicals as an intrinsic defence against insects, and it also indirectly affects the tri-trophic interaction (Ruther *et al*., 2002).

In-plant plants, varieties of secondary metabolites are presented constitutively and expressed for different functions. Therefore, plants under continuous insect pest attacks can better invest in constitutive defences (Wittstock and Gershenzon, 2002). Furthermore, the concentration of constitutive secondary phytochemicals varies within the same plant tissues (Howe and Jander, 2008). Constitutive plant defences might also be present at specialised conductive tissues, acting as constitutive and inducible plant defence (Agrawal and Konno, 2009).

Sustained production of defence secondary metabolites from plants only sporadically subjected to herbivory will constantly divert resources from growth. Hence, plants under limited exposure to herbivores must set up time-confined defences to

minimise defensive costs (Herms and Mattson, 1992). Furthermore, plants can distinguish herbivore feeding from mere abiotic wounding (Howe and Jander, 2008).

The diversity of insects and their damage is a pointer to how plants can differentiate between herbivores inflicting the damage. To accomplish these plants, respond to so-called elicitors' compounds eliciting plant defence responses that are either insect-derived or plant produced compounds modified by insects (Howe and Jander, 2008).

2.8 Gall formation in plants

Naturally, cells in plants are encoded with genes that are expressed in the plant phenotypic structure in order for the plant to perform some physiological roles (Oliveira and Isaias, 2010). Their functionality, as well as development, generates tissues and organs needed for plant growth and reproduction. Meristematic alteration in plant tissue development through the enlargement and disparity of its cells leads to gall formation (Oliveira and Isaias, 2010; Isaias *et al*., 2011; Isaias *et al*., 2014). Galls are like natural micro laboratories cum sophisticated models in studying the fate of plant cells (Isaias *et al*., 2014). Plant galls are formed as a result of cell division and differentiation. It is a product of the repetitive pattern of these biochemical processes, which later culminates to form a new organ. In galling in a plant, the pattern of cells polarisation and expansion of host plant organs are often ruptured. This process causes cell re-differentiation that changes their functionality (Isaias *et al*., 2014). Consequently, these gall tissues provide the psyllid with nutrients, protection and shelter from harsh environmental conditions.

Galls induction by insects causes plant cells to undergo differentiation and alteration, becoming visible as changes in the host organ morphology (Oliveira and Isaias, 2009). Cells are genetically reprogrammed toward a new and different path (Lee and Schiefelbein, 2002). According to Meyer and Maresquelle (1983), galls can simply be identified based on their distinct morphological and histological features. Gall formation in plants by an insect is believed to be stimulated by chemical substances injected into the plant (Malpighi, 1675; Plumb, 1953). However, there are divergent views on the specific chemical substance injected into plant tissue during gall induction. Several specific inorganic chemicals, amino acids, and adenine-containing compounds are suspected of causing galling in a plant (Mani, 1964). Auxins have been connected in the galling process

because abnormal growth caused by them are like natural gall in plant cells and tissues (Overbeek, 1966; Nitsch, 1968). Phytohormones, rather than signals that influence them, have been hypothesised to be possible gall-inducing factors (Hori, 1992).

Plant hormones discovered in insects' saliva and salivary sheath and plant hormones derived from insects are suspected of partaking in galling (Hori 1992). However, the origin of hormones responsible for gall formation is still difficult to distinguish.

2.9 Gall Formation mediated by Chemical interaction

Over the years, the plant's gall formation and growth mechanism have been proposed to result from mechanical stimulation, but current evidence has shown a chemical signal hypothesis (Hori, 1992; Sopow *et al*., 2003; Matsukura *et al*., 2009). Chemical secretions have been implicated to be responsible for gall induction and development in a plant, but the nature of the gall-inducing signals has not been understood. Plant hormones such as auxin and cytokinins focused on gall induction (Hori, 1992). More so, traces of hormones identified in insects' salivary sheaths have been hypothesised as the significant stimulant in galling (Matsukura *et al*., 2009). Similarly, the presence and abundance of plant hormones in gall-forming insects have implicated hormones in the process. This suggested gall-forming can elevate hormonal concentration in plants (Tooker and Helms, 2014). On the other hand, cytokinins are abundant in insects, contributing to gall development (Davies, 2004). It has also been suggested that cytokinins help initiate gall growth and maintain gall morphogenesis (Yamaguchi *et al*., 2012).

During gall formation, it is presumed that the gall formers manipulate its host's metabolic activities in such a way as to stimulate the quality and accumulation of nutrient and defence substances (Hartley and Lawton, 1992; Motta *et al*., 2005). Several authors have also reported the chain of chemical interaction during galling and its development. Isaias *et al*. (2014) reported alterations in the nutrient and chemical defences around the larvae chamber. This is evident in the biochemical bioassay of healthy and galled tissues. Price *et al*. (1987) posited that the galls serve as shelter and source of nutrients for the gall former.

Plant biochemical constituents with defensive potentials, such as the phenolic compounds, have been evaluated using histochemical or phytochemical screening (Motta

et al., 2005; Isaias *et al*., 2014). Their activities are not persistent in the galling series due to variability in their constituent over time and their reliance on prevailing conditions of the environment (Formiga *et al*., 2009; Detoni *et al*., 2011b). Thus, phenolic compounds responsible for defence during galling in the plant differs. Histochemical determination of phytocompounds in plant gall has indicated various phytochemical compounds (Carvalho *et al*., 2005; Oliveira *et al*., 2006; Moura *et al*., 2008). These phytochemical compounds vary in their activities and functions (Oliveira *et al*., 2006).

Galled plant nutritive tissues differ in the characters of their cytology and biochemical compounds, and this is fundamentally dependent on the taxonomic classification of the gall former (Bronner, 1992). According to Isaias *et al*. (2014), a different class of gall-forming insects induce the accumulation of biochemical compounds on their nutritive tissues. Similar results have been reported for other categories of insects (Oliveira and Isaias, 2010b; Motta *et al*., 2005; Vieira and Kraus, 2007).

Histochemical staining and spectroscopy have elucidated aspects of the chemical interactions between plant and gall former cum gall development. In addition, the determination of volatiles in plant galls is efficient in understanding insect behaviours and their metabolic pathway (Isaias *et al*., 2014).

2.10 Functions of primary metabolites in *Milicia excelsa*

Metabolism in plants is a supplementary effect of all chemical activities structured into discrete pathways, all embodied in a web of chemical reactions (Mysore *et al*., 2014). It is classified as anabolism, the product production and storage of chemical energy and catabolism, which reverse anabolic activities. The growth and development of plants are hinged on a seamless equilibrium between anabolism and catabolism.

Primary metabolites in plants are macromolecules such as sugar, protein and other compounds involved in the metabolic pathway, growth, development, and reproduction (Mysore *et al*., 2014). Similarly, they are essential for maintaining the active physiology of the plant. They can be categorised into alcohols, lactic acid, certain amino acids and citric acid groups.

Plant – insect interactions cause numerous changes in the metabolism and fitness benefits. Insects interact with plants in diverse ways to manipulate plants functions and

physiological processes (Jander *et al*., 2015). For example, its feeding habits on plants significantly influence and induce changes in plant primary metabolism.

Plant protects its organs from insect attack by relying on its immune system supported by metabolic processes (Mysore and Ryu, 2004). However, there is a dearth of information on the metabolism activities necessary for plant growth and development and defence responses to an insect pest. Among other observed effects, insect attacks can elevate or suppress plant photosynthetic efficiency, remobilise carbon and nitrogen resources, and alter plant growth (Jander *et al*., 2015). Plants are often prone to attacks, which has made them evolve over the year by building different mechanisms to recognise these attackers and protect themselves. A common way plants respond to insect attacks is through the withdrawal and diversion of photosynthetic products from the region of attacks, thereby impeding the allocation of vital growth nutrients needed by the plant during the attack period. Energy saved through this diversion is used in building up defences. However, this response does not apply to other plant species when attacked by insects (Jander *et al*., 2015). In some cases, insects influence the production of plant primary metabolites for their use, and this makes it difficult to establish in most cases if the modifications are responses to attack or otherwise (Baldwin *et al*., 1998; Jander *et al*., 2015).

Insect pest infestation often induces movement of stored and new primary biochemical compounds between induction and complete plant tissues. Translocation of plant biochemical compounds to the region of insect attack is deduced as the re-enforcement of the plant defence system through the provision of mineral substrates and the management of the plant metabolic functions by the herbivore. Conversely, translocation of plant biochemical compounds produced through primary metabolism from the region of insect attack or/and induction has been postulated to deprive the insect of food, aiding plant ability to recuperate by storing food needed for regrowth as well as to instigate insect parasitism (Sturm and Chrispeels, 1990; Zhang *et al*., 1996; Ehness *et al*., 1997; Ohyama and Hirai, 1999; Allison and Schultz, 2005; Schultz *et al*., 2013). Baldwin *et al*. (1994), Hartmann and Ober (2000) and Ziegler and Facchini (2008), in their separate findings, established that during insect attacks on a plant, biochemical compounds containing nitrogen are being translocated from one section of the plant to another.

Nitrogenous compounds are being produced in the plant as pre-synthesised compounds required for plant resistance, unlike carbon, which are synthesised as carbohydrates needed to produce defence compounds. This is true because carbohydrates are cheaply produced in a plant grown under adequate sunlight compared to nitrogenous compounds. In addition, nitrogen is usually a growth-dependent nutrient for most insects (Holland *et al*., 1996; Lu *et al*., 2015). Reordering plant biochemical compounds impede insect pest activities such that the plant would become nutrient deficient for the insects to feed on (Tao and Hunter, 2013; Lu *et al*., 2015). In some cases, insect-induced reordering of plant primary biochemical compounds could be strategically carried out in order for the plant to regrow after infestation by the insect has lapsed, coupled with the un-nutritive tissue manipulation of the host plant (Millard and Grelet, 2010).

During insect attacks in plants, plants are usually at a crossroads on the direction to choose, to either divert their metabolites to growth or defence (Herms and Mattson, 1992). This challenge arises from the fact that growth and defence are resource-limited processes. The production of secondary metabolites competes with growth; plants must have strategies that allow them to minimise the cost/benefit ratio of their chemical defences to allocate resources to growth processes (Fuenzalida, 2015). As a result, plants subjected to frequent insect attacks usually present high levels of constitutive defences, whereas those under sporadic attacks respond in a more inducible and less expensive fashion (Wittstock and Gershenzon, 2002).

2.11 Functions of secondary metabolites in *Milicia excelsa*

Small organic compounds believed not to have any specific growth and development functions in plant tissue are referred to as secondary metabolites. They generally aid in plant defence against predators, insects and microorganisms (Harbone *et al*., 1999). These compounds, which are under research, have been described as detrimental to the survival and feeding of herbivores (Nwokeji *et al*., 2016). Production of some secondary plant's biochemical compounds, such as the terpenoids group, which are generally toxic to insect pests, have been reported as the mechanism in which most plants defend themselves from insect pests (Nwokeji *et al*., 2016). Secondary metabolites, in most cases, are considered as research compounds, and they are being classified to the

carotenoids and polyphenols groups (Heneman and Zidenberg-cherr, 2008). Secondary metabolites are intriguing for their structural multiplicity and inherent chemical compounds, making them good candidates in manufacturing drugs and natural pesticides. Their structural multiplicities are astounding as it becomes difficult to invent or synthesise them. Early studies have reported that secondary metabolites are residue produced from primary metabolism and their functions (Bennett and Wallsgrove, 1994). This showed that their functions were basically on its application without recourse. Secondary metabolites play a critical role in the interface between different organisms (Bennett and Wallsgrove, 1994; Hartmann, 2007). The dynamics of secondary metabolites among different plants is very complex and, as such, tagged as the wonders of the natural world (Jeffrey *et al*., 2018). A focal point of investigation on the changes of secondary metabolites is on the plant-herbivores relationship and the induced defence substance produced by the plant during the interaction.

Plant synthesises secondary metabolites in manifolds, and some of them could be isomers with the same function, and this multiplicity can be very voluminous in a single plant species (Romeo, 2014). The diversity of the secondary metabolites can be determined as the abundance of phytochemicals using an entropy ratio of the spectroscopic peaks concerning the richness and relative abundance of a plant. Changes in plant secondary metabolites multiplicity can significantly detrimental effects on insect pest activities on the plant (Richard *et al*., 2015). Knowledge of the evolutionary connection between varieties of biochemical compounds in plants elucidates the patterns of evolutionary medications plant-insect interactions. The seepage entails the evolution of new plant defence compounds in reaction to herbivores induced discriminatory pressures. The central role of secondary phytochemical compounds in the interface between plant and insect involves many plant chemical compounds that act as substances for defence purposes (Arnason *et al*., 2004).

Generally, plants contain different numbers of biosynthetic groups of secondary metabolites, which in most cases include many structurally related analogues and derivatives. There are perplexing collections of biochemical substances and their mode of action in higher plants such as mahogany. In the different parts of the tropical mahogany tree, there is an extraordinary range in the amounts of phytocompounds present. This

concerns the neem tree (*Azadirachta indica*) with great insecticidal potential as an insect antifeedant and growth deterrent (Isman *et al*., 1996). Intensive study of some plants has shown that they contain many volatile compounds in their tissues (Isman *et al*., 1996).

Tropical forest tree species such as *M. excelsa*, *Khaya spp*. are somewhat less well studied, and a few have been investigated. However, the phytochemical compounds are well known to indigenous peoples who valued them and used them for health care and other purposes (Pennington *et al*., 1981).

A study with *Sitophilus zeamais* showed that secondary metabolites in plants contributed to the reduced food consumption of the insect when fed with treated diets (Omar, 2000). Phytochemical compounds which act as antifeedants to insects are found in host plants part at different amounts necessary to cause a visible effect on herbivores. However, the number of tropical tree parts screened for phytochemical compounds is scanty, and few investigations are reported on the insecticidal potentials and methods of defences of these plants.

Results of different tree parts extract with potentials to reduce insect larvae growth activity of lepidopterans have been reported, as well as the prospects of these phytochemical compounds to be easily isolated from lots of others (Xie *et al*., 1994; Wheeler *et al*., 2001). However, there is a dearth of information on the chemical interactions between tropical tree species and insects in the forest ecosystem. Knowledge of the chemical interface between plants and insects will help manage insects pest. For example, *Hipsipyla grandella*, a specialist and stem boring insect which causes shoot damage to mahogany trees, have been reported to infest closely related trees such as those in the genus Toona. However, in cases where shoots of the infested plant are grafted onto the rootstock of *Toona ciliata*, the tree tends to withstand the insect attack due to the translocation of phytocompounds inherent in Toona root (da Silva *et al*., 1999).

2.12 Role of fungi in gall formation

Microorganisms such as fungi are reported to be the significant contributor in galling insect activities, and microbes isolated from plant gall have been documented as the leading cause of mortality of the insect host (Gange *et al*., 2002). An early study by Richter-Vollert (1964) opined that microbes such as fungi isolated from plant galls act as

inquiline, which does not participate in gall formation and development processes. Instead, gall-forming insects induce galls by manipulating plant tissues to their benefit (Shorthouse and Rohfritsch, 1992).

Galls formed by insects are usually to the advantage of the former in the provision of nutriment and protection from predators and harsh environmental conditions (Oliveira *et al*., 2015). In addition, healthy cells inside the galls help the nymph develop, and it is a prominent contributing protagonist to gall organisation (Rohfritsch, 2008). Intriguingly, some gall-forming insect obtains nutrition through their intimate relationship with fungi (Kehr and Kost, 1999; Rohfritsch, 2008).

Bronner (1992) reported that in some gall-forming insects, nutritive tissue induced by larva or nymphs are vague and cytochemical features of a classic nutritive tissue in the form of a fungal mycelium are present. Similarly, some gall formers usually take advantage of this relationship to degrade the hemicellulose compounds in plant and causes damage synergistically on a plant with the aid of saprophytic fungi (Mamaev, 1975). The relationship between some gall-forming insects and their associated fungi has been well-thought-out to be a genuine symbiotic relationship, a situation where they are mutually dependent for their survival (Rohfritsch, 1992b; Rohfritsch, 2008).

2.13 Oxidative stress and reactive oxygen species in *Milicia excelsa*

Hypersensitivity is caused due to plant reaction to oxidative stress, which causes the production of superoxide anion or hydrogen peroxide, referred to as reactive oxygen species (ROS). Reactive oxygen species such as superoxide anions and hydroxyl radicals are free radicals' molecules different from the non-radical molecules such as hydrogen peroxides and singlets oxygen. Reactive oxygen species can also be classified as molecules derived from oxygen and are synthesised as oxygen by-products. Therefore, its role in cell signalling and homeostasis, which causes significant damages to plant cells structure, is of great importance. Initially, ROS were believed to be toxic residues derived from aerobic metabolism and discharged by antioxidant and an antioxidative enzymatic process. However, these molecules' concentration in plant cells is sacrosanct to the deleterious and beneficial roles. In plants, cells are synthesised by the unavoidable leakage

of electrons onto oxygen and an offshoot of numerous metabolism in different cells compartments of plants (Bolwell and Wojtaszek, 1997).

Stress due to biotic and abiotic factors stimulates the production of ROS in plant cells due to the interference of the plant cells' homeostatic pattern, which at a very high amount are injurious to the cells (Bolwell, 1999). In situations where plant defence mechanisms are overwhelmed by excessive ROS production, the plant cells are adjudged to undergo oxidative stress. The continued production of ROS due to oxidative stress can lead to the death of the plant cells (Frahry and Schopfer, 1998; Bolwell, 1999). However, in most cases, plant cells death could be averted due to the inherent ability of the cell to regulate the amount of ROS produced closely to avoid cell damage. Scavenging of excessive oxidative molecules is achieved by a pragmatic antioxidative system of different antioxidants (Richberg et al., 1998).

Several reports have been presented on higher activities of numerous enzymes to combat induced oxidative stress using an antioxidant defence system. The scavenging capability is related to improved plant tolerance to oxidative stress (Bolwell, 1999). Reactive oxygen species places significant roles in mediating the growth and development of plant and their response to stressors.

The identification of ROS-generating enzymes has led to the assertions that plant cells can start and increase ROS production (Liu *et al*., 2010). The main principle of ROS signalling is in control of the fragile balance between productions and scavenging. ROS's confined and chronological synthesis is life-threatening in ROS's cellular and intracellular transduction signals (Bailey-Serres and Mittler, 2006).

2.14 Role of reactive oxygen species in plant defence

Reactive oxygen species are part of plants' primary defence system (Mittler *et al*., 2004). In some cases, its high toxicity and reactiveness can cause plant cells to suffer from oxidative destruction (Asada and Takahashi, 1987). The rapid accumulation of ROS molecules at induction points is otherwise known as oxidative burst. It often leads to hypersensitivity that is an upshot to plant cells' death (Gechev *et al*., 2006). On the other hand, its production could benefit plants in situations where it acts as a signal that could trigger another defence mechanism into action (Dat *et al*., 2000; Grant and Loake, 2000).

For example, most NADPH-dependent oxidases in plants are also connected with hydrogen peroxide production in response to insect attack and wounding (Sagi and Fluhr, 2001; Razem and Bernards, 2003).

Lesions related to hypersensitive reaction indications have been reported in plants under insect infestation, thereby creating doubt in the potential ROS to defend against insect attack (Chen, 2008).

Insect attack usually leads to mechanical wounding of a plant at the point of induction, and this invariable causes medications of the plant response to the wound (Kessler and Baldwin, 2002). Shortly after being wounded, plant amasses reactive oxygen species whose role in signalling in plant defence response activation is well studied. According to Van Breusegem *et al*. (2001), plants tissues start to synthesise reactive oxygen species at the point of induction and around the plant after induction. It has also been reported that the amount of ROS amass in plants is positively linked to the host's ability to resist attack (Moloi and van der Westhuizen, 2006). Reactive oxygen species in plants denote a normal physiological reaction in which, under stress, large quantities can be produced. It is reported to take part in plant defence response and contributes negatively to plant health.

2.15 Insect-plant interaction defence strategy

Insect attacks are usually characterised by removing resources from the plant, which are often less detrimental to the producer (Fuenzalida, 2015). Plants that tolerate herbivory owe their ability to improve photosynthesis, boost its growth and profuse stem branching and ability to allocate and re-allocate food between the shoot and root and vice versa during and after damage (Strauss and Agrawal, 1999).

Plant tolerance and insect attack resistance are often considered alternative evolutionary responses to herbivory not always negatively correlated and not usually considered mutually exclusive (Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999). This non-exclusivity arises from the fact that traits involved in plant tolerance against herbivores are most times adaptive for other selective pressures (Rosenthal and Kotanen, 1994). Comparatively, tolerance and resistance are not mutually exclusive, but a recognised resource trade-off has important implications for the co-evolutionary theory of

plant and insect (Strauss and Agrawal, 1999). While plants resistant to insect attack engage in an evolutionary arms race, tolerance, on the other hand, does not represent a selection pressure and hence leads to the development of more stable insect-plant relations over evolutionary time (Rosenthal and Kotanen, 1994; Strauss and Agrawal, 1999). While plants resistant to herbivore attack may not be selected for tolerance because they experience minor damage, shifts to tolerant genotypes might occur when resistance traits become ineffective (Strauss and Agrawal, 1999). Conversely, tolerant plants might shift towards a resistance strategy if pressure by generalists increases. This is true that specialist insects spring forth to generalist insects and vice versa (Futuyma and Agrawal, 2009).

Generally, it is proposed that tolerant and resistant plant strategies and specialist and generalist herbivores remain in equilibrium through evolution. However, although specialists are much more abundant than generalists amongst herbivore insects, resistance is also more common than tolerance as a plant defence strategy (Schoonhoven *et al*., 2005). This is because a weak resistance due to the specialisation of herbivores may lead to the development of tolerance and new and more effective resistance traits. Thus, resistance becomes a more common strategy than tolerance (Fuenzalida, 2015).

2.16 Management of *Phytolyma fusca* on *Milicia excelsa*

In pest management, preventive measures in which the build-up of pests is stopped by suitable means and remedial action by which control strategies are adopted after the attack has taken place are usually adopted (Nair, 2007). The attainment of preventive measures solely lies in identifying the causes of pest build-up. Preventive measures aim to keep the numbers of the pest below the threshold where economic damages can be done and to stop them from advancing into an outbreak. It is hinged on the knowledge of remote causes for pest build-up. Lots of interacting influences are involved in ascertaining the size of a pest population, and it is usually cumbersome to categorise which influences are responsible for causing large scale pest build-up.

Preventive strategies in pest management are feasible in some instances where the pest population is identified. This strategy encompasses silvicultural interventions targeted at the improvement of tree health.

Remedial strategies are often targeted at reducing the abundance of the pest by exterminating the insect using different control measures. Many remedial strategies have been propounded and used against the insect. The most typical and active way of exterminating insects has been applying chemical control. Chemical control using insecticides are either prophylactic or remedial. To sustainably manage insect pests in the tropical forest ecosystem in such a way as to avert a resurgence of secondary pest, accumulation of toxic residues in the food chain, integrated pest management (IPM) practices was developed. This practice entails adopting numerous strategies such as controlling biological organisms, chemical, botanical, and cultural practices. The IPM practices are targeted at lowering the pest population level below the economic injury and not at the complete extermination of the insect.

2.16.1 Chemical control

The use of chemicals in insect control has been well thought out as essential in managing insect pests, and severe considerations have been outlined in its application under the sustainable integrated pest strategy. Chemical control of insect pests remains a cornerstone that will persist for many years ahead. The practical and judicious application of chemical control is a complex practice that entails detailed information on the insect population changes and their efficiency and efficacy. Factors for the judicious use of chemical insecticide are considered to offer a synopsis of the benefits and constraints experienced in it.

Synthetic insecticides are proactive in exterminating targeted insects. However, if the insecticide mode of action and use is carefully and meticulously, insecticides remain an efficient and economical means of insect control.

The supposedly benefit of insecticide to local farmers is still portrayed to be acceptable when considering the cost, total crop yield and the supposed pest risk reduction, even when the direction for the use of these insecticides is not complied with. This has made its use more popular as a quick means of insect pest control. However, there is no doubt that synthetic insecticides are harmful to humans and the environment.

Several chemicals have been evaluated in some West African countries to manage the 'Iroko' gall former. White (1966) reported favourable outcomes in using some selected

insecticides to control 'Iroko' gall former in Nigeria. The application of Lambda-cyhalothrin on *M. excelsa* seedling under plantation conditions showed a significant difference compared to non-insecticide applied. However, the effectiveness of these insecticides could easily be reduced by single rainfall, making it difficult to impact insects (Olajuyigbe *et al*., 2015; Ugwu and Omoloye, 2014). Therefore, chemical insecticides were suggested for use only on a small scale and in the nursery (Cobbinah, 1993). However, concerns for protecting the environment from heavy elements and harmful substances and the economic burden attached to the use of chemical insecticides, its use is being discouraged, especially on insect pests that are present throughout the year.

2.16.2 Biological control

Biological control of insect pests can be referred to as the science of insect population ecology. It is an interface between predator and prey populations. This interface between insects and their natural enemies which contributes to the regulation of the insect population is an ecological process.

Prospective pest populations may develop unrestricted and high growth in their numbers when this interface chain is destroyed. Introducing an alien insect into a new area can lead to pest outbreaks, especially when the insect pest's natural enemies' populations are exterminated (Price, 1987). Similarly, the dissociation of insects from their natural enemies due to habitat modification can make an insect attain pest status. Biological control of insect pests is concerned with equalising the imbalances caused due to extermination of insect natural enemy population.

The strategies adopted in using biological organisms in pest control are the classical strategy, inundation, inoculation, conservation of the insect's natural enemy, and augmentation. The classical strategy approach has been the most commonly used in managing insect pests. This approach entails the conservation of natural enemies, and this can mend the establishment of introduced natural enemies. In some situations, augmentation with the laboratory-reared organism is used to support one or two biological control approaches to allow for the sufficient build-up of natural enemies to control the pest. This biological control approach, which is temporary, usually acts by suppressing the pest population below the economic damage level during their critical growth stage. In

situations where native pest populations are expanding and moving beyond their range to dissociate from their natural enemies, inoculative release approaches are usually adopted (Greathead and Waage, 1983). Entomopathogens has also been used to subdue pest outbreak, and it has been adjudged to be safe to use, environmentally friendly and host-specific. This is encouraged for adoption over chemical control because it preserves the integrity of another biological organism.

In the classical approach to biological control of insect pests, insect population ecology is believed to be improved, but there is less proof that the theory of population ecology is promoted (Waage, 1989). Practically, the assessment of this approach is seldom carried out before it releases due to technical constraints. Therefore, biological control is an essential aspect of insect pest management. An ecological approach to biological control fundamentally impacts the sustainable management of insect pests.

2.16.3 Use of Botanicals

Plants have been reported to contain the chemical substance of great pesticide potential. The application of plant extract in controlling insect attacks is well documented. Before the advent of synthetic insecticides, plant extracts were predominantly used to manage insect attacks o plants (Owen, 2004). Pesticides of biochemical origin, which include those derived from plant and plant parts, can inhibit insects' growth, reproductive capacity, and oviposition. The number of flora possessing biochemical insecticidal substances are vast. These have elevated research interest over the years on the screening and trials of plants with insecticidal potentials. According to the findings of Jacobson (1989), more than 2000 species of plant possesses the potential to be used as an insecticide and more are still explored (Jacobson, 1989).

The discovery of new and spectacular biochemical compounds with insecticidal activities is significant in combating the alarming rate of pest resistance. Plants and plant parts containing active biochemical compounds that can control insect attacks are more favourably used to manage the drawbacks of chemical insecticides. There is an urgent need for more studies to explore new plant active ingredients with diverse mechanisms of action in insect pest control. Plant secondary metabolites are believed to act as a defence substance as an intruder (Rattan, 2010).

The use of botanicals have been adjudged to be environmentally friendly, biodegradable, and, when applied for the control of a specific insect, does not harm non-targeted organisms. In addition, the use of botanicals, unlike chemical control based on active ingredients, comprises a collection of phytochemicals that interfere with the behavioural and physiological processes of the target organisms. Therefore, the likely possibility of the target organisms showing signs of resistance is difficult (Saxena, 1987).

2.16.4 Cultural Control

This method involves deliberate human interference to the insect pest environment, such as creating a harsh condition that could threaten their survival and lead to death. Different approaches to this control method interfere with the pest's ability to attack a plant to cause damages and hinder their reproductive potential. Different techniques such as adopting different cropping and storage systems have been used to manage insect pests under cultural control. This method is usually considered the first-ditch defence around building other control measures (Ugwu, 2013). In practice, this insect pest control method can reduce insect attacks below the economic threshold when combined with other insect pest controls. However, its efficiency is hinged upon the techniques and practices adopted. This makes it a vital approach to ineffective insect pest management.

The ecology of the interface between herbivore and plant is one of the challenges encountered in understanding the mechanism and process of cultural control as a pest management strategy. These challenges, if surmounted, would aid entomologists to forecast effectively more precisely the potential value of similar control approaches.
The study of cultural control as a sustainable pest management method revolves around evaluating different practices and methods for specific crop systems.

2.16.5 Silvicultural Control

The activity of insect pests and their population can be unsympathetically disturbed by simple manipulation of the insect's environment. For example, different farming and planting techniques such as inter-cropping planting under shaded environments have been reported to cause adverse negative effects on some insect pests. An example is a reduction in the population of *P. lata* and numbers of gall formed on *M.*

excelsa seedlings when planted in the field as an understorey plant or as a plantation (Wagner *et al*., 1996; Ofori and Cobbinah, 2007).

Shading as a silvicultural approach for the control of gall-forming insects such as *P. lata* has been reported to affect galls' reduction positively. This reduction is based on how dense the shading effect is arranged. However, according to Wagner *et al*. (1996), the adoption of silvicultural control *P. lata* on the 'Iroko' plant showed positive results, but silvicultural control on its own cannot effectively control the insect pest beyond economic damage. Though the adoption of two or more controls is more likely to have a significant effect on the population of the insect but practices within an Integrated Pest Management programme is most promising.

2.16.6 Use of Physical barriers

A physical barrier can be described as a device constructed to prevent insect pests from entering a plant. It implies protecting the plant from the insect with materials that will hinder their passage and accessibility to the plant. Agyeman (1994), in the adoption of the use of physical barrier, reported on the effects of this control strategy on seedling growth of Milicia excelsa. He posited that the plant's relative growth rate was high at a total irradiance of 42 %. However, the use of a physical barrier must consider the influence of solar radiation on the plant. Conversely, the adoption and use of Physical barrier strategy in the control and management of *P. lata* on *M. excelsa* seedlings are best suitable and practical at the nursery stage (Olajuyigbe and Adegeye, 2012).

2.16.7 Fertilisation Control

Soil nutrition management has been affirmed as the most vital option for optimum crop production, and it often affects plants' response to insect attacks. The ability of plants under intensive soil nutriment to resist insect attack is excellent based on the physicochemical properties of well-nourished soil (Chau and Heong, 2005). Furthermore, a study of plant response to fertiliser application showed that crops planted on manured soil had increased phytochemical content and activity (Sinha *et al*., 2018).

Specific minerals fertiliser application in soil has been proved to boost the accumulation of plant roots significantly and shoots biochemical compounds (Lu *et al*.,

2013). Improved circulation of soil nutrient elements towards plant phytochemical compounds is evidence in their growth and development and optimum biomass accumulation. However, applying some specific mineral nutrients such as nitrogen to soil is said to aid insects feeding on a plant (Strong *et al*., 1984: Schoonhoven *et al*., 1998).

Well-nourished soil is characterised by perfect soil fertility and enriched with beneficial micro and macro-organisms of great benefits. Plants cultivated on manure showed an extra advantage in tolerating and resisting insect attacks (Magdoff and Van, 2000). Availability of soil nutrients and their assimilation is not solely responsible for the amount of damage caused by insect pests but rather its aptitude to withstand and recover from such attacks. Fertilisation has a tremendous influence on the physiological vulnerability of plants to insect attack either through the alteration of the plant acceptability to insects or by activating the individual plant resistance instinct.

According to Zhong-Xian *et al*. (2007), nitrogen is an essential factor that aid in the development and abundance of insects. Therefore, its application aids insects' nefarious activities on the plant. Nitrogen and other macronutrients are required for optimum plant growth and development, metabolism, and good yield.

Mineral nutrients, when used excessively, lead to wastages as well as causing pest problems such as high insect fecundity, longer life span and general overall fitness of the insect. Invariably, fertilisation directly impacts the level of damage an insect pest can cause (Sétamou *et al*., 1995). Crops grown on soil enhanced with sufficient nitrogen are influenced by insect interactions and abundance (Fallahpour *et al*., 2015). They have better chances of survival after being attacked by the insect when compared to crops grown at low nitrogen levels (Tanzubil *et al*., 2006). Improved fertiliser application on soil has been reported to boost plants' susceptibility to insect attack. This is evident in the activities of insects of crops grown on soil with high nitrogen content such as high fecundity, shorter life cycle and long adulthood (Fallahpour *et al*., 2015). Pest management using organic fertiliser is expected to influence insect pest control, but few reports have shown a reduced insect population growth rate after enhanced nitrogen nutrient application. This suggests that extreme nutrient application can hinder the population growth rate of insects (Zehnder and Hunter, 2009).

Other macronutrients such as potassium have been linked to boosting plants' disease and insect pest resistance. Potassium is required in developing a healthy and well-anchored plant rooting system and the stimulation of nitrogen and other mineral nutrient uptake. The design of an utmost sustainable production system is hinged on the knowledge of the interaction between the status of soil fertility and insect pest. Therefore, knowledge of the relations between soil fertility status and insects has become the centre of interest for designing the most sustainable production system.

2.17 Ecological approach in Pest Control

This pest control strategy is more concerned with the awareness of the environment and the need to protect nature to control insect pests (Castle and Prabhaker, 2011). It is a comparative idea in which an insect pest control strategy is observed and executed with high consciousness to environmental protection. Harmful activities such as those that will affect natural resources, ecosystem stability, biodiversity, food security, and life on earth are carefully considered. Since years back, the continued and unregulated use of chemical control such as insecticides tends to discourage the use of other environmentally friendly and harmless methods, which have been in practice in pest management for ages (Castle and Prabhaker, 2011).

Despite the drawbacks such as pest resistance secondary pest outbreaks, among others from the use of chemical controls, manufacturers of chemical pesticides are continuously flooding the market with new chemicals to exterminate headstrong pests. Many authors have reported the pending danger due to the increasing use of this pesticide (DeBach and Bartlett, 1951; Castle and Prabhaker, 2011). All over the world, pest control practices are philosophically guided by conscious environmental awareness. This is evident in the creation of government bodies for pesticide use regulation because of the grass-roots campaign against its use, depicting earth poisoned by pesticides. The ecological approach in pest control also aids the encouragement of integrated pest management as a substitute for chemical control in pest management. A critical interrogation before the society and policymaker is whether there should be the integration of biotechnological advances to pest management which is perceived to de-emphasise cultural practices that have been in use for centuries (Paarlberg, 2009).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study area

The studies consisted of Laboratory and Nursery experiments. Experiments were carried out at the Entomology Laboratory of Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan (UI), Basel Convention Coordinating Centre for Africa (BCCC)-Geo-Environmental Research Centre (GRC) Laboratory, Department of Chemistry, Pharmaceutical Chemistry Laboratory, HistoPathology Laboratory of Department of Veterinary Micro-pathology and Plant Pathology Laboratory of Department of CPEB, UI and Biological Laboratory of National Institute of Science Laboratory Technology (NISLT) Sanmoda, Ibadan.

The study experiments were carried out at the Evergreen Tree Planters (BISROD Furniture Co. LTD) Forest Demonstration site, Ijari in Ijebu-Northeast Area of Ogun State, from February 2019 to March 2020. The Evergreen Tree Planters Forest Demonstration site is located on latitude 8°29.81 7'N and longitude 3° 24.25 4'E and altitude of 277m. The area is characterised by high rainfall, ranging from 1600 mm to over 2600 mm. It has a dry season of 2-5 consecutive months with a mean temperature of 18 °C (Oyedepo *et al*., 2011).

Poultry Droppings (PD), Cattle Dungs (CD), and Pig Faeces (PF) were collected from Oslot Animal farms in Ijebu-ode. The manures were kept in the open (cured) for two months before use. Fertiliser, NPK (15:15:15) was collected from the stock at the Chemical/reagents store, Department of CPEB, University of Ibadan.

Topsoil (sandy loam) was collected from the forest floor at Oke Eri in Ijebu-Ode, Ogun State. The soil was sieved to remove debris and large stone particles using a sieve net of size 2.5 mm.

3.2 Source of *Milicia excelsa* fruits and seeds extraction

Matured fruits of *M. excelsa* were harvested from a fruiting tree near the Obafemi Awolowo hall of residence in the campus at the University of Ibadan, Ibadan (Lat.

7°26'58.20" - 7°26'58.08" N and Long. 3°53'48.56" - 7°53'48.48 "E). Fruits collected from the tree were processed by soaking in water for 48 hours and, after that, macerated to remove seeds. The soaked fruits were macerated and seeds were separated from the chaff using a sieve.

3.3 Raising of seedlings in the Nursery

Seeds of *M. excelsa* were raised following Ugwu and Omoloye (2013) method at the Evergreen Tree Planters Forest Plantation nursery.

The sowing medium, coarse river sand (CRS) was obtained from a perennial stream and sieved to obtain 0.6 -2mm grain size. Then they were sterilised using a sterilising pot by heating at 80ºC for 8 hours allowed to cool before use. The CRS was packed in germination baskets. Seeds were sown in the germination basket and covered sparingly using CRS. Misting with water was done using an atomiser spray daily (Plate 3.1).

After three weeks, the seedlings were moved into plastic bags (13 cm by 15 cm by 20 cm) packed with sandy loam soil. *Milicia excelsa* seedlings were transferred into the screen cage (dimension: 6 m x 8 m x 8 m) and kept protected against *P. fusca* and other insect pests (Plate 3.2). The screen cage was constructed at the Nursery section, Evergreen Tree Planters, Forest Demonstration Site, Ijebu-Ode, Ogun State.

3.4 Analysis of phytochemical constituents in the galled leaf of *Milicia excelsa* attacked by *Phytolyma fusca*

Analysis of phytochemical constituents in the galled leaf of *M. excelsa* attacked by *P. fusca* was done following Amin Mir *et al.* (2012). Twenty 8 months old uninfested seedlings kept in the screen cage were collected and used for this experiment. Ten seedlings were exposed to *P. fusca* attack for 28 days in the open (outside the screen cage), while the other ten seedlings were kept protected in the screen cage. Healthy and galled fresh leaf samples were collected from the top 3-5 leaves. Leaf collection was done at the 8th and 16th months and analysed for phytochemical constituents. Galled and healthy leaves collected were washed thoroughly in distilled running water and air-dried. The leaves were further dried in an oven for 3 days at 45°C and later ground into fine powder. The leaves powder was kept in a cool, dry place for further use.

Plate 3.1: **Fruits (1), Soaked fruits (2), Extracted seeds (3) sprouted seedlings (4) of *Milicia excelsa***

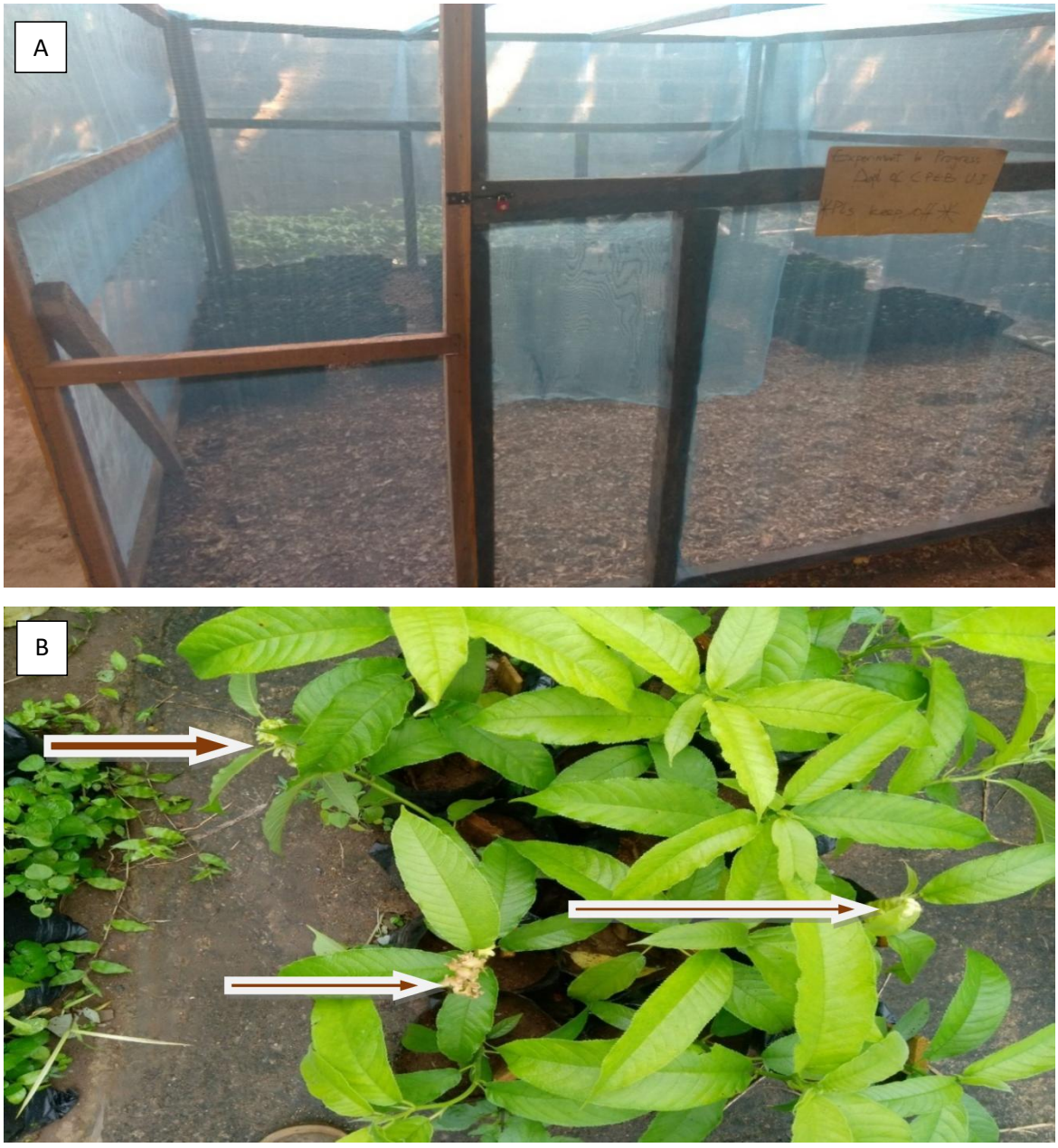

Plate 3.2: ***Milicia excelsa*** **seedlings growing in the (A) Screen Cage, (B) Open**

3.4.1 Qualitative phytochemical analysis of healthy and galled *M. excelsa* leaves

The phytochemical constituents of the healthy and galled leaves samples of *M. excelsa* were analysed following standard procedures.

1. **Saponins:** Two grammes of powdered leaf samples were boiled with 20 mL of ionised water (H2O) and filtered. The filtrate, 10 mL, was mixed with 5 mL of distilled H2O in a test tube and briskly shaken to get an even persistent froth. This was then mixed with 3 drops of olive oil to form an emulsion that indicates saponins' presence.
2. **Tannins:** Powdered samples (0.5 g) of the leaves were boiled in 20 mL of distilled water in a test tube and filtered. After that, Iron chloride ($FeCl_3$), 0.1 %, was added to the filtered samples and observed for brownish green or a blue-black colouration which showed the presence of tannins.
3. **Alkaloids**: Marquis Reagent, 1 mL, concentrated Sulphuric acid, 2 mL and a few drops of 40% formaldehyde were added and mixed to 1 mL of the leaf extract. A dark orange or purple appearance indicated the presence of alkaloids.
4. **Flavonoids:** A few drops of 1 % NH_3 solution were added to the leaf sample's aqueous extract in a test tube. Yellow colouration is observed if flavonoids compounds are present.
5. **Steroids:** Leaf sample extract, 1 mL was dissolved in 10 mL of chloroform, and an equal volume of concentrated H_2SO_4 was added carefully slanted. Steroids were noticed when the upper layer turned red and the sulphuric acid layer turned yellow with green fluorescence.
6. **Coumarins:** To 3 mL of 10% NaOH was added 2 mL of extract. The formation of yellow colour indicate the presence of coumarins.
7. **Quinone:** Extract (1 mL) was mixed with concentrated sulphuric acid (H_2SO_4). The appearance of a coloured formation signified that quinone was present.
8. **Glycosides:** Concentrated H_2SO_4 of 1 mL was prepared in the test tube; 5 mL of extract from each sample was mixed with 2 mL of glacial CH_3CO_2H containing 1 drop of $FeCl_3$. The above mixture was carefully added to 1 mL of concentrated H_2SO_4 so that the concentrated H_2SO_4 was underneath the mixture. The presence of cardiac glycoside in the sample showed a brown ring.

9. **Terpenoids**: Aqueous extract of 5 mL of each leaf sample was mixed with 2 mL of $CHCl_3$ in a test tube; 3 mL of concentrated H_2SO_4 was carefully added to the mixture to form a layer. An interface with a reddish-brown colouration was formed if the terpenoids constituent was present.
10. **Anthocyanins:** To 2 mL of the extract, 2 mL of 2N HCl and ammonia was added. The appearance of pink-red turned blue-violet indicated the presence of anthocyanins.
11. **Anthraquinone:** Few drops of magnesium acetate solution were added to 1 mL of the extract; the formation of the pink colour showed the presence of Anthraquinone.

3.4.2 Quantitative phytochemical analysis of healthy and galled *Milicia excelsa* leaf

Collection of leaf sample

Leaf samples were collected, and visual observation for gall infestation on the plant leaves was carried out. Leaf samples were categorised into healthy and galled leaves.

1. **Moisture content:** Moisture content in leaf samples was quantified following the method of Vijayan *et al*. (1997). Five replicates of Leaf samples were harvested separately and cleaned. After that, the fresh weight was determined. Then, the leaves were kept open for 6 hours and then oven-dried at 80 °C for 48 hours until the constant dry weight was attained. Average leaf moisture content was calculated using the equation.

$$\text{Leaf moisture content (\%)} = \frac{fresh\ weight\ \ of\ leaves - dry\ weight\ of\ leaves}{fresh\ weight\ of\ leaves}\ x\ 100$$

2. **Chlorophyll:** Chlorophyll content in the healthy and galled leaves was estimated following the method of Plummer (1971). Leaf sample, 1 g, was standardised with acetone (10 mL). The solution was centrifuged at 3000 rounds per minute for 10 minutes. The supernatant was retrieved in another tube, and the process was repeated until the desired pigment was observed. Optical density (OD) was measured at 645 and 663 nm, and chlorophyll was computed.

3. Proteins: Protein in the leaf sample was measured following Lowry *et al.* (1951). Protein in dried leaf samples was extracted using ethanol (80%) and 10% of Trichloro-acetic acid. Quantification of the protein content was later estimated.

4. Sugar: Hot ethanol was used to extract sugar in the dried leaf powder of the sample and was quantified using the method of Plummer (1971). The OD was measured at 625 nm then sugar content was calculated following the standard method.

5. Phenol: Phenol in leaf samples was extracted using ethanol (80%) following Sadasivam (1992) method. Phenol was quantified using a reaction mixture containing 0.1mL of the extract, and its volume was made up to 3 mL with twice distilled water in a clean test tube. FCR, 0.5 mL was added. Then after 2 min, 1 mL of 20% sodium carbonate was added and thoroughly mixed. The mixture was kept in a boiling water bath for about 5 minutes and allowed to cool. The absorbance was measured against blank at 650 nm. The quantity of phenol in the sample was expressed in mg/g.

6. Alkaloid: This was carried out using Harborne (1973) method. Five gramme of leaf samples were weighed into a beaker. Then, 200 mL of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. The mixture was filtered and concentrated in a water bath to one-quarter of the original volume. After that, concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle, and the precipitate was retrieved and washed with dilute ammonium hydroxide and then filtered. The residue, alkaloid, was dried and weighed.

7. Tannin: This was done using Van-Burden and Robinson (1981) method. Leaf samples (5g) were weighed into a 50 mL plastic bottle. 50 mL of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. Then 5 mL of the filtrate was pipetted into a test tube and mixed with 2 mL of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10minutes.

8. **Saponins:** The method of Obadoni and Ochuko (2001) was adopted. To 100 cm^3 of 20% aqueous ethanol, 25 g of leaf samples was added in a conical flask and heated at 55°C for 4 hours. The mixture was filtered and the extraction process repeated with the residue with double the volume and concentration of the solvent. The total filtrate was hot water bathed to reduce its volume to 40 mL and later transferred into a funnel, 250 mL. Later, 20 mL of diethyl ether was added and shaken vigorously. The filtrate was purified twice, and 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. The samples were dried in the oven to a constant after evaporation to give the saponins content.
9. **Flavonoids:** Boham and Kocipai-Abyazan (1994) method were used in this experiment. Leaf samples (10g) was extracted repeatedly with 100 mL of 80% aqueous methanol at room temperature. The solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible, evaporated into dryness over a water bath, and weighed constant.
10. **Terpenoids:** Terpenoids in the leaf samples were quantified following Indumathi *et al*. (2014). Dried leaves extract 100mg (wi) was collected and soaked in 9 mL of ethanol for 24 hours. The filtrate was extracted with 10mL of petroleum ether using a separating funnel. The ether extract was separated into pre-weighed glass vials and allowed to dry up (wf). Ether was evaporated, and the yield (%) of terpenoids contents was measured using the formula;

$$\text{Terpenoids (\%)} = \frac{Wi - Wf}{Wi} \; x \; 100$$

3.5 Analysis of healthy and galled leaves of *Milicia excelsa* using Gas Chromatography-Mass Spectroscopy (GC-MS)

Healthy leaves were collected from seedlings stock protected in the screen cage, while galled leaves were collected from seedlings exposed to *P. fusca* attack. The leaf samples were thoroughly washed in distilled water. Leaf samples were cut into smaller sizes before oven drying at 60 °C before pulverising. Powdered samples, 500 g was macerated in n-Hexane for 3 days and occasionally stirred. After 3 days, the extract was

filtered using Whatman No. 1 filter paper. Extracts were later concentrated using a rotary evaporator.

Leaf samples were collected consecutively from the same seedlings at 8 and 16 months old. Gas Chromatography-Mass Spectrometry analysis of the healthy and galled leaves extract was carried out in Agilent Technologies GC-MS instrument 7820 A GC system with electron impact ionisation (70eV). The specification of the column used was Agilent 19091 S-433: 325°C: 30m by 250 μm by 0.25μm, and the column was packed with (5% phenyl)-methyl polysiloxane. Helium was used as carrier gas at a constant flow of 1mL per minute. The oven temperature was maintained at 50-280°C at a rate of 10°C per minute. The split ratio was 1:5, and the injector volume was 1μL. Identification of individual components of the sample was performed by computerised matching the acquired mass spectra with those stored in the Wiley/NIST 0.8 mass spectral library of the GC-MS data system. The percentage composition of the different components of the samples was calculated from the peak area integrated by the analysis programme.

3.6 Characterisation and Quantification of reactive oxygen species

After six weeks of planting, the response of *M. excelsa* seedlings to oxidative stress was determined using histochemical staining techniques.

3.6.1. Mechanical wounding and histochemical staining

Twelve healthy *M. excelsa* seedlings aged 6 months were collected and used in determining plant response to mechanical wounding. The seedlings were grouped into healthy and wounded seedlings. The 3rd and 4th leaves from the top of the seedlings grouped as stressed were mechanically pierced using a sterilised perforator to mimic *P. fusca* mode of attack. The control group was not pierced.

3.6.2 Detection of superoxide anion radicals in leaves of *Milicia excelsa* using Nitro Blue Tetrazolium (NBT) stain

The wounded third and fourth leaves were excised and immersed facing down in 0.1 % (w/v) NBT staining solution, 100 mL consisting of 10 mM Sodium azide, 50 mM Potassium phosphate and pH of 6.4. The leaves were infiltrated with the solution by building a vacuum (100 mbar for 2 minutes), after which they were released gently, and the process was repeated severally until infiltration of the leaves was completed.

The leaves were later incubated in 10 mL of 0.1% NBT for 15 minutes at ambient temperature under cool fluorescent light. After that, leaves were bleached to remove chlorophyll by washing it several times in fresh ethanol. The appearance of a blue stain in leaves indicated the presence of superoxide ions. The stained leaves were photographed.

3.6.3 Detection of hydrogen peroxide in leaves of *Milicia excelsa* using 3, 3-Diaminobenzidine (DAB) stain

Leaves were detached and submerged in 1 mg/mL of the staining solution at a pH of 3.8. The leaves were vacuum infiltrated at 100 mbar for 2 min and released gently. The process was repeated until complete infiltration was observed. Brown precipitation was later observed in the leaves after incubation in a plastic box at high humidity for six hours. The leaves were bleached with repeated washes in ethanol. Then the leaves were later mounted in a solution of ethanol: lactic acid: glycerol at ratio 3:1:1, respectively and photographed.

3.6.4 Quantification of hydrogen peroxide in the leaf of *Milicia excelsa*

The quantity of hydrogen peroxide (H2O2) in the leaf of *M. excelsa* leaf was carried out following Kotchoni et al. (2006). Whole leaves (third leaf from top) taken from 6-month-old M. excelsa seedlings were wounded mechanically using a sterile perforator (diameter = 0.55cm). The wounded third leaves were collected and placed in 1 mg/mL of DAB solution and pH 7.5. Incubation of the leaf samples was done in light for 3 hours, and leaves were later bleached by applying heat at 80°C in 80 % ethanol. After bleaching, the leaves polymerised product was expected to show brown colour. The samples were standardised five times the volume with leaf weight in 0.2 M Perchloric acid. The blend was incubated for 5 minutes on ice and then centrifuged for 10 minutes at 10000 rmp and 4°C. Samples supernatant absorbance were read in a UV/VIS spectrophotometer at 450 nm. Estimation of H2O2 concentration was done for 1 g of the leaf tissue of M. excelsa using standard calibration with 0.2 M Perchloric acid containing 100 µM, 1 mM, 5 mM, 10 mM, 25 mM and 50 mM respectively, the concentration of hydrogen peroxide.

3.7 Identification of fungi associated with *Phytolyma fusca* during gall formation on *Milicia excelsa* seedlings

Plant materials, healthy and galled (un-ruptured and ruptured) leaves of *M. excelsa* were collected from seedlings kept in the screen cage and open field. The collected plant samples were immediately kept in sample bottles and taken to the laboratory. The plant materials were cut into small pieces. Nymphs were removed from un-ruptured galled leaves. The healthy leaves, un-ruptured galled leaves, ruptured galled leaves were cut into smaller pieces (3 cm) using flame-sterilised forceps. After that, the samples were transferred to sterile Petri dishes containing 10% hypochlorite solution for surface sterilisation. The plant materials, including the insects, were transferred to 5 replicates of Potato Dextrose Agar (PDA) plates prepared under aseptic conditions and kept in a Laminar Air Flow Chamber. Fungi were observed after 7 days of incubation at 30°C of samples. The fungi were purified using the hyphal tips technique on PDA medium and then subculture separately on slant medium. Fungi were identified accordingly using their cultural characters as described by Gilman (1957), Barnett and Hunter (1972) and Nelson et al. (1982). The initial identification was based on visual colony characteristics and was later examined under a compound microscope in which slides were made by mounting fungal material in lactophenol solution.

3.8 Assessment of morphological response of *Milicia excelsa* leaf to *Phytolyma fusca* attack

Assessment of the morphological response of the leaves of *M. excelsa* to attack by *P. fusca* was carried out using the method of Kraus *et al.* (1998) with slight modification. Healthy and galled leaves of *M. excelsa* were collected from six months old seedlings raised at the Evergreen Tree Planters central nursery, Ijebu-ode. Leaf samples were placed in buffered neutral formaldehyde and CRAF III. The materials were later embedded in wax and sectioned at 7 µm with a rotary microtome. The sectioned waxed samples were stained with sass saffarin (0.5%), washed in water and counterstained in 0.25% fast green FCF. The section was observed with an Olympus SZ-HT electronic microscope for internal structures of the leaves.

3.9 Evaluate the effects of soil amended with manure on the growth and gall formation of *Milicia excelsa* seedlings during *Phytolyma fusca* attack.

The effect of the addition of poultry droppings (PD), cow dung (CD) and pig faeces (PF) amended on topsoil was evaluated. Manures (2.5 kg) were added to 5 kg topsoil and filled into polythene pots of size 17.5 cm x 20 cm. The experiment was replicated 60 times and arranged in a complete randomised design.

3.9.1 Physico-chemical analysis of soil and manure

Soil and manure used for the study were analysed for physico-chemical properties following the methods described by the International Institute for Tropical Agriculture (1979). Analysis was carried out on the soil and manure separately to determine their pH, organic carbon and other mineral nutrients.

3.9.2 Effect of poultry droppings, cattle dung and pig faeces on the growth and gall formation of *Milicia excelsa*

This experiment used two hundred and forty (240) uniformly growing *M. excelsa* seedlings obtained from the screen cage. A completely randomised design experiment was set up with four treatments (Table 3.1). The treatments were replicated sixty times.

Initial data were collected two weeks after transplanting, while subsequent data were collected fortnightly for 22 weeks. The following data were collected.

1. **Seedling Height (cm):** This was measured from the base of the plant to the terminal bud using a measuring tape.
2. **Collar Diameter (cm):** This was measured using Vernier mini-caliper; measurement was at the base of the plant.
3. **Numbers of Leaves:** This was done through counting.
4. **Seedling Damage (survival):** This is computed by dividing the total infested seedling by all the sampled seedlings.
5. **Number of Galls:** This was done through counting.
6. **Numbers of rupture gall:** This was done through counting.

Table 3.1: Effects of poultry droppings, cattle dung and pig faeces on growth and gall formation of *Milicia excelsa* seedlings

Treatment	Mixture ratio (5kg: 2.5 kg)
T1 (control)	No Manure
T2	Poultry Droppings
T3	Cattle Dung
T4	Pig Faeces

3.9.3 Effect of solid and liquid poultry droppings and NPK fertiliser on growth and gall formation of *Milicia excelsa*

This study was conducted as a follow-up to Study in Table 3.1; to investigate the comparative effect of liquid poultry droppings and solid poultry droppings on the growth and gall formation of *M. excelsa* during *P. fusca* attack. To achieve this, four hundred and eighty (480) uniformly growing seedlings without galls were obtained from the stock in the screen cage.

A completely randomised design experiment was set up with seven (7) treatments (Table 3.2). The experiment was replicated sixty times.

Initial data were collected two weeks after transplanting, while subsequent data were collected fortnightly for 24 weeks. The following data were collected.

1. **Seedling Height (cm):** This was measured from the base of the plant to the terminal bud using a measuring tape.
2. **Collar Diameter (cm):** This was measured using Vernier mini-caliper; measurement was at the base of the plant.
3. **Numbers of Leaves:** This was done through counting.
4. **Seedling Damage (survival):** This is computed by dividing the total infested seedling by all the sampled seedlings.
5. **Number of Galls:** This was done through counting.
6. **Numbers of rupture gall:** This was done through counting.

Table 3.2: Effects of solid and liquid poultry droppings and NPK fertiliser on growth and gall formation of *Milicia excelsa* treatments combinations

Treatment/Level	Mixture ratio
T1L1	Liquid Poultry droppings (0.1 litres)
T1L2	Liquid Poultry droppings (0.2 litres)
T2L1	Solid Poultry droppings (0.1 kg)
T2L2	Solid Poultry droppings (0.2 kg)
T3L1	NPK (15:15:15) (0.1 kg)
T3L2	NPK (15:15:15) (0.2 kg)
T4	No manure (control)

3.9.4 Effect of poultry droppings, cattle dung, and pig faeces compost on growth and gall formation of *Milicia excelsa*

A total of one hundred (100) healthy *M. excelsa* seedlings were used for this study. Seedlings were transplanted to polythene pots containing topsoil with varying manure composts (Table 3.3). After transplanting, the seedlings were allowed to stabilise for two weeks before initial data collection; after that, data was taken at two weeks intervals for 12 weeks. The experimental design used was a Complete Randomise design with five treatments replicated twenty times.

Data collected were on seedlings' growth and gall formation.

1. **Seedling Height (cm):** This was measured from the base of the plant to the terminal bud using a measuring tape.
2. **Collar Diameter (cm):** This was measured using Vernier mini-caliper; measurement was at the base of the plant.
3. **Numbers of Leaves:** This was done through counting.
4. **Seedling Damage (survival):** This is computed by dividing the total infested seedling by all the sampled seedlings.
5. **Number of Galls:** This was done through counting.
6. **Numbers of rupture gall:** This was done through counting.

Table 3.3: Effects of poultry droppings, cattle dung and pig faeces composts on growth and gall formation of *Milicia excelsa* treatment combinations

Treatments	Mixture ratio
T1	Pig + Cattle + Poultry compost manure
T2	Pig + Cattle compost manure
T3	Pig + Poultry compost manure
T4	Poultry + Cattle compost manure
T5	No manure (control)

Data Analysis

Data collected for studies 3.9.2, 3.9.3 and 3.9.4 were analysed using Descriptive statistics and Analysis of Variance (ANOVA). Significantly different means were separated using Duncan Multiple Range Test (DMRT) at ($P<0.05$)).

CHAPTER FOUR
RESULTS

4.1 Qualitative and Quantitative phytochemical compounds in healthy and galled leaves of 8 month-old *Milicia excelsa*

The phytochemical compounds present in the healthy and galled leaves of 8 months old *M. excelsa* are presented in Tables 4.1 and 4.2. The healthy and galled leaves contained saponins, tannins, alkaloids, flavonoids, steroids, and terpenoids. However, there was no coumarins, quinone, glycoside, anthraquinone and anthocyanin (Table 4.1). Results showed that moisture contents in healthy leaves of *M. excelsa* were 51.98%, while that of the galled leaves was 67.45% in the galled leaf. Similarly, the presence of chlorophyll, sugar, protein, phenols, saponins, terpenoids, alkaloid, tannins and flavonoids showed that the healthy leaves had 0.48±0.01 mg/g, 1.21±0.23 mg/g, 4.88±0.16 %, 5.64±0.01 mg/g, 0,09±2.08 mg/g, 0.55±0.01 mg/g, 2.12±0.03 mg/g, 0.44±0.45 mg/g, 0.49±0.08 mg/g respectively while the galled leaf 0.18±0.003 mg/g, 2.16±0.003 mg/g, 0.1±0.04 %, 4.27±0.12 mg/g, 0.01±0.01 mg/g, 0.16±0.07 mg/g, 2.44±0.02 mg/g, 0.23±0.06 mg/g, 0.02±0.01 mg/g of chlorophyll, sugar, protein, phenols, saponins, terpenoids, alkaloid, tannins and flavonoids in the galled leaves respectively.

The percentage moisture content and total sugar in *M. excelsa* leaf after *P. fusca* attack increased by 15.47% and 0.95 mg/g, respectively. *Phytolyma fusca* attack caused a decrease in the leaves chlorophyll content from 0.48±0.01 mg/g to 0.18±0.003 mg/g, protein (4.88±0.16% to 0.1±0.04%) and phenol (5.64±0.01 mg/g to 4.27±0.16 mg/g) between the healthy leaves and galled leaves respectively. Results also showed increase in alkaloids (2.12±0.03 % to 2.44±0.02 %) compounds and decrease in saponins (0.09±2.08 mg/g to 0.01±0.01 mg/g), terpenoids (0.55±0.01 mg/g to 0.16±0.07 mg/g), tannin (0.44±0.45 mg/g to 0.23±0.06 mg/g) and flavonoids (0.49±0.08 mg/g to 0.23±0.06 mg/g) compounds in the galled leaves after *P. fusca* attack.

4.1: Qualitative Phytochemical Compounds Present in healthy and galled leaves of *Milicia excelsa*

		Healthy Leaves	
		8th Month	**16th Month**
1	Saponins	+	+
2	Tannin	+	+
3	Alkaloid	+	+
4	Flavonoid	+	+
5	Steroids	+	+
6	Coumarins	_	_
7	Quinone	_	_
8	Glycoside	_	_
9	Terpenoids	+	+
10	Anthocyanins	_	_
11	Anthraquinone	_	_
		Galled Leaves	
		8th Month	**16th Month**
1	Saponins	+	+
2	Tannin	+	+
3	Alkaloid	+	+
4	Flavonoid	+	+
5	Steroids	+	+
6	Coumarins	_	_
7	Quinone	_	_
8	Glycoside	_	_
9	Terpenoids	+	+
10	Anthocyanins	_	_
11	Anthraquinone	_	_

+ indicates phytochemicals present and - indicates phytochemicals absent.

4.2: Quantitative Phytochemical Compounds in the healthy and galled leaves of *Milicia excelsa*

		Healthy Leaves	
		8th Month	**16th Month**
1	Moisture Content (%)	51.98	70.38
2	Chlorophyll (Mg/g)	0.48±0.01a	0.76±0.01b
3	Sugar (Mg/g)	1.21±0.23b	1.54±0.23b
4	Protein (%)	4.88±0.16c	9.68±0.16a
5	Phenols (Mg/g)	5.64±0.01cd	7.96±0.01a
6	Saponins (Mg/g)	0.09±2.08e	0.17±2.08c
7	Terpenoids (Mg/g)	0.55±0.01ab	0.70±0.31b
8	Alkaloid (%)	2.12±0.03f	3.78±0.13d
9	Tannin (Mg/g)	0.44±0.45a	0.84±0.85b
10	Flavonoid (Mg/g)	0.49±0.08a	0.73±0.39b
		Galled Leaves	
		8th Month	**16th Month**
1	Moisture Content (%)	67.45	85.66
2	Chlorophyll (Mg/g)	0.18±0.003c	0.58±0.01d
3	Sugar (Mg/g)	2.16±0.003b	2.07±0.01c
4	Protein (%)	0.1±0.04d	8.83±0.06b
5	Phenols (Mg/g)	4.27±0.12a	9.52±0.03b
6	Saponins (Mg/g)	0.01±0.01f	0.25±0.16e
7	Terpenoids (Mg/g)	0.16±0.07cd	0.54±0.43d
8	Alkaloid (%)	2.44±0.02b	6.30±0.14a
9	Tannin (Mg/g)	0.23±0.06c	0.57±0.65d
10	Flavonoid (Mg/g)	0.02±0.01f	0.50±0.19d

4.2 Qualitative and Quantitative phytochemical compounds in healthy and galled leaves of 16 month-old *Milicia excelsa*

The phytochemical compounds present in the healthy and galled leaves of 16 months old *M. excelsa* are presented in Tables 4.1 and 4.2. The healthy and galled leaves contained saponins, tannins, alkaloids, flavonoids, steroids, and terpenoids. However, there were no coumarins, quinone, glycoside, anthraquinone or anthocyanin.

The quantitative moisture content in healthy leaves of *M. excelsa* was 51.98%, while the galled leaves were 67.45%. The chlorophyll content, sugar, protein, phenols, saponins, terpenoids, alkaloid, tannins and flavonoids present in the healthy leaves were 0.76±0.01 mg/g, 1.57±0.23 mg/g, 9.68±0.16 %, 7.96±0.01 mg/g, 0.17±2.08 mg/g, 0.70±0.31 mg/g, 3.78±0,03 mg/g, 0.84±0.85 mg/g and 0.73±0.39 mg/g respectively while the galled leaves had 0.58±0.01 mg/g, 2.07±0.01 mg/g, 8.83±0.06 %, 9.52±0.03 mg/g, 0.25±0.16 mg/g, 0.54±0.43 mg/g, 6.30±0.14 mg/g, 0.57±0.65 mg/g, 0.50±0.19 mg/g of chlorophyll contents, sugar, protein, phenols, saponins, terpenoids, alkaloid, tannins and flavonoids respectively.

There was an increase in moisture content, total sugar, and total phenol in the galled leaf by 15.28%, 0.53 mg/g and 1.56 mg/g after attack by *P. fusca. Phytolyma fusca* attack led to a decrease in the chlorophyll content from 0.76±0.01 mg/g to 0.58±0.01 mg/g and total protein from 9.68±0.16 % to 8.83±0.06 % between the healthy and galled leaves, respectively.

There were increases in saponins (0.17±2.08 mg/g to 0.25±0.16 mg/g) and alkaloids (3.78±0.13 % to 6.30±0.14 %) compounds and decrease in terpenoids (0.70±0.31 mg/g to 0.54±0.43 mg/g), tannin (0.84±0.85 mg/g to 0.57±0.65 mg/g) and flavonoids (0.73±0.39 mg/g to 0.50±0.19 mg/g) compounds in the galled leaves, respectively.

4.3 Phytochemical compounds in healthy and galled leaves of 8-month-old *Milicia excelsa* using Gas Chromatography-Mass Spectroscopy

The results of phytochemical compounds present in healthy and galled leaves of 8-month-old *M. excelsa* are presented in Tables 4.3 and 4.4. The chromatogram peaks were integrated and compared with the database spectrum of known compounds deposited in the GC-MS Library (NIST08.L).

Results indicated the presence of twenty-two (22) and nineteen (19) heterocyclic compounds in the healthy and galled leaves of *M. excelsa*. However, at a minimum quality of 80, the chromatogram indicated the presence of twenty (20) and eighteen (18) compounds in the healthy and galled leaves, respectively. For healthy *M. excelsa* leaves, results showed that *1-butyl hexyl-benzene* had the highest peak area value, 9.20, while *1-ethyl octyl-benzene* and *1-methyl nonyl-benzene* had the lowest peak area values, 1.80. The highest peak area value for the galled leaves was 8.75 (1-butylheptyl-benzene), while the lowest peak area value was 1.78 (*1-methyl nonyl-benzene*).

Comparing the results of the healthy and galled leaves showed that the same compound was identified in the healthy and galled leaf except for compounds such as *1-propyl heptyl-benzene, 1-hexyl heptyl-benzene* and *Phytol*. There was also the production of a new compound (*1-pentyl-octyl-benzene*) in the galled leaves.

4.4 Phytochemical compounds in healthy and galled leaves of 16-month-old *Milicia excelsa* using GC -MS

Results of compounds present in the leaf samples are presented in Tables 4.5 and 4.6. The peaks in the chromatogram were integrated and compared with the database spectrum of known phytochemicals stored in the GC-MS Library (NIST08.L).

Healthy and galled leaves showed twenty-four (24) and twenty-two (22) heterocyclic compounds, respectively. At the minimum quality of 80, eighteen (18) and fifteen (15) compounds were identified in the healthy and galled leaves, respectively. Phytochemical compounds in the galled leaves indicated the presence of Phytol while *1-Ethyloctyl-, 1-Methylnonyl-, 1-Pentyhexyl-, 1-Butylnonyl-, and Methyl-dodecyl- Benzenes* present in galled leaves were absent due to *P. fusca* infestation.

In healthy leaves, *Dihydropinene*: a new compound, was produced at the highest peak area value of 9.62, while *1-butyl-hexyl-benzene* produced had the lowest peak area value, 1.86. *Phytol* was indicated at two peak area values of 3.76 and 5.45, respectively. *1-pentyl-octyl-benzene* had the highest peak area value, 9.07 for the galled leaves, while *3-phenylbenzene* had the lowest peak area value (1.61). The presence of *Phytol* was also indicated at a peak area of 8.50.

Table 4.3: Phytochemical compounds identified in the healthy leaves of 8-month-old *Milicia excelsa* by GC-MS Peak Report TIC

Peak	RT	PA	N of C	MF	MW
1	21.626	2.56	5-decyl-benzene	$C_{16}H_{26}$	218
2	21.943	2.04	1-propyl heptyl-benzene	$C_{16}H_{26}$	218
3	22.629	1.80	1-ethyl octyl-benzene	$C_{16}H_{26}$	218
4	23.974	1.80	1-methyl nonyl-benzene	$C_{16}H_{26}$	218
5	25.119	4.80	1-pentyl hexyl-benzene	$C_{17}H_{28}$	232
6	25.261	9.20	1-butyl heptyl-benzene	$C_{17}H_{28}$	232
7	25.617	6.85	1-propyl octyl-benzene	$C_{17}H_{28}$	232
8	26.348	6.40	1-ethyl nonyl-benzene	$C_{17}H_{28}$	232
9	27.674	6.60	1-methyl decyl-benzene	$C_{17}H_{28}$	232
10	28.619	7.99	1-pentyl heptyl-benzene	$C_{18}H_{30}$	246
11	28.793	7.54	1-butyl octyl-benzene	$C_{18}H_{30}$	246
12	29.194	5.42	1-propyl nonyl-benzene	$C_{18}H_{30}$	246
13	29.938	5.14	1-ethyl decyl-benzene	$C_{18}H_{30}$	246
14	31.329	5.10	1-methyl undecyl-benzene	$C_{18}H_{30}$	246
15	32.196	8.14	1-hexyl heptyl-benzene	$C_{19}H_{32}$	260
16	32.461	4.96	1-butyl nonyl-benzene	$C_{19}H_{32}$	260
17	32.985	4.00	1-propyl decyl-benzene	$C_{19}H_{32}$	260
18	33.988	3.30	1-ethyl undecyl-benzene	$C_{19}H_{32}$	260
19	34.492	2.67	Phytol	$C_{20}H_{40}O$	296
20	35.896	3.05	1-methyl dodecyl-benzene	$C_{19}H_{32}$	260

RT=Retention Time, PA=Peak Area, N of C= Name of Compound, MF=Molecular Formula, MW=Molecular Weight.

Table 4.4: Phytochemical compounds in the galled leaves of 8-month-old *Milicia excelsa* by GC-MS Peak Report TIC

Peak	RT	PA	N of C	MF	MW
1	21.943	1.87	1-butyl hexyl-benzene	$C_{16}H_{26}$	218
2	22.622	1.79	1-ethyl octyl-benzene	$C_{16}H_{26}$	218
3	23.968	1.78	1-methyl nonyl-benzene	$C_{16}H_{26}$	218
4	25.125	4.96	1-pentyl hexyl-benzene	$C_{17}H_{28}$	232
5	25.268	8.75	1-butyl heptyl-benzene	$C_{17}H_{28}$	232
6	25.624	6.90	1-propyl octyl-benzene	$C_{17}H_{28}$	232
7	26.355	6.51	1-ethyl nonyl-benzene	$C_{17}H_{28}$	232
8	27.681	6.88	1-methyl decyl-benzene	$C_{17}H_{28}$	232
9	28.625	8.39	1-pentyl heptyl-benzene	$C_{18}H_{30}$	246
10	28.800	7.67	1-butyl octyl-benzene	$C_{18}H_{30}$	246
11	29.201	5.80	1-propyl nonyl-benzene	$C_{18}H_{30}$	246
12	29.945	5.43	1-ethyl decyl-benzene	$C_{18}H_{30}$	246
13	31.329	5.47	1-methyl undecyl-benzene	$C_{18}H_{30}$	246
14	32.202	8.74	1-pentyl octyl-benzene	$C_{19}H_{32}$	260
15	32.474	5.64	1-butyl nonyl-benzene	$C_{19}H_{32}$	260
16	32.985	3.95	1-propyl decyl-benzene	$C_{19}H_{32}$	260
17	34.000	3.66	1-ethyl undecyl-benzene	$C_{19}H_{32}$	260
18	35.889	3.40	1-methyl dodecyl-benzene	$C_{19}H_{32}$	260

RT=Retention Time, PA=Peak Area, N of C= Name of Compound, MF=Molecular Formula, MW=Molecular Weight.

Table 4.5: Phytochemical compounds identified in the healthy leaves of 16-month-old *Milicia excelsa* by GC-MS Peak Report TIC

Peak	RT	PA	N of C	MF	MW
1	21.581	1.86	1-butyl hexyl-benzene	$C_{16}H_{26}$	218
2	25.171	8.25	1-buyl heptyl-benzene	$C_{17}H_{28}$	232
3	25.533	5.54	1-propyl octyl-benzene	$C_{17}H_{28}$	232
4	26.270	5.75	3-phenyl undecane	$C_{17}H_{28}$	232
5	27.609	5.06	1-methyl decyl-benzene	$C_{17}H_{28}$	232
6	28.528	7.10	1-pentyl heptyl-benzene	$C_{18}H_{30}$	246
7	28.696	6.73	5-phenyl dodecane	$C_{18}H_{30}$	246
8	29.110	4.65	1-propyl nonyl-benzene	$C_{18}H_{30}$	246
9	29.861	4.37	3-phenyl dodecane	$C_{18}H_{30}$	246
10	31.245	4.21	2-phenyl dodecane	$C_{18}H_{30}$	246
11	32.086	7.40	7-phenyl tridecane	$C_{19}H_{32}$	260
12	32.351	3.76	1-butyl nonyl-benzene	$C_{19}H_{32}$	260
13	32.487	9.62	Dihydropinene	$C_{10}H_{18}O$	138
14	32.881	3.62	4-phenyl-benzene	$C_{19}H_{32}$	260
15	33.574	3.76	Phytol	$C_{20}H_{40}O$	296
16	33.891	2.58	3-phenyl-benzene	$C_{19}H_{32}$	260
17	34.389	5.45	Phytol	$C_{20}H_{40}O$	296
18	35.799	1.92	1-methyl dodecyl-benzene	$C_{19}H_{32}$	260

RT=Retention Time, PA=Peak Area, N of C= Name of Compound, MF=Molecular Formula, MW=Molecular Weight.

Table 4.6: Phytochemical compounds identified in the galled leaves of 16-month-old *Milicia excelsa* by GC-MS Peak Report TIC

Peak	RT	PA	N of C	MF	MW
1	21.568	1.98	5-decyl-benzene	$C_{16}H_{26}$	218
2	25.158	8.01	1-butyl heptyl-benzene	$C_{17}H_{28}$	232
3	25.533	6.00	1-propyl octyl-benzene	$C_{17}H_{28}$	232
4	26.264	6.18	3-phenyl undecane	$C_{17}H_{28}$	232
5	27.603	6.38	1-methyl decyl-benzene	$C_{17}H_{28}$	232
6	28.522	7.80	1-pentyl heptyl-benzene	$C_{18}H_{30}$	246
7	28.690	7.15	5-phenyl-dodecane	$C_{18}H_{30}$	246
8	29.104	5.45	4-phenyl-dodecane	$C_{18}H_{30}$	246
9	29.854	5.10	3-phenyl-dodecane	$C_{18}H_{30}$	246
10	31.238	5.26	3-phenyl-dodecane	$C_{18}H_{30}$	246
11	32.086	9.07	1-pentyl octyl-benzene	$C_{19}H_{32}$	260
12	32.862	4.21	4-phenyl-benzene	$C_{19}H_{32}$	260
13	33.871	1.61	3-phenyl-benzene	$C_{19}H_{32}$	260
14	34.175	8.50	Phytol	$C_{20}H_{40}O$	296
15	35.779	2.71	1-methyldodecyl-benzene	$C_{19}H_{32}$	260

RT=Retention Time, PA=Peak Area, N of C= Name of Compound, MF=Molecular Formula, MW=Molecular Weight.

There were changes in the phytochemical compounds present in the healthy leaves as the plant matured. There were 20 phytochemical compounds in the healthy leaves at eight-month-old while 18 phytochemical compounds were identified at sixteen-month-old. Results showed the production of phytochemical compounds; *Dihydropinene* and two *Phytol* groups at the 16-month-old. Phytochemical compounds; *1-propyl-heptyl-benzene, 1-ethyl-octyl-benzene, 1-methyl-nonyl-benzene*, and *1-pentyl-hexyl-benzene* present in the healthy leaves at the 8-month-old were absent at 16 months.

Phytochemical compounds in the galled leaves at 16-month-old indicated the presence of *Phytol* while *1-Ethyloctyl-, 1-Methylnonyl-, 1-Pentyhexyl-, 1-Butylnonyl-, and Methyl-dodecyl- benzenes* were absent.

4.5 Reactive Oxygen Species in the leaves of *Milicia excelsa*

4.5.1 Superoxide anion radicals in the leaves of *Milicia excelsa*

Plate 4.1 showed wounded (Pierced) third leaf (from top) excised from the *M. excelsa* seedling at six months old. Detection of superoxide anion accumulation in the leaves after wounding, using the Nitro-blue tetrazolium staining method, showed no blue stains at the point of wounding or around the leaf tissue. Wounded and injury-free (control) leaves issues showed no sign of colouration (blue colour) around and at the point of the wound (hole) and across major veins of the leaves tissue (Plate 4.2).

4.5.2 Hydrogen peroxide in the leaf of *Milicia excelsa*

In wounded leaf, brown 3,3- diamino-benzidine stain was primarily all over the leaf tissue. Results showed brown colouration at accumulation zones of hydrogen peroxide in the leaf tissue. The leaf sample showed an appearance of brown colour at the point of wounding and across leaf veins (Plate 4.3A). There were no brown colouration on the control (injury-free) leaf tissues (Plate 4.3B).

Spectra of different concentration of hydrogen peroxide from spectrophotometry readings were 0.126, 0.227, 0.294, 0.401 and 0.460 at concentrations of 1mM, 5Mm, 10mM, 25mM and 50Mm, respectively. The spectra of hydrogen peroxide extracted from the injured leaf tissue were 0.112, 0.131 and 0.166. For injury-free leaves issues (control), there was no indication of brown stain on the tissue. Hence, the spectra reading of the leaf

Plate 4.2: *Milicia excelsa* leaf pierced with sterile perforator (hole diameter= 0.55cm)

Plate 4.3: Leaf of *Milicia excelsa* after treatment with NBT stain (A) wounded leaf, (B) injury-free leaf (control)

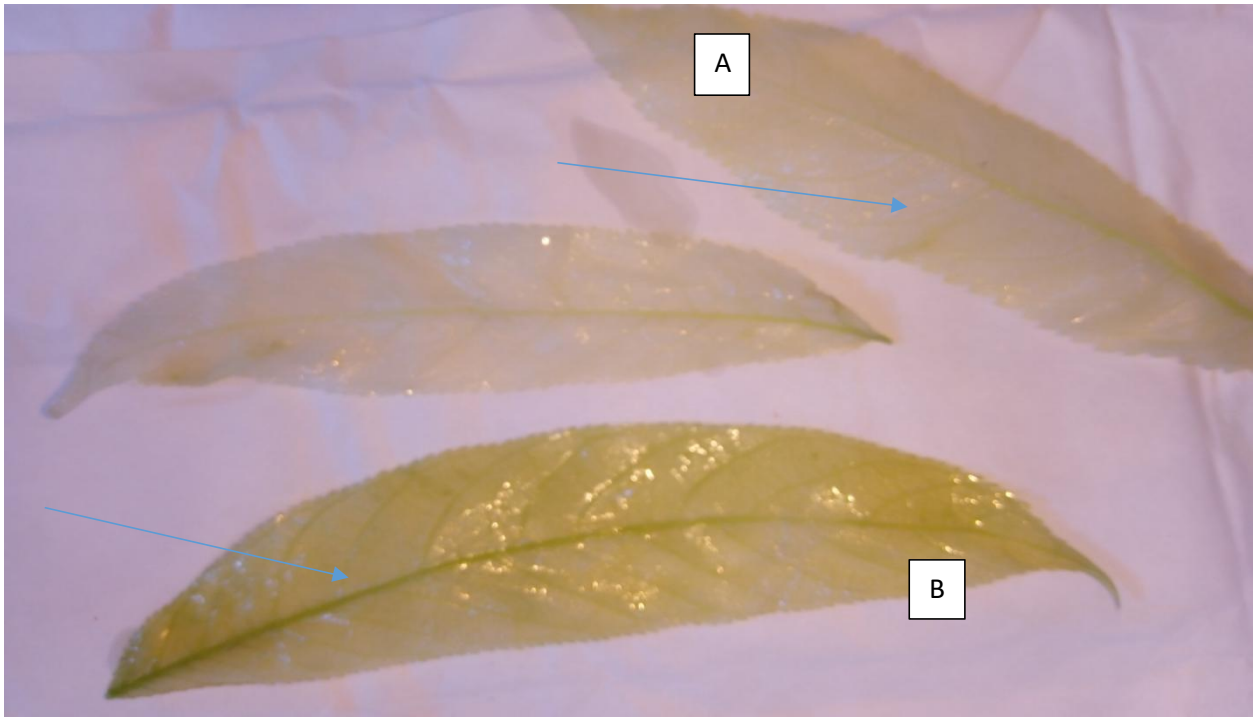

Plate 4.4: **Leaf of *Milicia excelsa* after staining with DAB, wounded leaf (A), injury-free leaf (B)**

extracts was not read for all the samples. Results from this study showed that the concentration of hydrogen peroxide in 1g of *M. excelsa* injured leaf tissue were between $1mMgFW^{-1}$ and $5mMgFW^{-1}$ (Figure 4.1).

4.6 Fungi identified from ruptured, un-ruptured galls of *Milicia excelsa* and nymphs of *Phytolyma fusca*

Fungi were isolated from healthy leaves, galled leaves (un-ruptured), and galled leaves (ruptured) of *M. excelsa* seedlings and nymphs of *P. fusca* collected from inside the gall (Table 4.7).

Four fungi species were isolated from the samples collected. Fungi isolated and identified in the specimens include *Fusarium solani, F. oxysporum, Aspergillus niger*, and *Collectotricum coccodes* (Table 4.7). *Fusarium spp* was more predominant in all the samples collected, followed by *C. coccodes and Aspergillus niger*, respectively. *Fusarium solani, F. oxysporum, C. coccodes* and *A. niger* were isolated from the healthy leaves, *F. solani* and *F. oxysporum* were isolated from the un-ruptured galled leaves while *F. oxysporum, C. coccodes* and *A. niger* were isolated from the ruptured galled leaves. *F. oxysporum* and *F. solani* were isolated (Plates 4.5). *Fusarium solani* and *F. oxysporun* had the highest frequency of occurrence (4) while *C. coccodes* and *A. niger* had the lowest frequency of occurrence of 4.

Pure cultures of *Collectotricum sp.* isolated was white fluffy and woolly; its appearance was initially bright but later turned dull and dry as it matured. *Aspergillus niger* isolated had a characteristic carbon black colour. It grew rapidly, covering the Petri dish within three days. The *Fusarium sp.* isolated formed larger colonies in PDA. *Fusarium oxysporum* isolated had a colour changing from white, cream and violet. *Fusarium solani* had a similar resemblance with *F. oxysporum* forming a cylinder-like macroconidium without curvature in its cortex (Plate 4.5).

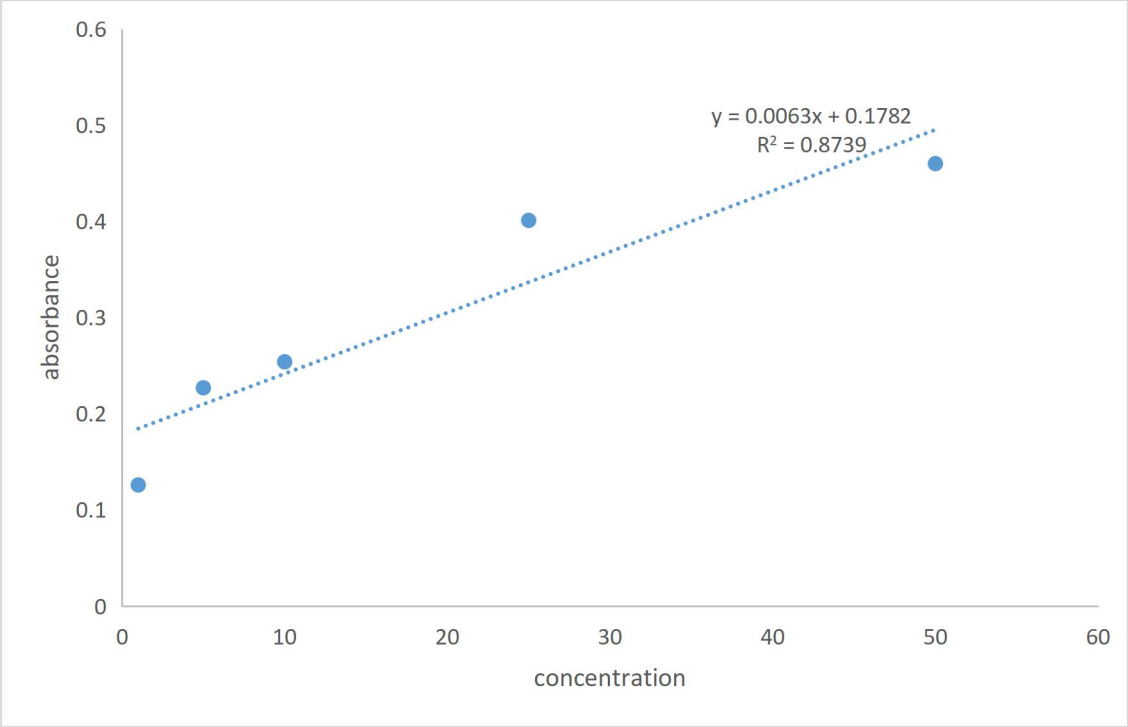

Figure 4.1: Hydrogen peroxide calibration curve

Table 4.7: Fungi identified in galls of *Milicia excelsa* seedlings and nymphs of *Phytolyma fusca*

S/No.	Plant parts	Fungi	Frequency of occurrence
1	Healthy leaf	*Fusarium solani*	4
		Fusarium oxysporum	4
		Collectotricum coccodes	2
		Apergillus niger	2
2	Un-ruptured galled leaf	*Fusarium solani*	4
		Fusarium oxysporum	4
3	Ruptured gall leaf	*Fusarium oxysporum*	4
		Collectotricum	2
		Aspergilus niger	2
4	Nymph	*Fusarium oxysporum*	4
		Fusarium solani	4

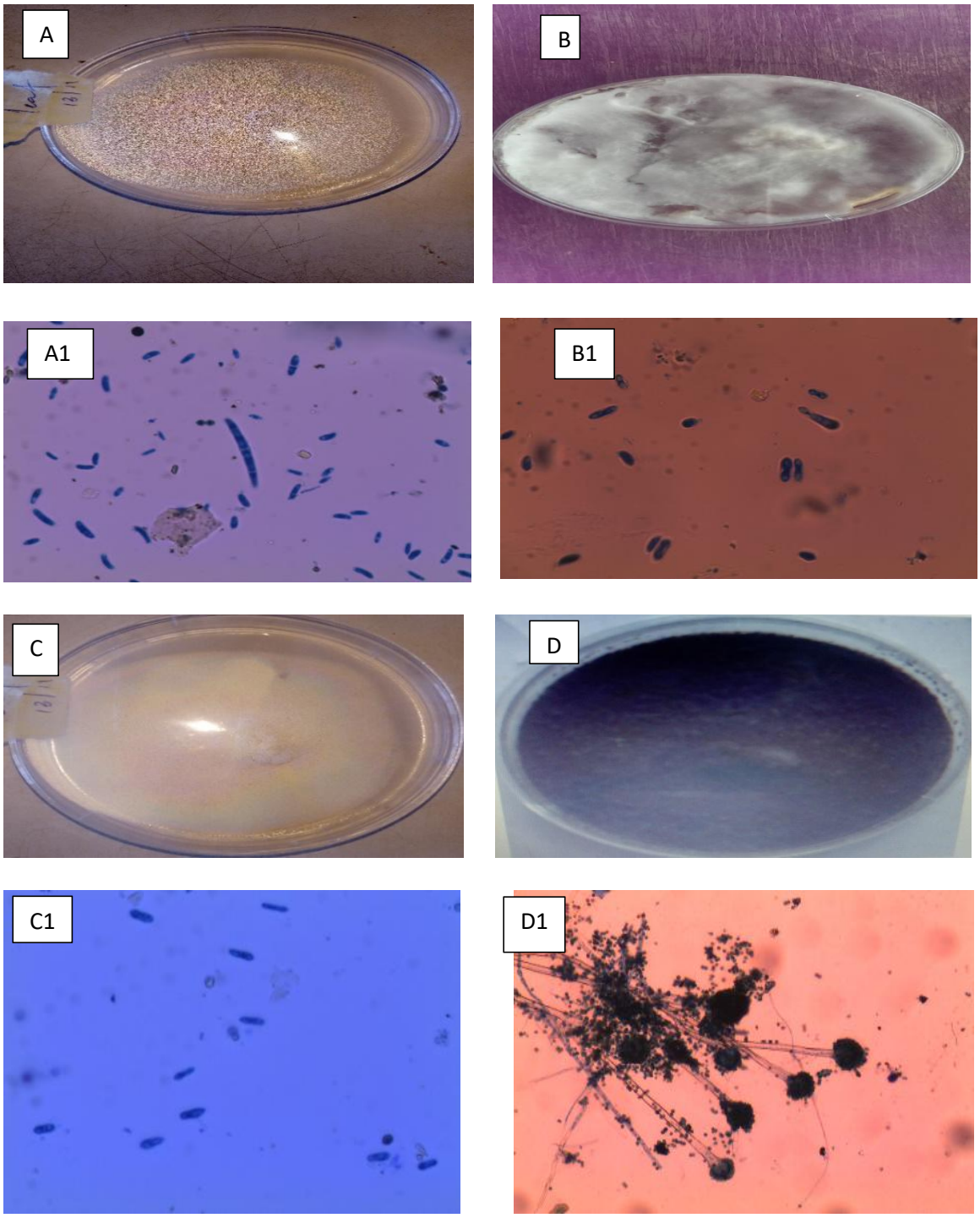

Plate 4.5: **Pure cultures of fungi isolates and their respective microscopic structure and spore appearance showing *Fusarium oxysporum* (A, A1), *Colletotricum coccodes* (B, B1), *Fusarium solani* (C, C1) and *Aspergillus niger* (D, D1)**

4.7 Histology of gall formed in leaves of *Milicia excelsa*

4.7.1 Morphology of gall formed in the leaves of *Milicia excelsa* by *Phytolyma fusca*

Phytolyma fusca induced gall on the lateral buds, apical meristem and leaves of *M. excelsa*. Galls were induced completely, expanded, and located on the subapical and apical portions of the plant (Plate 4.5). Inside some gall, a single nymph was observed in the nymph chamber. The insect larvae punctured and burrow through the epidermal cell, palisade parenchyma cells and spongy parenchyma cells, as well as the vascular bundles of the leaves. This process of *P. fusca* attack results in the swelling phase in the gall development. The swelling phase was characterised by cell differentiation and thickening of plant tissues, thus creating the nymph chamber where the immature insect gets its nutrition as it develops.

After 14 days, gall was observed to dehisce; this process sets in when the nymph has grown into immature adults, and it is usually characterised by the opening of the slit created at the point of entrance. At this stage, the nymph was exposed, paving the way for the immature adult to exit the gall; the colour of the gall remained green. After the immature P. fusca adult has exited, the gall begins to senesce, forming a putrefying mass that initially turns grey and then black. This putrefying mass usually remains attached to the plant branch for several weeks, even after drying up. In other cases, this putrefying mass is colonised by pathogens, especially fungi, which causes an outbreak of secondary infection on the plants (Plate 4.6).

4.7.2 Anatomy of gall formed in the leaves of *Milicia excelsa* by *Phytolyma fusca*

The Healthy *M. excelsa* plant leaves section showed the characteristic feature of plant leaves. The lamina showed epidermis with trichome, and the mesophyll consisted of spongy parenchyma cells situated amid two palisade parenchyma cells. Within the spongy parenchyma cells were sited the collateral vascular bundles, and the midrib areas were constituted of the epidermis, collenchyma, cortical parenchyma and vascular bundle apparatus . The vascular bundle apparatus contains elements of xylem and phloem.

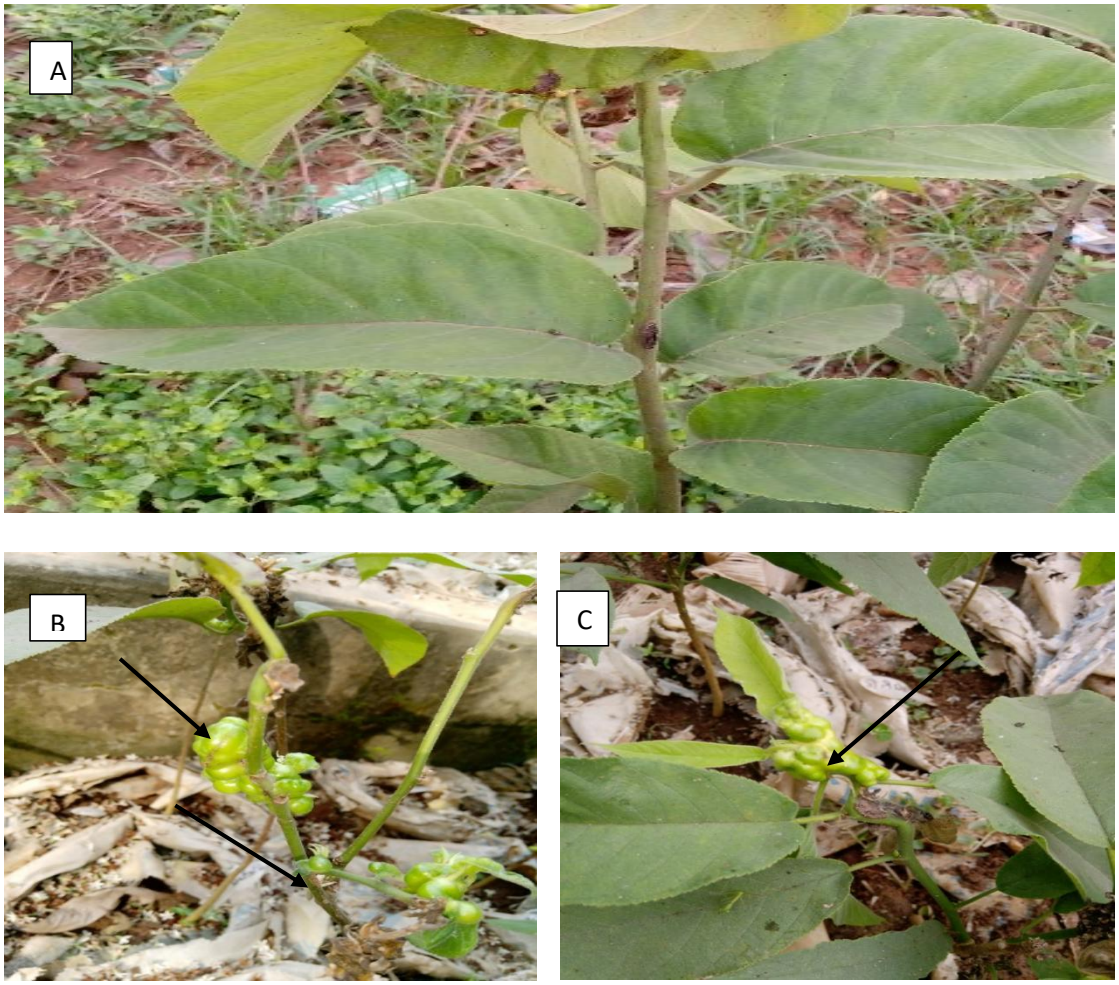

Plate 4.6: **Gall induced by *Phytolyma fusca* on *Milicia excelsa* leaves: Morphology of gall: (A) seedling of *Milicia excelsa* without gall, (B) gall development on lateral bud (C) gall development on Apical meristem**

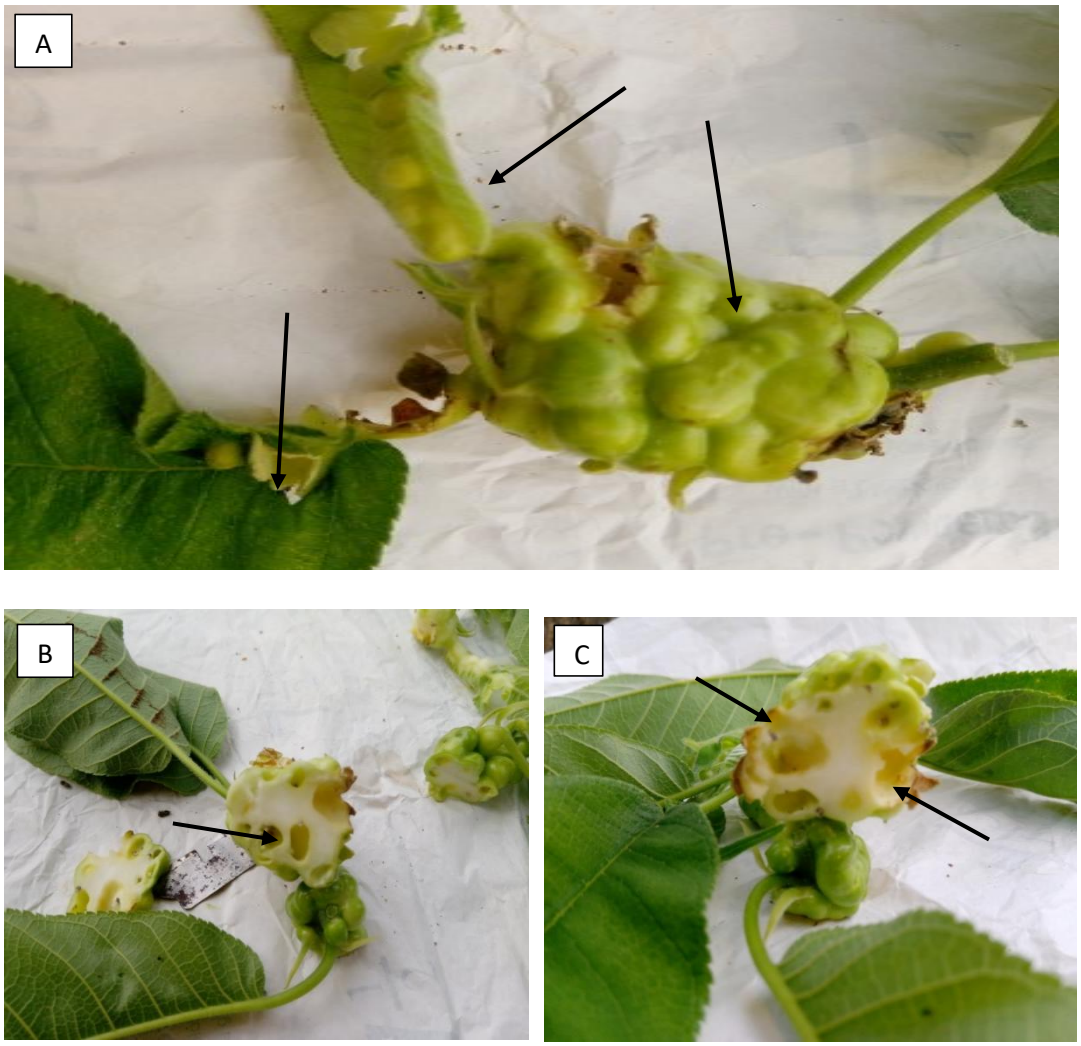

Plate 4.7: **Gall induced by *Phytolyma fusca* on *Milicia excelsa* leaves: Morphology of gall: (A) gall development (ruptured and un-ruptured) on apical meristem and midrib of leaf (B) Transverse section of gall showing Nymph chamber with immature insect (C) Transverse section of gall showing point of exit after gall ruptured**

However, an attack by *P. fusca* caused deformation in the features. Gall thickness increased in the swelling phase during gall formation, whereas the amounts of parenchyma cells appeared to be plenty in the mesophyll. The presence of chloroplast was noticed around the epidermis and appeared very minute with a dark tone stain (Plate 4.7). Vascular tissues looked distorted, showing features like a short wedge with the midrib of the gall. There was evidence of nutritive tissues around the nymph chamber showing features and actions of fungi mycelia down to the leaf epidermis. Results also showed a breakdown of plant tissue around the nymph chamber (Plate 4.8). The appearance of the gall was retained till the swelling phase. The appearance after that changed during the opening of the slit suspected to be caused by gall tissue hypertrophy. Necrotic tissues were noticed in the gall during senescence. It started from the area where the slit is located down to the internal portion of the gall cells.

4.8 Physico-chemical constituents of soil and manure used to manage *Phytolyma fusca* attack on *Milicia excelsa* seedlings

The proportion of the nutrient elements in the amendments (poultry droppings, pig faeces and cattle dung) used in the management of *P. fusca* on *M. excelsa* seedlings showed that the manures were rich in organic matters needed for plant growth and the ability to recover from insect attack. Pig faeces had the highest proportion K (2.31%), P (0.002%), followed by cattle dung (N) 1.94%. Poultry droppings had the least proportion of N (1.63 %). Poultry droppings and cattle dung had an equal proportion of P (0.001%), while cattle dung had the highest proportion of K (1.311%). The proportion of K in pig faeces and cattle dungs were 0.196 % and 0.195 %, respectively.

There was a high proportion of Magnesium, Mg (0.196 %), Sodium, Na (0.944 %) and total carbon (80.5 %) in pig faeces. This was followed by poultry droppings (Mg, 0.118 %, Na, 0.539 %), while cattle dung had the least proportion of Mg (0.227 %) and Na (0.180 %). Cattle dung had the highest proportion of total carbon (39.5 %).

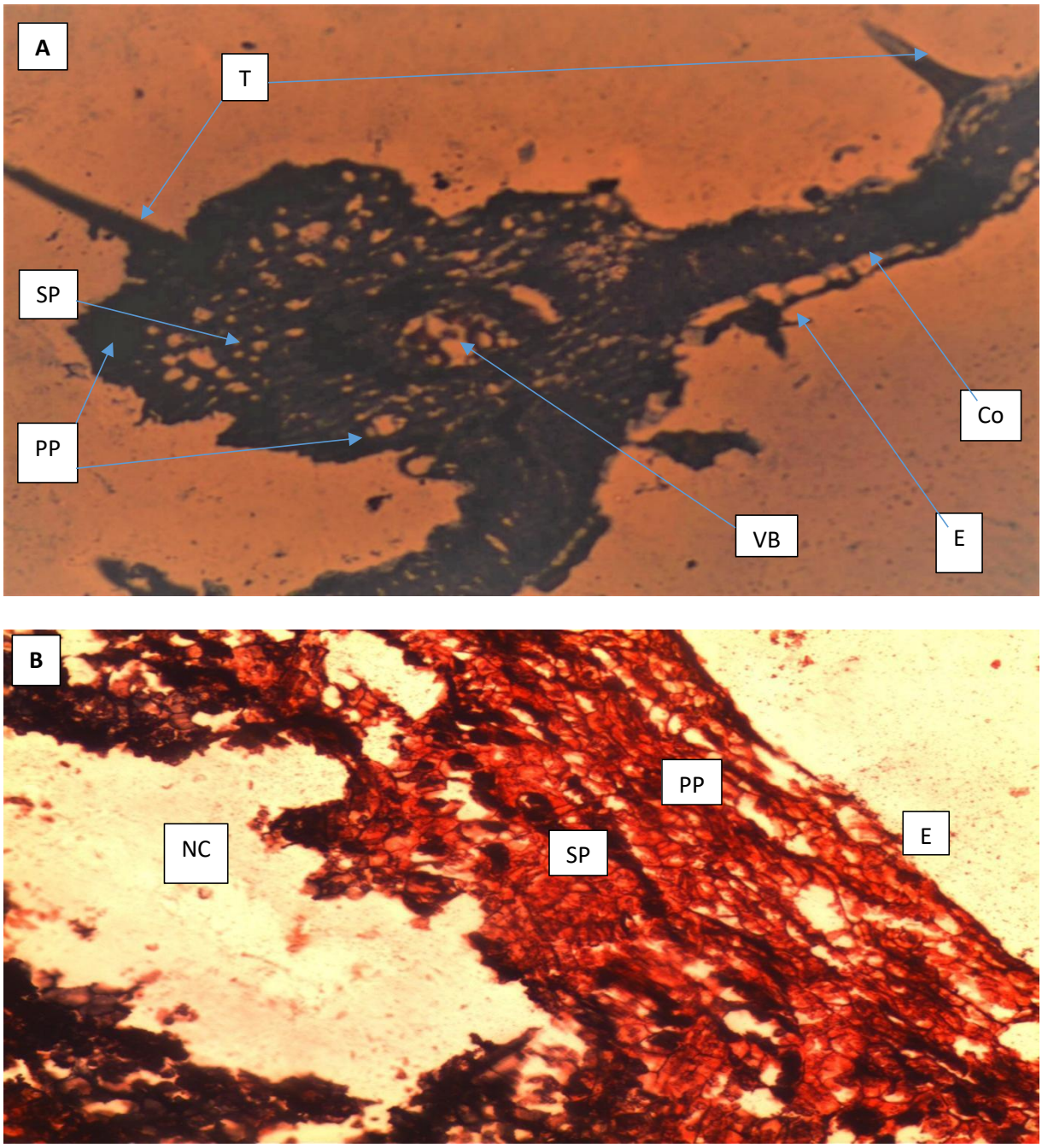

Plate 4.8a: **Anatomical features of *Milicia excelsa* leaves: A (healthy leaf mid-rib) and B (galled leaf mid-rib) showing Trichome (T), Spongy parenchyma (SP), Palisade parenchyma (PP), Vascular bundle (VB), Collenchyma cell (Co.) and Epidermal cell (E)**

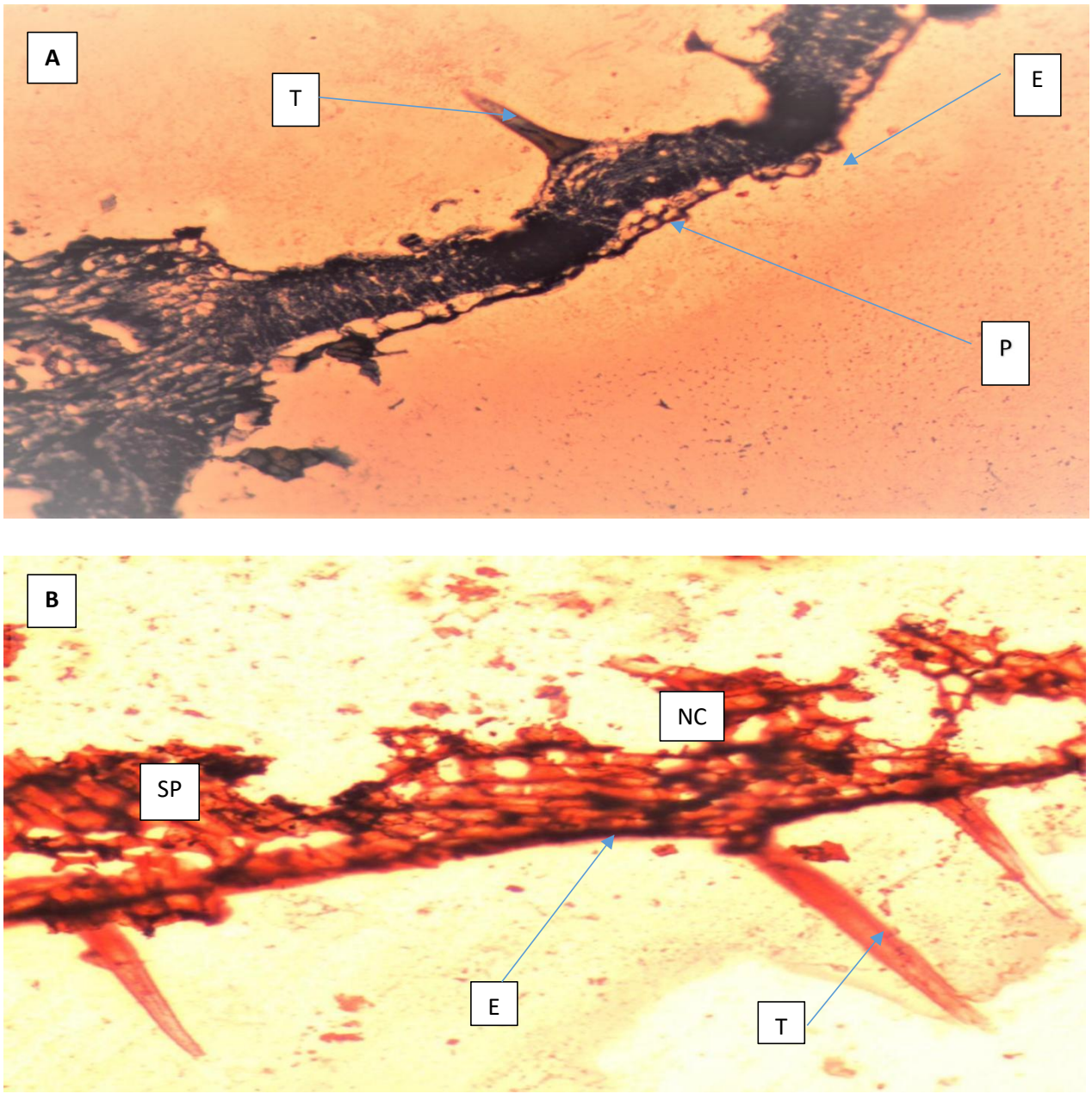

Plate 4.8b: **Anatomical Features of *Milicia excelsa* leaves: A (healthy leaf lamina) and B (galled leaf lamina) showing Trichome (T), Parenchyma cells (P), Epidermal cell (E) and Nymph Chamber**

4.9 Effects of manures on growth and gall formation of *Milicia excelsa*

4.9.1 Effects of manures on seedling height

Table 4.8 showed results on the effect of adding different manure on the seedling height of *M. excelsa* during infestation by *P. fusca* studied for 22 weeks. At two weeks after transplanting, T2 (18.27±1.15cm) had the highest height, followed by T4 (15.46±0.86cm) and T1 (10.33±0.46cm) while T3 had the least (5.67±0.21 cm).

Seedlings' height increment was steady from week 2 to week 12, but at week 14, height growth decreased across all the treatments.

At the end of the study, T4 (46.21±2.59cm) had the highest mean seedlings height while T1 had the least (23.4±0.82 cm). Treatments T2 and T3 had a mean seedling height of 30.44±4.08 cm and 41.76±1.63 cm, respectively. The decrease in seedlings' height in T1, T2 and T4 at week 14 extended to week 20 except for T3 seedlings. Treatments: T2, T3 and T4 showed exponential height increment, unlike T1, where seedling height increment was gradual. Results showed significant differences in treatments on the seedling height at $p = 0.05$ (Figure 4.2).

4.9.2 Effects of manures on seedling collar diameter

The result of the comparative effect of the addition of Poultry Droppings (T2), Cattle Dung (T3) and pig faeces (T4) on the Collar diameter of *M. excelsa* seedlings during the attack by *P. fusca* two weeks after showed that T2 (0.35±0.02cm) and T4 (0.35±0.02cm) had the highest mean collar diameter followed by the control, T1 (0.24±0.01cm) while T3 had the least (0.17±0.01cm) (Table 4.9).

Collar diameter increased among the treatments at two (2) weeks after planting for twenty-two (22) weeks. However, there was a decrease in the mean value of collar diameter at week 14 for T1 and T4.

Treatment, T2 at the end of the study, had the highest mean collar diameter (0.77±0.03cm), followed by T4 (0.74±0.04cm) and T3 (0.67±0.02cm) while T1 had the least (0.46±0.02cm). Results showed that there were significant differences in the treatments at $p=0.05$ (Figure 4.3)

Table 4.8: Effects of different manure on the height of *Milicia excelsa* seedlings attacked by *Phytolyma fusca*

	Seedling Height				
Weeks	**T1(cm)**	**T2(cm)**	**T3(cm)**	**T4(cm)**	**P-value**
2	10.33±0.46	18.27±1.15	5.67±0.21	15.46±0.86	0.00
4	10.37±0.45	18.58±1.13	5.67±0.21	15.46±0.86	0.00
6	15.85±0.68	38.43±2.06	12.34±0.39	29.93±1.56	0.00
8	19.46±0.70	53.00±2.32	23.09±0.73	41.13±1.71	0.00
10	21.89±0.75	60.60±2.45	35.06±1.10	44.64±2.21	0.00
12	24.05±0.79	68.60±2.61	44.79±1.41	51.39±2.21	0.00
14	22.80±1.12	60.79±3.09	41.90±2.08	46.65±2.69	0.00
16	21.63±0.99	54.40±2.79	42.93±1.50	45.97±2.70	0.00
18	19.95±1.23	51.97±3.35	39.03±2.23	42.44±3.01	0.00
20	18.57±1.38	29.75±3.92	42.19±1.57	38.18±3.24	0.00
22	23.40±0.82	30.44±4.08	41.76±1.63	46.21±2.59	0.00

P=0.05

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

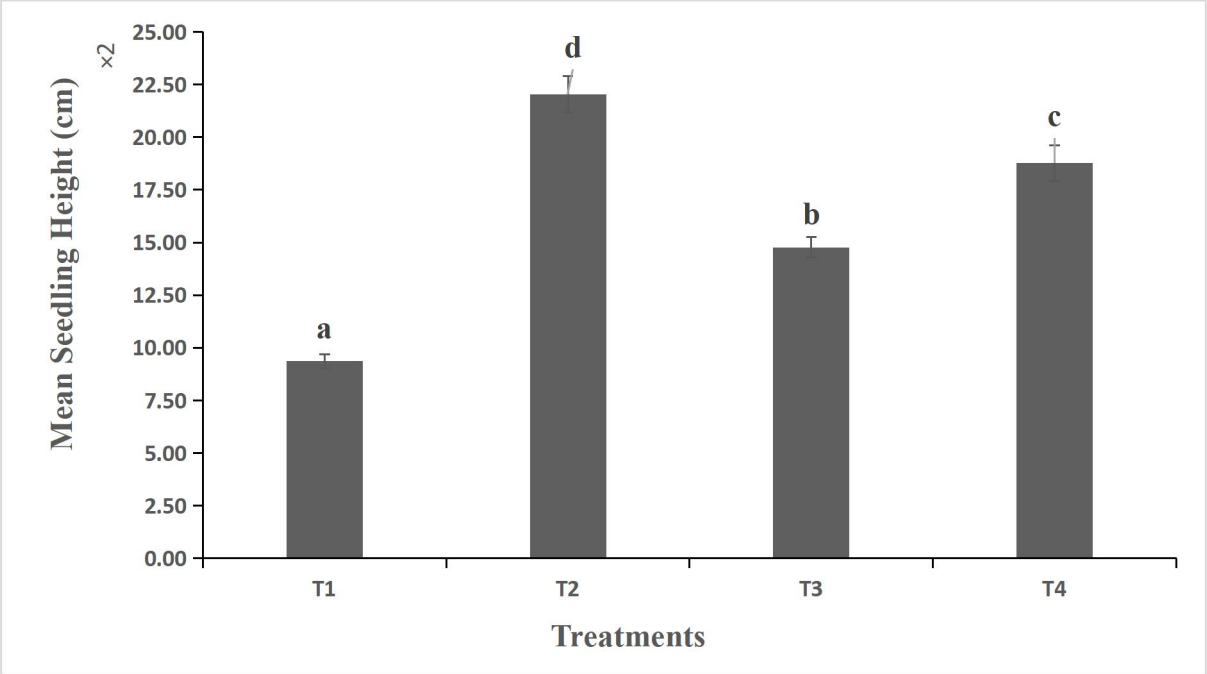

Figure 4.2: Effects of different manure on the height of *Milicia excelsa* seedlings attacked by *Phytolyma fusca*

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

Table 4.9: Effects of different manure on collar diameter of *Milicia excelsa* seedlings during attack by *Phytolyma fusca*

	Collar diameter				
Weeks	**T1(cm)**	**T2(cm)**	**T3(cm)**	**T4(cm)**	**P-value**
2	0.24±0.01	0.35±0.02	0.17±0.01	0.35±0.02	0.00
4	0.24±0.01	0.36±0.02	0.17±0,01	0.35±0.02	0.00
6	0.33±0.01	0.55±0.02	0.29±0.01	0.47±0.02	0.00
8	0.34±0.01	0.65±0.02	0.37±0.01	0.57±0.02	0.00
10	0.4±0.01	0.71±0.02	0.48±0.01	0.65±0.03	0.00
12	0.46±0.01	0.78±.03	0.55±0.01	0.74±0.03	0.00
14	0.24±.01	0.78±.03	0.61±.03	0.59±0.04	0.00
16	0.46±0.02	0.77±.03	0.67±.02	0.74±0.04	0.00
18	0.46±.03	0.75±0.02	0.64±.02	0.74±0.03	0.00
20	0.53±.01	0.82±0.05	0.67±.02	0.8±0.04	0.00
22	0.53±.01	0.82±.05	0.7±.02	0.85±0.03	0.00

P=0.05

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

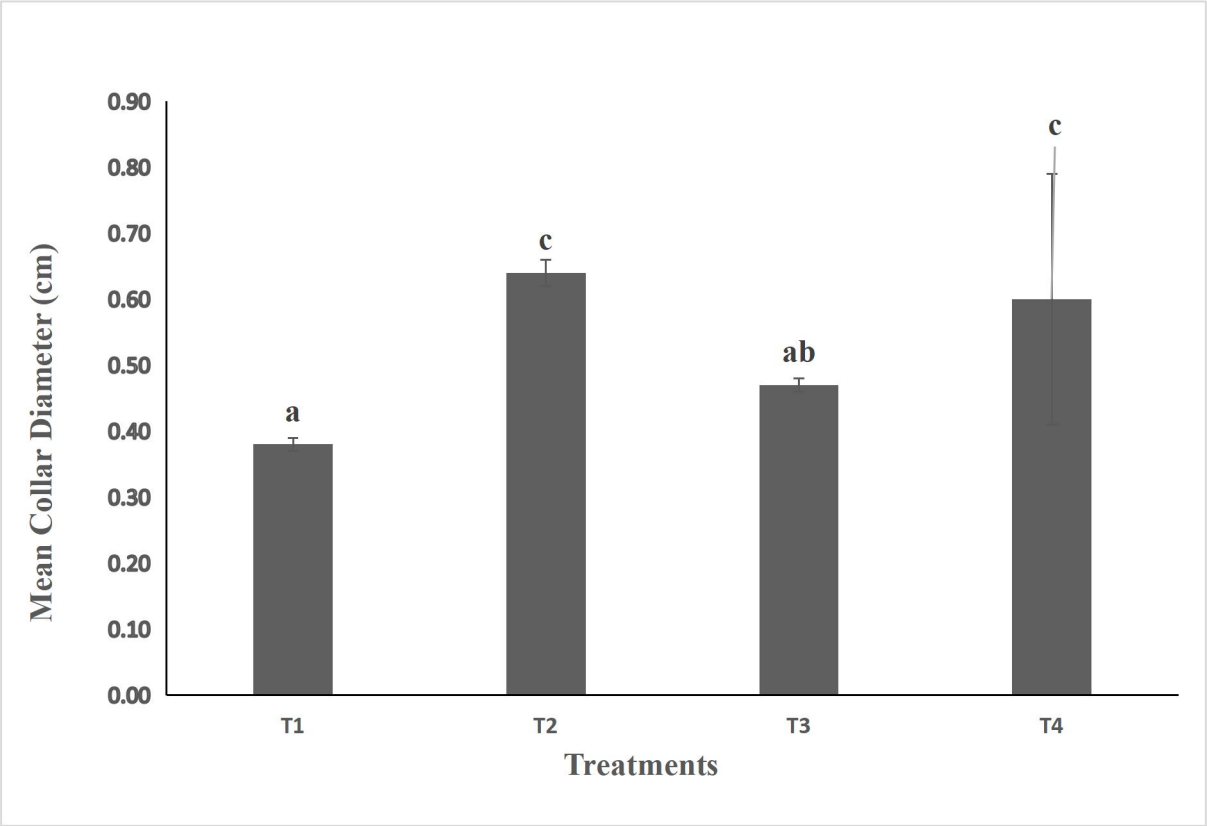

Figure 4.3: Effects of different manure on collar diameter of *Milicia excelsa* seedlings

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

4.9.3 Effects of manures on seedlings number of leaves

The effect of topsoil (control) (T1) and different manures: poultry droppings (T2), cattle dung (T3) and pig faeces (T4) on the number of leaves of *M. excelsa* seedlings studied for 22 weeks is presented in Table 4.10. Two weeks after transplanting treatment, T4 had the highest mean number of leaves (15.2±0.79), followed by T2 (14.42±0.70) and T1 (9.62±0.36), while T3 had the least (8.69±0.23).

The number of leaves increased from the 2nd week to the 12th week after transplanting for all the seedlings treatment. There was a decrease in the mean number of leaves at the 12th week after transplanting, which extended to week 22. During the *P. fusca* attack, there was a progressive increase in the number of leaves from week 16 to week 22 across all the treatments.

At the end of the study, T3 had the highest mean number of leaves (10.48±1.32), while T1 had the lowest mean number of leaves (6.5±0.83). Results showed a significant difference in the treatments at p=0.05 (Figure 4.4).

4.9.4 Effects of manures on gall formation

The effect of poultry droppings (T2), cattle dung (T3) and pig faeces (T4) on the gall formation on *M. excelsa* seedlings during the attack by *P. fusca* for 22 weeks are presented in Table 4.11. Gall formation was observed four (4) weeks after transplanting on 90% of the seedlings. There was no gall on T1 within the first 10 weeks of the study, while the incidence of gall was observed on treatments T2, T3 and T4. Galls were observed on the control treatment (T1) 12 weeks after transplanting. There was no incidence of gall formation on all the seedlings at week 14 till the termination of the experiment.

Treatment, T1 had the highest percentage of seedling survival (80%), followed by T3 (78%) and T4 (70%), while T2 had the least (50%) at the termination of the study. Treatment, T2 had the highest mean number of un-ruptured (3.87±0.32) and rupture galls (1.60±0.18).

Incidence of un-ruptured gall was high in the control, T1 (1.25±0.09) followed by T4 (0.75±0.06) while T3 had the least (0.75±0.06). Results showed that there were no significant differences in treatments.

Table 4.10: Effects of different manure on number of leaves of *Milicia excelsa* seedlings during attack by *Phytolyma fusca*

Number of leaves					
Weeks	**T1**	**T2**	**T3**	**T4**	**P-value**
2	9.62±0.36	14.42±0.70	8.69±.23	15.2±0.79	0.00
4	9.62±0.36	14.66±0.67	8.69±.23	15.2±0.79	0.00
6	11.95±.32	16.52±0.60	15.02±.64	16.98±.68	0.00
8	11.95±.35	13.92±0.45	14.95±.68	14.91±.56	0.00
10	11.45±.38	14.46±0.39	13.23±.43	13.2±0.52	0.00
12	11.3±.40	11.38±0.53	12.3±.54	12.2±0.53	0.35
14	7.58±.48	6.27±0.68	6.32±.44	5.90±.50	0.13
16	4.88±0.47	5.56±0.81	3.54±.45	4.73±0.53	0.11
18	5.09±0.55	2.96±0.72	2.96±.72	4.00±0.57	0.15
20	5.65±0.71	4.48±1.10	9.48±1.24	7.36±1.08	0.01
22	6.5±0.83	7.27±1.25	10.48±1.32	9.98±1.20	0.03

P=0.05

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

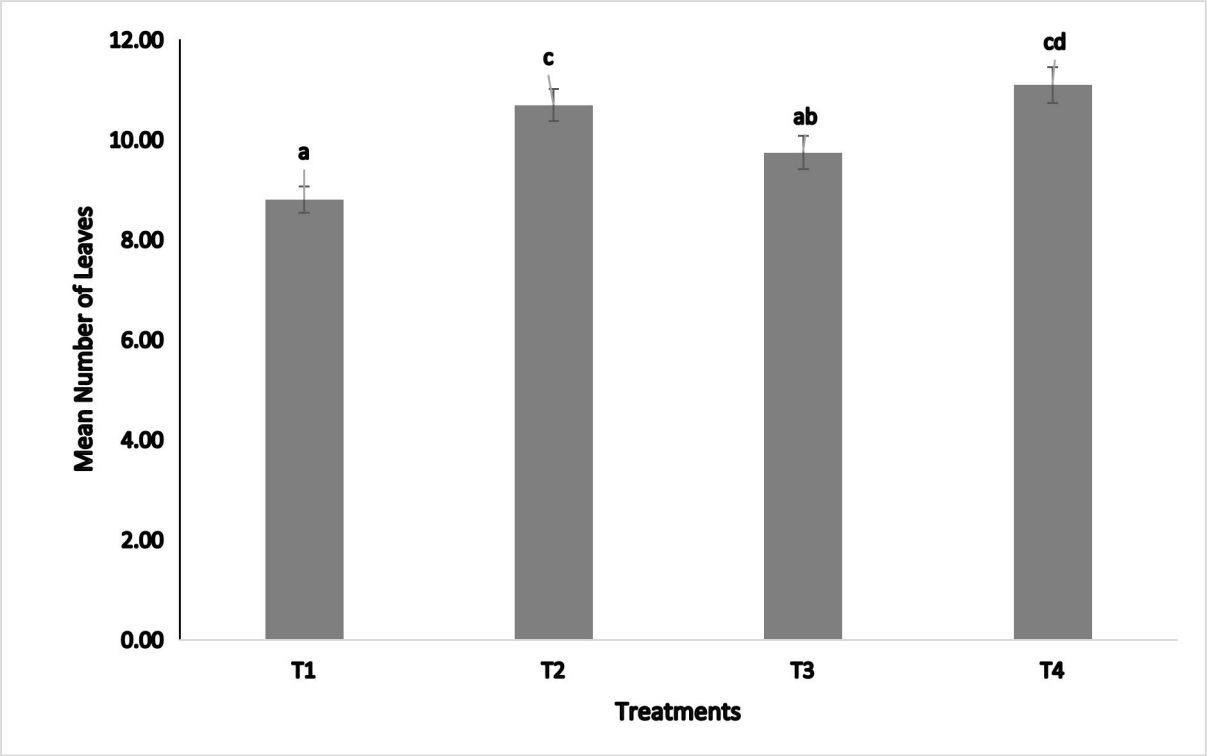

Figure 4.4: Effects of different manure on the number of leaves on *Milicia excelsa* seedlings

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

Table 4.11: Effects of different manure on gall formation of *Milicia excelsa* seedlings during attack by *Phytolyma fusca*

			Number of un-ruptured galls	Number of ruptured galls
Treatments	**N**	**% Survival**	**Mean**	**Mean**
1	60	80	1.25±0.09ab	0.28±0.5a
2	60	50	3.87±0.32c	1.60±0.18b
3	60	78	0.75±0.06a	0.27±0.04a
4	60	70	0.97±0.1a	0.25±0.04a

P=0.05

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

4.9.5 Relative Growth Rate of seedling height and collar diameter

The relative growth rate of seedling height and collar diameter showed that T2 and T3 had the highest value, 0.2, after 4 weeks. After 8 weeks, T3 (0.2) had the highest value while T2 and T 4 had 0.16 and 0.14 cm, respectively (Figures 4.5 and 4.6)

At the 6th week after transplanting, there was a decrease in growth among all the treatments. T1 had the lowest mean value of 0.05, while T3 had the highest of 0.1. Treatments T2 and T4 equally had a mean value of 0.08.

The RGR of collar diameter results showed that T3 had the highest mean (0.14) while T4 had the least (0.07) at week 4 after transplanting. At week 8, there was an increase in the growth of T4. In the 16th week, T2 and T4 were 0.06 and 0.06, respectively. Treatment, T1 had the lowest mean (0.055), and T3 had the highest (0.09).

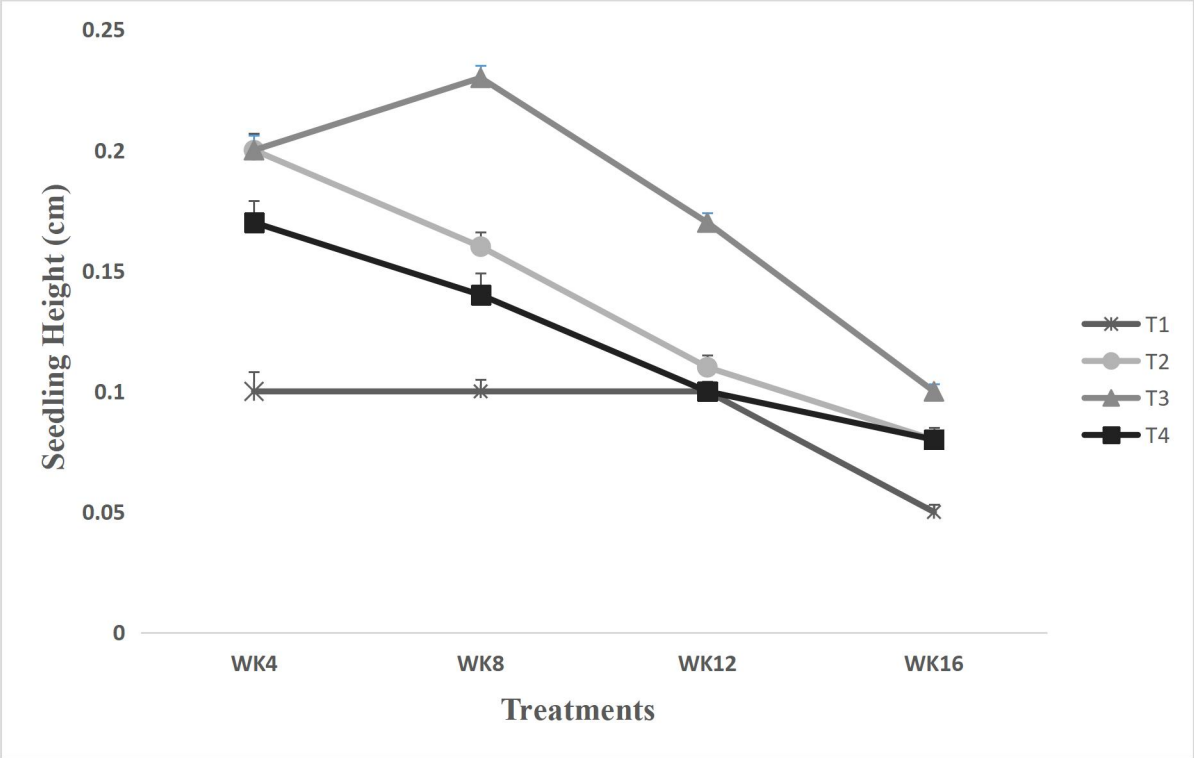

Figure 4.5: Effects of different manure on Relative Growth Rate of seedling height of *Milicia excelsa* during attack by *Phytolyma fusca*

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

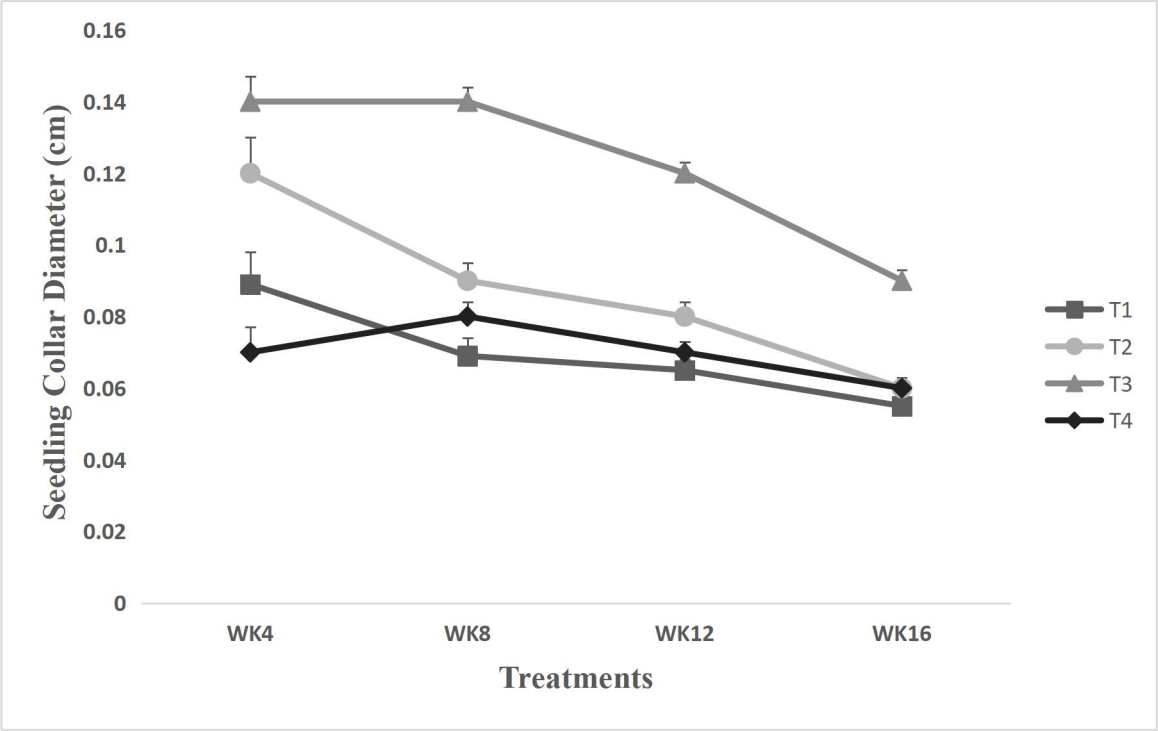

Figure 4.6: Effects of different manure on Relative Growth Rate of collar diameter of *Milicia excelsa* during attack by *Phytolyma fusca*

T1= Control, T2=Poultry droppings, T3=Cattle dung, T4=Pig faeces

4.10 Effects of solid and liquid poultry droppings (PD) and NPK on the growth and gall formation of *Milicia excelsa* during *Phytolyma fusca* attack.

4.10.1 Effects of solid and liquid poultry droppings and NPK on seedling height

Table 4.12 showed the performance of *M. excelsa* seedlings under solid Poultry droppings (T1), Liquid poultry droppings (T2) and NPK (15:15:15) (T3) amendments at different levels and topsoil only (control) (T4) studied for 24 weeks (Table 4.12. Results showed that for level 1, T3 had the highest seedling height (17.98±8.13cm) followed by T2 (15.44±5.61cm) and T1 (14.62±7.85cm) while T4 had the least (13.94±7.5cm) at week 2. For level 2, T1 (15.75±6.13cm) had the highest seedling height followed by T2 (13.92±5.81cm) and T4 (13.9±7.5cm) while T3 had the least (12.74±8.92cm).

At week 24, results for level 1 showed that T1 (52.19±1.61cm) had the highest seedling height followed by T4 (48.04±1.64cm) and T2 (46.71±1.53cm) while T3 had the lowest seedling height of 46.74±1.53cm. For level 2, T1 had the highest seedling height (52.80±1.49cm) while T3 (43.98±1.70cm) had the least. Treatments T2 and T4 had seedling height values of 50.93±1.52cm and 45.74±1.59cm, respectively.

Results also showed significant differences among the treatments and across levels at p=0.05 (Figure 4.7).

4.10.2 Effects of solid and liquid poultry droppings and NPK on seedling collar diameter

Collar diameter of *M. excelsa* grown on solid poultry droppings (T1), Liquid poultry droppings (T2) and NPK (15:15:15) (T3) amendments at different levels and topsoil only (control) (T4) studied for 24 weeks are presented in Table 4.13. Results showed that at the start of the study for level 1, T1 and T4 had the highest collar diameter of 0.2±0.02cm and 0.2±0.02cm, respectively, while T2 (0.16±0.02cm) and T3 (0.16±0.02cm) had the lowest collar diameter. For level 2, T1 (0.23±0.02cm) had the highest collar diameter while T3 (0.16±0.02cm). Treatments, T2 (0.20±0.02cm) and T4 (0.20±0.02cm) had the same values for collar diameter.

Table 4. 12: Effects of different levels Poultry droppings and NPK on *Milicia excelsa* seedlings height

	Seedling Height (cm)							
	Level 1				Level 2			
Weeks	T1	T2	T3	T4	T1	T2	T3	T4
2	14.62±7.85	15.44±5.61	17.98±8.13	13.94±7.5	15.75±6.13	13.92±5.81	12.74±8.92	13.90±7.5
4	14.57±1.05	15.45±1.05	15.3211.05	14.36±1.21	17.48±1.05	15.58±1.05	14.40±1.05	14.18±1.21
6	16.63±1.27	17.18±1.27	17.48±1.27	16.83±1.27	19.82±1.27	17.83±1.27	15.78±1.27	17.13±1.27
8	17.933±1.31	17.83±1.31	17.60±1.31	17.27±1.51	22.93±1.31	20.52±1.31	16.35±1.31	17.90±1.85
10	20.43±1.56	20.85±1.56	19.56±1.56	20.53±1.80	27.03±1.56	24.65±1.56	18.75±1.56	20.76±1.80
12	21.77±1.67	22.05±1.67	21.44±1.67	23.16±1.93	29.40±1.67	27.23±1.67	19.65±1.67	23.51±1.93
14	24.05±1.85	24.75±0.85	21.91±1.85	25.18±2.14	31.42±1.85	27.97±1.85	22.13±1.85	25.98±2.14
16	27.77±2.10	28.60±2.10	24.33±2.10	26.92±2.10	38.7±2.10	33.67±2.10	24.63±2.10	27.28±2.10
18	31.35±2.14	29.95±2.14	29.80±2.14	26.87±2.14	39.02±2.14	36.02±2.14	23.85±2.14	27.76±30.43
20	35.35±2.33	34.37±2.33	34.22±2.33	30.70±2.32	43.68±2.33	40.43±2.33	27.27±2.33	30.14±2.41
22	36.95±2.04	36.13±2.04	35.98±2.04	32.23±2.04	49.80±2.12	47.93±2.16	41.05±2.42	31.74±2.26
24	52.19±1.61	46.91±1.53	46.74±1.53	48.04±1.64	52.80±1.49	50.93±1.52	43.98±1.70	45.74±1.59

T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

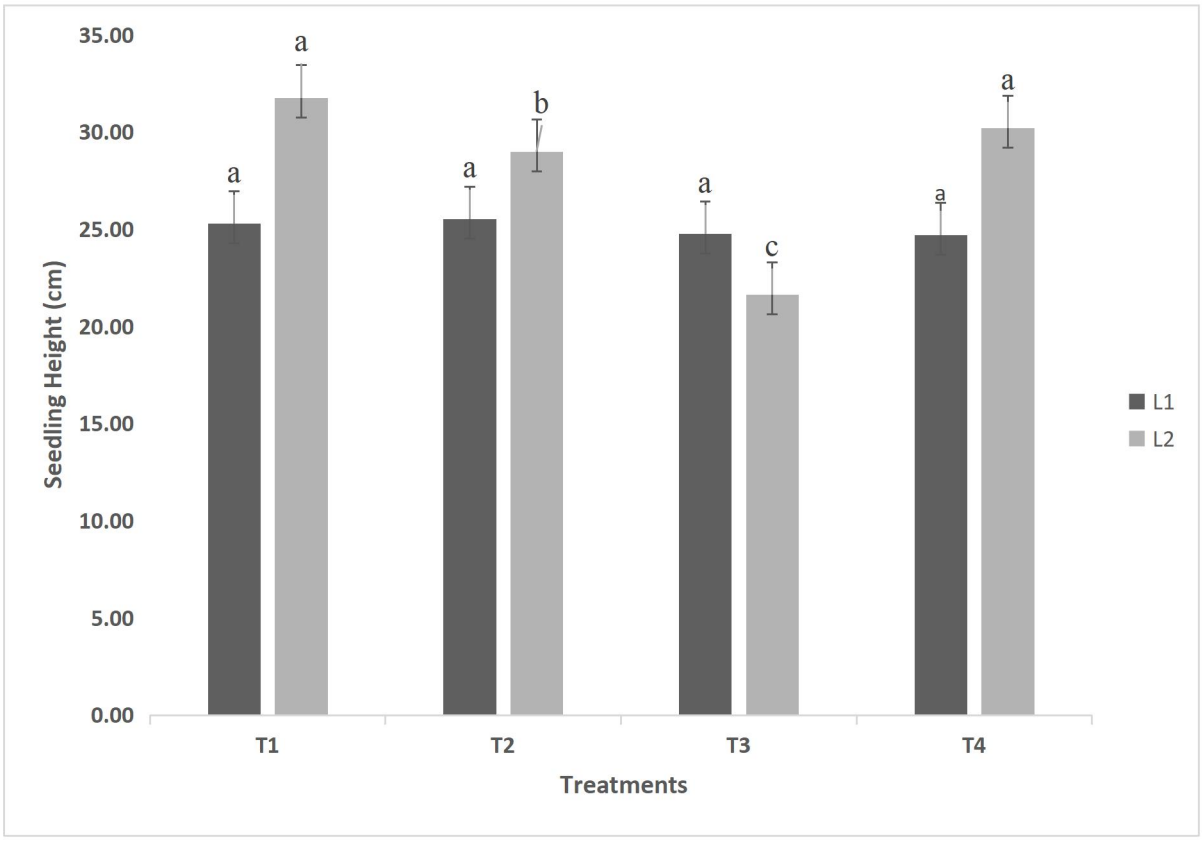

Figure 4.7: Effects of solid and liquid Poultry droppings at different levels and NPK on Height of *Milicia excelsa* seedlings

T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

Table 4.13: Effects of different levels Poultry droppings and NPK on Collar diameter of *Milicia excelsa* seedlings collar diameter

	Collar Diameter (cm)							
	Level 1				Level 2			
Weeks	T1	T2	T3	T4	T1	T2	T3	T4
2	0.20±0.02	0.16±0.02	0.16±0.02	0.20±0.02	0.23±0.02	0.20±0.02	0.16±0.02	0.20±0.02
4	0.23±0.04	0.27±0.04	0.42±0.04	0.28±0.05	0.33±0.04	0.26±0.04	0.25±0.04	0.27±0.05
6	0.22±0.01	0.22±0.01	0.24±0.01	0.18±0.01	0.21±0.01	0.21±0.01	0.17±0.01	0.19±0.01
8	0.38±0.03	0.37±0.03	0.38±0.03	0.41±0.04	0.54±0.03	0.42±0.03	0.35±0.03	0.39±0.04
10	0.46±0.04	0.47±0.04	0.41±0.04	0.48±0.04	0.66±0.04	0.55±0.04	0.41±0.04	0.47±0.04
12	0.44±0.04	0.40±0.04	0.42±0.04	0.53±0.04	0.68±0.04	0.54±0.04	0.39±0.04	0.52±0.04
14	0.64±0.06	0.57±0.06	0.44±0.06	0.58±0.07	0.76±0.06	0.64±0.06	0.49±0.06	0.57±0.07
16	0.55±0.44	0.54±0.44	0.45±0.44	0.46±0.44	0.85±0.44	0.66±0.44	1.66±0.44	0.44±0.44
18	0.64±0.05	0.53±0.05	0.50±0.05	0.45±0.05	0.75±0.05	0.61±0.05	0.41±0.05	0.45±0.05
20	0.72±0.05	0.62±0.05	0.59±0.05	0.53±0.05	0.95±0.05	0.81±0.05	0.61±0.05	0.51±0.05
22	0.72±0.04	0.62±0.04	0.59±0.04	0.53±0.04	1.10±0.05	0.97±0.05	0.87±0.05	0.54±0.05
24	1.09±0.04	0.90±0.04	0.87±0.04	0.89±0.04	1.21±0.04	1.08±0.04	0.97±0.04	0.86±0.04

T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

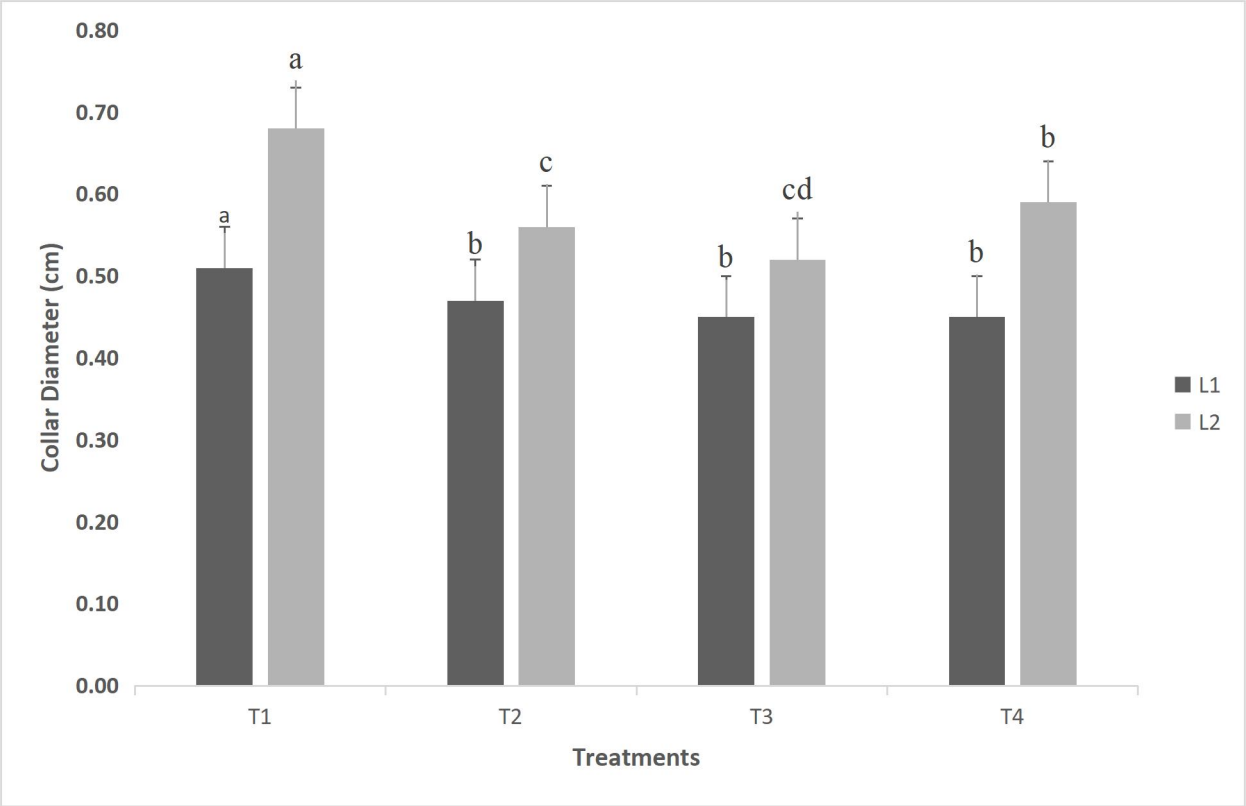

Figure 4.8: Effects of solid and liquid poultry droppings at different levels and NPK on collar diameter of *Milicia excelsa* seedlings

T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

There was a progressive and steady increase in collar diameter across the treatment at all levels except at week 4. At week 24, level 1, T1 (1.09±0.04cm) had the highest collar diameter, while T3 (0.87±0.04cm) had the least. Treatments T2 and T4 had a collar diameter of 0.09±0.04cm and 0.89±0.04cm, respectively. For level 2, T1 (1.21±0.04cm) had the highest collar diameter followed by T2 (1.08±0.04cm) and T3 (0.97±0.04cm), while T4 had the lowest collar diameter value of 0.86±0.04cm. There were significant differences in mean collar diameter for treatments at level 2 at p=0.05 (Figure 4.8).

4.10.3 Effects of solid and liquid poultry droppings and NPK on seedling number of leaves

Result of the effect of solid poultry droppings (T1), Liquid poultry droppings (T2) and NPK (15:15:15) (T3) amendments at different levels and topsoil only (control) (T4) on the number of leaves of *M. excelsa* seedlings under P fusca attack studied for 24 weeks are presented in Table 4.14.

At the start of the study (week 2) for level 1, T2 (8.42±0.55) had the highest mean number of leaves while T3 (6.88±0.47) had the least. Treatments T1 and T4 had a mean number of leaves of 7.17±0.48 and 7.23±0.46, respectively. For level 2, T1 (8.93±0.48) had the highest mean number of leaves treatment, T2 and T4 had 8.13±0.47 and 7.87±0.46, respectively, while T3 (6.60±0.53) had the lowest.

At the end of the study (week 24), results across treatments at both level 1 and level 2 showed that T1, level 2 had the highest mean number of leaves value of 26.96±0.54, followed by T4, level 2 (25.70±0.57) and T2, level 2 (25.16±0.54) respectively. The lowest mean number of leaves was recorded for T3, level 1 (17.93±0.55), while T4, level 1, T2, level1, T1 level1 and T3, level 2 had a mean number of leaves of 18.98±0.9, 19.08±0.55, 20.96±0.58 and 22.96±0.64, respectively. Results also showed a significant difference in the treatments across levels at p=0.05 (Figure 4.9).

Table 4.14: Effects of different levels Poultry droppings and NPK on number of leaves of *Milicia excelsa* seedlings

	Numbers of Leaves							
	Level 1				Level 2			
Weeks	T1	T2	T3	T4	T1	T2	T3	T4
2	7.17±0.48	8.42±0.55	6.88±0.47	7.23±0.46	8.93±0.48	8.13±0.47	6.60±0.53	7.87±0.46
4	7.52±0.59	8.63±0.59	7.62±0.59	7.70±0.69	9.97±0.59	8.98±0.59	7.43±0.59	8.97±0.68
6	7.32±0.62	7.48±0.62	6.77±0.62	6.03±0.62	7.85±0.62	7.77±0.62	6.80±0.62	7.56±0.62
8	6.75±0.58	8.33±0.58	6.87±0.58	8.00±0.67	10.27±0.58	8.32±0.58	7.20±0.58	9.27±0.82
10	8.41±0.65	9.02±0.65	7.17±0.85	9.20±0.75	11.15±0.65	9.30±0.65	7.50±0.65	10.73±0.75
12	9.00±0.71	9.75±0.71	8.82±0.71	9.80±0.82	11.88±0.71	10.30±0.71	8.30±0.71	12.17±0.82
14	9.34±0.68	9.87±0.67	8.20±0.67	9.87±0.77	11.82±0.67	10.45±0.67	7.50±0.67	11.07±0.77
16	10.32±0.71	10.12±0.71	8.53±0.71	9.37±0.71	11.98±0.71	11.17±0.71	7.42±0.71	11.37±0.73
18	10.37±0.68	9.78±0.68	8.77±0.68	8.32±0.68	11.37±0.68	10.57±0.68	6.85±0.69	10.45±0.71
20	13.57±0.77	13.32±0.77	12.30±0.77	11.48±0.77	17.37±0.77	16.57±0.77	12.73±0.77	16.45±0.80
22	13.57±0.73	13.32±0.73	12.30±0.73	11.48±0.73	21.97±0.75	21.16±0.76	18.60±0.83	21.70±0.80
24	20.96±0.58	19.08±0.55	17.93±0.55	18.98±0.59	26.96±0.54	25.16±0.54	22.96±0.61	25.70±0.57

T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

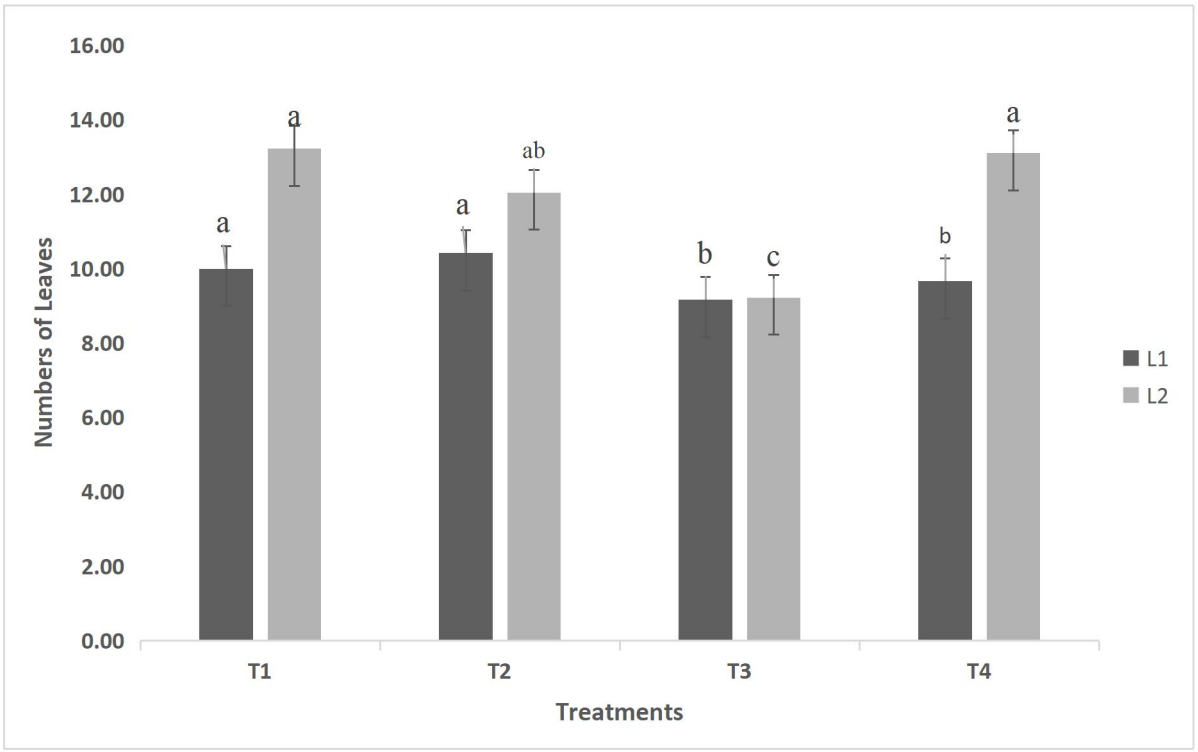

Figure 4.9: Effects of solid and liquid Poultry droppings at different levels and NPK on number of leaves of *Milicia excelsa* seedlings

T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

4.10.4 Effects of solid and liquid poultry droppings and NPK on gall Formation

Results on Table 4.15 showed the effect of Solid poultry droppings (T1), Liquid poultry droppings (T2) and NPK (15:15:15) (T3) at different levels and topsoil only (control) (T4) on gall formation of *M. excelsa* seedling during an attack by *P. fusca* studied for 24 weeks.

Treatments, T2 and T3 had the highest percentage *M. excelsa* seedlings survival of 88 % and 88 %, respectively, followed by T1 (80 %) at level 1. Treatment, T4 had the lowest survival percentage of 77 %. Results on gall formation showed that T4 had the highest mean number of un-ruptured and ruptured gall of 40.05±4.69 and 29.29±2.66 respectively, followed by T1 (39.12±4.69 (un-ruptured), 19.80±2.30 (ruptured)), T3 (31.73±4.69 (un-ruptured), 15.95±2.30 (ruptured)) while T2 had the lowest mean number of un-ruptured gall value of 31.53±4.69 and ruptured gall value of 15.95±2.30, respectively.

For level 2, T1 had the highest percentage of *M. excelsa* seedlings survival of 93 %, followed by T2 (90 %) and T4 (82 %), while T3 had the lowest seedling survival value of 73 %. Results on gall formation (number of un-ruptured and ruptured gall) showed that T1 had the highest mean number un-ruptured gall value of 40.03±4.69 followed by T4 (19.86±4.82) and T3 (8.15±4.69) while T2 had the lowest mean number of un-ruptured gall value of 8.12±4.69. For ruptured gall, T4 had the highest mean value of 4.44±2.41 followed by T1 (4.30±2.30) T3 (1.87±2.30), while T2 had the lowest mean number of un-ruptured gall value of 1.67±2.30. Results also showed a significant difference in gall formation (un-ruptured and ruptured gall) among treatments and across levels at p=0.05.

Table 4.15: Effects of different levels Poultry droppings and NPK on Survival and gall formation of *Milicia excelsa* seedlings

				Number un-ruptured galls	Number of Ruptured galls
	Treatment	**N**	**% Survival**	**Mean**	**Mean**
Level 1	**1**	60	80	39.12±4.69a	19.80±2.30ab
	2	60	88	31.53±4.69a	15.85±2.30a
	3	60	88	31.73±4.69a	15.95±2.30a
	4	60	77	48.05±4.69b	29.29±2.66b
Level 2	**1**	60	93	40.03±4.69a	4.30±2.30ab
	2	60	90	8.12±4.69a	1.67±2.30a
	3	60	73	8.15±4.69a	1.87±2.30a
	4	60	82	19.86±4.82b	4.44±2.41b

P= 0.05
T1= Solid poultry droppings, T2= Liquid poultry dropping, T3= NPK (15:15:15), T4= Control
Level 1= 0.1 L/ 1 Kg (PD/soil), Level 2= 0.2 L/ 2 Kg (PD/soil)

4.11 Effects of Poultry droppings, pig faeces and cattle dung composts on the growth and gall formation of *Milicia excelsa* seedlings

4.11.1 Effects of different manure composts on seedlings height

Results from this study showed the effect of different manures compost: Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and control (topsoil) (T5) on the height of *M. excelsa* seedlings during an attack by *P. fusca* studied for 12 weeks (Table 4.16).

Two weeks after treatment application, results on seedling height showed that T1 (19.90±1.30 cm) had the highest while T2 (14.65±0.94 cm) had the lowest. T3, T4 and T5 had mean seedling height values of 16.80±1.39 cm, 15.55±1.29 cm and 16.85±0.79 cm, respectively (Table 4.16).

This study showed increases in the mean value of seedlings' height at week 2 to week 6 and a decline at week 8 across all the treatments (figure 4.10).

Results at week 12 showed that treatment, T1 had the highest mean seedling height value of 46.40±1.06 cm followed by T3 (41.65±1.86 cm), T4 (28.80±1.41 cm) and T2 (25.05±1.45 cm), while T5 had the lowest mean seedling height value of 23.05±1.31 cm. There was showed significant difference among the treatment at p=0.05.

4.11.2 Effects of different manure composts on collar diameter

Results on the effect of different organic manure composts: Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5) on the collar diameter of *M. excelsa* seedlings during an attack by *P. fusca* studied for 12 weeks is presented in Table 4.17.

Table 4.16: Effects of different manure composts seedling height of *Milicia excelsa*

Weeks	T1(cm)	T2(cm)	T3(cm)	T4(cm)	T5(cm)
2	19.90±1.30b	14.65±0.94a	16.80±1.30ab	15.55±1.29a	16.85±0.79ab
4	23.65±1.22b	16.20±0.86a	22.40±1.29b	17.25±1.30a	17.90±0.79a
6	37.65±1.88c	20.70±0.88a	29.05±1.34b	20.45±1.24a	19.85±0.76a
8	36.00±2.09c	18.95±0.97a	26.75±1.26b	17.55±1.08a	17.45±0.75a
10	48.95±1.20d	25.80±1.44b	35.35±1.41c	27.15±1.49b	20.80±0.50a
12	46.40±1.06d	25.05±1.45ab	41.65±1.86c	28.80±1.41b	23.05±1.31a

P= 0.05

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

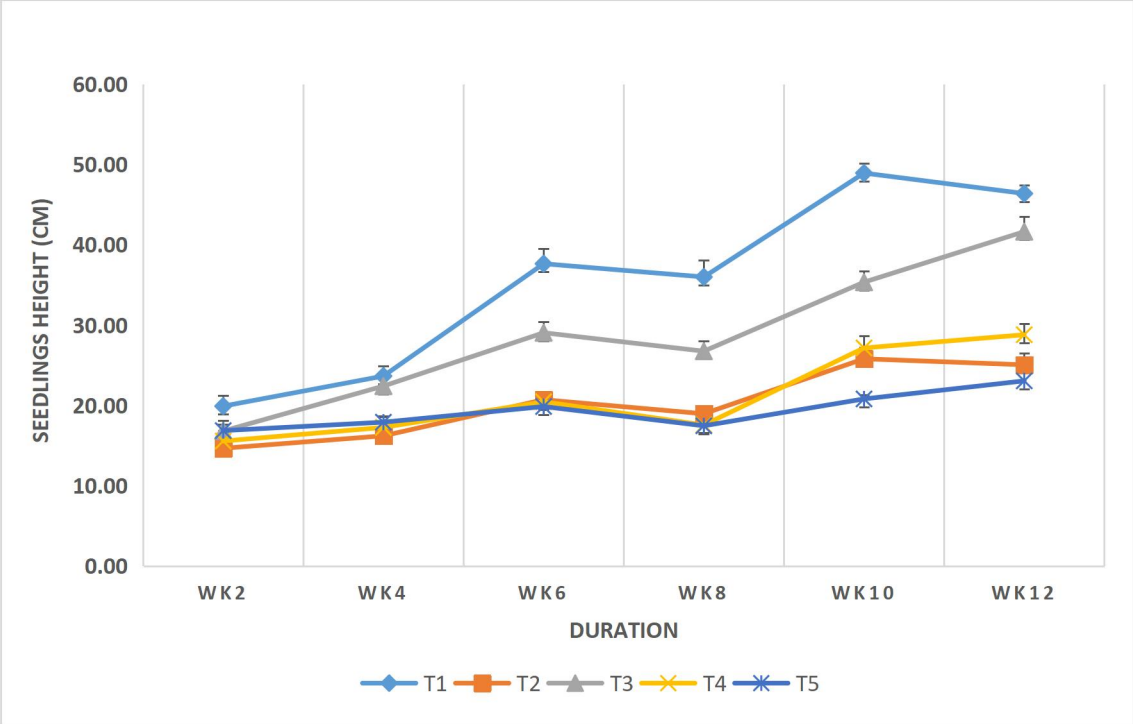

Figure 4.10: Effects of different composts on the height of *Milicia excelsa* seedlings

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

Table 4.17: Effects of different manure composts on seedling collar diameter of *Milicia excelsa*

Weeks	T1(cm)	T2(cm)	T3(cm)	T4(cm)	T5(cm)
2	0.28±0.02c	0.21±0.02b	0.24±0.02bc	0.19±0.01b	0.13±0.01a
4	0.42±0.02d	0.24±0.02b	0.35±0.02c	0.24±0.02b	0.13±0.01a
6	0.46±0.02d	0.25±0.02b	0.35±0.02c	0.25±0.01b	0.20±0.01a
8	0.46±0.02d	0.25±0.02b	0.35±0.02c	0.25±0.01b	0.20±0.01a
10	0.52±0.02d	0.35±0.02b	0.39±0.01c	0.32±0.01ab	0.29±0.02a
12	0.52±0.02d	0.35±0.02b	0.39±0.01c	0.32±0.01ab	0.29±0.02a

P= 0.05

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

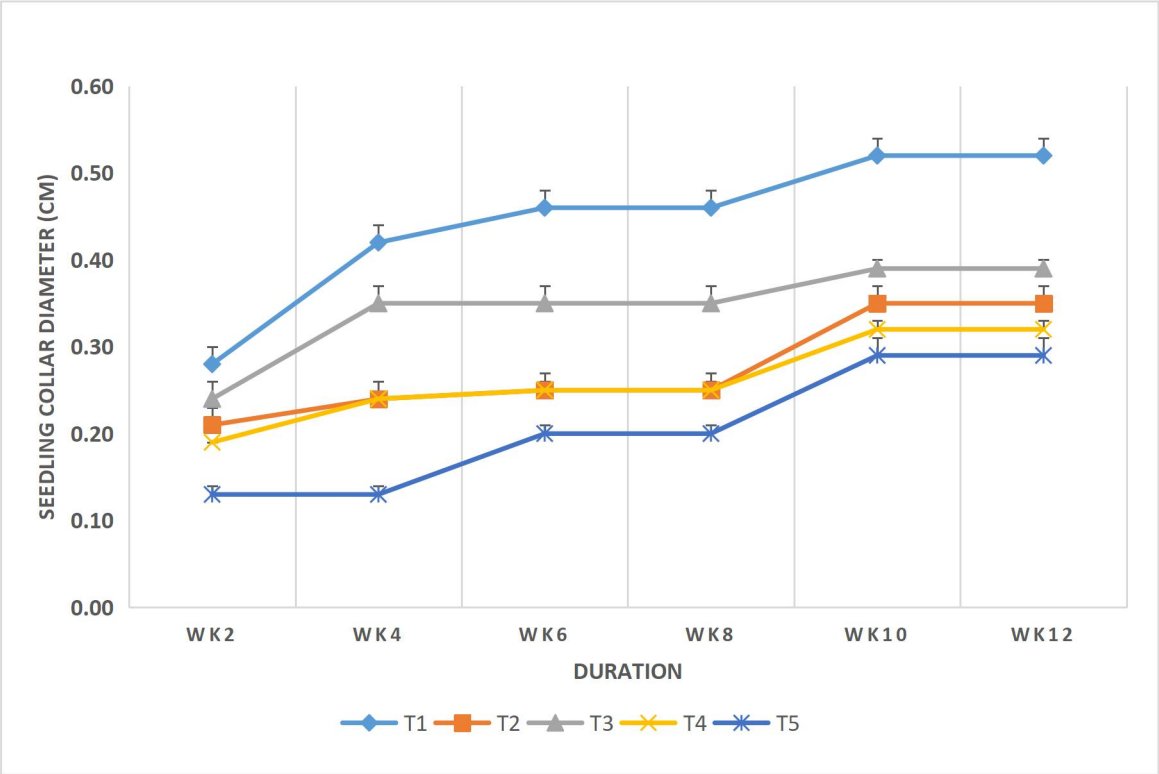

Figure 4.11: Effects of different manure composts on seedling collar diameter of *Milicia excelsa*

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

Results on the effect of the treatments on seedling collar diameter showed a steady increase in seedling collar diameter across all the treatments at week 2 to week 12 (Figure 4.11).

Week 2 showed that T1 (0.28±0.02 cm) had the highest mean collar diameter while T5 (0.13±0.01 cm) had the lowest. Treatments T2, T3, and T4 had mean collar diameter values of 0.21±0.02 cm, 0.24±0.02 cm and 0.19±0.01 cm, respectively.

At week 12, T1 (0.52±0.02 cm) had the highest mean collar diameter, followed by T3 (0.39±0.01 cm), T2 (0.35±0.02 cm) and T4 (0.32±0.01 cm), while T5 (0.29±0.02 cm) had the lowest. Results from this study showed that there was a significant difference among treatments at p=0.05.

4.11.3 Effects of different manure composts on number of leaves

Results on the effect of different organic manure composts: Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4), and control (topsoil) (T5) on the number of leaves of *M. excelsa* seedlings during the attack by *P. fusca* studied for 12 weeks is presented in Table 4.18.

Results at week 2 showed that T1 had the highest mean number of leaves value of 9.60±0.68 followed by T4 (9.25±o.68), T5 (8.55±0.53) and T3 (8.35±0.47), while T2 had the lowest mean number of leaves value of 7.75±0.84.

This study showed a gradual increment in the mean number of leaves at week 2 to week 6 during the experiment. At week 8, there was a drop in the mean value of the number of leaves across all treatments and a subsequent increase after week 10 (Figure 4.12).

At week 12, T1 had the highest mean number of leaves value of 15.20±1.81 while T3 (10.50±0.74) had the least. Treatments T2, T4 and T5 had a mean number of leaves values of 11.15±1.34, 11.80±0.61 and 13.75±0.96, respectively (Table 4.18).

Results showed that there was a significant difference in the treatments at p=0.05.

Table 4.18: Effects of different manure composts on number of leaves of *Milicia excelsa* seedlings

weeks	T1	T2	T3	T4	T5
2	9.60±0.68a	7.75±0.84a	8.35±0.47a	9.25±0.68a	8.55±0.53a
4	12.50±0.75b	9.70±1.04a	9.15±0.47a	9.25±0.68a	8.55±0.53a
6	17.40±1.62b	10.20±1.02a	9.15±0.47a	9.25±0.68a	10.45±0.79a
8	11.20±0.74c	7.55±0.52a	7.45±0.66a	8.95±0.71ab	9.90±0.44b
10	13.10±0.88b	9.00±0.32a	9.35±0.37a	10.55±0.39a	12.55±0.70b
12	15.20±1.81b	11.15±1.34a	10.50±0.74a	11.80±0.61ab	13.75±0.96ab

P= 0.05

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

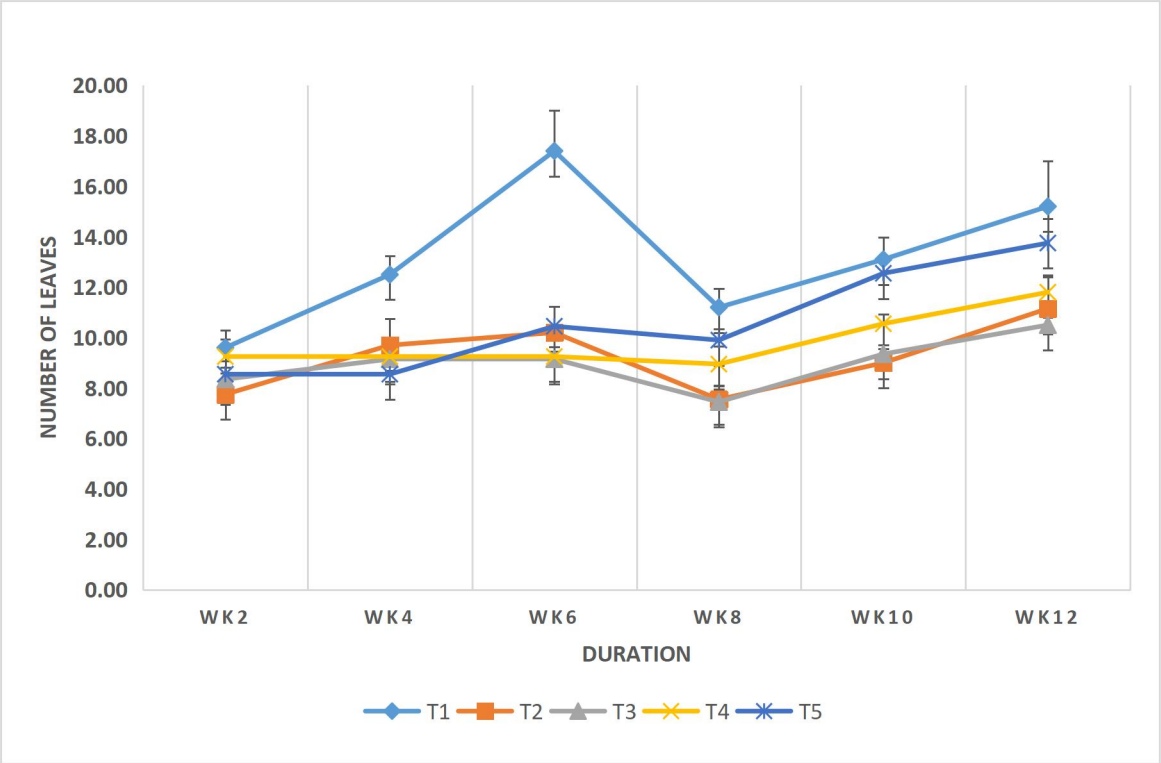

Figure 4.12: Effects of different manure composts on number of leaves of *Milicia excelsa* seedlings

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

4.11.4 Effects of different manure composts on gall formation

Results from this study showed the effect of different organic manures composts: Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and control (topsoil) (T5) on survival and gall formation (un-ruptured and ruptured gall) of *M. excelsa* seedling during an attack by *P. fusca* studied for 12 weeks (Table 4.19).

Results showed no mortality in *M. excelsa* seedling used in this experiment between February and May 2020 among all treatments.

There was the incidence of gall formation in all the treatments and treatment, T1 had the highest (5.65±0.83), followed by T3 (3.45±0.88), T5 (2.40±0.69) and T2 (1.6±0.40) while T4 had the least (1.50±0.55). For the number of galls ruptured, T1 had the highest mean value of 3.60±0.64, while T5 had the lowest value of 1.25±0.40. Treatments T2, T3 and T4 had mean values of ruptured galls of 2.25±0.51, 2.65±0.61 and 1.35±0.67, respectively. There were significant differences in gall formation among all treatments at p=0.05 (Table 4.19).

Table 4.19: Effects of different manure composts on gall formation of *Milicia excelsa* seedlings

			Number of un-ruptured galls	Number of ruptured galls
Treatments	**N**	**% Survival**	**Mean**	**Mean**
1	20	100	5.65±0.83b	3.60±0.64a
2	20	100	1.60±0.40a	2.25±0.51ab
3	20	100	3.45±0.88a	2.65±0.61ab
4	20	100	1.50±0.55a	1.35±0.67b
5	20	100	2.40±0.69a	1.25±0.40b

P=0.05

Pig faeces + Cattle dung + Poultry droppings (T1), Pig faeces + Cattle dung (T2), Pig faeces + Poultry droppings (T3), Poultry droppings + Cattle dung (T4) and Control (topsoil) (T5)

CHAPTER FIVE

DISCUSSION

5.1 Phytochemical constituents in healthy and galled leaves of *Milicia excelsa*

This study showed that *P. fusca* is attracted to the constitutive phytochemical constituents (primary and secondary metabolites) present in *M. excelsa*. On the other hand, *M. excelsa* continuously exposed to *P. fusca* attacks induces the production of phytochemical compounds qualitatively and quantitatively. This confirmed the report of Bruce and Pickett (2011) that metabolites in plants are important compounds used by insects for locating host plants for feeding and mating. This study showed that phytochemical compounds in healthy and galled *M. excelsa* leaf contained Saponins, Steroids and terpenes (Monoterpenes and Diterpenes) at the 8th month 16th month of growth, respectively. This contradicted the findings by Vereecke *et al.* (1997) that phytochemical compounds constituent differs in healthy and infested plant tissue.

In this study, results showed the presence of the same phytochemical constituents in the healthy and galled leaves of *M. excelsa* over time but in different quantities. The quantitative phytochemical constituents of healthy and galled leaves vary in the amount of some compounds present in the plant. Galled tissue growth is connected to the amount and variation in secondary metabolites in the host plant. The quantitative analysis showed drastic decreases in the terpenoids, tannin and flavonoids contents in the galled leaves, unlike the healthy ones. Terpenoids are the most abundant secondary metabolites with an incredible structural diversity of compounds. They are very volatile and usually found in different species of plants. They are known for their characteristic flavour and odour in plant tissue where they are present (Lichtenthaler *et al.*, 1997; Lichtenthaler, 1999). Monoterpenes, a class of terpenoids, are relatively weak odorants that an insect often uses for smell (Lichtenthaler, 1999).

The phytochemical analysis of healthy and galled leaves of *M. excelsa* using Gas Chromatography- Mass Spectrometry revealed the abundance and nature of compounds present in the healthy and galled plant. The presence of twenty and eighteen compounds in

the healthy and galled leaf at the 8th month eighteen and fifteen compounds at 16th months, respectively, showed that the insect uses up phytochemical compounds present in the plant. This corroborated the report of Dangl and McDowell (2006) that plants produce various chemical substances which are of importance to herbivores. These could serve as food or/an attractant to the insect pest. Most of the compounds present in the healthy and galled leaves were identified as saponins. Glycosides of triterpenes and steroids are usually called saponins (Mugford and Osbourn, 2013). The presence of these phytochemicals in high-to-low concentration in the healthy to galled leaves suggested that they may be responsible as an attractant to the matured insect, as well as a source of food for the nymphs.

Aside from saponins and steroids, *M. excelsa* galled and healthy leaves contained Phytol and Dihydropinene, a form of Diterpenes and Monoterpenes, respectively, which act as antifeedant to insect pests. This corroborated the report of Mugard and Osbourn (2012) that there is a link between the host plant and its phytochemical compounds that determines the ability of an insect to locate it. In contrast, Agudelo *et al*. (2018) reported that some plant defence compounds could be reduced due to gall formation in some plants. It was also reported that galls caused by insects could manipulate the plant to produce and translocate mineral nutrients to the gall instead of the plant defence substance. It was also postulated that gall former is notorious for controlling the development of their host to their advantage, especially for feeding purposes. This was contradicted by Nyman and Julkunen (2000), that the number of secondary metabolites with defence potential in the plant was more in gall tissues compared to healthy tissue. In their separate study, Kot *et al*. (2017) concluded that gall formers could manipulate the biochemical compound in their host when comparing the phytochemical compositions of plant galls with that of healthy plant tissue.

The production of 1-pentyl-octyl benzene and other heterocyclic compounds (an induced compound) could be responsible for the toughness and colour change (green to yellowish-green leaves colour) in *M. excelsa*. This could lead to the build-up of resistance to *P. fusca* persistence attack on *M excelsa*. It could also be attributed to the absence of Phytol, as evident in the plant at 16 month-old. This supports the report of Castro-Diez *et al*. (2000) that variability in resistance in the plant during insect attack may be due to the

distribution of chemicals, which by implication could affect the morphological structures of the leaves, thereby making it unpalatability, tougher and harder for the insect to feed on. This also justified the findings of Strauss and Agrawal (1999) that plants could either show resistance or/and tolerance to insect attack, and a shift to tolerance might occur when resistance traits become ineffective, as reported with specialist insects.

Milicia excelsa seedlings protected against *P. fusca* (healthy leaves) showed a significant increase in phytochemical compounds such as Phytol and subsequent production of Dihydropinene, unlike that of the galled leaves. This is evident in the plant at 16-month old with no significant physical change in plant leaf texture and colour. Renwick (2002) reported similar findings that for a plant to adjust due to an attack by an insect, one of the direct defence strategies is for it to structurally adjust through the production of metabolites and leaf texture thickening. Studies revealed that the concentrations of some chemical compounds increase during gall development. Kraus and Spiteller (1997) also reported that insect attack leads to changes in plant phytochemical compounds, especially at the attack site.

5.2 Reactive oxygen species during *Phytolyma fusca* attack on *Milicia excelsa*

At the point of attack, insects create wounds that appear in different forms on plant tissue. Wound and specific insects stimulate diverse signalling pathways in plants, including the generation of reactive oxygen species (de Bruxelles and Roberts, 2001; Kessler and Baldwin, 2002; Rakosy-Tican *et al*., 2016).

The qualitative analysis of superoxide anion and hydrogen peroxides accumulation in *M. excelsa* leaves tissue using Nitroblue-Tetrazolium and Diaminobenzidine staining techniques, respectively, indicated the accumulation of hydrogen peroxide molecules in the leaf tissue while superoxide anion was not detected. This finding corroborated the report of Orozco-Cardenas and Ryan (1999) on the circulation of hydrogen peroxides in tomato leaf after wounding. Wounding causes plant tissue to generate ROS locally and systemically throughout the plant (Kessler and Baldwin, 2002). This study, however, gave an insight into the type of ROS produced in *M. excelsa* leaf during *the P. fusca* attack.

The role and type of ROS produced in *M. excelsa* have not been reported, and from this study, it was observed that wounding (piercing) does not necessarily lead to gall

formation in *M. excelsa* leaf but could generate a hypersensitivity reaction. This is in line with the proposal of Bi and Felton (1995) that the accumulation of reactive oxygen species interferes with the plant-insect interface. Mechanical wounding of plant tissue is often experimented with to examine the defence responses of plants to insect attack (de Bruxelles and Roberts, 2001). It can also be used to investigate the process of Hypersensitive Reaction that may lead to the formation of abnormal plant tissues such as galls.

5.3 Fungi associated with *Phytolyma fusca* in *Milicia excelsa* gall

This study isolated identified fungi in *M. excelsa* and *P. fusca*. Agyeman *et al.* (2009) reported that gall formation in *M. excelsa* by *P. fusca* is usually accompanied by a disease outbreak inside the gall. Determination of remote causes of disease outbreaks caused by fungi and their mechanisms is very significant in understanding the interaction between plant and insect (Twumasi *et al*., 2014).

Four fungi species were identified in the plant samples and the nymph of *P. fusca.* This implicated *P. fusca* as a possible vector that introduces fungi into the plant tissue. This corroborated the report of Rohfritsch (2008) that during oviposition, some hemipterous insects lay eggs together with fungal conidia on the leaf surface. Mamaev (1975) also reported that plant tissues could be broken down by insect nymphs using a saprophytic medium garnered with fungal mycelia. It was purported that gall formers (psyllids) damage plant tissues synergistically with the help of saprophytic fungi. Sperber and Collevatti (1996) reported that endo-parasites are usually attached to the nymphs of the most psyllid. These parasites are reported to mummify the nymphs in the gall chamber usually. In some other plant galls, fungi mycelia have been reported to be attached to the gall chamber interiors, providing the insect with food through the induction of lysis and rupturing of vascular bundle channels (Rohfritsch, 2008). Results from this study showed evidence of these features and characteristics.

Galls formed due to insect attacks have been reported to accommodate certain fungi. This act has made the relationship between plant and insect complex and uncertain (Batra and Lichtwardt, 1963). This study identified *Fusarium solani, Collectotricum coccodes, Aspergillus niger* and *Fusarium oxysporum* in the gall of *M. excelsa* as well as

the nymphs of *P. fusca*. Fusarium species are widespread pathogens that cause cortical rots and vascular wilts and are also involved in the dieback decline of *M. excelsa* seedlings (Apetorgbor *et al*. 2001; Mancini *et al*. 2001).

Fungi on healthy plant leaves showed no sign of damage on the plant. However, this study's role in this fungus is not limited to the process that led to secondary infestation on *M. excelsa* seedling after gall rupture, a stage when the insect had exited the gall, thereby creating a conducive environment for the fungi to be virulent. Conversely, the presence of fungi in the un-ruptured galled of *M. excelsa* without symptoms of dieback diseases is likely an indication that the insect has a relationship with fungi. The role of the fungi in this relationship from this study is suspected to function as the gall tissue organiser and nutritive device. These saprophytic fungi are suspected of breaking down the plant tissue while the insect, through its specialised mouthpart, gets nutriment from the fungi mycelia inside the leaves tissue. The result showed tissue degradation around the nymph chamber (nutritive tissues).

5.4 Gall morphology of *Milicia excelsa*

This study affirmed earlier reports that *P. fusca* forms gall on *M. excelsa* plant parts, especially the leaves. The insect mainly attacks young and vigorously growing seedlings, typical for gall-forming hemipterans. *Phytolyma fusca* form galls in *M. excelsa* leaf tissue (the portion of active growth) after the female insect oviposit its egg(s) on the leaf surface and the egg(s) hatches and larvae pieces through the leaf epidermis to the parenchyma cells (Ugwu and Omoloye, 2015). Galls were observed to be formed in a single or groups on the leaves, and they coalesce depending on the number of larvae that emerge or pierced through the leaf epidermis. The galling process is first started with the swelling of parenchyma cells, thereby creating a space inside the leaf tissue (gall chamber), and this chamber is connected to the outside by a constricted opening. At the start, the colour of gall formed on plant leaf tissue is green and juicy till maturity before it ruptures. *Phytolyma fusca*, just like other insects in the order Hemiptera, can induce galls in their host plant (Wool *et al*., 1999; Raman, 2003). According to Arduin *et al.* (2005), class Psyllidae has almost about 350 species of gall formers.

Histological analysis of *M. excelsa* gall suspected that galling is induced through hypersensitive reaction, which led to the consistent production of hydrogen peroxides and swelling of plant tissue at the point of attack. *Phytolyma fusca*, just like other psyllids, have five nymphal instars, and they display mechanisms that keep them safe from harsh environmental conditions. This is true because harsh or unfavourable environmental conditions such as high humidity negatively affect these insects' activities (White, 1960). The presence of ostiole on the transverse section of the gall showed that the insect, after penetrating the leaf tissue, may cause the plant to produce wax to seal the point of entrance partially. Christiano (2002) reported that *Pseudophacopteron sp*., a gall-forming insect on *Aspidosperma austral* uses ostiole to maintain linkage with the immediate external environment.

Milicia excelsa leaf receives lots of leaf tissue damages due to gall formation by *P. fusca*. On the gall epidermal cells, this alteration is evident as seen in the folding or bending of the leaf to form a mass and the neoformation of the ostiole trichome. This supported the findings of Meyer and Maresquelle (1983) that trichomes act as a structural defence for the ostiole in different galls.

5.5 Gall Anatomy of *Milicia excelsa*

Generally, plant leaf resources are believed to be drained by gall-forming insects through the gall formed (Larson, 1998; Nyman and Julkunen-tiito, 2000). Anatomical features of the healthy and galled leaf of *M. excelsa* indicated that the insect larvae penetrated through the leaf epidermal cell to create a nymphal chamber where it gets shelter and nutrition. This is true for gall-forming insects whose feeding mouthparts are for sucking.

They insert their stylets deep down into the internal cells, such as phloem of the plant tissue, injecting viscose materials, thereby forming a salivary sheath (Rohfritsch and Anthony, 1992). According to Crew *et al.* (1998), the function of gall-forming insect salivary sheath is still being investigated but could be suspected to help the insect to insert its proboscis into the plant tissue or prevent the plant from unleashing defence substance in order to resist the damage inflicted upon it. In *M. excelsa* gall, the salivary sheath was observed almost in every cell area of the gall sections. It was evident that the nymph was

feeding on plant nutrients around the parenchyma cell, xylem and vascular bundle. Crew *et al*. (1998) observed that hemipterous insects suck plant nutrients from the xylem. Rohfritsch (1992) and Raman (2003) also acknowledged that penetration by Hemiptera is intercellular with the breaking down of different plant tissue cells, especially the middle lamella. According to Raman (2003), first instar nymphs gets nutriments from fluid in the cells tissue while the conducting tissue remains intact, unlike in the character of the free-feeding sucking insects. However, this is not the case for *Phytolyma fusca*, as observed from this study. The expansion of salivary sheaths to the endothelial cells is an indication that the *Phytolyma fusca* nymphs get nutrients from their content. The nutrient finding activity can justify this act because chambers in the swelling phase in gall development are empty. The vascular system seems to be altered by *P. fusca* nymphs to divert plant nutrients directly to the nymphal chamber. Wool *et al*. (1999) reported similar traits in other plant galls. Anatomical features of gall induced by *P. fusca* on *Milicia excelsa* supplement existing information on gall biology and reveal the plasticity of plant tissues stimulated by biotic factors.

5.6 Effect of Manure on the Growth and Gall Formation of *Milicia excelsa*

This study's data confirmed that application of manure; Poultry dropping, Cattle dung and Pig faeces stimulated the growth of *M. excelsa* seedlings and *P. fusca* attack on *M. excels*a, causing gall formation. This finding corroborated the report of Jahn (2004) that soil amendment aids in the production of broad, fresh and soft leaves, which are of high preference needed for oviposition by insects on plants. Setamou *et al.* (1993; 1995) also confirmed that fertilisation usually increases the number of damages caused by insects on the plant. In this study, there were low to relatively reduced *P. fusca* damage on *M. excelsa* seedlings grown on soil without fertilisation to seedlings grown with manure during the study period (March – October 2019). Seedlings not treated with manure had the highest chances of survival and stunted growth. This indicated that *M. excelsa* is a good source of food and nourishment for *P. fusca*, and the insect strives better on healthy and high vigour growing *M. excelsa* seedlings. This finding contradicted the report of Godase and Patel (2001) that the application of manure causes the reduction of the insect population. Similarly, Miguel and Clara (2003) detected that plants grown on

manures amendments showed less insect attack, which may be due to the reduced amount of nitrogenous elements in manures.

Findings from this study also indicated that seedlings treated with poultry droppings and pig faeces had the potential to survive *P. fusca* attack under active growth over time better than seedlings treated with cattle dung and those unfertilised. This could be true due to the plant's ability to synthesise or produce phytochemicals that will boost its immunity and build plant resistance against the insect pest and any possible outbreak of secondary infestation. The relative growth rate among the various treatments indicates that amendment with manure has an important impact on *M. excelsa* seedlings' growth and gall formation. In this study, soil amendment using manure boosted the growth and survival of *M. excelsa* and increased the number of galls formed. Soil amendments positively impacted the plants' growth compared to plants grown in marginal soil. This supported the report of Mochiah *et al*. (2011) that soil amendments are crucial for plant growth and vigour and must be strategically adopted. Also, pest controls should be strategically carried out in such a way as to lessen insect pest damages to the productivity of plants.

5.7 Effect of solid and liquid poultry droppings and NPK on the Growth and Gall Formation of *Milicia excelsa*

This study evaluated the response of *M. excelsa* seedlings to soil amended with solid and liquid forms of poultry droppings and NPK fertilisers. Soil macronutrients are essential nutrient elements required for plant growth and development. They play important functions in plant metabolism and energy production.

Application of organic and synthetic fertilisers increased *P. fusca* galls on *M. excelsa* seedlings. There were low numbers of galls formed on *M. excelsa* seedlings grown with liquid poultry dropping and NPK than on those grown with solid poultry droppings during the study period (July 2019 – February 2020). This contradicted the report of Sureka and Rao (2001) that fertilisation using vermicompost showed promising results in the effective reduction of insect pest population in the okra plant. Similarly, Yardim and Edwards (2003) reported the effect of fertiliser sources on insects and their predator's associates on tomatoes plant.

Findings from this study indicated that both solid and liquid poultry dropping (organic fertiliser) and NPK (inorganic fertiliser) do not reduce *P. fusca* attacks but can aid the plant to build resistance over time. According to Sétamou *et al*. (1993; 1995), soil amendments often intensify the number of damages caused by insects on plants. Fallahpour *et al*. (2015) also reported that the excessive nitrogen supplement to soil stimulates plant-insect interface and automatically attracts more insect pests and insect reproduction. Sinha *et al*. (2018) also reported that the amendment with lots of nitrogen fertiliser conspicuously increases the oviposition potential of the Asian corn borer on maise leaves.

Milicia excelsa seedlings treated with solid and liquid poultry dropping grow better than seedlings treated with NPK fertiliser and seedlings growing on soil not amended. Seedlings treated with solid poultry dropping responded better to all other treatments. This contradicts the findings of Borokini *et al.* (2011) that nutrient is taken up and assimilated by plant roots in liquid dissolved form than those treated in solid forms. However, Decker (1993) noted that liquid manure act as a growth stimulus under unique and difficult condition. The quantity of manure applied to the soil does not significantly affect the seedlings' growth except for seedlings treated with different dosages of NPK fertiliser. This corroborates the findings of Uhart and Andrade (1995) that leaf area, leaf area index and crop photosynthesis increase under increased nitrogen supplements, which invariably leads to waste and pest problems such as increased reproduction, longevity, and overall fitness of the insect.

It was observed that seedlings treated with liquid poultry droppings were advantaged over those with solid poultry droppings and responded differently to nutrient availability which invariably affected the gall formation on the seedlings. This could be that solid poultry droppings need to decompose further before the plant can absorb the nutrient. Generally, the solid and liquid poultry droppings and NPK treatments recorded a high percentage of survival of *M. excelsa* seedling at all levels, but at a higher dosage, seedlings treated with NPK had a low survival percentage. Hosseini *et al*. (2015) reported an improved rate of natural increase by aphids on plants grown on soil amended with full capacity of nitrogen elements. Ukwungwu (1985) and Setamou *et al.* (1993; 1995) demonstrated that crop damage is a function of improved soil amendments. Soil

amendment with manure and NPK fertiliser increased gall formation in *the M. excelsa* plant. This is probably due to the amendment's increased supply of nitrogen elements.

5.8 Effect of different combinations of Poultry droppings, pig faeces and cow dung on the Growth and Gall Formation of *Milicia excelsa* seedlings

This study showed that *P. fusca*, a major insect pest of *M. excelsa*, is present throughout the year, and it attacks healthy and vigorously growing *M. excelsa* seedlings. There were severe attacks on *M. excelsa* seedlings treated with different combinations of manure. The abundance of the insect, as evident in its gall formation, is suspected to be dependent on the nutrition status of the young growing seedling. This confirmed the report of Sinha *et al.* (2018) that nitrogen deposits hypothetically stimulate the increase in insect population and availability of plant resources.

Hosseini *et al*. (2015) also reported that crops grown on soil with high nitrogen levels tend to assist insect population growth. Wale *et al*. (2006) also corroborated the hypothesis of the influence of nitrogen fertiliser on insect abundance and reproduction and damages done. Therefore, the effect of soil amendment is directly proportional to its inherent nutrient status and the ability of the insect to cause damage.

A severe attack rate (gall formation) was observed for *M. excelsa* seedling treated with manure composed of poultry droppings, cattle dung and pig faeces, whereas seedlings planted on topsoil showed a minimum level of attack (gall formation). Rousselin *et al.* (2016) submitted that enhanced nitrogen nutrient fertilised soil stimulated plant growth and vigour, fostering insect pest population.

The application of manure compost has been identified to cause a great impact on a plant's ability to resist or be susceptible to pest infestation (Sinha *et al*., 2018). This attribute is stringently connected to the soil's best Physico-chemical properties and biological characteristics. Soils rich in organic matter and minerals are the epitome of fertile soil with a complex food web and beneficial organisms. Magdoff and Van (2000) also opined that organically grown crops have high tolerance and resistance to insect pests. The effect of composts of poultry droppings, pig faeces, and cattle dung showed seedlings growing on amended soil performed better than those without poultry droppings and

unfertilised. *Milicia excelsa* seedlings treated with the combination of poultry droppings performed better in growth variables. Moreover, the insect *P. fusca* attacked healthier, nutritious, and succulent leaves. It was observed from this study that leaf production (leaf number) was seriously affected by gall formation two weeks after the experiment was set up.

Therefore, this study showed that the availability of sufficient essential nutrients in the soil determines the amount of damage received by *M. excelsa* by *P. fusca* and the ability of the plant to recover or/and withstand such damage.

This supports the report of Sinha *et al.* (2018) that soil amendments affect plant physiological susceptibility to insect attack through building plant resistance or manipulating its susceptibility.

Soil nutrition management practices are the basic contributing factor for attaining high plant yield and plant response to insect attack.

Understanding this practice with insect pests is the basic system principle for high yield production (Chau and Heong, 2005).

CHAPTER SIX
CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Phytochemicals constituent in *M. excelsa* leaf is important to *P. fusca* for host plant location, especially as a feeding and mating sites. These phytochemical compounds are suspected of aiding in host finding by *P. fusca.* Compendium of phytochemicals identified from *M. excelsa* vary considerably, and the ratios in which they are released is important. The study suggested for the first time that the presence and abundance of Phytol and Dihydropinene and other secondary metabolites explains the attraction of the *P. fusca* to *the M. excelsa* plant. The presence and abundance of these compounds may not necessarily be the only determining factor explaining *P. fusca* preference to *M. excelsa* host. However, the presence and abundance of these chemical compounds in the healthy leaf indicate that the midge utilises this compound for its advantage.

From this study, it could be inferred that Dihydropinene and Phytol, a monoterpene and diterpene, respectively, is utilised by *P. fusca*. The quantitative phytochemical constituent in healthy and galled leaves showed changes in the vital cellular content of *M. excelsa*. Gall tissue development is linked to variation in the number of phytochemical compounds present in the plant. From the quantitative analysis, there were drastic decreases in the terpenoids, tannin and flavonoids contents in the galled leaf compared to the healthy plant leaves.

Plant galls are tumour-like growths produced on plant organs by insects and other pathogens alike. This study affirmed that *P. fusca*, a hemipteran, induces gall on *M. excelsa* seedlings. The breakdown of plant tissue around and away from the nymph chamber indicated the presence of saprophytic fungi inside the gall.

The presence of fungi in the gall is an indication that the insect has a relation with fungi. The fungi are suspected to function as an organiser of gall tissues and a nutritive device. The fungi are suspected of breaking the plant tissue while the insect gets nutriment through its specialised mouthpart.

Findings revealed that the incidence of secondary infestation (dieback diseases) on *M. excelsa* is because of the introduction of fungi into the plant tissues by *P. fusca*. It provides practical information on the association between pathogen and *P. fusca* on *M. excelsa* and its role.

Soil amendment with manure stimulated the growth of *M. excelsa* seedlings and *P. fusca* attack. The relatively reduced populations of *P. fusca* on *M. excelsa* seedlings grown without fertiliser to seedlings grown with manure during the study period showed the feeding relationship between the duos.

This indicated that *M. excelsa* is a good source of food and nourishment for *P. fusca*, and the insect strives better on healthy and high vigour growing *M. excelsa* seedlings. Findings from this study also indicated that seedlings treated with poultry droppings and pig faeces had the potential to survive *P. fusca* attack under active growth over time better than seedlings treated with cow dung and seedling growing on topsoil (no manure).

6.2 Recommendation

1. Further experiments should be carried out to investigate the role of terpenoids in the interaction between *P. fusca* and *M. excelsa* seedlings.

2. Seedlings of *M. excelsa* should be allowed to grow to the sapling stage of about eight to 16 month-old in the screen house before transplanting to the field to boost their chances of survival.

3. Insecticides combined with fungicides is highly recommended for use in the control of *P. fusca* on *M. excelsa* due to the incidence of fungi infestation after the insect attack.

4. A holistic management of *M. excelsa* using organic amendments and the application of insecticides and fungicides should be adopted for the effective and efficient management of the insect pest and to boost the chances of survival of the plant.

5. Organic amendments should be applied in the season when the insect population is scanty.

6.3 Major contribution to Knowledge

1. Terpenes are the predominant phytochemical constituent in *M. excelsa* leaves.
2. *Phytolyma fusca* attack on *M. excelsa* seedlings lead to the loss of terpenes (Phytol and Dihydropinene) in the plant leaves.
3. Hydrogen peroxide was produced in *M. excelsa* leaves as a response to oxidative stress due to the piercing mouth part of *P. fusca*.
4. *Phytolyma fusca* attacked healthy growing *M. excelsa* seedlings succulent plant parts.
5. Soil amendment with poultry droppings or pig faeces boosted the growth of *M. excelsa*, causing *P. fusca* to be attracted to healthy and vigorously growing seedlings.
6. Major fungi associated with *P. fusca* gall formation on *the M. excelsa* plant are *Fusarium solani* and *Fusarium oxysporum*

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APPENDIX

Appendix 1: Physico-chemical composition of soil used in the management of *Phytolyma fusca* on *Milicia excelsa*

S/N	Chemical Composition	Soil
1	Ph	6.60
2	% Sand	83.0
3	% Organic Carbon	1.86
4	% Clay	7.0
5	Exch. Acidity	0.30
6	Total Nitrogen	0.21
7	% Salt	10.0
8	P (Mg/Kg)	58.50
9	Ca (Cmol/Kg)	13.64
10	Mg (Cmol/Kg)	0.95
11	K (Cmol/Kg)	0.13
12	Na (Cmol/Kg)	1.53
13	Mn (Mg/Kg)	0.31
14	Fe (Mg/Kg)	9.52
15	Cu (Mg/Kg)	1.86
16	Zn (Mg/Kg)	0.50

Appendix 2: Nutrient Composition of manures used in the management of *Phytolyma fusca* on *Milicia excelsa*

S/N	Nutrient elements	Poultry Droppings	Pig Faeces	Cattle Dung
1	% Total Carbon	31.5	80.5	39.5
2	% Total Nitrogen	1.63	2.31	1.94
3	% Total Phosphorus	0.001	0.002	0.001
4	% Ca	0.246	0.207	0.321
5	% Mg	0.118	0.196	0.117
6	% K	0.195	0.196	1.311
7	% Na	0.539	0.944	0.180
8	Mn	1.05	4.82	0.75
9	Fe	0.05	0.015	0.005
10	Cu	13.64	9.38	5.36
11	Zn	13.08	4.48	2.54